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## **ANIMAL PRODUCTION**

## USING FACILITIES OF APILARNIL (BEE DRONE LARVAE) IN POULTRY NUTRITION

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*Preliminary communication*

### Summary

The use of hormones in poultry production has not been allowed in order to protect consumer health in the United States and EU countries for more than 50 years. In Turkey, the administration of hormones and hormone-like substances to poultry diets was banned in 1973. This perspective has stimulated many scientists to search for new and safe alternatives.

Honey bees and bee products have been used in treatment of human, animal and plant diseases as “Apitherapy Applications” for a long time. In recent years, some studies have been conducted to determine the availability of honey, royal jelly, pollen, bee venom and propolis in livestock animals. In poultry studies, it was investigated the antioxidants and antimicrobial effects of bee products and discussed in their usable potential as natural growth enhancers. In this context, apilarnil is an another bee products, which is currently being examined.

Apilarnil, is a drone larvae extract, which also contains small amounts of royal jelly, bee bread, honey and propolis. Usually it is not utilised and honeycombs with apilarnil are cut and discarded by bee-keepers. However, apilarnil has many useful properties: it is an antiviral, immune system enhancer, anabolic stimulator, and increases appetite, the body’s energy, vitality and regenerative power. Moreover, because it comes mainly from a male structure, apilarnil is very rich in androgenic hormones, so it stimulates the spermatogenesis in men. Therefore, it is expected that apilarnil would have both androgenic and anabolic effects and can be considered as a natural alternative to drugs and chemicals to stimulate sexual development.

In this review, it will discuss the potential for using apilarnil, a natural bee product, instead of banned anabolic compounds.

**Key words:** *apilarnil (bee drone larvae), poultry production.*

### INTRODUCTION

Naturally occurring hormones in human and animals are essential for various physiological processes. Steroids hormones play important roles in growth, development, reproduction and social behavior (Passantino, 2012). Gonadal steroids affect development either by increasing protein synthesis through bounding directly to special intracellular receptors or by indirectly stimulating the excretion of growth hormone and other anabolic hormones (Fennell *et al.*, 1996; Lawrence and Fowler, 2002). For this reason, gonadal hormones have been used for long years in mammals, especially in cattle and sheep, in order to increase meat yield (Lawrence and Fowler, 2002; Scanes, 2003). The six hormone types most widely used in meat production include three natural hormones (oestradiol 17 $\beta$ , testosterone, and progesterone) and three synthetic substances (trenboloneacetate mimics testosterone, melengestrol acetate mimics progesterone and zeranol mimics estradiol) (Passantino, 2012). However, the use of anabolic compounds in livestock and poultry is now prohibited in the EU countries in order to protect animal welfare and carcinogenic potential including cancer of breast, ovary, testes, prostate and colon (Scanes, 2003). Unlike EU, poultry and beef cattle industries operate under different regulation in USA. While the use of growth hormones has not been allowed for rearing poultry in the 1950s, it is legal and accepted in beef cattle production. In Turkey, the administration of hormones and hormone-like substances to animal diets was banned in 1973. Following these developments, it has began to search for new and safe alternatives. In this review, it will discuss the potential for using apilarnil, a natural bee product, in poultry production as feed additives instead of banned hormones and hormone-like substances.

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## HORMONES USE IN POULTRY INDUSTRY

Synthetic or natural androgens stimulate breeding performance, sexual behaviors and secondary sex characteristics as well as muscle development in mammals (Lawrence and Fowler, 2002; Frandson *et al.*, 2009). In the limited number of previous studies carried out on poultry, some androgens have been reported to have mainly anabolic effects (stimulating muscle development) and that some androgens have mainly androgenic effects (stimulating male breeding performance) (Fennell and Scanes, 1992a; Scanes, 2003). Testosterone is a major androgen (Scanes, 2003) and has equal anabolic and androgen effects (Fennell and Scanes, 1992a). Fennell and Scanes (1992a) determined that androgen implantation (testosterone, 5  $\alpha$ -dihydrotestosterone, 19-nortestosterone) increased body and muscle development; reduced feed:gain ratio and abdominal adipose tissue weight, yet did not affect shank-toe length in female and male turkeys. Similarly, Maruyama *et al.* (1996) determined that growth rate increased when testosterone pellets were implanted in castrated and intact male turkeys. These positive effects of androgen administration on turkeys could not be detected in chickens. Fennell and Scanes (1992b) determined that androgen administration in chickens did not stimulate growth, yet increased comb and wattle development. Similarly, in another study carried out by Fennell *et al.* (1996), it was determined that body development (body weight, average daily gain, shank-toe length and breast muscle weight) and bursa of Fabricius weight showed a decrease; however, comb weight increased in roosters that were administered testosterone in 2-6 weeks period. According to Holst-Schumacher *et al.* (2010), steroid hormones do not constitute a good growth promoter in broilers. The reason for this suggestion is that steroid hormones are very short-body in the bloodstream of non-laying birds since they have a higher metabolic clearance rate than in laying birds. All these reports point out that androgens have an androgenic effect rather than an anabolic effect on chickens (Fennell and Scanes, 1992b).

## APILARNIL (BEE DRONE LARVAE)

Apilarnil, a honey bee product, is a drone larvae extract, which also contains small amounts of royal jelly, bee bread, honey and propolis. The extract from drone larvae and bee brood has a very complex composition, similar to royal jelly. It is a “luxury” food rich in proteins. It was reported that apilarnil contains 25-35% dry matter, 9-12% proteins, 6-10% carbohydrates, 5-8% lipids, 2% ash and 3% unidentified substances (Matsuka *et al.*, 1973; Stangaciu, 1999). Usually it is not utilized and honeycombs with apilarnil are cut and discarded by beekeepers because of Varroa (bee parasites) attractance. Apilarnil is obtained by extracting from the special drone larvae comb cells followed by; trituration (crushing, grinding), homogenisation, filtration and lyophilisation. Only after these mentioned processing can apilarnil be stored and/or used properly (Stangaciu, 1999).

## APILARNIL USE IN POULTRY NUTRITION

There is growing public dissatisfaction with the use of synthetic supplements in poultry production. Therefore, natural products (probiotics, prebiotics, organic acids, enzymes, herbs and aromatic plant extracts etc.) can be considered as growth promoters to improve production performance. In recent years, some studies have been conducted to determine the availability of honey, royal jelly, pollen, bee venom and propolis in livestock animals (Tolon *et al.*, 2002; Çelimli, 2005; Han *et al.*, 2009; Guo and Ding, 2010; Öztürk *et al.*, 2010; Arpášová *et al.*, 2013; Kačániiová *et al.*, 2014; Seven *et al.*, 2014). In the studies conducted with poultry, it was investigated the antioxidants and antimicrobial effects of bee products especially in propolis (Seven *et al.*, 2009; Tekeli *et al.*, 2010) and discussed in their usable potential as natural growth enhancers (Açıkgöz *et al.*, 2005; Shalmany and Shivazad, 2006; Seven and Seven, 2008; Seven *et al.*, 2008).

Apilarnil is an another bee products, which is currently being examined. It has many properties such as, anabolic stimulator, antivirals, immune system enhancers which also increase the appetite, increase the body's energy, vitality and regenerative power (Iliescu, 1993; Stangaciu, 1999). Moreover, because it comes mainly from a male structure, apilarnil is very rich in androgenic hormones, so it stimulates the spermatogenesis in men (Constantin, 1989; Iliescu, 1993). Therefore, it is expected that apilarnil would have both androgenic and anabolic effects and can be considered as a natural alternative to drugs and chemicals to stimulate growth and sexual development.

Few studies conducted with broilers to determine the effects of apilarnil administration on growth performance and sexual development have been reported in the literature. Yücel *et al.* (2011) found that administration of 4

g/day apilarnil to male broilers during growth period (from 22 to 42 days) did not have a beneficial effect on the body weight, feed intake and feed conversion ratio. However, 4 g/day oral apilarnil administration stimulated the development of secondary sex characteristics (length of comb and length and width of wattle) of male broilers. These results imply that apilarnil has mainly androgenic (testosterone-like) effects rather than an anabolic effect on male chickens. In a later study by Altan *et al.* (2013), low (2.5 g/bird) and high (7.5 g/bird) doses of apilarnil administration did not cause a positive effect on growth performance of male and female broilers. Birds receiving apilarnil suppressed blood glucose and cholesterol concentrations and remained immobile for a shorter period in a tonic immobility test and showed less home-cage avoidance responses suggesting a lower level of fearfulness. They were also observed increases in testicular weight, testosterone concentration and comb growth in males receiving apilarnil implied that it may be possible to stimulate precocial sexual development and early sperm production with apilarnil administration at an early age. According to these authors, early sexual maturation by apilarnil administration may provide an opportunity for extension of reproductive lifespan and decreased generation time in males of elite stock, thereby, also reducing costs in the poultry sector.

## CONCLUSION

Apilarnil is a natural bee product rich in androgenic hormones which stimulate growth and sexual development. Because of this properties, it has been recently began to investigate on its usability as feed additives in poultry production. Especially, more detailed studies are required to determine potential androgenic effect of apilarnil. Therefore, carrying out such studies with male broiler breeders will be more beneficial. In addition, preparation of apilarnil preperats, which can be added to feed, will facilitate its commercial usability.

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## INFLUENCE OF PREGNANCY STAGE ON BLOOD BIOCHEMICAL PARAMETERS IN ALPINE GOATS

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*Original scientific paper*

### Summary

The aim of this paper was to determine the influence of pregnancy stage on biochemical parameters in the blood of Alpine goats. The research included 21 French Alpine goats who were divided per 7 in the group according stage of pregnancy (up to 3 months, from 3 to 4 months, and more than 4 months). Feeding of goats was with a mixture of grains and meadow hay. In the blood of goats were determined concentrations of the biochemical parameters (urea, glucose, total proteins, albumins, globulins, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, NEFA- non esterified fatty acids and creatinine as well as enzyme activity: CK-creatinine kinase, GGT- $\gamma$ -glutamyl transferase and ALP- alkaline phosphatase). As pregnancy progressed a significant decrease in the content of total protein (from 77.73 to 65.87 g/L), albumin (from 31.70 to 28.18 g/L) and globulin (from 46.03 to 37.69 g/L) as well as activity of GGT (from 41.12 to 29.04 U/L) and an increase concentrations of urea (from 2.29 to 3.11 mmol/L), triglycerides (from 0.27 to 0.50 mmol/L) and NEFA (from 0.03 to 0.06 mmol/L) were determined. Comparing determined biochemical parameters with reference values in the blood of goats during various stages of pregnancy, significant differences were not evident, except concentration of urea and albumin, which were at the lower limit and concentration of globulin, which were at the upper limit of reference values for goats. Mentioned biochemical parameters changes in the blood of goat in late pregnancy (urea and albumin) indicate a lack of protein in the feed and the need to include stages of pregnancy and breed as important factors in quality monitoring of herd.

**Key words:** *biochemical parameters, enzyme activity, blood, Alpine goat*

### INTRODUCTION

In Croatia, according to a Croatian Agricultural Agency, during 2014 are rearing around 65000 breeding goats. Breeding and selection work was conducted on 6480 breeding stock at 96 growers on 7 goat breeds. Analysing the proportion of all goat breeds in the Republic of Croatia on which control of production characteristic is carried out, the most grown is Alpine (4784), which is 73.83% of the total goat number that are under selection coverage. Alpine goat in Croatia are rearing more than 30 years. Accomplished production results alpine goat under selection coverage (index of kidding 1.00; litter size: 1.12; total quantity of milk: 628.14 L as well as average birth weight: 3.71 kg; HPA, 2015) indicate the quality breeding. It is known that pregnancy is a very demanding and stressful period for the animal and it should be given considerable attention. During pregnancy may happen significant metabolic changes that can lead to health disturbances and productivity of animals. The nutrition requirements of goats increase during late pregnancy due to the rapid growth of the foetus. If small ruminants do not receive at least half of the energy during this period, fat depots are mobilised in large quantities (Firat and Ozpinar, 2002). In high pregnant goats with more foetuses can often get to the metabolic diseases (Rook, 2000). Monitoring the concentration of biochemical parameters in the blood of small ruminants give us a clearer picture of their nutritional and health status before the changes are visible on the animal (Antunović *et al.* 2002 and 2009). In the available literature can find it quite of papers on the change of biochemical parameters in pregnant goats of different breeds, while about alpine there is no available research. Research on biochemical indicators in the blood of Danish Landrace dairy goat spent Mbassa and Poulsen (1991), in the blood of pregnant Sahel goats Waziri *et al.* (2010) and Sandabe *et al.* (2004), in the blood of pregnant German Improved Fawn goats (Samardzija *et al.*, 2013). The aim of this paper is to determine the influence of pregnancy stage on biochemical parameters in the blood of Alpine goats.

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## MATERIALS AND METHODS

The research included 21 French Alpine goats. Goats were 4 years old, healthy and in good condition. Goats were in the third pregnancy with a single fetus and fertilized with the same buck. The research was conducted during the winter feeding season on the family farm in Slavonia. Goats were divided according to the stage of pregnancy per 7 in the group as follows: up to 3 months, from 3 to 4 months and more than 4 months of pregnancy. Goats were housed together in groups in boxes, depending of the pregnancy. Feeding of goats was with a mixture of grains (50% corn and 50% barley) at the quantity of 1 kg and meadow hay *ad libitum*. Goats were also consuming salt and water *ad libitum*.

Blood from pregnant goats was collected from the jugular vein into both serum Vacutainer tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium). After that, the serum was separated by centrifugation (10 min) at 3000 revolutions/min on centrifuge Rotofix 32 A (Hettich). In the blood serum concentrations of the biochemical indicators (urea, glucose, total proteins, albumin, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, NEFA-non esterified fatty acids and creatinine as well as enzyme activity: CK-creatine kinase, GGT- $\gamma$ -glutamyl transferase and ALP- alkaline phosphatase) were determined. Globulin concentrations were calculated according to the formula total protein-albumin and the ratio of albumin/globulin by dividing these values. All biochemical parameters were determined with Olympus System Reagents (OSR), manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland, manufactured for Olympus Diagnostic GmbH, Hamburg, using Olympus AU 400 apparatus.

The results were statistically evaluated using LSD test (Statistica, 2008) to determine the effect of stage of pregnancy goats on biochemical parameters. Results were expressed as mean (mean) and standard deviation (s). Differences were considered as significant at the level of ( $P < 0.05$ ) or less.

## RESULTS AND DISCUSSION

A significant decrease in the concentration of total protein, albumin, globulin and increase of urea were determined as the pregnancy progressed (Table 1).

Tab. 1. Influence stage of pregnancy Alpine goats on blood biochemical indicators

Parameters, mmol/L	Pregnancy stage (Mean $\pm$ s)			Reference ranges and mean*
	< 3 months	3-4 months	> 4 months	
Urea	2.80 $\pm$ 0.65 <sup>ab</sup>	2.29 $\pm$ 0.21 <sup>a</sup>	3.11 $\pm$ 0.90 <sup>b</sup>	2.80-7.20 (4.80)
Glucose	3.06 $\pm$ 0.36	2.99 $\pm$ 0.31	2.83 $\pm$ 0.34	2.30-3.60 (3.00)
Cholesterol	1.85 $\pm$ 0.30	1.90 $\pm$ 0.16	2.15 $\pm$ 0.56	1.60-3.70 (2.40)
HDL-cholesterol	1.10 $\pm$ 0.17	0.92 $\pm$ 0.42	1.19 $\pm$ 0.23	-
LDL-cholesterol	0.63 $\pm$ 0.17	0.76 $\pm$ 0.35	0.77 $\pm$ 0.29	-
Triglycerides	0.27 $\pm$ 0.14 <sup>a</sup>	0.50 $\pm$ 0.18 <sup>b</sup>	0.42 $\pm$ 0.20 <sup>ab</sup>	0.10-0.50 (0.20)
NEFA	0.04 $\pm$ 0.02 <sup>ab</sup>	0.03 $\pm$ 0.01 <sup>a</sup>	0.06 $\pm$ 0.02 <sup>b</sup>	-
Total protein, g/L	77.73 $\pm$ 5.09 <sup>Aa</sup>	71.02 $\pm$ 4.56 <sup>b</sup>	65.87 $\pm$ 7.21 <sup>B</sup>	59.00-82.00 (72.00)
Albumine, g/L	31.70 $\pm$ 0.54 <sup>Aa</sup>	28.38 $\pm$ 1.36 <sup>B</sup>	28.18 $\pm$ 2.89 <sup>b</sup>	29.00-40.00 (32.00)
Globuline, g/L	46.03 $\pm$ 4.82 <sup>A</sup>	42.92 $\pm$ 4.62 <sup>AB</sup>	37.68 $\pm$ 6.07 <sup>B</sup>	27.00-41.00 (36.00) <sup>1</sup>
Ration Alb/glob	0.70 $\pm$ 0.08	0.67 $\pm$ 0.09	0.77 $\pm$ 0.14	0.60-1.30 <sup>1</sup>
Creatinine, $\mu$ mol/L	66.34 $\pm$ 4.42	65.98 $\pm$ 8.54	63.80 $\pm$ 4.84	39.00-67.00 (52.00)

\*Tschour *et al.* (2008); <sup>1</sup>Smith (2002); <sup>A, B, C</sup>- means with different superscript letters differ significantly ( $P < 0.01$ ); <sup>a, b</sup>- means with different superscript letters differ significantly ( $p < 0.05$ ); s –standard deviation; NEFA- non esterified fatty acids

A significant increase of triglycerides was observed in goats during pregnancy from 3<sup>rd</sup> to 4<sup>th</sup> month and later a smaller decline in late pregnancy, but with no significant difference ( $P > 0.05$ ). NEFA concentrations varied so that there was a significant increase in late pregnant goats compared to the earlier stage of pregnancy. Also, a smaller decrease in serum glucose and creatinine concentrations as well as increase of cholesterol concentration were found as pregnancy progress, but the differences were not significant ( $P > 0.05$ ).

Comparing established biochemical indicators in the blood of goats during various stages of pregnancy with reference values, significant differences were not determined, except concentration of urea and albumin. Concentration of urea in serum is an indicator of feed protein status (Kohn *et al.*, 2005). In present investigation we determined that serum urea concentration were on lowest reference range while concentration

of NEFA increased in blood of pregnant goats. Higher urea concentration in blood of late pregnant goats can be a result of muscle protein catabolism when large amounts of body reserves are mobilized (Whitney *et al.*, 2009). Khatun *et al.* (2011) determined a similar trend of urea increase in the blood of goats as pregnancy progresses. If an animal is unable to consume enough forage to meet maintenance requirements, it uses body reserve, resulting in increased concentrations of serum NEFA and urea due to adipose and protein catabolism (Caldeira *et al.*, 2007). These authors discovered that increase concentrations of NEFA was the first reaction of undernutrition in ewes. High levels of NEFA are indicative of lipid mobilization and fatty acid oxidation (Wathes *et al.*, 2009. Sakha *et al.*, 2006). Albumin concentrations were at the lower limit of the reference values (Table 1), which implies some disbalance between the needs for protein and an increase needs in goat foetus. Specifically, concentrations of albumin are generally an indicator of long-term supply of protein in animal (Payne, 1987). It is known that the albumins are a very important source of amino acids for the necessities of the foetus and the mother (Jainudeen and Hafez, 1989). Decrease of total protein over the pregnancy and lactation could be explained by a rapid extraction of immunoglobulin from the plasma during the last few months of pregnancy when colostrum is being formed in the mammary gland (Kaneko *et al.*, 2008) as well as the increased needs for proteins for the foetus development (Castillo *et al.*, 1997). Also the albumin/globulin ratio parameters in the blood of pregnant goats, was at the lower limit of the reference values, although this ratio should refer to the current changes in the total protein concentration. Globulin concentrations in the blood of goats were at the upper limit of the reference values. All this indicates inadequate nutrition of pregnant goats and a lack of protein in their diets. Similar results in the feeding of sheep fed diets deficient in protein was found by Sahoo *et al.* (2009). Caldeira and Portugal (1991) found that the decrease in the concentration of albumin in the blood of sheep is associated with inappropriate feeding. We observed increase in serum triglyceride during late pregnancy. This is in agreement with the findings of Castillo *et al.* (2003) in research with cows. Blood cholesterol concentrations significantly increased as pregnancy advanced in Sahel goats (Waziri *et al.*, 2010). Reasons for these fluctuations in triglycerides and cholesterol may be because endocrine profiles changes and lipolysis and lipogenesis are regulated to increase lipid reserve during high pregnancy and these reserves are utilizes during parturition and the beginning of lactation (Roche *et al.*, 2009). Lower blood concentration of glucose in pregnancy of sheep correlated is with foetus development, mobilization of maternal glucose to foetal blood circulation (Jacob and Vadodaria, 2001). Similar findings for glucose and cholesterol concentration in Sahel goats with our results, but with significantly difference found Sandabe *et al.* (2004).

Enzyme activities are predictive of the health status of goats (Mahgoub *et al.*, 2008). This increase of GGT activity as pregnancy was coming to the end indicated the preparation of goats for lactation (Table 2). In fact, GGT activity is important in the mammary gland because GGT is freed when the membrane of alveolar cell break and goes into colostrum whose activity become higher than in serum (Sobiech and Wiczorek, 1981).

Tab. 2. Influence of pregnancy stage of goats on blood enzymes activity in organic breeding

Enzymes, U/L	Pregnancy stage (Mean ± s)			Reference ranges and mean*
	< 3 months	3-4 months	> 4 months	
CK	90.50 ± 28.75	87.60 ± 25.88	110.57 ± 54.56	96-268 (182.00)
ALP	106.58 ± 96.33	216.01 ± 157.87	203.81 ± 188.26	31-1941 (508.40)
GGT	41.12 ± 6.87 <sup>Aa</sup>	29.04 ± 4.99 <sup>B</sup>	30.07 ± 6.56 <sup>b</sup>	26-63 (44.00)

\* Tschour *et al.* (2008); <sup>a, b</sup> - means with different superscript letters differ significantly ( $P < 0.05$ ); s –standard deviation; CK-creatin kinase; GGT- $\gamma$ -glutamyl transferase; ALP-alkaline phosphatase

The increase in ALP and CK activities were determined in goats as pregnancy progressed, but the differences were not significant ( $P > 0.05$ ). In blood of sheep Khatun *et al.* (2011) was determined similar ALP activity as stage of pregnancy advanced. Increase or decrease of ALP activity during pregnancy or lactation may be associated with foetal demands and secretion into the milk (Mbassa and Poulsen, 1991). Decrease protein uptake causes muscular destruction and then increases muscular enzyme activities (Fischbach, 2000). In this respect, high levels of serum CK activity is evidence supporting protein deficiency at late pregnancy. Similar results were obtained Gürgöze *et al.* (2009) in Awassi ewes.

## CONCLUSION

Stage of pregnancy significantly affected on some biochemical parameters in the blood of goats. As pregnancy progress concentrations of urea, triglycerides, NEFA significantly increased while concentration of total protein, albumin and GGT activity decreased.

Mentioned changes indicate a lack of protein in the feed of late pregnant goats, and the need to include pregnancy stages and breed as an important factors in the quality monitoring of herd and taking timely correction in rearing goats.

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## THE EFFECTS OF DIFFERENT BROILER GENOTYPES AND ADDING OXYGEN IN INCUBATOR AT HIGH ALTITUDE (1700 M) ON POSTHATCH PERFORMANCE

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*Scientific paper*

### Summary

The objective of this study was to evaluate the effect of oxygen supplement in incubator at high altitude (1700 m) and different genotypes on growth performances of male broiler chicks. All eggs (984 numbers) from two genotypes eggs which were produced by breeders of 42 or 44 wk of age were distributed randomly into two hatching cabinets which were operated. The first cabinet was oxygenated at 25% whereas no oxygen added to the second cabinet between days 18 and 21 incubation and called control group. The number of chicks hatching was recorded day 21. At hatch the chicks were transferred to the broiler rearing facilities at the research farm of Agricultural Faculty. Body weight of day 42 and FCR (Feed Conversion Ratio) was affected by oxygen Supplement and genotype. In this study we observed a reduced feed conversion and a higher weight at the 6th weeks of age when oxygen was added. Final body weight was heavier in Hubbard genotype than in Ross genotype. In Conclusion, it is concluded that extra O<sub>2</sub> supplementation at high altitudes has to be considered in order to improve subsequent performance of broiler.

Key words: *Broiler, incubation, high altitude, oxygen supplement*

### INTRODUCTION

Poultry in the livestock sector has developed rapidly compared with the others and become industrial structure. The most important reasons of this situation, poultry has a high productive power and is convenient to mechanization, poultry meat costs are low and production can be made regardless of climate and soil. Profitability which is the main aim in commercial broiler production is directly affected by saleable number of chicks after incubation; concordantly the quality of chicks acquired has importance, too. There are many factors as parental age, management and nutrition of parents, genotype, altitude, egg weight, maternal immunity and storage conditions before the incubation as well as general incubation conditions (temperature, humidity, ventilation and turning) affect the quality of chicks. Hypoxia can be formed because oxygen amount alter with altitude in nature. Oxygen amount decreases with high altitudes and affects hatching time and hatchability (Visschedijk, 1980). Detrimental effects on embryonic development and hatchability above 984 m have been observed under industry conditions during the artificial incubation of both fowl and turkey eggs At an altitude of 1640 m, it is generally accepted that there will be a depression in hatchability of eggs of eggs type fowls (Moreng, 1983). Embryonic metabolism in last stage incubation is controlled by adequate exchange of oxygen (Rahn *et al.*, 1974). Respiratory gas exchange from the egg in the plateau phase, is determined by the oxygen conductance and the partial pressure gradient of the gas between the ambient air and the inner side of the shell (Hamidu *et al.*, 2007). Oxygen conductance is specific for breed (Janke *et al.*, 2004; O'Dea *et al.*, 2004). Tona *et al.* (2004) showed that broiler breeder genotypes may have different embryonic development and subsequent broiler performances.

The purpose of this study was to examine the effect of oxygen supplement in incubator at high altitude (1700 m) and different genotypes on growth performances of male broiler chicks.

### MATERIAL AND METHODS

A total of 984 hatching eggs obtain from two commercial farms (Elazığ and Malatya province, Turkey) from two modern broiler genotypes (Ross and Hubbard) at 42 and 44 flock ages respectively. Both flocks were reared with similar management and feeding regimens in the same area of Turkey. The experiment was conducted at high altitude (1700 m). Two incubators (Çimuka) were used and 4 replicates by genotype were randomly divided into each incubator. As one of the incubators was supplemented with oxygen (O<sub>2</sub> group) at  $24.5 \pm 0.5\%$

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between d 15 and 21 of incubation, the other was not (Control group) and measured 19% oxygen concentration in the cabinet. The percentage of oxygen was measured with an oxygen meter and flow rates from oxygen or nitrogen storage tanks were adjusted hourly to maintain the desired oxygen level. The eggs were placed in to incubators which were operated at  $37.5 \pm 0.5^{\circ}\text{C}$  dry bulb temperature and 60% RH and were turned twice per hour through d 18 of incubation. The hatcher conditions were given below.

Tab. 1. Incubator profile in the hatcher

(day:hour)	Temperature ( $^{\circ}\text{C}$ )	RH (%)
18:00	37.4	67.3
18:06	37.3	67.5
18:12	37.2	67.5
19:00	37.1	68
19:06	37	68
19:12	37	68
20:00	37	68
20:06	36.9	68
20:12	36.8	67
20:23	36.8	65

After the hatching all chicks were weighted and done feather sexing determination individually. All male chicks were wing-banded and divide in to each replication (30 chicks/group per replicate). Body weight and feed intake were recorded at weekly during the experiment. Mortalities were determined daily during the study. The photoperiod was 23L:1D, and a starter diet (3080 kcal metabolizable energy of kg, 24% crude protein) from d 0 to 10, a grower diet (3190 kcal metabolizable energy of kg, 21% crude protein) from d 11 to 21, a finisher diet (3300 kcal metabolizable energy of kg, 18.5% crude protein) from d 22 to 42 and water were available ad libitum. Broilers were slaughtered at 42 days of age. The data were subjected to ANOVA using the GLM procedure (SAS Institutes, 1998). Statistical significance of differences among means was assessed by Duncan's multiple range test.

## RESULT AND DISCUSSION

The effects of oxygen supplementation and different genotypes on body weight at high altitude were given table 2. Oxygen supplementation and genotype did not have a significant effect on body weight at 1 weeks of age. Although genotype has a significant effect on body weight at 2 weeks of age, oxygen supplementation did not. Both oxygen supplementation and genotype have significant effect on body weight from 3 weeks of age to 6 weeks of age. Body weight at 6 weeks of age was higher in oxygen supplement when compared to those control; 2165.82 g and 2055.40 g respectively. Body weight at 6 weeks of age for the Ross and Hubbard genotypes were 2044.40 g and 2208.19 g, respectively. The difference between the genotypes was statistically significant ( $P < 0.01$ ). Liu *et al.* (2009) reported that hypoxia have detrimental effects for the embryo when the eggs were incubated at high altitude. Wei and Wu (2005) indicated that the hatchability of the sea-level breed was only 37% at a high altitude of 2,900 m compared with the normal hatchability of 90% at sea level. The findings that oxygen supplementation had effect on body weights of broiler is in agreement with a previous reports (Celen *et al.*, 2009; Sahan *et al.*, 2006). Sahan *et al.* (2006) reported that oxygen supplementation from 18 to 21 d of incubation increased chick weight and final body weight of broilers. In this study, body weight during the experiment was not affected by the interaction between the oxygen supplementation and genotypes.

Tab. 2. Weekly body weight (g) of broilers hatched from incubated eggs with oxygen supplement and different broiler breeders

<i>Main effects</i>	Weeks					
	1 $\bar{x} \pm S\bar{x}$	2 $\bar{x} \pm S\bar{x}$	3 $\bar{x} \pm S\bar{x}$	4 $\bar{x} \pm S\bar{x}$	5 $\bar{x} \pm S\bar{x}$	6 $\bar{x} \pm S\bar{x}$
O <sub>2</sub>	147.35±0.93	339.19±3.33	725.87±10.95 <sup>a</sup>	1094.37±11.65 <sup>a</sup>	1677.27±17.48 <sup>a</sup>	2165.82±27.02 <sup>a</sup>
Control	146.08±1.14	328.93±4.27	696.31±10.12 <sup>b</sup>	1048.23±12.10 <sup>b</sup>	1633.66±17.39 <sup>b</sup>	2055.40±20.75 <sup>b</sup>
<i>P</i>	NS	NS	*	*	*	**
Ross	145.59±0.85	328.86±3.22 <sup>b</sup>	690.25±7.95 <sup>b</sup>	1039.60±9.96 <sup>b</sup>	1605.07±14.08 <sup>b</sup>	2044.40±24.74 <sup>b</sup>
Hubbard	148.25±1.25	341.51±4.46 <sup>a</sup>	740.87±13.52 <sup>a</sup>	1115.87±13.53 <sup>a</sup>	1723.70±19.98 <sup>a</sup>	2208.19±22.01 <sup>a</sup>
<i>P</i>	NS	*	**	**	**	**
<i>Interaction effects</i>						
O <sub>2</sub> X Ross	146.31±1.20	336.56±4.04	706.61±9.83	1060.14±13.74	1618.81±18.66	2073.91±38.40
O <sub>2</sub> X Hubbard	148.76±1.47	342.77±5.64	752.17±21.78	1141.60±18.40	1757.94±29.23	2297.12±26.80
Control X Ross	144.73±1.20	319.59±4.92	669.88±12.58	1013.82±13.74	1587.84±21.40	2006.84±27.38
Control X Hubbard	147.70±2.05	340.18±7.03	728.36±15.25	1088.49 ±19.31	1687.28±26.37	2113.46±29.79
<i>P</i>	NS	NS	NS	NS	NS	NS

\*\* P<0.01; \* P<0.05; NS: Not Significant

The effects of oxygen supplementation and different genotypes on feed conversion ratio at high altitude were shown table 3. Oxygen supplementation did not have a significant effect on feed conversion ratio at 1-5 weeks of age. Feed conversion ratio of broilers at 6 weeks of age was better in oxygen supplement when compared to those control; 1.97 and 2.07 respectively. This result agrees with that of Sahan *et al.* (2006), who stated that feed conversion ratio values of the oxygen supplemented group were significantly lower than the control group values at the 6th week of age. As stated by Vanhooser *et al.* (1995) reported that low oxygen in the cabinet atmosphere drastically decrease feed efficiency of broiler chicks. Genotypes did not influence feed conversion ratio of broilers during experiment significantly. There was not a significant affect by oxygen supplementation and genotypes interaction for feed conversion ratio.

Tab. 3. Weekly feed conversion ration of broilers hatched from incubated eggs with oxygen supplement and different broiler breeders

<i>Main effects</i>	Weeks					
	1	2	3	4	5	6
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
O2	1.36±0.01	1.56±0.01	1.65±0.02	1.85±0.03	1.84±0.02	1.97±0.01 <sup>b</sup>
Control	1.37±0.01	1.56±0.01	1.69±0.02	1.90±0.01	1.86±0.02	2.07±0.01 <sup>a</sup>
<i>P</i>	NS	NS	NS	NS	NS	**
Ross	1.36±0.01	1.57±0.01	1.69±0.03	1.89±0.02	1.86±0.02	2.04±0.03
Hubbard	1.38±0.01	1.55±0.01	1.65±0.03	1.86±0.03	1.83±0.02	2.00±0.03
<i>P</i>	NS	NS	NS	NS	NS	NS
<i>Interaction effects</i>						
O2 X Ross	1.35±0.00	1.57±0.01	1.67±0.02	1.88±0.03	1.85±0.02	1.98±0.01
O2 X Hubbard	1.37±0.02	1.55±0.03	1.63±0.06	1.82±0.04	1.83±0.05	1.96±0.02
Control X Ross	1.37±0.01	1.58±0.02	1.71±0.05	1.90±0.01	1.88±0.04	2.09±0.01
Control X Hubbard	1.38±0.02	1.55±0.01	1.67±0.02	1.89±0.04	1.84±0.01	2.04±0.01
<i>P</i>	NS	NS	NS	NS	NS	NS

\*\* P<0.01; \* P<0.05; NS: Not Significant

Both oxygen supplement and genotypes had no effect on livability during the experiment (Table 4). Livability was 95.95% in the oxygen supplementation, compared with 93.38% in the control at the end of experiment.

Tab. 4. Weekly livability (%) of broilers hatched from incubated eggs with oxygen supplement and different broiler breeders

<i>Main effects</i>	Weeks					
	1	2	3	4	5	6
	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
O2	100.00±0.0	100.00±0.0	100.00±0.0	96.67±1.60	96.67±1.60	95.95±0.95
Control	100.00±0.0	100.00±0.0	96.22±1.71	94.38±2.50	94.38±2.50	93.38±1.87
<i>P</i>	NS	NS	NS	NS	NS	NS
Ross	100.00±0.0	100.00±0.0	98.30±0.99	95.05±2.05	95.05±2.05	95.05±2.05
Hubbard	100.00±0.0	100.00±0.0	97.92±2.07	96.00±2.31	96.00±2.31	94.28±1.10
<i>P</i>	NS	NS	NS	NS	NS	NS
<i>Interaction effects</i>						
O2 X Ross	100.00±0.0	100.00±0.0	100.00±0.0	97.20±0.00	97.20±0.00	97.20±0.00
O2 X Hubbard	100.00±0.0	100.00±0.0	100.00±0.0	96.15±3.85	96.15±3.85	94.70±1.50
Control X Ross	100.00±0.0	100.00±0.0	96.60±0.30	92.90±4.00	92.90±4.00	92.90±4.00
Control X Hubbard	100.00±0.0	100.00±0.0	95.85±4.15	95.85±4.15	95.85±4.15	93.85±2.15
<i>P</i>	NS	NS	NS	NS	NS	NS

\*\* P<0.01; \* P<0.05; NS: Not Significant

## CONCLUSION

Data from this study showed that oxygen adding at the last stage of incubation improved body weight and feed conversion ratio of broiler at the end of study. Genotype has a significant effect on body weight.

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## EFFECT OF PASTURE-BASED FEEDING SYSTEMS ON THE LAMB MEAT QUALITY

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*Review paper*

### Summary

A review of the literature was undertaken to assess the current state of knowledge regarding the effect of pasture-based feeding systems on lamb carcass and meat quality, with particular focus on fatty acids and sensory characteristics. The sheep breeders, besides being profitable, need to satisfy consumer demands for safe meat of high quality. Quality of carcass and meat can be influenced by different factors, with feeding system being one of the main. While many authors have investigated differences between pasture- and concentrate-based feeding systems on lamb meat quality, there is still a lack of knowledge on how different pastures can influence main quality characteristics of lamb meat. Some differences in carcass, fatty acid composition and sensory characteristics have been reported on the meat of lambs grazing different artificial and permanent pastures. Farmers should consider grazing at mountain pastures to be a successful strategy for production of high-quality lamb meat that consumers are willing to buy. To have more information and to confirm the results, further research is needed on this topic.

Key words: *lamb, feeding, carcass, fatty acid, flavour*

### INTRODUCTION

Sheep meat production should meet quality requirements of industry and consumers, quality being the determining factor in the agro-food chain (Ramírez-Retamal *et al.*, 2013). Quality of lamb carcass can be evaluated by considering weight, the level of fattening, conformation and proportion of different tissue components (muscle, bone and fat). On the other hand, meat quality can be determined more objectively through properties of the meat, such as pH, colour, chemical composition, fatty acids (FA) and sensory characteristics. All these variables are influenced by many factors where the main are age and sex of the animal, breed and type of feed (Guerrero *et al.*, 2013). Many studies have investigated the effect of feeding systems on the mentioned characteristics, comparing extensive (based on grazing) with intensive feeding system, where lambs are mainly fed concentrate and hay (e.g. Arousseau *et al.*, 2007; Borton *et al.*, 2005; Cividini *et al.*, 2014; Ekiz *et al.*, 2013; Fisher *et al.*, 2000; Santos-Silva *et al.*, 2002). It is reported that large supplies of concentrates, compared to grass-based feeding, results in lambs with higher carcass weights, better conformation and shorter period of reaching slaughter weight (Borton *et al.*, 2005; Carrasco *et al.*, 2009; Ekiz *et al.*, 2013; Jacques *et al.*, 2011; Priolo *et al.*, 2002).

World Health Organisation (WHO) recommends moderate red meat consumption, because its highly saturated fatty acid (SFA) content is associated with higher risk of obesity, diabetes and cardiovascular disease. The ratios between polyunsaturated fatty acid (PUFA) and SFA (PUFA/SFA) and between n-6 and n-3 FA (n-6/n-3) are considered two important indices for nutritional evaluation of fat. The recommended PUFA/SFA ratio should be increased to higher than 0.4, which is hard to achieve in ruminants due to biohydrogenation in the rumen that significantly, reduces the PUFA content in muscle and adipose tissues. At the same time, the recommended n-6/n-3 ratio should be below 4.0. Higher levels of n-3 FA, which are considered to be positive in the human diet (Williams *et al.*, 2000), can be found in higher amounts in grazing lambs (Cividini *et al.*, 2014, Ekiz *et al.*, 2013; Nuernberg *et al.*, 2008; Wood *et al.*, 2003) improving both PUFA:SFA and n-6/n-3 ratios.

FA composition plays an important role in the definition of meat quality, as it can affect sensory characteristics, especially flavour (Wood, 2003). Preferences of lamb meat are very subjective and vary across the world, depending on what flavours consumers are accustomed to, but also on lamb production system and other factors (Montossi *et al.*, 2013). In general, trained assessors reported that lambs fed on pastures have more off-odours, off-flavours and more rancid and livery flavour than lambs fed on concentrate (Borton *et al.*, 2005; Priolo *et*

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*al.*, 2002). When information on the animal rearing system is provided, consumers preferred lamb meat from pasture-based feeding system (D'Alessandro *et al.*, 2012), because it is perceived as more natural, healthier and it ensures better conditions for animal welfare.

While many studies have investigated differences between lambs from the above mentioned feeding systems, there is still a lack of information on how different pastures can affect carcass characteristics and meat quality of lambs. Thus, the objective of this work is to determine what is known up to date on the effect of artificial and mountain pastures on carcass characteristics, fatty acid composition and sensory characteristics of lamb meat.

For this review, 'pasture-based feeding system' is the term used to identify system in which lambs are strictly reared on pastures without any provided supplementation.

## EFFECT OF DIVERSE ARTIFICIAL PASTURES

Just few papers analysed the effect of diverse botanical pastures on lamb carcass and meat quality. Most of them investigated the influence of artificial pastures of grass or clover, while alternative species such as forbs and other legumes, except clover, have been investigated less.

Lambs often grow slower under ordinary pasture-based feeding systems than those fed on concentrates (Jacques *et al.*, 2011; Priolo *et al.*, 2002). This could be improved by use of pastures with plant species and varieties, which could better meet animal seasonal nutrition requirements. Lambs grazing on legumes typically grow faster than grass-fed lambs, because of more rapid digestion, which allows higher feed intakes (Fraser *et al.*, 2004; Golding *et al.*, 2011; Young *et al.*, 1994). Fraser *et al.* (2004) compared production performance of grazing lambs finished on red clover (*Trifolium pratense*), lucerne (*Medicago sativa*) or perennial ryegrass (*Lolium perenne*) swards. They found that lambs grazing red clover had a higher live weight gain than those grazing the other two forages, while those grazing lucerne also gained live weight at significantly higher rate than those grazing perennial ryegrass. In the same study, there was no effect of forage treatment on carcass weight. According to Petron *et al.* (2007) lambs that grazed on botanical diverse pasture, composed from creeping bentgrass (*Agrostis stolonifera*), soft brome (*Bromus hordeaceus*), thistle (*Carduus* sp.) species and timothee (*Phleum pratense*), along with various herb species, had significantly lower slaughter weight and carcass yield than the lambs that were grazing ryegrass or leguminous rich pasture. Very low average daily gain is explained as the result of low protein content of mentioned pasture.

Consumption of fresh herbage at pasture is positively linked to the presence and the content of PUFA in meat. Green plants contain high levels of linolenic acid (C18:3n-3), but due to the bio-hydrogenation in the rumen, those levels are not transferred to meat. Lambs finished on legume forage swards have higher proportions of PUFA, in particular linoleic (C18:2n-6) and linolenic acids, and higher PUFA/SFA and n-6/n-3 ratios than lambs finished on perennial ryegrass sward (Fraser *et al.*, 2004).

Fraser *et al.* (2004) did not find any significant differences in sensory characteristics between treatments when lambs were finished on red clover, lucerne or perennial ryegrass swards. Young *et al.* (1994) investigated meat sensory characteristics of lambs grazing single species swards of perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*), phalaris (*Phalaris aquatica*), lucerne (*Medicago sativa*), chicory (*Chicorium intybus*) and prairie grass (*Bromus willdenowii*). Judging by panellist comments, the cocksfoot treatment produced the best tasting meat, while phalaris pasture the least acceptable because the meat had a significantly stronger strange flavour and it was perceived as "rancid" and possibly "alkaline".

## EFFECT OF MOUNTAIN PASTURES

Over the past decade, there has been a renewed interest in mountain farming probably stimulated, among other reasons, by the increased request for high-quality animal products obtained from extensive production systems (Povoio *et al.*, 2013).

Many studies have shown that meat, milk and cheese obtained from grazing cattle in mountain areas, are of high quality and often related to higher health-giving properties for humans (e.g. Collomb *et al.*, 2002; De Noni and Battelli, 2008; Steinshamn *et al.*, 2010). On the contrary, there is still a lack of research on quality of lamb meat raised on mountain pastures. Just a few papers (Ådnøy *et al.*, 2005; Fisher *et al.*, 2000; Lind *et al.*, 2009; Willems *et al.*, 2013, 2014) investigated carcass characteristics and meat properties of lambs reared on mountain pastures. It was found that in different production systems, lamb growth performance is lower on the mountain than in lowland pastures (Lind *et al.*, 2009; Willems *et al.*, 2013). The mentioned results were associated with the walk long distances on often steep slopes in the mountain areas (Hays *et al.*, 1978) and to the resulting different body conformation of lambs, compared to those reared in the lowlands. Besides that,

nutritionally poor forage (Schubiger *et al.*, 1998) and more extreme climate conditions, including hypoxia (Christen *et al.*, 2006), may have influenced the observed characteristics.

Willems *et al.* (2013) compared growth performances of lambs of two breeds grazing lowland and three alpine pasture types diverse in altitude, botanical composition and nutrient content. How the high forage quality of one of the three alpine pastures was comparable to the lowland one, growth and slaughter performance of lambs did not differ much between them. Because of lower quality of forage, in combination with less favourable topographic pasture conditions, differences in lamb growth performances were found for the other two alpine pastures. In this study, lambs from all alpine pasture types had poor to moderate carcass conformation and fat cover was lower than the demands of the market. This shows that summer grazing on poor alpine pastures may result in carcasses that do not satisfy market needs. In this case, lambs are often sent from mountains to lowland pastures for a finishing period.

In this regard, Lind *et al.* (2009) reported that finishing period on mountain pastures has a positive effect on the amount of unsaturated fat in lamb meat. More particularly, lambs raised and fattened on a permanent mountain pasture had a higher level of PUFA when compared with lambs raised on the same mountain pasture, but fattened on an artificial lowland pasture. However, the same author points out that these results were not congruent with same experiment performed at another location, as the meat from lambs grazing the artificial lowland pasture was influenced by finishing system and had higher amount of PUFA than lambs grazing mountain pastures, while in the following year, no effects were found. The mentioned results could be connected to the effect of the year and, in particular, to the forage yield and quality characteristics that probably were not appropriately taken into account. Ådnøy *et al.* (2005) also found a higher level of PUFA in meat from mountain lambs compared to those from the lowlands. Fatty acid composition from different studies is reported in Table 1.

Although more than 85% of dietary PUFA are hydrogenated in the rumen (Doreau and Ferlay, 1994), some dietary PUFA escape rumen biohydrogenation. In particular, phenolic compounds present in some plants, can prevent ruminal biohydrogenation thus increasing the accumulation of alpha-linolenic and linoleic acid in the tissues of animals grazing those plants (Vasta *et al.*, 2010). Willems *et al.* (2014) proved that in an alpine environment, different pasture species can lead to differences in the intramuscular and adipose tissue fatty acid composition. Furthermore, they demonstrated that levels of C18:2n-6 and C18:3n-3 FA are positively associated with the contents of phenolic compounds in the vegetation.

In addition to others, phenols and terpenes are compounds that can influence meat flavour because they are directly transferred from pasture to animal tissue. Presence of those compounds in animal tissues strongly depends on pasture botanical composition, so they are often used as potential biomarkers of diet for authentication of products derived from pastures (Moloney *et al.*, 2014; Prache *et al.*, 2005; Priolo *et al.*, 2003).

According to Ådnøy *et al.* (2005) and Lind *et al.* (2009), meat from mountain pastures is less hard and less juicy, but tender than meat from the artificial pasture. Meat from the mountain pasture was also less fatty and with higher intensity of metallic flavour than that from artificial pasture. Results from Hersleth *et al.* (2012) revealed that both Norwegian and Italian consumers would buy meat from lamb grazing on the mountain rather than on lowland pastures. An explanation could be found in appreciation of mountain pasture as more natural.

Tab. 1. Effect of lowland (L) and mountain (M) pastures on fatty acid composition (% of total fat) in lamb meat.

Reference	Breed <sup>1</sup>	Sample <sup>2</sup>	SFA		MUFA		PUFA		n-6/n-3	
			L	M	L	M	L	M	L	M
Ådnøy <i>et al.</i> (2005)	NCS	LD	45.3	45.9	40.3	38.2	4.4	6.9	-	-
Lind <i>et al.</i> (2009)	NCS	SFLD	56.1	54.3	39.5	40.9	2.9	3.6	-	-
Willems <i>et al.</i> (2014)	ES, VS <sup>3</sup>	LD	39.9	39.4	40.4	38.2	14.6	17.2	1.88	1.70

<sup>1</sup> NCS - Norwegian Crossbreed Sheep; ES - Engadine Sheep; VS - Valasian Black Sheep

<sup>2</sup> LD - M. *Longissimus dorsi*; SFLD - Subcutaneous fat over the M. *Longissimus dorsi*

<sup>3</sup> Average across breeds and two experimental years

## CONCLUSIONS

Despite the lack of literature dealing with lamb performance, fatty acid composition and sensory characteristics, this review shows that it is possible to obtain good-quality carcasses and meat with high amount of desirable PUFA through the use of high-quality pastures.

Even the reported differences in sensory characteristics of lamb meat from different pastures are either absent or very small, consumers declared to prefer lamb meat from mountain pastures because it was perceived as more natural and thus healthier.

Therefore, farmers should consider grazing on mountain pastures as a successful strategy for production high-quality lamb meat that consumers are willing to buy. Further research is required to achieve a systematic knowledge basis about the lamb carcass and meat quality, as affected by pasture-based feeding system, especially in mountain pastures where free ranging animals mainly graze on different vegetation types.

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## FATTY ACID COMPOSITION OF DRUMSTICK AND THIGH MUSCLE OF BROILERS FED BY DIETS CONTAINING MIXTURE OF RAPESEED, LINSEED AND FISH OILS THROUGH VARIOUS LENGTH OF FATTENING PERIOD

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*Original scientific paper*

### Summary

A study was conducted with aim to evaluate effect of different oils added in broiler diets on fatty acids profile of drumstick/thigh meat. 200 chickens of ROSS 308 provenience randomly divided in four experimental treatments different in diets oils composition as well as in length of feeding: control group fed sunflower oil through 21 day of experiment and three groups containing mixture of 3% of rapeseed oil +1.5% linseed oil and 1.5% of fish oil (RLFO). Broilers were fed by this diet through last 7, 14 and 21 days of fattening period. In the first 21 day all experimental groups received same starter diet. Total and individually saturated fatty acid (SFA), monounsaturated (MUFA), polyunsaturated fatty acid (PUFA) and different groups of fatty acids ratio were used as fatty acid profile parameters. Differences among experimental diets determinate by ANOVA. Linear and logarithmic regression models were fitted to the relationship between length of feeding by RLFO and fatty acids deposition in muscle of drumstick and thigh meat. Generally, RLFO diet increased content of myristic acid and palmitic acid, all n-3 PUFA, and total MUFA but decreased all examined n-6 PUFA content in muscle of drumsticks and thighs. Deposition on n-3 PUFA in meat had linear or logarithmic function indicating possibilities of higher n-3 PUFA deposition in broilers meat in accordance with the length of feeding.

Key words: *broilers, fatty acids profile, oils mixture, length of feeding*

### INTRODUCTION

Due to the specific digestion of poultry, adding different fats or oils in the broiler chickens diet resulting in changing the profile of fatty acids in the meat. On the other hand, modification of fatty acids profile of broilers' meat may have adverse effects that are reflected in reduced oxidative stability and undesirable sensory characteristics of the meat. Appropriate choice of oil as a source of fatty acids, their concentration in feed and adding anti-oxidants were the subjects of the increased interest in order to obtain optimal product. Fish oil, flaxseed oil and rapeseed oil contain high concentrations of polyunsaturated fatty acids. Fish oil is one of the best sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) but its use in poultry feeding is limited (up to 1-2%) because of the negative effect on sensory characteristics of the final product (Lopez-Ferrer *et al.*, 2001). Many other investigations (Bedekovic *et al.*, 2012; Skrtic *et al.*, 2009; Haug *et al.*, 2007; Kralik *et al.*, 2007; Blanch *et al.*, 1995; Ki-TaegNam *et al.*, 1997) indicate that adding flaxseed oil rich in alpha-linolenic acid and rapeseed oil rich in oleic and alpha-linolenic acid in the diet of broilers can significantly increase the concentration these fatty acids in meat. Enrichment of chicken meat with polyunsaturated fatty acids, especially omega-3 fatty acids and antioxidants is the subject of many investigations in the world. The effect of length of feeding with such diets on quality of broiler meat is considerably less studied. Therefore, the aim of this study is to determine the effects of adding different oils in the diet of broilers, supplemented with higher doses of vitamin E, as well as to determine the influence of the length of feeding by such diets on broilers' meat quality with emphasis on the fatty acids profile in nutritionally most valuable parts of the carcass. The investigations were based on the hypothesis that by defining of an appropriate system of broiler nutrition (diets with appropriate concentrations of fish oil and linseed oil combined with rapeseed oil supplemented with vitamin E), it is possible to achieve an optimal meat quality from the structural point of fatty acids, without negative effects on productive performance of broiler chickens.

### MATERIAL AND METHODS

Two hundred unsexed chickens of ROSS 308 provenience randomly divided in four experimental treatments different in diets oils composition as well as in length of feeding: control group fed sunflower oil (SO) through

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last 21 day of the fattening period and three groups fed through 7, 14 and 21 day by diet (OM) containing 3.0% of rapeseed oil +1.5% linseed oil and 1.5% of fish oil. Previously all broilers were fed by same starter diet (Figure 1).

Treatment	Days				
	0.	21.	28.	35.	42.
C	starter			SO	
OM7	starter		SO		OM
OM15	starter	SO		OM	
OM21	starter			OM	

Fig. 1. Plan of the experiment

Up to 21 days of the experiment, all chicks received the same commercial starter diet containing 22% crude protein and 12.5 MJ ME / kg. From 21 to 42 d. birds received grower experimental diets according to the plan of the experiment. Structure and composition of the diets are shown in Table 1.

Tab. 1. Structure and chemical composition of experimental diets

Ingredients, %	Experimental diets	
	SO	OM
Corn	41.00	41.00
Wheat	17.00	17.00
Soybean meal	22.00	22.00
Sunflower meal	10.35	10.35
Sunflower oil	6.00	-
Rapeseed oil	-	3.00
Linseed oil	-	1.50
Fish oil	-	1.50
Limestone	1.20	1.20
Dicalcium phosphate	1.40	1.40
Salt	0.235	0.235
Methionine	0.14	0.14
Lysine	0.16	0.16
Premix*	0.50	0.50
Vitamin E	0.015	0.015
Chemical composition		
Crude protein, %	19.51	
Crude fat, %	8.35	
Crude fiber, %	4.78	
Crude ash, %	5.52	
ME, MJ/kg	13.06	
Ca, %	0.91	
P, %	0.70	
Lysine, %	1.01	
Methionine + cysteine, %	0.73	

\*in 1 kg: vitamin A 2400000 IU, vitamin D3 480000 IU, vitamin E 6000 mg, vitamin K3 500 mg, vitamin B1 300 mg, vitamin B2 1200 mg, nicotinamid 7000 mg, calcium pantothenate 2000 mg, vitamin B6 800 mg, vitamin B12 3000µg, folic acid 150 mg, biotin 10 mg, cholinechlorid 80000 mg, methionine 100000 mg, J 200 mg, Mn 16000 mg, Zn 10000 mg, Co 20 mg, Fe 6000 mg, Cu 1000 mg, Se 30 mg, antioxidant 20000 mg, flavophospholipol 600 mg, coccidiostatic 20000 mg.

The content of fatty acids in thighs with drumsticks and feed was determined by gas chromatography. Meat without skin was used for fatty acids determination. Extraction of lipids was performed on 0.5 g samples according to Folch (1957). Lipids were dissolved in heptane and methylated using Na-methoxide and methanolic HCl 3N (Supelco, PA, USA). Methyl esters of fatty acids were analyzed using a Carlo Erba 8130 gas chromatograph equipped by 100m capillary column (CP-sil 88 WCOT, 100-mx 0.25 mm, Chrompack, Middelburg, The Netherlands). Starting temperature was 70°C, and after 4 minutes the temperature was increased to 13 degrees per minute until reaching 175°C and this temperature was maintained 27 minutes after which the temperature is further increased to 4°C per minute until reaching 215°C and maintained 31 minutes. The temperature is again increased to 4°C per minute until reaching a final temperature of 225°C. The flow of

gas is was 1.62 ml / minute, with 1: 34 ratio in the flame-ionizing detector. Fatty acids were converted to amounts of fatty acids expressed g/100g fatty acids (Ackmana and Sipos, 1964). A cod liver oil was used as standard for every tenth sample. The experiment data was analyzed using analysis of variance and regression analysis by SPSS16.0.

## RESULTS AND DISCUSSION

### Fatty acids composition of the experimental diets

The FA composition of the experimental diets is shown in Table 2. Adding mixtures of fish oil, linseed oil and rapeseed oil considerably increased total n3 fatty acids as well as EPA, DPA and DHA (docosahexaenoic acid) and decreased total n6 fatty acids in the OM diet.

Tab. 2. Fatty acids content in experimental diets (g/100g of total fatty acids)

Fatty acids	Starter	Experimental diets	
		SO	OM
C14:0	0.39	0.09	0.89
C16:0	11.07	7.22	8.55
C18:0	3.51	2.95	2.44
C18:1n-9	22.90	24.58	35.56
C18:2n-6	54.06	60.62	29.99
C18:3n-3	3.66	1.34	13.99
EPA	0.54	0.15	1.36
DPA	0.08	0.08	0.50
DHA	0.77	0.06	1.48
$\sum$ n3	5.05	1.63	17.33
$\sum$ n6	54.21	60.71	30.32
n6/n3	10.73	37.25	1.75
SFA	14.97	10.26	11.88
MUFA	23.23	24.66	36.64
PUFA	59.72	63.00	48.00
SFA/MUFA	0.64	0.42	0.32
SFA/PUFA	0.25	0.16	0.25

Decreasing the polyunsaturation level of the OM diet and keeping fairly equal level of saturated fatty acids in both experimental diets resulted in higher SFA/PUFA ratio in the OM diet. Thus, dietary PUFA (polyunsaturated fatty acids) to SFA (saturated fatty acids) ratio increased with the dietary polyunsaturation level. At same time, n6/n3 ratio was extremely decreased by adding fish oil, linseed oil and rapeseed oil in OM diet.

### Effect of increasing dietary n3 FA on FA profile in broiler meat tissue

The broiler drumstick and thigh muscle fatty acid content data are presented in Table 3. It is established that in comparison with SO (control) group OM diet led to higher C14:0 (myristic acid), C18:1-n-9 (oleic acid), C18:3-n-3 ( $\alpha$ -Linolenic acid-ALA), EPA, DPA, DHA, n3 and total MUFA (monounsaturated fatty acids). At same time C18:2-n-6 (linoleic acid), total n6, total PUFA as well as n6/n3 and SFA/MUFA ratios were decreased.

Tab. 3. Fatty acids content (% of total fatty acids) in drumstick and thigh muscles

Fatty acids	Experimental diets				P OM vs. SO
	SO	OM7	OM15	OM21	
C14:0	0.374 $\pm$ 0.021	0.492 $\pm$ 0.051	0.572 $\pm$ 0.033	0.693 $\pm$ 0.017	0.000
C16:0	16.43 $\pm$ 0.52	15.93 $\pm$ 0.65	15.932 $\pm$ 0.646	17.443 $\pm$ 1.154	ns
C18:0	7.650 $\pm$ 1.079	7.328 $\pm$ 0.558	7.026 $\pm$ 0.495	7.025 $\pm$ 0.340	ns
C18:1n-9	26.314 $\pm$ 0.82	26.246 $\pm$ 1.57	28.948 $\pm$ 0.97	30.538 $\pm$ 1.07	0.000
C18:2n-6	35.404 $\pm$ 1.878	32.026 $\pm$ 2.891	27.276 $\pm$ 1.249	22.468 $\pm$ 1.055	0.000
C18:3n-3	1.042 $\pm$ 0.100	2.888 $\pm$ 0.446	4.224 $\pm$ 0.282	5.313 $\pm$ 0.509	0.000
C20:4n-6	4.963 $\pm$ 0.928	4.443 $\pm$ 0.292	4.012 $\pm$ 1.009	2.902 $\pm$ 0.483	0.012

EPA	0.060 ±0.012	0.450 ±0.092	0.718 ±0.046	0.893 ±0.139	0.000
DPA	0.302 ± 0.068	0.920 ±0.249	1.186 ±0.180	1.340 ±0.075	0.000
DHA	0.422 ±0.094	1.404 ±0.258	1.930 ±0.340	2.150 ±0.290	0.000
∑ n3	1.826 ±0.257	5.662 ±0.504	8.058 ±0.595	9.695 ±0.434	0.000
∑ n6	38.940 ±2.093	36.266 ±2.748	30.478 ±1.313	25.058 ±1.260	0.000
n6/n3	21.556 ±2.107	6.464 ±0.967	3.796 ±0.258	2.588 ±0.151	0.000
SFA	24.454 ±1.577	23.730 ±1.550	23.530 ±0.675	25.160 ±1.337	ns
MUFA	30.848 ±1.40	30.710 ±1.780	34.008 ±1.140	36.270 ±0.880	0.000
PUFA	41.194 ±2.296	42.406 ±2.567	39.014 ±1.684	35.150 ±1.415	0.042
SFA/MUFA	0.796 ±0.083	0.774 ±0.068	0.692 ±0.034	0.695 ± 0.042	0.004
SFA/PUFA	0.596 ±0.071	0.562 ±0.067	0.606 ±0.038	0.718 ±0.066	ns

As expected with decreasing polyunsaturatuion in diets, PUFA content in meat tissue generally slightly decreased, primarily owing to decreasing n6 family of polyunsaturated fatty acids. A similar response was observed in certain n6 PUFA, particularly in linoleic acids (C18:2n-6). Simultaneously, n3 fatty acids in meat increased much more (more than 5 times), but much larger quantities of n6 compared to n3 fatty acids caused the decreasing n6/n3 ratio on muscle tissues of broilers. Deposition of EPA, DPA and DHA in broilers' meat significantly increase with including rapeseed, linseed and fish oil in the diet. Also, according to Lopez-Ferrer *et al.* (2001) increasing level of ALA (C18:3n-3) in diets increases the concentration of EPA and DHA in the thighs. According Zelenka (2008) same can be said for the relationship ALA - EPA, but not for the ratio ALA - DHA because of the limited capacity of elongation and desaturation of ALA in poultry. Deposition of fatty acids in meat of broilers drumstick and thigh as function of length of feeding by diet rich in n3 fatty acids had linear or logarithmic pattern (Table 3).

Tab. 3. Fatty acids deposition in drumstick/thigh muscle as function of experimental diets feeding length

Fatty acids	Model	R <sup>2</sup>	P	b <sub>0</sub>	b <sub>1</sub>
C14:0	y= b <sub>0</sub> + b <sub>1</sub> x	0.839	0.000	0.386	0.014
C16:0	y= b <sub>0</sub> + b <sub>1</sub> x	0.277	0.053	14.935	0.105
C18:0	y= b <sub>0</sub> + b <sub>1</sub> x	0.077	0.336	7.437	-0.023
C18:1n-9	y= b <sub>0</sub> + b <sub>1</sub> x	0.698	0.000	24.256	0.310
C18:2n-6	y= b <sub>0</sub> + b <sub>1</sub> x	0.286	0.049	0.702	-0.012
C18:3n-3	y= b <sub>0</sub> + b <sub>1</sub> x	0.872	0.000	1.710	0.174
C20:4n-6	y= b <sub>0</sub> + b <sub>1</sub> x	0.493	0.005	5.362	-0.112
EPA	y= b <sub>0</sub> + b <sub>1</sub> x	0.805	0.000	0.242	0.032
DPA	y=b <sub>0</sub> + (b <sub>1</sub> ln(x))	0.511	0.004	0.176	0.383
DHA	y=b <sub>0</sub> + (b <sub>1</sub> ln(x))	0.577	0.002	0.070	0.692
∑ n3	y= b <sub>0</sub> + b <sub>1</sub> x	0.914	0.000	3.752	0.290
∑ n6	y= b <sub>0</sub> + b <sub>1</sub> x	0.870	0.000	41.890	-0.802
n6/n3	y= b <sub>0</sub> + b <sub>1</sub> x	0.857	0.009	8.198	-0.281
SFA	y= b <sub>0</sub> + b <sub>1</sub> x	0.174	0.138	22.759	0.097
MUFA	y= b <sub>0</sub> + b <sub>1</sub> x	0.768	0.000	28.075	0.400
PUFA	y= b <sub>0</sub> + b <sub>1</sub> x	0.727	0.049	46.100	-0.517
SFA/MUFA	y= b <sub>0</sub> + b <sub>1</sub> x	0.305	0.040	0.802	-0.006
SFA/PUFA	y= b <sub>0</sub> + b <sub>1</sub> x	0.564	0.002	0.544	0.008

OM diet contained about 50% lower amount total n6 fatty acids and consequently less linoleic acid (C18:2n-6) which is dominant n6 FA comparing to SO diet. On the other hand, chickens fed by OM diet deposited smaller amounts of arachidonic (AA) and linoleic acid (C20:4n-6) compared to chickens SO meal with a tendency of linear decline by length of feeding (Table 3).

Since the diets do not contain arachidonic acids its presence in meat of drumstick and thigh can be explained by the fact that during the metabolism linoleic acid, through processes desaturation and elongation, converted into arachidonic acid. Confirmation of this is the negative linear trends, the most arachidonic acids (-0.112) and then linoleic (-0.012) in meat related to length of feeding by OM diet (Table 3).

Many studies indicate the possibility of conversion of ALA to EPA, EPA and DHA which has been confirmed by this experiment. Regression response of ALA content in tissues of thigh and drumsticks on increasing of

ALA content in the diets has a linear trend while DPA and DHA deposition in the tissue showed logarithmic pattern.

This is consistent with studies of Zelenka *et al.* (2008) and Schneider *et al.* (2007) that showed limited capacity fatty acids desaturation and elongation at poultry. n6/n3 ratio in tissue of drumsticks and thighs linearly decreased with length of broilers feeding with OM with significant coefficient of the model determination ( $R^2=0.857$ ).

## CONCLUSION

Results of the study indicated that broilers feeding by mixture of rapeseed, linseed and fish oil is efficient and effective way of modifying the fatty acid profile of drumstick/thigh. With longer broilers feeding with diets rich in desirable fatty acids (mainly those n3 family) comes to increasing of their concentrations in the muscle tissues. Thus, the manipulation of the oil composition in poultry diets and combination of different length of broilers feeding may be a good approach in desirable fatty acid profile designing of the meat for human consumption.

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## STATE OF HEALTH AND SAFETY IN ANIMAL HUSBANDRY IN TURKEY

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*Review paper*

### Summary

The safety and security applications conducted in work places in Turkey are under the control of the Occupational Health and Safety Act No. 6331, which was put into effect on 30 June, 2012. This regulation primarily focuses on the prevention of occupational accidents and diseases.

Agricultural work can bring about a variety of health problems. Agricultural workers are faced with risks of cancer, respiratory diseases, injuries and accidents. Their working environments are full of “physical hazards” that may come from air, land, fire and machines and “toxicological hazards” that may stem from pesticides, fertilizers and fuels and dust.

Women, children, young workers, the disabled, the elderly are regarded as risk groups in working life. To the people in livestock business care should be given according to the work they do. The ministry of Labour and Social Security in Turkey states in a decree passed on 26 December, 2012 that the work places where cattle are raised and milked and sheep, goats, and poultry products are manufactured are classified within “dangerous” work places. Also, employees engaged in beekeeping activities and silkworm breeding are placed within risk groups. Workers should be warned about the possible dangers of animal waste in the work area and especially in closed spaces and necessary precautions should be taken to prevent the spreading of poisonous gas from fertilizer waste. Great importance should be attached to the use of protective equipments against infectious diseases and workers should be provided with first-aid training against physical traumatic risks that may occur during the cleaning of the animal shelters, during the care and transportation of animals.

The purpose of this study is to emphasize the importance of occupational health and safety and shed light on the risks in livestock breeding sector.

**Keywords:** *Occupational health, safety, animal, risk, hazard*

### INTRODUCTION

Throughout the world, as a result of increasing importance attached to man in general, workers' health and safety have become a more important issue to be considered by every work place. Thus, importance attached to the prevention and reduction of work place accidents and diseases has increased.

When the research conducted on work accident and occupational diseases is examined, it is seen that the amount of such accidents and diseases observed in agriculture and livestock breeding sectors is remarkable.

The most effective way of preventing work accidents and diseases is adopting approaches to identify problems and solve them. The current study aims to discuss the importance of safety and security at workplace by emphasizing the potential risks in the livestock sector.

#### 1-The Importance of Workers' Health and Safety

Health and safety are important for both employees and employers. This is more important for workers as their life and future are under risk at their workplaces. On the other hand, in a modern society, the employer is expected to provide working conditions required to protect the health and ensure the safety of the employee. Thus, the employer can fulfill the social responsibility towards his/her workers and increase the efficiency in the workplace. Act of protecting the health and ensuring the security of the employee entails the construction of a working environment that provides protection for the employee against environmental pollution, high noise levels, machines, radiation etc. (Dursun, 2006).

When work security and safety are neglected, it is highly likely that the incidences of occupational accidents and diseases will increase. And those most negatively affected from this will be workers because first the income

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level of the worker's family will decrease. The employer losing his/her health and job may experience some psychological problems. As a result, both the employee and the society will be adversely affected (Yiğit, 2005).

Making efforts to prevent work-related accidents and diseases that cause important economic and social problems has become a must for both the state and organizations. Losses resulting from work accidents and occupational diseases not only negatively affect the efficiency of the organization but also its competitiveness in international markets (Yılmaz, 2010).

World Health Organization (WHO) and International Labor Organization (ILO) define occupational health and safety as "Ensuring the highest physical, spiritual and societal health and welfare of workers; elimination of the health risks that may stem from working conditions and manufactured goods; elimination of the risk factors that may result in accidents and injuries; placements of workers in positions suitable for their physical and spiritual conditions; and establishment of a working environment that meets the physical and spiritual needs of the worker" (Özçer, 1988). Organizations attaching importance to the improvement of its human resource should first ensure the construction of a secure environment to work.

## **2-Health and Safety of the Population Working In Agriculture**

Agricultural works may bring about various health problems. Agricultural workers can be confronted with many health problems such as cancer, respiratory diseases, injuries and accidents. As agricultural lands are not close to urban areas in general, some difficulties may be experienced in obtaining emergency health care services.

In agriculture sector, working environments are full of "physical hazards" that may come from air, land, fire and machines and "toxicological hazards" that may stem from pesticides, fertilizers and fuels and dust (Şimşek, 2015).

Agriculture is one of the most dangerous sectors in terms of occupational diseases and early deaths. The main features of health and safety applications in agricultural works differentiating them from other works are;

- a- Working place and living place are in the same area and in general, all the family members live and work together,
- b- Because of the smallness of farm lands, unhealthy living conditions are created for short terms,
- c- Agricultural production is seasonal and most of the works are completed in a short time; thus, most of the workers in the sector are undeclared workers,
- d- Agricultural activities require intense labor force and this labor force is in a state of constant movement,
- e- High majority of the works should be done in open areas,
- f- Difficulties are experienced in the arrangement of working schedules,
- g- Because of the life mostly spent on the edge of the field, difficulty in meeting basic needs such as clean drinking water and electricity,
- h- Problems experienced in the disposal of human and animal wastes,
- ı- Intense interaction with soil, animals and plants,
- j- Uncontrolled increase in the use of chemicals and lack of control on the use of pesticides,
- k- Difficulties in the construction of health care services suitable for rural areas,
- l- Lack of standards and regulations in relation to occupational health and safety or difficulties experienced in their application,
- m- Workers' low level of education and resulting health problems,
- n- Lack of training given to workers on occupational health and safety (Donham and Thelin, 2006).

In addition to above-mentioned features of the agriculture sector, the causes of diseases and early deaths may vary depending on the worker's personal traits, time and place, type of agricultural production, and whether animal breeding is performed together with agricultural activities (Şimşek, 2015).

High majority of the families working in agriculture sector are also engaged in animal breeding or work in places where contact with animals is quite likely. In such cases, infectious diseases are more widely seen. The size of agricultural organizations is also found to be closely associated with early deaths and diseases (Arcury *et al.*, 2010).

## **3- Diseases and Accidents in Livestock Sector**

### **3.1. Zoonotic (Zoonoz) Diseases**

These diseases can be defined as diseases that can be transmitted by humans and animals to each other. Many zoonotic diseases can be transmitted by cows, sheep, rabbits, birds, cats, dogs, monkeys, mice etc and result in

serious problems. Zoonoz can be caused by bacteria, parasites or can be viral or fungal-induced. Moreover, their transmission can be mediated by arthropods such as ticks and fleas and they are defined as arthropodal. These elements cause diseases in animals in different ways and can be transmitted to humans in various ways (Anonymous, 2015a).

### **3.2. Respiratory Diseases**

The research carried out in developed countries reveals that 25% of the workers in animal breeding sector suffer from some kind of respiratory disease. Thus, respiratory diseases are the most frequently seen diseases among the employees working in animal breeding sector.

Gases make up the most important cause of respiratory diseases in animal breeding sector. In large closed areas where cattle and other animals are fed and in chicken sheds, the ammoniac levels are high and this poses serious threats to human health. Used as fertilizer, anhidr ammoniac causes both acute and long-term respiratory diseases. In fertilizer storage units, sudden exposure to hydrogen sulfide may lead to deaths (Anonymous, 2015a).

### **3.3. Skin Diseases**

Skin diseases can be classified as contact dermatitis, sun-induced, infection-induced and insect-related diseases. Nearly 70% of the diseases seen in animal breeding and agriculture sectors are skin diseases. Contact dermatitis is the most common one. Fertilizers, plants, animals feeds, pesticides and antibiotics added to animal feeds cause skin diseases. Long exposure to sunlight may result in serious skin diseases. Actinic Keratoses and skin cancers are among the most serious ones.

When protective equipments not complying with the standards of occupational health and safety regulations are used, this may result in many different diseases resulting from direct contacts such as allergic contact dermatitis, allergic rhinitis, bites and kicks, poisonous animal stings, asthma, trauma and scratches. Moreover, when the necessary precautions are not taken, health problems resulting from organic elements such as agrochemical poisoning, resistance towards antibiotics, chronic bronchitis, contact dermatitis may be encountered.

### **3.4. Accidents**

Remarkable amount of work accidents in animal breeding sector results from direct physical contact with animals. Kicking, pushing, attacking and biting animals may lead to serious injuries. According to data reported by National Traumatic Injury Surveillance of Farmers (NIOSH) in America, in all agricultural sectors including animal breeding, an important part of injuries results from direct contacts with animals and injuries caused by only cows and sheep account for 18% of all the injuries. Such injuries are the injuries resulting in the greatest amount of temporary labor losses.

In addition to these, there are some health problems stemming from physical elements such as hearing loss, traumas caused by machines, musculoskeletal system disorders.

## **4. CLOSE CONTACT RISKS IN ANIMAL HUSBANDRY**

### **4.1. Risks related to Close Contact in Cattle and Sheep Breeding and their Precautions**

Some efforts should be invested to raise the awareness of workers to prevent infectious diseases in barns and sheep pens. Vaccination and feed controls should be conducted by vets authorized by the Ministry of Food, Agriculture and Livestock at certain intervals. When something unusual is noticed such as unusual look, smell of behavior, vet should be called immediately without having contact with the animal and without staying in the same environment with it. Besides animals, vaccination of the workers against biological risks should be strictly controlled.

The level of the dust in environments where workers are with animals should be measured. Suitable protective equipments should be used by workers to reduce pathogenic microorganism and parasite contacts. For instance, in environments where microbiological intensity is high, air filtrating masks in stead of dust filtrating masks must be used.

Suitable lightning should be provided in animal shelters; thus, related risks of trauma can be reduced. Against physical trauma risks that may occur during the cleaning of barns, care and transportation of animals such as fall, fracture, soft tissue trauma, head trauma, workers should be given first-aid training (Anonymous, 2015b).

### **4.2. Close Contact Risks in Poultry Farming Sector**

If necessary precautions are not taken in poultry farming, some health problems may be encountered. To prevent such health problems, first sufficient lighting should be provided in poultry houses. Moreover, dust measurements should be regularly conducted. Dry feces, feed, underlay, hair and feathers, skin and equipments are the main sources of dust in the chicken shed. The size of the particle of the dust is as important as its source for human health. Those having a size smaller than 5 microns are called “dust in the smoke form” or “dust that

can be inhaled". These small dust particles can reach the smallest units of the lung, alveoli, and they can even penetrate into blood. Breathing of animals and microbial activities inside the shed lead to the formation of gases such as ammoniac, carbon dioxide, carbon monoxide, methane and hydrogen sulfur. For these gases, in-shed standard levels must be supervised.

Personal protective equipments that must be used by workers while working in the field should be provided and controlled. Periodical health checks of workers should be carefully conducted including those for microbial diseases (Hepatitis B, Salmonella spp., Lysteria spp. etc.). In addition to these, exposure to low temperature and high moisture in slaughter houses may cause diseases (Anonymous, 2015b).

#### **4.3. Close Contact Risks in Beekeeping and Silkworm Breeding and their Precautions**

For the production of honey, beeswax, pollen and royal jelly, beekeeping is a widely practiced in many regions of Turkey. Beekeepers should use proper equipments such as work suits, gloves, hats and workers should be informed about bee sting and bee-related diseases to be protected from such diseases.

For the manufacturers to be protected against silkworm-related diseases, employees responsible for the cleaning of cocoon cleaning should be provided with the required protective equipments. Workers should be informed about the chemicals used during the disinfection of worm raising rooms and great important should be attached to the ventilation of these rooms. (Anonymous, 2015b).

#### **4.4. Health and Safety Precautions for Workers of Animal Husbandry Sector**

Almost all of the zoonotic diseases are quite dangerous and serious health problems. Therefore, some precautions must be taken against them. Vaccination of animals and workers, taking required safety precautions in the work environment and hygiene of animal products can reduce the risk of being infected with diseases. By using the correct antibiotics, the development of bacteria can be prevented and this is very important for the prevention of these diseases. Workers need to be informed about the factors leading to the emergence of these diseases.

Use of personal protective equipments is of great importance for the health and safety of the workers in the sector. Effective ventilation in closed areas and regular cleaning will considerably reduce the amount of dust. By using equipments that can enable healthy breathing, the dangers posed by gases and dusts can be reduced. Dust-filtrating masks are not adequate to be protected from some poisonous and deadly gases. More sophisticated equipments and masks are required to be protected against such gases.

In animal husbandry sector, skin diseases are quite common. Fertilizers, plants, feeds, pesticides, weed, insects, the sun, microbes, bacteria and viruses cause skin diseases. Thus, proper protective equipments and clothes should be provided for workers.

## **5. RESULTS AND SUGGESTIONS**

In animal husbandry sector, work health and safety should be defined clearly. Animal farming policies should be developed in such a way as to foster both large-scale and small-scale organizations. Specific programs and strategic action plans need to be developed to prevent occupational risks for workers in animal husbandry sector. Furthermore, occupational health-related applications should be incorporated into the framework of basic health care services.

Works to be done in animal husbandry may bring about various health problems. Workers of animal husbandry sectors are vulnerable to many hazards such as cancer, respiratory diseases, injuries and accidents. Their working environments are full of "physical hazards" that may come from air, land, fire and machines and "toxicological hazards" that may stem from pesticides, fertilizers and fuels and dust. In animal husbandry, for the health and safety of workers, following precautions should be taken.

-Workers should be informed about the ways through which infectious diseases are transmitted and they should be given detailed information about effective hygiene.

- Vaccination and feed controls should be conducted by vets authorized by the Ministry of Food, Agriculture and Livestock at certain intervals. When something unusual is noticed such as unusual look, smell of behavior, vet should be called immediately without having contact with the animal and without staying in the same environment with it.

- The level of the dust in environments where workers are with animals should be measured.

- Workers should be provided with appropriate personal protective equipments to reduce the likelihood of contact with pathogenic microorganisms and parasites. For instance, in environments where microbiological intensity is high, air-filtrating masks should be used in stead of dust-filtrating masks.

-Another measure to be taken for the prevention of infectious diseases is the regular change of work clothes and washing of them in washing machines only used for this purpose.

- By providing enough lighting in animal shelters, trauma risks can be reduced.
- Against physical trauma risks that may occur during the cleaning of barns, care and transportation of animals such as fall, fracture, soft tissue trauma, head trauma, workers should be given first-aid training.
- The amount of epidemiologic research on the health of employees working in animal husbandry sector should be increased.
- Risk evaluation guidelines should be prepared and proper controls should be made.
- Professional training should be given to workers of animal husbandry sector and they should be made recorded workers.
- Educational materials suitable for the socio-demographic structure of the workers in animal husbandry sector.
- By means of legal regulations, workers should be encouraged to get some training about diseases and early deaths in animal husbandry sector.

There are some serious shortcomings in animal husbandry sector in relation to work health and safety. There is no sensitivity towards animal husbandry sector and work health. The Ministry of Labor and Social Security and the Ministry of Food, Agriculture and Livestock should work in cooperation on the issue of occupational health and safety. When the necessary precautions are taken, accidents and diseases can be reduced in animal husbandry sector to a great extent.

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## AN ALTERNATIVE OPPORTUNITY FOR SMALL FARMERS IN TURKEY: DAIRY GOAT FARMING

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*Professional paper*

### Summary

As a result of the information given up to now it is possible to say that the demand for goat products increases gradually both in the world and Turkey. Goat breeding in Turkey has been performed either within an agricultural facility or in form of village herd or migratory herd. There are important investments made by Private Sector to this area in recent years. The main purpose of this study is to analyze the economic aspects of dairy goat farming in Turkey and to evaluate its conditions and opportunities as a alternative investment area for entrepreneurs and farmers in rural area. Statistical data for 2005-2014, used in the study have been obtained from FAOSTAT and TurkStat. Data obtained have been shown in the tables issued by the use of percentage and index calculations. If technical and economic problems encountered by farmers are solve by short- and long-term precautions, goat breeding will make important contributions to the economy of both region and country.

Keywords: *dairy goats farming, goat milk, goat cheese, goat milk marketing.*

### INTRODUCTION

Small farms represent a significant proportion of the total Turkish farms, and current trends in agriculture pose new challenges for their viability and survival. To meet such challenges, there is a need for introducing high-value non-traditional alternative enterprises. Small farms are facing uncertainty and additional risks in today's competitive world. It is necessary to acquire and use risk management tools and possess other marketing skills to survive in this competitive environment (Muhammed *et al.*, 2009).

According to the data of FAOSTAT for 2013 there are approximately 1.01 billion goats in the world. Production of goat milk in the world was 17.8 million tons in 2012. Goat milk production between 2005 and 2012 has increased 18.37%. The share of Turkey in the global goat population and goat milk production is 0.73% and 1.79%, respectively (FAOSTAT, 2015).

Many studies on the technical aspects of goat breeding in Turkey have been performed by Veterinary Surgeons and Zoo Technicians (Taskin *et al.*, 2000; Kaymakci and Taskin, 2005; Oral and Altinel, 2006; Koyuncu *et al.*, 2006; Bilginturan and Ayhan, 2008; Takma *et al.*, 2009; Gunlu and Alasahan, 2010). Some studies on economical of aspects of dairy goat farming in Turkey have been done (Dellal *et al.*, 2002; Tan and Dellal, 2004; Dellal and Dellal, 2005; Dellal *et al.*, 2008; Keskin *et al.*, 2008; Paksoy and Ozcelik, 2008; Demircan *et al.*, 2008; Akturk *et al.*, 2009; Engindeniz and Ucar, 2014a, 2004b). But these studies should be repeated in the course of time and solutions should be produced for related problems.

The main purpose of this study is to analyze the economic aspects of dairy goat farming in Turkey and to evaluate its conditions and opportunities as a alternative investment area for entrepreneurs and farmers in rural area. Statistical data for 2005-2014, used in the study have been obtained from FAOSTAT and Turkish Statistical Institute (TurkStat). Data obtained have been shown in the tables issued by the use of percentage and index calculations.

### THE NUMBER OF GOATS IN TURKEY

According to the data of TurkStat for 2014 there are still 10.35 million goats in Turkey. Hair goats which are available in every region of Anatolia constitute approximately 98% of goat population. Hair goats are bred commonly in the inner parts and on the side of forests. Other than Hair and Angora goats in Turkey comparatively a little number of Maltase goats and cross breeds and Kilis cross breeds have been produced respectively at Western Anatolia coastline and in Kilis and its vicinity. In the recent years, Saanen cross

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breeding have been observed to be developed in Aegean and Marmara Regions (Engindeniz and Ucar, 2014a).

In 2005-2014 period population of Hair goats has increased 61.82% and population of Angora goats has decreased 23.67%. Share of goats in the population of small ruminant in 2005 and 2014 became 20.45% and 24.96%, respectively (Table 1).

Tab. 1. The Goat Population in Turkey

Years	Hair goats (head) (1)	Angora goats (head) (2)	Total goats (head) (1+2)	Index (2005=100)	Share of goat population in small ruminants (%)
2005	6,284,498	232,966	6,517,464	100.00	20.45
2006	6,433,744	209,550	6,643,294	101.93	20.59
2007	6,095,292	191,066	6,286,358	96.45	19.80
2008	5,435,393	158,168	5,593,561	85.82	18.92
2009	4,981,299	146,986	5,128,285	78.69	19.08
2010	6,140,627	152,606	6,293,233	96.56	21.42
2011	7,126,862	151,091	7,277,953	111.67	22.53
2012	8,199,184	158,102	8,357,286	128.23	23.35
2013	9,059,259	166,289	9,225,548	141.55	23.96
2014	10,169,348	177,811	10,347,159	158.76	24.96

Source: TurkStat (<http://www.tuik.gov.tr>).

Considering the regional distribution of goat population in Turkey in 2014 Hair goats are observed to exist in Mediterranean Region at most (27.49%) and Southeastern Anatolia Region (22.19%) and Aegean Region (12.77%). Angora goats are observed to exist in Western Anatolia Region at most (62.19%) and Eastern Marmara Region (15.40%) and Western Black Sea Region (5.35%) are seen to follow it.

### GOVERNMENT SUPPORTS TO DAIRY GOAT FARMS IN TURKEY

Various precautions have been taken by the Ministry of Food, Agriculture and Livestock in order to solve the problems of and to develop goat breeding in recent years. Following supports have been applied in 2014 pursuant to the decree no. 2014/6091 taken by the council of ministers on the supports to be made to Agriculture Sector and the notification no. 2014/22 on the codes of practice on Animal Production Supports;

- 7 € per brood stock to the producers who feed breeding sheep and goats and are the members of the unions of Goat and Sheep Breeders and the breeders whose animals are registered in the systems of the Ministry of Food, Agriculture and Livestock.

- 0.07 €/l for goat milk to the breeders who sell the raw milk produced to milk processing facilities within the scope of “the Regulation on the Registration and Approval Transactions for Food Establishments” published in the official gazette dated and numbered respectively 17/12/2011 and 28145 and is the member of Animal Breeding Organization which has completed its top organization duly at national level as stated in Article 10 of this decree

- 0.18 € per animal for Alum and Brucellosis Vaccine to the Practitioners for the programmed vaccinations determined by the Ministry of Food, Agriculture and Livestock within the frame of the Struggle with Animal Diseases.

- 28 € per animal in ovine breeding for protection and development periods to the breeders taken to the scope of the project applied by the Ministry of Food, Agriculture and Livestock in order to protect the genetic resources of animal onsite and to develop them,

- Furthermore it has been decided to make payment per animal in addition to supports for animal breeders raising organic sheep and goats.

Investment and working capital loans are offered to breeders who want to establish an organization with a capacity of minimum 50 animals for sheep or a capacity of minimum 25 animals for goats (Saanen, Kilis, Angora goat breeding and cross breeding with stated races for the eugenics of Hair goats) or want to increase its installed capacity to the said capacities.

### GOAT MILK AND CHEESE PRODUCTION IN TURKEY

The number of milked goats between 2005 and 2014 has increased 82.61%. Hair goats and Angora Goats constitute 98.23% and 1.77% of 4.40 million goats milked in 2014. 99.41% and 0.59% of 463,394 ton milk

produced in 2014 have been produced respectively from Hair goats and Angora goats. Average milk productivity per milked animal in 2012 was 106.54 kg for Hair goats and 35.43 kg for Angora goats. Total goat milk produced in 2014 constitutes 2.50% of total milk production (Table 2).

Tab. 2. Goat milk production in Turkey

Years	Hair goat milk (ton) (1)	Angora goat milk (ton) (2)	Total goat milk (ton) (1+2)	Index (2005=100)	Share of goat milk in total milk production (%)
2005	250,246	3,513	253,759	100,00	2.28
2006	250,594	3,165	253,759	100,00	2.12
2007	234,883	2,604	237,487	93,59	1.93
2008	207,385	2,185	209,570	82,59	1.71
2009	190,286	1,924	192,210	75,74	1.40
2010	270,476	2,335	272,811	107,51	2.01
2011	318,273	2,315	320,588	126,34	2.13
2012	367,208	2,221	369,429	145,58	2.17
2013	413,444	2,299	415,743	163,83	2.28
2014	460,642	2,752	463,394	182,61	2.50

Source: TurkStat (<http://www.tuik.gov.tr>).

According to data of FAOSTAT for 2012 457,401 tons goat cheese has been produced in the world. Turkey takes 0.02% share in the world with its production of 92 tons. Production of goat cheese has increased 46.03% in Turkey between 2005 and 2012 (Table 3).

Tab. 3. Development of goat cheese production in Turkey and the World (tons)

Years	Cheese production of the World (ton)	Cheese production of Turkey (ton)	Index (2005=100)	Share of Turkey in the World (%)
2005	468,176	63	100.00	0.01
2006	462,527	63	100.00	0.01
2007	460,650	59	93.65	0.01
2008	458,852	52	82.54	0.01
2009	463,345	48	76.19	0.01
2010	475,124	68	107.94	0.01
2011	368,526	80	126.98	0.02
2012	457,401	92	146.03	0.02

Source: FAO (<http://www.fao.org>).

## MARKETING OF GOAT MILK AND PRODUCTS IN TURKEY

Goat milk products produced generally for family consumption in rural area become wanted today upon the concentration of urbanization and developments in tourism. Firms processing the milks collected from goat farms in the integrated plants manufacture pasteurized milk, cheese, strained yoghurt and butter and put them into market. Furthermore some firms use goat milk in ice-cream production (Savran *et al.*, 2011).

Turkish entrepreneurs have imported Saanen and Boer goats from Australia, Togenburgs from Swiss and USA, Damascus Goats from Syria and created a new market of 425 million € in the farms that they have established. Goat farms give the entrepreneurs to take the return of investment in 6-8 years since the amount of investment is little and the price of goat milk is two-and-half times more than the price of cow's milk. Together with recent developments the share of goat milk exceeds 5% in the market of milk and milky products of 5 billion €. Furthermore most preferred goat type in the farms is the Saanen goats which take origin from Australia and may triple milk productivity (Engindeniz and Ucar, 2014b).

According to the data TurkStat the goat milk price per litre received by farmers in Turkey are € 0.49 in 2005, € 0.66 in 2010, € 0.60 in 2014, respectively (Table 4). However, according to the data FAOSTAT for 2012 the goat milk price obtained by farmers in Turkey is 657.80 €/tons. In this sense Turkey has a similar position with Latvia (611.59 €/tons), Norway (596.14 €/tons), Greece (592.88 €/tons) and is over Albania (561.36 €/tons), Azerbaijan (556.21 €/tons) and Spain (541.67 €/tons) (FAOSTAT, 2015).

Tab. 4. Milk prices received by farmers in Turkey

Years	Goat milk (€/kg)	Sheep milk (€/kg)	Cow milk (€/kg)
2005	0.49	0.49	0.40
2006	0.48	0.48	0.39
2007	0.56	0.55	0.44
2008	0.51	0.49	0.37
2009	0.53	0.55	0.37
2010	0.66	0.63	0.44
2011	0.59	0.57	0.33
2012	0.66	0.63	0.38
2013	0.56	0.58	0.32
2014	0.60	0.64	0.37

Source: TurkStat (<http://www.tuik.gov.tr>).

Total production value of goats (428 million €) in Turkey formed 2.74% of total animal production value in 2014. Further, goat milk production value was 61.14% of total production value of goats (Table 5).

Tab. 5. Production value obtained from goats in Turkey (2014)

Goat products	Total value (1000 €)	%
Goat milk	262,000	61.14
Goat meat	160,800	37.52
Goat skin	3,005	0.70
Goat hair	2,004	0.47
Goat mohair	738	0.17
Total (1)	428,547	100.00
Animal production value (1000 €) (2)	15,665,196	-
% (1/2)	2.74	-

Source: TurkStat (<http://www.tuik.gov.tr>).

There is no any data of FAOSTAT and TurkStat on web pages related to goat milk and products foreign trade of Turkey. But according to data of TurkStat, live goat import value of Turkey were € 118,810 in 2010, € 209,431 in 2011, € 247,990 in 2012, € 1,155,458 in 2013, € 549,223 in 2014, respectively. Turkey import goats mostly from Australia. Recently, Turkey have exported live goats to Azerbaijan. Live goat export value of Turkey were € 189,844 in 2013, € 60,229 in 2014, respectively (TurkStat, 2015).

## CONCLUSION

Goat milk is also in demand as a gourmet item and a healthy food. Therefore, the goat industry has potential as an alternative enterprise for many small, part-time, and limited resource farmers. An increase in domestic production, import, and ethnic population indicates that prospects for the goat milk production in Turkey are promising.

Today production of special and quality goat cheese from goat milk is handled seriously by European countries and related production is performed commonly in first France and Spain, Italy, and Greece. Such kind of products may be produced in Turkey with similar conditions and economically considerable earnings may be obtained. Considering the aspects of Turkey regarding agriculture and animal breeding milked goat and production of goat milk are known to have a great potential as in Mediterranean countries.

If technical and economic problems encountered by farmers are solve by short- and long-term precautions, goat breeding will make important contributions to the economy of both region and country. However in the event that safety product range to be exported is enlarged, Turkey will be able to compete with other countries. EU has important deficits in goat products and this deficit can be met by Turkey in most rational way. This goat breeding in Turkey should be supported directly and indirectly in accordance with EU standards.

Structural changes are necessary in the medium and long terms as well as the continuance of the supports by price in order to increase the production in goat breeding. For this purpose growth and merge of little and dispersed enterprises and conversion of the enterprises to enterprises specialized in ovine breeding for milk production and related inputs for these enterprises should be provided.

An organizational model should be prepared based on horizontal and vertical integration within the period from the production of goat products to consumption by consumers. This model will be formed by cooperatives. Cooperatives will be determinative in not only production stage but also in processing and marketing the products. Producers should be promoted in this direction.

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## EFFECT OF ENERGY AND PROTEIN LEVELS ON WATER HOLDING CAPACITY OF GOAT MEAT

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*Original scientific paper*

### Summary

An experiment was conducted to evaluate the effects of three levels of metabolisable energy (2, 2.4 and 2.8 Mcal/kg DM) and three levels crude proteins (12.6, 14 and 16.8 percent) on water holding capacity (WHC) to identify the optimum levels of dietary energy and protein of indigenous kid meat. For this case were taken meat samples of 27 kids fattened carcass under complete randomize design with a factorial experiment of 3×3 (Three levels of energy × three levels of protein) for 4 months fattening period. Three kids of each group (9×3 equal 27 kids) randomly slaughtered and then after 24 hours were sampled of longissimus dorsi (MLD), muscle. Water holding capacity of the MLD muscle included measurements for drip loss and cooking loss. The muscles were vacuum-packaged and put on a plastic hurdle (about 30 g) and conditioned for 1, 7 and 14 days in a chiller at 4°C for measuring drip loss and cooking loss. Juiciness of meat is directly related to the intramuscular lipids and moisture content of the meat. Chevon is reportedly less juicy than lamb, a quality attributed to the lower fat content of chevon. Effects of different levels of energy and proteins were not significant on drip loss and cooking loss of kid meat. In the present experiment the least square means for the percent of drip loss and cooking loss, as well as the effect of the time of display on the cooking loss in the MLD muscle were not affected ( $P > 0.05$ ) by the treatments. However, the drip loss increased ( $P < 0.05$ ) by days 7 and 14 of the time of display. The post-mortem aging time were significant effect on drip loss and cooking loss in most of groups ( $P < 0.05$ ). In general, it can be concluded that the diet with 2/4 Mcal/kg DM metabolisable energy and 14 percent crud protein (energy to protein ratio of 1:1) suggested as an appropriate diet.

**Key words:** *Indigenous kids, protein and energy levels, water holding capacity.*

### INTRODUCTION

Water is the major component of lean muscle accounting for approximately 75% of its weight (Aberle *et al.* 2001). There are three types of water in muscle; each differing in degree of its freedom, most of the water in muscle is held within the cell structures include inside myofibrils and inter-myofibrillar spaces, between myofibrils and cell membranes, between muscle cells and between bundles of muscle cells (Kolczak *et al.*, 2007). The bound water has reduced mobility and is very resistant to freezing and evaporation by heat. This water changes very little in post rigor muscles (Offer and Knight, 1988). The second type of water found in skeletal muscle is called immobilized water. A Nuclear Magnetic Resonance study indicated that the amount of water which was tightly trapped in protein networks increases with ageing (Bertram *et al.*, 2004). The ability of fresh meat to retain both inherent and added water content is termed WHC (Aberle *et al.*, 2001). Drip loss is an important quality criterion for the meat processing industry and the consumer. Offer and Knight (1988) described drip loss as a reddish fluid mainly consisting of water and proteins. It can be expelled from cut surfaces of muscles or pieces of meat without any mechanical force other than gravity. Drip loss occurs from cut surfaces of meat. High drip loss is undesirable because it detracts from the appeal of the meat, and valuable proteins and flavor compound are lost in the exudates (Varnam and Sutherland, 1995). Drip loss is normally in the order of 3% in beef but may be exacerbated by very low pH and by freezing and thawing to as much as 15% (Offer and Knight, 1988). In the past, the assessment of drip loss was done by several methods. For example the filter paper-press method and the bag method of Honikel (1998). Cooking losses from chevon are of interest because, the water that remains in the cooked product is the major contributor to the sensation of juiciness and chevon cooking losses are often close to or over 35percent (Webb *et al.*, 2005). High cooking losses not only reduce the size of the meat portion but also result in reduced juiciness, tenderness and loss of flavor. Cooking loss of the meat is possibly exacerbated by its limited fat content (Huff-Lonergan and Lonergan, 2005).

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## MATERIALS AND METHODS

The 27 kid meat samples were taken of fattened carcasses under complete randomized design with a factorial experiment of 3×3 with 9 groups for 4 months fattening period. Control group and other experimental groups of 1, 2, 3, 4, 5, 6, 7, 8, and 9 were fed by ration of energy to protein ratio of 1:1, 1:1.2, 1:0.8, 1.2:1, 1.2:1.2, 1.2:0.8, 0.8:1, 0.8:1.2 and 0.8:0.8 respectively by total mixed ration. Three levels of metabolisable energy (2, 2.4 and 2.8 Mcal/kg DM) and three levels crude proteins (12.6, 14 and 16.8 percents) on water holding capacity (WHC) to identified the optimum levels of dietary energy and protein of indigenous kid meat. After slaughtered and post mortem 24 hours were sampled of longissimus dorsi (MLD) muscle. The muscle was vacuum-packaged and put on a plastic hurdle (about 30 g) and conditioned for 1, 7 and 14 days in a chiller at 4°C for measuring drip loss and cooking loss. At the 0, 4 (Second experiment only), 7 and 14 days post mortem and longissimus dorsi muscle sample was weighed (approximately 30 g) and put on a plastic hurdle. Then both items (meat samples on the plastic hurdle) were put into sealed polyethylene bags hermetically closed to prevent surface evaporative loss. After a 24h storage period at 4°C, the meat samples were removed from the bag and reweighed. The difference in the weight of the samples, before and after storage, divided by the sample weight before storage × 100 accounted for the % drip loss (Honikel, 1998).

The measurement of cooking loss was conducted on the longissimus dorsi (MLD) (between the 6th and 13th thoracic vertebrae) samples were weighed (about 30g) and held in plastic bags and immersed in a 80°C water-bath until the internal temperature reached 78°C as monitored with a needle thermometer. Then, the bags were cooled under running tap water for 30 min, blotted dry with paper towels, and reweighed. Cooking loss, as percentages, were then calculated from the difference between the weights (Honikel, 1998).

## RESULTS AND DISCUSSION

Effect of different levels of energy and protein on drip loss, cooking loss in most of groups were not significant, but post-mortem aging time of different levels of energy and protein were significant ( $P < 0.05$ ). In this experiment the least square means for the percent of drip loss and cooking loss in the MLD muscle was not affected ( $P > 0.05$ ) by the treatments. The drip loss increased ( $P < 0.05$ ) by days 7 and 14 of the time of display. The post-mortem aging time were significant effect on drip loss and cooking loss in most of groups ( $P < 0.05$ ). The cooking loss increased ( $P < 0.05$ ) by 14 days of the time of display. The post-mortem aging time were significant effect on cooking loss in most of groups ( $P < 0.05$ ). Leheska *et al.* (2002) reported that dietary treatments had not influence on drip loss and cooking loss. According to Trout (1988), cooking loss is more dependent on final pH, sarcomere length and cooking conditions. Kadim *et al.* (2003) reported ageing had a significant influence on percent cooking loss of the selected muscles. Dietary treatment comprising different level of energy and protein or post mortem aging period did not have any effect on the cooking loss of MLD muscle (Kannan *et al.*, 2006). In general, it can be concluded that the diet with 2/4 Mcal/kg DM metabolisable energy and 14 percent crud protein (energy to protein ratio of 1:1) suggested as an appropriate diet.

Tab. 1. Drip loss and cooking loss in Longissimus dorsi muscle of goats under different treatments and post mortem aging period (n=27)

Treatments		Post mortem aging periods					
		Drip loss			Cooking loss		
		1 day	7 day	14 day	1 day	7 day	14 day
Longissimus dorsi (MLD)	1	1.35 <sup>x</sup>	3.65 <sup>y</sup>	3.89 <sup>y</sup>	23.4 <sup>x</sup>	26.2 <sup>xy</sup>	28.7 <sup>y</sup>
	2	1.42 <sup>x</sup>	3.73 <sup>y</sup>	3.84 <sup>y</sup>	24.1 <sup>x</sup>	26.5 <sup>xy</sup>	29.6 <sup>y</sup>
	3	1.33 <sup>x</sup>	3.48 <sup>y</sup>	3.54 <sup>y</sup>	23.2 <sup>x</sup>	25.8 <sup>xy</sup>	28.2 <sup>y</sup>
	4	1.35 <sup>x</sup>	2.59 <sup>xy</sup>	3.18 <sup>y</sup>	23.6 <sup>x</sup>	26.9 <sup>xy</sup>	29.2 <sup>y</sup>
	5	1.42 <sup>x</sup>	3.05 <sup>y</sup>	3.66 <sup>y</sup>	25.1 <sup>x</sup>	28.3 <sup>xy</sup>	30.4 <sup>y</sup>
	6	1.09 <sup>x</sup>	2.46 <sup>xy</sup>	3.03 <sup>y</sup>	25.3 <sup>x</sup>	28.5 <sup>xy</sup>	30.8 <sup>y</sup>
	7	1.55 <sup>x</sup>	3.26 <sup>y</sup>	3.76 <sup>y</sup>	23.1 <sup>x</sup>	25.7 <sup>xy</sup>	28.9 <sup>y</sup>
	8	2.14 <sup>x</sup>	3.42 <sup>xy</sup>	4.13 <sup>y</sup>	22.8 <sup>x</sup>	25.6 <sup>x</sup>	27.4 <sup>y</sup>
	9	1.15 <sup>x</sup>	2.78 <sup>y</sup>	3.53 <sup>y</sup>	23.3 <sup>x</sup>	29.1 <sup>y</sup>	31.3 <sup>y</sup>
±SEM		0.42	0.71	0.69	1.59	1.81	1.73

x ,y Means within rows with different superscripts are different among post mortem aging periods ( $P < 0.05$ ).

## CONCLUSION

It can be concluded that drip loss and cooking loss in Longissimus dorsi muscle of goats were influenced by post-mortem aging period of goat meat, whereas changing levels of energy and protein had no significant effect on water holding capacity of goat meat, but the appropriate diet was 2/4 Mcal/kg DM metabolizable energy and 14 percent crude protein (energy to protein ratio of 1:1).

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## FACTORS AFFECTING ANIMAL WELFARE DURING TRANSPORT IN CATTLE

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*Professional paper*

### Summary

Whereas welfare and health of animals can be substantially affected by transport, recently livestock transportation has become a routine management practice in export or import. Transportation is source of stress for animals. During transport, many factors such as loading, unloading, loading density, transport duration, water and feed needs of animals and movements of vehicle directly effect on animal welfare, health, and carcass and meat quality. Therefore, physical needs of the animal at the different stages of life must be accommodated within each stage of transportation and psychological responses of cattle must be considered with good management before, during, and after transport.

Keywords: *Transportation, animal transport, welfare, cattle, handling*

### INTRODUCTION

Welfare defines the state of an animal as regards its attempts to cope with its environment (Fraser and Broom, 1990) so animal welfare is directly related with the environment where animal in it. These environments could be a farm, a house, a garden, a street or a vehicle. Welfare is very important for farm animals. Poor welfare include the following: reduced life expectancy, impaired growth, impaired reproduction, body damage, disease, immunosuppression, adrenal activity, behavior anomalies, and self-narcotization (Broom, 1991). Farmed animals pass through three distinct stages in their lives: production, transport and slaughter (Harris, 2005) and transporting is one of the important stress factor on animals and affects animal welfare.

### TRANSPORTING AND WELFARE IN CATTLE

Transport of the farm animal is a necessary apart of its life. Transporting of livestock farm to farm or farm to slaughter is routine herd management practice in all over the world. Recently, transportation of cattle husbandry has become a routine management practice as livestock export or import has become more common.

Tab. 1. A brief survey on cattle transportation in Europe (Anonymous, 2002)

	inside the Europ. Union	imports from other countries	exports to other countries	<b>Total</b>
Breeding cattle	134315	4747	101812	240874
Calves	1092512	316880	64	1409456
Cattle	2018210	196788	228780	2443778
Slaughter cattle	-	-	-	22070695
Slaughter calves	-	-	-	5793395
<b>Total</b>	<b>3245037</b>	<b>518415</b>	<b>330656</b>	

Although cattle transportation has been occurring for hundreds of years, the same problems still occur these days as occurred when animals were first being moved. Handling, loading, transporting and unloading of animals can have very substantial effects on their welfare (Broom, 2003). During transport livestock animals are exposed to a variety of potential stressors. If adaptation does not occur and the stressors persist, the

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physiological changes in the animal might lead to a pathological state in the long run and have deleterious effects on health, well-being, performances and, ultimately, product quality (von Borell, 2001).

### FACTORS AFFECTING WELFARE DURING TRANSPORT

Direct handling, constraint and novel housing environment during transport are short stressful events for animals. In particular, the motion of the lorry, noise, vibration, quickly changing light, temperature and humidity conditions, poor air quality, shortage or lack of water and feed are grouped as physical stressors and are recognized as the reasons of reduced welfare and health of the animals which can decrease product quality and may even cause death (Zanardi *et al.*, 2007).

Loading, characteristics of loading and unloading ramp, loading density, vehicle characteristics (eg: suspension, height, ventilation), road and climatic conditions are direct effect on animal welfare (Zanardi *et al.*, 2007). Also transport and transport practices play a pivotal role in the animal welfare and protection during transport. Tarrant and Grandin (2000) reported that 80% of the losses of balance of animals on the means of transport are due to sharp braking and too rapid change of speed. But it can be said that some factors previously mentioned; loading density, travelling time, loading and unloading ramps, vehicle design more important the other factors because of their effects on product quantity and quality.

#### *Loading Density*

The amount of space allowed for an animal during transport is one of the most important factors influencing its welfare. Space allowances have two components. The first component is the floor area available to the animal to stand or lie in. This equates to what is usually referred to as stocking density. The second component is the height of the compartment in which the animal is carried. Absolute minimum space allowances are determined by the physical dimensions of animals (Anonymous, 2002). However, acceptable minimum allowances will be dependent on other factors as well. These include the ability of the animals to thermoregulate effectively, ambient conditions, particularly environmental temperature, and whether the animals should be allowed enough space to lie down if they so wish.

Equation that suggested by the UK Farm Animal Welfare Council (1991) for the minimum acceptable area for all types of animal:

$$A = 0.021 W^{0.67}$$

where A is the minimum floor area required by the animal in m<sup>2</sup> and W is the weight of the animal in kg. The constant in the equation (0.021) depends on the shape of the animal, in particular the ratio of its body length to its body width (Anonymous, 2002). The space allowed per animal in the vehicles calculated according to number of animals and capacity of the vehicle, and these calculations were compared with the standards of EU (Table 1) (RSPCA, 2010).

Tab. 2. Allowed space for each animal during transport in EU standards

	Weight (Kg)	Area per head (m <sup>2</sup> )
Small calves	50	0.30–0.40
Medium calves	110	0.40–0.70
Heavy calves	200	0.70–0.95
Medium cattle	325	0.95–1.30
Heavy cattle	550	1.30–1.60
Very heavy cattle	>700	>1.60

Also loading densities are determined according to the average live weight, condition, size, shape and horn status of the cattle. 5% fewer cattle should be loaded if they are horned.

#### *Travel Time*

One of the factors that affect animal welfare is also travel time.

A journey is considered to start when the animal is first loaded at its place of departure and be completed when the animal has been unloaded at its destination.

Time spent on Roll-on/Roll-off (RO-RO) vessels counts towards the journey time.

Time spent by animals in pens on specialist livestock vessels does not count towards total journey time, provided certain conditions are met.

Time whilst on the aircraft during air transport does not count towards total journey time (Anonymous, 2009). Long journeys may put their welfare at risk. Within the European Union, maximum journey times to protect the welfare of farmed animals transported for commercial purposes are regulated by Council Regulation (EC) No. 1/2005. These regulations restrict journey times for cattle to a maximum of 8 h, except when certain additional requirements are met. These additional requirements include provisions for: sufficient bedding, a ventilation system that is capable of maintaining the temperature between 5 and 30°C within the vehicle when it is moving or stationary, the provision of sufficient and appropriate feed together with any specific feeding equipment; a water supply and devices capable, when necessary, of providing drinking water instantly to each animal while they are on the vehicle and a navigation system that records the route and position of the vehicle. The journey times, rest periods, and watering and feeding intervals when using road vehicles that meet the additional requirements are described in the regulations as follows:

Unweaned calves, lambs and kids that are still on a milk diet, and unweaned piglets must, after 9 h of travel, be given a rest period of at least 1 h, and sufficient for them to be given liquid and, if necessary, fed. After this rest period, they may be transported for a further 9 h.

All cattle, sheep and goats other than unweaned animals must, after 14 h of travel, be given a rest period of at least 1 h sufficient for them to be given liquid and, if necessary, fed. After this rest period, they may be transported for a further 14 h. After the above journey times, the animals must be unloaded, fed and watered and be rested for at least 24 h (Cockram, 2007).

Journey times and rest periods according to rules are shown in Table 3.

Tab. 3. Maximum transporting and resting periods (Hour) for cattle

	Basic standard	Higher standard vehicle			
		vehicle	Travel	Rest	Travel
Cattle	8		14	1	14
Unweaned calves	8		9	1	9

### ***Loading and Unloading Ramp***

Loading and unloading livestock onto or off a vehicle can be the most stressful part of animal transportation. Loading and unloading facilities typically need to be compatible with a range of vehicles. This will mean that some premises may need to have more than one loading or unloading ramp. Whilst a gentle incline is appropriate for loading bays, unloading bays should always aim for the ramp created by the tailgate and the unloading bay to be as level as possible. There is a variety of different designs for unloading bays. These range from flat concrete or raised steps (Figure 1) to more complex designs which have hydraulic systems that move up or down depending on the height of the lorry (Figure 2).



Fig. 1. Flat concrete or raised steps



Fig. 2. Hydraulic systems

Whatever design is used, the side gates should provide a secure barrier and preferably be solid in construction to prevent distractions (Cockram, 2007).

The gradient of ramps should preferably be 8-10° and should never be steeper than an angle of 20° to the horizontal for calves. Cattle can climb 20-25° ramps but cleats at 30 cm intervals are needed. Ramps steeper than 20° are not recommended because of the risks if animals panic (Anonymous, 2015).

### **Vehicle Design**

There are various types of vehicles used for cattle transportation especially on shorter transports. For those legal requirements are more or less only defined regarding the space allowance. Commonly used in long distance cattle transport are double decked semitrailers, trailers and road trains, each with four or five pens. In most modern lorries the upper floor is movable (Anonymous, 2015).

All vehicles used to transport animals must comply with the 'basic standards' specified within the order but animals must not be transported for more than 8 h in these vehicles. Vehicles which are to be used to transport animals for periods in excess of 8 h must comply with the additional requirements for 'higher standard' vehicles set out in the Order.

Transport vehicles, especially those used for long journeys, (longer than 8 hours) must have a suspension system that is at least as good as air suspension on all axles.

The walls and roof of the vehicle should be insulated to minimize over-heating or excessively cold conditions. There must be ventilation openings and forced ventilation which can operate independently of the vehicle engine.

Forced ventilation must be used when the vehicle is stationary.

The height of the compartment for cattle should be at least 20 cm above the top of the head of each animal when it is standing in a comfortable position. This figure applies for all vehicles (Anonymous, 2002).

The floors of all vehicles must be covered with sufficient bedding in order to provide comfort and reduce the likelihood of injury (RSPCA, 2010).

### **CONCLUSION**

Problems such as rising mortality, deformation on carcass, losing weight, decreasing meat quality that occurred resulting from unheeded animal welfare during transport, show that importance of animal welfare regulations. Moving animals to herd to herd or herd to slaughter is routine in production processes and before, during or after transport, various circumstances such as loading density, travel time, loading and unloading ramp and vehicle design effect on animal welfare.

In conclusion, knowing stress factors that will able to occur during transport and their effects on animals, animal needs according to race, species, sex, age, slaughtered or breeding and because of that reduce these effects, basic precautions that will be applied as part of regulations are very important.

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## PROPOSALS FOR DEVELOPMENT STRATEGIES OF LIVESTOCK SECTOR IN CENTRAL AFRICAN REPUBLIC

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*Original scientific paper*

### Summary

The Central African Republic (CAR) is a landlocked country with no access to the sea and a total area of 622,980 km<sup>2</sup>. According to the General Census (2009), CAR's current population is estimated at about 5 million. 56% of Central Africans live in rural areas. The Central African economy is essentially based on agriculture (55%). Agricultural production and livestock systems are identified, from north to south, by the dominant bioclimatic conditions in such manner as cropping systems are highly dependent on rainfall. Climate variability and weather events therefore have a direct impact on agro pastoral production. The livestock sector in Central African Republic consists mainly of cattle, and to a lesser extent of sheep, goats, pigs and poultry. It is estimated that nearly 300 000 cattle are marketed each year including 250 000 cattle consumed within the country and 50,000 cattle exported abroad. Beef is particularly appreciated by the population. It's the major source of animal protein in CAR and is involved in the composition of many dishes. An ordinary citizen eats beef about 3 to 4 days at least a week. This meat is consumed in several forms: cooked, fried, stewed, dried, smoked ... The annual per capita consumption over the whole territory is about 17 kg and over 30 kg in the capital Bangui. Unfortunately, recent socio-political troubles have destroyed this balance. These disorders have caused the fall of the national herd of almost half of its headcount before the conflict. It is therefore necessary to find and to invent new strategies to –(i)- meet the needs of the constantly growing population –(ii)- to develop this sector which is a potential source of foreign exchange for the country and –(iii)- to preserve the gene pool that is in danger today. These are the objectives referred to in this article.

Keywords: *Livestock, Central African Republic*

### INTRODUCTION

The Central African Republic (CAR) has a favorable environment for the development of cattle breeding. Weather conditions, low population density (5.30 inhab./Km<sup>2</sup>), abundance of surface waters and the presence of good quality pastures in the country are enormous potential for the development of this sector (Kadekoy, 2003). Cattle farming is not a very long time business in Central African Republic. The first drovers farmers (Fulani Mbororo) have entered the territory in the 1920s, from neighboring Cameroon. In the 1930s, they extended their route to the Land of Bossembélé, located in the centre of the country. In 1937, a staff of 200 000 animals were counted (Boutrais, 1988). In the 60s, external inputs have started up coming from Chad and Sudan. Meanwhile, the flow of cattle to Central Cameroon, Congo and Nigeria become important (Sarniguet *et al.*, 1967; Coste *et al.*, 1993) until the eve of the recent socio-political troubles in the country. The armed violences, cattle thefts, endemic diseases such as trypanosomiasis and various disturbances of the sector have caused the fall of the national cattle herd, the soaring of prices and starvation among the population. But quite a while now, several attempts to reorganization of this sector have been tried by the Central African authorities, NGOs, associations and other stakeholders involved in this activity. This article is on the same dynamic and offers some strategies to quickly remedy this situation and provide the population with a balanced diet, rich in good quality protein. Our hypothesis is simple and unique: despite the damage recorded in the cattle rearing, we believe that the potential of the country for breeding remained intact. CAR can still retake his place of first exporting country of cattle in the Central Africa sub-region.

### METHODOLOGY

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The approach chosen for this study has been to conduct with surveys to the stakeholders of cattle sector. More so, looking for archives and interviewing resource persons have enable to complete the collection of survey data. Several articles and official documents on the subject were viewed and sorted. The online database, FAO's archives were also involved. This has permitted to highlight some figures and tables useful purposes for our goals. The analysis of the existing allowed us initially to identify problems and then to assess the needs and finally to make concrete proposals for solutions. The results thus directly proposed development strategies of livestock while presentation of the existing will be subject of another article.

## RESULTS AND DISCUSSION

The results are presented in order of importance according to their effects on the country. Thus, our analysis has allowed us to make the following classification by focus area: security, education-research, production-commercialization, investment and the genetic improvement.

### Restoration of security

Ankogui *et al.* (2010) worked on the influence of insecurity on regional movements of cattle in Cameroon, Central African Republic and Chad. According to their studies, hostage taking, rackets, attacks on camps and murders are a manifestation of the insecurity. The economic crisis, the political and military troubles and the open trafficking of weapons have been identified as the major causes. Those responsible for the insecurity (highwaymen, uncontrolled armed groups, even the armed forces and some civil servants...) have close links with the communities concerned and the livestock traders. This phenomenon is responsible for displacing people to areas that are still stable, increasing poverty among herdsmen and problems with the supply of meat and milk in the three countries. More than 600,000 cattle are estimated to have migrated to Nigeria. This insecurity has meant that livestock production, whether transhumant or sedentary (figure 1), is in danger of extinction in the villages that have suffered from the atrocities. This presents major challenges to national governments in the framework of regional integration policies to combat highwaymen, who generally hide in the areas situated on the borders. Figure 1 gives an overview on the traditional livestock areas and movements. Insecurity in Central Africa has led farmers to flee at first CAR and then the Central African sub-region to go to Nigeria in West Africa (figures 2 and 3).

To restore security in the country, it's necessary to restore the rule of law. Impunity must be punished in accordance with the rules. The establishment of that state requires the introduction and the observance of democracy, respect to the laws of the nation, the equitable distribution of national resources, the implementation and practice of justice that gives to everyone what he deserves, setting up of credible elections that reflect the will of the population and the defense of national territory against external attacks. Otherwise, conflicts of interest will continue to bleed this beautiful and young country.

### Education and Research

The fields of education and Research that always go together have to be improved. New strategies of education have to be created and practiced. Our analyzes allow us to say that this sector has not always received the consideration it deserved (Table 1). For a long time, the sector has been neglected. For how can we understand

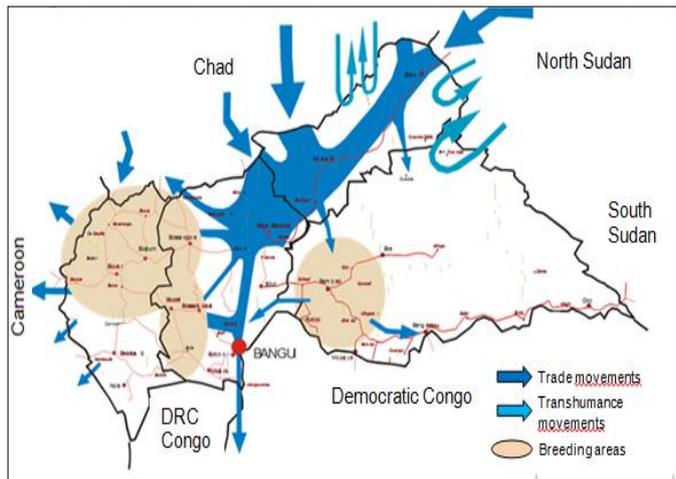


Fig. 1: Traditional livestock areas and movements (Tyc and Sarniguet, 1991).

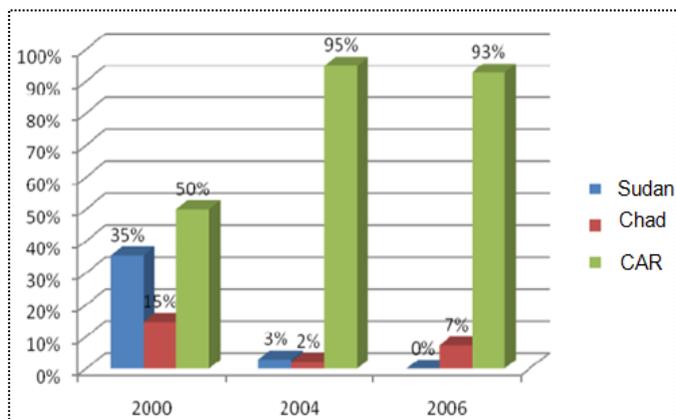


Fig. 2: Evolution of live cattle deals on the market of PK 13 in Bangui (ANDE, 2007)

that after 60 years of independence, the CAR is not able to train doctors in the field of general agronomy? Then the institutions that train technicians and engineers are in short supply: only one high school to train engineers, few which make agricultural technicians. This does not mean that nothing is done for some expended efforts are commendable. But we must do more.

Tab. 1: Comparison of gross literacy ratios (%) in the world (BAD, 2009)

Education Indicators	Year	CAR	Africa	Developping countries	Developped countries
Primary - Total	2008	81.7	99.6	106	101
Primary - Girls	2008	69.5	92.1	103	101
Secondary - Total	2007	36.2	43.5	60	101.5
Secondary - Girls	2002	...	40.8	58	101
Adult illiteracy - Women (%)	2003	...	47	27	1
Education expenditure as% of GDP	2006	1.4	4.5	3.9	5.9

We suggest the creation of agricultural vocational schools in areas of production and a major university in the country to meet the critical needs for training, restructuring of the education system in the field. CAR does not necessarily lack of hands work but of policies. For example many high school teachers have been forced to emigrate in other countries of the sub-region such as Gabon, for example, to be able to live of their profession. This brain drain largely explains the underdevelopment of the country. Also, the Central African diaspora has many engineers, renowned professors and competent staff. We therefore propose to the authorities to create policies to encourage their return. Research should not be overlooked. Nowadays, new methods of selecting productive and hardy breeds through biotechnology exist. The authorities must provide practitioners with new equipped laboratories to meet both the educational and production needs. The staff of state institutions involved in livestock must be trained, recycled and used to the new management methods. The fifties national infrastructures will have to be demolished and replaced by new buildings for breeders who for most often are not educated, we have to find simple and appropriate means for the transmission of knowledge to them. In this, the training workshops for farmers, their organization within community associations, national and sub-regional especially should be encouraged. A new demarcation of transhumance corridors must be made in light of the changing situation on the ground, the effects of global warming etc.

## Production

The table 2 gives an estimation of the bovine livestock of CAR.

Tab. 2: Estimation of the bovine livestock of CAR (Kadekoy, 2003)

Farming system	Breeders number	Cattle number (x1000)	Percentage
Transhumant cattle	20 000	3 784	93.60
Zebus agro-farming	3 000	120	3.00
Taurins agro-farming	730	12	0.30
System related to animal traction	5 600	18	0.40
Foreign seasonal transhumant	-	110	2.70
Total	-	4 044	100

According to the analysis of the previous table, there is no intensive farming in CAR. Several reasons may explain this state of affairs: the low density of the population, the vast territory, the profusion of pastures, hardiness animals, the contribution of livestock from neighboring countries and a more or less satisfactory management of the sector far as the outskirts of the 90s (Figure 3).

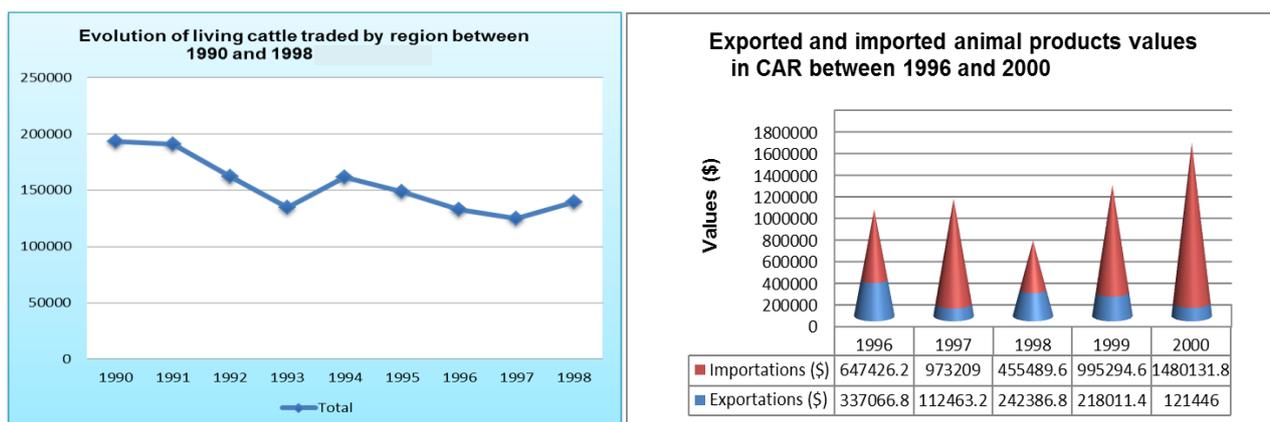


Fig. 3: Animal production supplies in CAR (MADR, 2003)

The rearing of cattle is limited to bovine breed zebu (*Bos indicus*) and taurine (*Bos taurus*) and their mestizo. We distinguish the following systems

- transhumant pastoralism,
- Livestock sedentary peasant,
- Livestock "model Ranching",
- the small village conditions,
- the small modern farming,

Excepted modern farming and ranching model, these systems are low of in or outputs. Other species of horse and donkey races of a reduced workforce are not supervised by a development program. In order to increase the national livestock, to bring down the price of cattle on the markets and to allow the population to cover its needs in meat products, we propose the following strategies:

- rehabilitation of rural roads to facilitate livestock marketing and transport,
- Construction of modern slaughterhouses to fight against the savage slaughter and expansion of epidemic / endemic diseases,
- Refurbish and build new rural and municipal markets,
- create intensive livestock breeding centers in partnership with universities and research stations, farmers and stakeholders in the development,
- Build in the all 16 prefectures of the country feed manufacturing factories to support production base. Develop monitoring farmers, agricultural extension so as to create new technologies adapted to local conditions in the country.

### Investment

Statistical calculations for 2001 show that the CAR livestock contributes to over 10.5% of national GDP. The lack of pastoral census for decades does not allow to work on reliable statistical data. According to a study realized by the African Development Bank in 2007 in CAR, agricultural sector in general and farming in particular has suffered too much political instability and lack of investments (less than 1% of state investment in five years). This explains the weak institutional capacity of supervisory structures and private operators, and the degradation of rural infrastructure. Over 15,000 km of rural and farm roads, only 16% are in good condition. Almost all agricultural markets and slaughterhouses are in disrepair. This has created a rural paradox productions that do not find buyers in the fields and food shortages in the cities. For instance, agricultural investment needs identified by the Government amounted to 97.3 billion FCFA (about \$ 200 million) for the period 2008-2010.

### Genetic improving

The observation made on local breeds revealed that in general, Central African cattle have low productivity by 45 to 50% meat and milk (2-4 liters per day). But experimental tests performed in success by artificial in situ insemination with French breeds (Salers, Limousin and Normand) in the sub-prefecture Kouï has shown good results with CAR breeds such as Akou, Goudali Jafun. The genetic improvement through artificial insemination or embryo transfer requires the active participation of producers in the project for better supervision and better monitoring. In the current context of production systems, sedentary cattle better meets the condition of success

pastoral cattle transhumance where local inter racial crosses can be made. Genetic improvement of small ruminants will also be considered for improving the production of meat and milk. This action can be done either by stations either in natural environments.

## CONCLUSION

As a conclusion, a consolidated summary of the above-proposed strategies in order of importance is given in Table 3. The higher is the scale, the greater the area concerned has priority.

Tab. 3: National priorities

RCA	Security	Status of animal genetic resources		National capacities			Cooperation	
	Borders security Internal security	Inventory Update of national statistics Livestock census	Management Development policies (use and conservation)	Infrastructures Buildings Roads Manufacturing factories	Formation Vocationnal school Universities Reserch center	Production Trading Exportations Agricultural extension	Bilateral	Regional
Order of importance	5	4	4	4	5	5	3	3

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## CHEMICAL COMPOSITION AND NUTRITIONAL CHARACTERISTICS OF SOME OATS AND RYE VARIETIES GROWN IN TURKEY

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*Original scientific paper*

### Summary

The experiment was conducted to determine the dry matter (DM), crude protein (DM), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), sugar, starch, neutral detergent fiber (NDF), acid detergent fiber (ADF), metabolizable energy for poultry (ME<sub>P</sub>), metabolizable energy for ruminant (ME<sub>R</sub>), crude ash (CA), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), beta glucan, total pentosan, soluble pentosan and viscosity in some oats and rye varieties grown in Turkey. Oats and rye samples were supplied from the western, southern and central part of Turkey. Total three oats and two rye varieties were used. Five samples of each variety were analyzed for all parameters. Experimental grain samples from each variety were ground through a 1 mm screen in preparation for chemical analysis. The differences between the oats varieties in DM, CP, EE, CF, NDF, ADF, ME<sub>R</sub>, CA, K, Mg, Ca, Mn, Cu and beta glucan contents; the rye varieties in CP, NFE, sugar, starch, NDF, ADF, ME<sub>P</sub>, K, Mg, P, Mn, Cu and viscosity contents were significant (P<0.05).

Keywords: *oats, rye, nutritional composition*

### INTRODUCTION

The composition of feedstuffs may vary widely due to differences in climate, soil conditions, maturity, cultivar, management, and processing factors. The knowledge of chemical features of feedstuffs is important in judging their nutritional consequences. It is important that the prediction of animal responses based on chemical analyses. Water-soluble pentosans form highly viscous solutions (Fernandez *et al.*, 1973; Fengler and Marquardt, 1988) and viscosity of different grain fraction was closely related to the content of water-soluble pentosans (Fengler and Marquardt, 1988). Viscosity is well established as a factor of great importance for the nutritional value of cereals like wheat, rye, barley and oats for humans, poultry and other non-ruminants. Several authors have found high correlations between viscosity of grains measured *in vitro* and nutritional value (Campbell *et al.*, 1989; Rotter *et al.*, 1989; Dusel *et al.*, 1997).

Oats (*Avena sativa* L.) are a major cereal grain worldwide as an energy feed and livestock feeding is the primarily use (70%) of oats (FAO, 2013). However, their chemical composition can vary widely, depending on variety, climate and fertilizer practices. Oats are much richer in gross energy than other cereals (19.5 MJ/kg), due to their relatively high oil content (3.5-7.5%). Oats contain more protein than maize (8-15%) but less than wheat and barley. Oats are much poorer in starch (about 40%) than maize, wheat and barley. The ADF content of oats is about 16% vs 3-4% for maize and wheat and 6% for barley. The degree of lignification of the hulls varies between varieties (Margan *et al.*, 1987; Crosbie *et al.*, 1985; Rowe *et al.*, 1988). Unlike other major cereals, oats have a very high fibre content due to the presence of hulls. The presence of hulls and their high fibre content make oats inferior to other cereal grains for high producing livestock, both due to their bulk and to the lower digestibility and energy density. The composition of oats depends on agricultural conditions: for example, protein, K content and NDF content were higher with optimal N fertilization than without N fertilizer (Givens *et al.*, 2004).

Oats are rich in pentosans (including beta glucans), which are polymers of pentose sugars that increase diet viscosity and can cause digestive problems in monogastric species and particularly sticky litter when fed to poultry (DAFS, 2012). Appropriate enzyme supplementation is required to avoid these adverse effects (Blair, 2008). Svihus *et al.* (2000), found that viscosity of different samples of oats vary considerably and that the viscosity values are affected by extraction method and heat treatment. Henry (1985) found that oats had a

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higher total pentosan content than wheat. Bhattu (1987) reported that the viscosity was influenced by the variety.

Rye (*Secale cereale* L.) are a minor cereal grain worldwide and annual production was less than approximately 1% of total cereal grain crop while rye grain is also used for feed, and more than approximately 40% of the world production was used for animal feeding (FAO, 2012). Rye is a winter-hardy annual or biennial grass mostly grown for its grain, particularly in Europe and North America, in areas where climate and soil are unfavourable for other cereals, or as a winter crop where temperatures are too low for winter wheat (Fuller, 2004). Rye contains about 10% of protein, has a low fibre content (about 2% CF) and a high starch content (about 62%). Rye contains high amounts of soluble arabinoxylans that increase viscosity and depress nutrient bioavailability in monogastric animals. This problem as well as the potential contamination with toxic ergot alkaloids tend to limit the use of rye grain in livestock feeding (Maner, 1987). Rye is also generally less palatable than other grains and is normally mixed with other grains or fodder components to increase its palatability (Dendy *et al.*, 2001).

The object of this study was to compare the varieties of oats and rye with respect to their chemical composition and ME values.

## MATERIALS AND METHODS

Cereal samples were supplied from the western, southern and central part of Turkey. Total three oats and two rye varieties were used. Five samples of each variety were analyzed for parameters. Experimental grain samples from each variety were ground through a 1 mm screen in preparation for chemical analysis. DM content was determined by oven-drying at 105°C for 16 h. The Kjeldahl method was used for the analysis of total nitrogen content of diets and CP was expressed as nitrogen x 6.25 (AOAC, 1980). EE content was obtained by the Soxhlet extraction using anhydrous diethyl ether. The CF content was determined using 12.5% H<sub>2</sub>SO<sub>4</sub> and 12.5% NaOH solutions (Nauman and Bassler, 1993). NFE calculated as 100 - % (moisture + CP + lipid + ash + CF). The samples were analyzed for the NDF and ADF according to the procedures of Goering and Van Soest (1970). The samples were analyzed for starch, sugar, CA according to the procedures of the AOAC (1980). Estimates for ME<sub>P</sub> were based on protein, ether extract, starch and sugar levels determined from the samples using a prediction equation (TSI, 1991):

$$ME_P, \text{ MJ/kg} = 0.3431 \times \text{fat \%} + 0.1551 \times \text{CP \%} + 0.1301 \times \text{total sugar \%} + 0.1669 \times \text{starch \%}$$

Estimates for ME<sub>R</sub> as MJ/kg in DM were based on protein, fiber and fat levels determined from the samples using a prediction equation (TSI, 1991):

$$ME_R, \text{ kcal/kg OM} = 3260 + 0.455 \times \text{CP} + 3.517 \times \text{EE} - 4.037 \times \text{CF}$$

CP, EE, CF quantities in OM (g/kg)

$$ME_R, \text{ MJ/kg DM} = ME_R \times 4.186$$

Atomic Absorption Spectroscopy (Ultrospec 2100 pro UV/visible spectrophotometer) was used determined K, Mg, Ca, P, Na, Zn, Fe, Mn, Cu concentrations.

Beta glucan content were determined enzymatically following the barley, wheat and triticale grains procedures of the commercial kits from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland); the beta-glucan protocol was AACC method 32-23. The total pentosans and soluble pentosans were analyzed according to the colorimetric methods of Hashimoto *et al.* (1987).

Viscosity of grains were determined according to Teitge *et al.* (1991) using a Brookfield Digital Viscometer (Model DV- II+PRO, Brookfield Engineering Laboratories, Stoughton, MA) maintained at 40°C.

Data were subjected to ANOVA using General Linear Models (SPSS, 1997). The model included varieties as main effects. The results obtained for the varieties were statistically analysed using Duncan's Multiple Range Test. Differences were considered to be significant, based on the 0.05 level of probability.

## RESULTS AND DISCUSSION

Nutrient composition and ME content of some oats and rye varieties are given in Table 1. The differences between the oats varieties in DM, CP, EE, CF, NDF, ADF, ME<sub>R</sub>, CA, K, Mg, Ca, Mn, Cu and beta glucan contents; the rye varieties in CP, NFE, sugar, starch, NDF, ADF, ME<sub>P</sub>, K, Mg, P, Mn, Cu and viscosity contents were significant (P<0.05). The average DM content (%) were obtained 91.23 for oats; 90.21 for rye; CP content (%) were obtained 16.50 for oats; 14.81 for rye; EE content (%) were obtained 5.10 for oats; 1.34 for rye; CF content (%) were obtained 7.17 for oats; 0.75 for rye; NFE content (%) were obtained 58.82 for oats; 71.83 for rye; sugar content (%) were obtained 5.15 for oats; 14.69 for rye; starch content (%) were obtained 39.45 for

oats; 54.12 for rye; NDF content (%) were obtained 26.48 for oats; 13.02 for rye; ADF content (%) were obtained 12.35 for oats; 2.45 for rye; ME<sub>P</sub> were obtained 11.56 for oats; 13.73 for rye; ME<sub>R</sub> were obtained 12.93 for oats; 13.81 for rye. The findings about nutrient composition and ME of some rye and oats varieties are consistent with the findings of the relevant studies in general (Batal *et al.*, 2011; DLG, 1991; Kearl *et al.*, 1979; NRC, 1994; 2001). Some of the differences between reports may be based on the difference between plant variety, soil structure and climate.

Tab. 1. Nutrient composition and metabolizable energy of some oats and rye varieties

Cereals	DM (%)	CP (%)	EE (%)	CF (%)	NFE (%)	Sug (%)	Stc (%)	NDF (%)	ADF (%)	ME <sub>P</sub> (MJ/kg)	ME <sub>R</sub> (MJ/kg <sup>DM</sup> )
Oats varieties											
Faik Bey	90.50 <sup>c</sup>	16.38 <sup>b</sup>	5.54 <sup>a</sup>	5.83 <sup>a</sup>	59.64	5.21	39.27	23.60 <sup>a</sup>	10.73 <sup>b</sup>	11.68	13.33 <sup>a</sup>
Seydişehir	91.31 <sup>b</sup>	15.28 <sup>c</sup>	4.39 <sup>b</sup>	8.79 <sup>a</sup>	58.69	5.27	39.82	25.01 <sup>b</sup>	17.22 <sup>a</sup>	11.20	12.42 <sup>c</sup>
Çekota	91.88 <sup>a</sup>	17.84 <sup>a</sup>	5.37 <sup>a</sup>	6.89 <sup>b</sup>	58.14	4.96	39.27	30.83 <sup>a</sup>	9.09 <sup>c</sup>	11.80	13.06 <sup>b</sup>
Means	91.23	16.50	5.10	7.17	58.82	5.15	39.45	26.48	12.35	11.56	12.93
SEM	0.18	0.33	0.20	0.39	0.33	0.15	0.33	0.96	1.08	0.11	0.11
Probability	0.001	0.001	0.026	0.001	0.181	0.711	0.769	0.001	0.001	0.053	0.001
Rye varieties											
Aslim	90.17	15.33 <sup>a</sup>	1.34	0.70	71.33 <sup>b</sup>	12.91 <sup>b</sup>	53.04 <sup>b</sup>	13.25 <sup>a</sup>	2.75 <sup>a</sup>	13.40 <sup>b</sup>	13.83
Populasyon	90.25	14.29 <sup>b</sup>	1.34	0.79	72.34 <sup>a</sup>	16.48 <sup>a</sup>	55.21 <sup>a</sup>	12.78 <sup>b</sup>	2.16 <sup>b</sup>	14.06 <sup>a</sup>	13.79
Means	90.21	14.81	1.34	0.75	71.83	14.69	54.12	13.02	2.45	13.73	13.81
SEM	0.07	0.22	0.06	0.04	0.27	0.63	0.41	0.10	0.13	0.14	0.01
Probability	0.622	0.005	0.986	0.338	0.049	0.001	0.001	0.009	0.008	0.007	0.304

<sup>a,b</sup> Means within a column in each variable with no common superscript differ significantly (P<0.05), SEM Standard error of means (Pooled), Sug sugar, Stc starch.

Mineral contents of some oats and rye varieties are given in Table 2. CA content (%) were obtained 3.62 for oats; 1.46 for rye; K content (%) were obtained 0.65 for oats; 0.60 for rye; Mg content (%) were obtained 0.19 for oats; 0.15 for rye; Ca content (%) were obtained 0.04 for oats; 0.03 for rye; P content (%) were obtained 0.26 for oats; 0.19 for rye; Na content (%) were obtained 0.02 for oats; 0.01 for rye; Zn content (ppm) were obtained 23.55 for oats; 25.37 for rye; Fe content (ppm) were obtained 56.98 for oats; 19.53 for rye; Mn content (ppm) were obtained 24.18 for oats; 11.75 for rye; Cu content (ppm) were obtained 10.18 for oats; 7.62 for rye. The finding about mineral contents of some rye and oats varieties are consistent with the findings of the relevant studies (Batal *et al.*, 2011; DLG, 1991, Kearl *et al.*, 1979; NRC, 1994; 2001). Some of the differences between reports may be based on the difference between plant variety, soil structure and climate.

Tab. 2. Mineral contents of some oats and rye varieties

Cereals	CA (%)	K (%)	Mg (%)	Ca (%)	P (%)	Na (%)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Oats varieties										
Faik Bey	3.10 <sup>c</sup>	0.68 <sup>a</sup>	0.17 <sup>b</sup>	0.032 <sup>b</sup>	0.26	0.020	22.22	63.77	15.75 <sup>b</sup>	15.14 <sup>a</sup>
Seydişehir	4.14 <sup>a</sup>	0.68 <sup>a</sup>	0.19 <sup>b</sup>	0.032 <sup>b</sup>	0.26	0.017	23.08	58.32	14.69 <sup>b</sup>	9.86 <sup>b</sup>
Çekota	3.62 <sup>b</sup>	0.60 <sup>b</sup>	0.21 <sup>a</sup>	0.050 <sup>a</sup>	0.27	0.011	26.24	47.50	44.22 <sup>a</sup>	5.55 <sup>b</sup>
Means	3.62	0.65	0.19	0.037	0.26	0.019	23.55	56.98	24.18	10.18
SEM	0.12	0.01	0.01	0.01	0.01	0.001	1.45	3.71	3.88	1.43
Probability	0.001	0.004	0.001	0.001	0.666	0.062	0.563	0.167	0.001	0.006
Rye varieties										
Aslim	1.46	0.62 <sup>a</sup>	0.14 <sup>b</sup>	0.02	0.20 <sup>a</sup>	0.01	27.06	20.58	8.74 <sup>b</sup>	11.26 <sup>a</sup>
Populasyon	1.47	0.56 <sup>b</sup>	0.17 <sup>a</sup>	0.05	0.17 <sup>b</sup>	0.01	23.68	17.78	15.50 <sup>a</sup>	3.06 <sup>b</sup>
Means	1.46	0.60	0.15	0.03	0.19	0.01	25.37	19.53	11.75	7.62
SEM	0.01	0.01	0.01	0.01	0.01	0.001	1.40	2.24	1.24	1.59
Probability	0.598	0.001	0.001	0.001	0.001	0.062	0.260	0.586	0.001	0.001

<sup>a,b</sup> Means within a column in each variable with no common superscript differ significantly (P<0.05), SEM Standard error of means (Pooled).

Beta glucan, total pentosan, soluble pentosan and viscosity of some oats and rye varieties are given in Table 3. Beta glucan contents (% w/w) were obtained 3.11 for oats; 1.73 for rye; total pentosan contents (%) were obtained 3.70 for oats; 3.99 for rye; soluble pentosan contents (%) were obtained 1.01 for oats; 0.99 for rye;

viscosity contents (cP) were obtained 0.79 for oats; 1.47 for rye. Although, the findings about beta-glucan, total pentosans, soluble pentosans and viscosity of some oats and rye varieties are consistent with the findings of the relevant studies, our findings are inconsistent with some of the previous studies. Svihus and Edvardson (2002) found that the average viscosity was obtained 6.9 cp for oats. Demirbas (2005) determined that beta glucan content of oats was the highest (5.7%) and the lowest (2.2%), rye was the highest (0.7%) and the lowest (1.5%) grown in Turkey. Kırkpınar *et al.* (1997) found that the average total pentosan content 9.83% for oats, viscosity 1.14 cp and not significant among oats varieties. Kırkpınar (2004) found that the average total pentosan content were obtained 9.73% for oats and the differences among the varieties were significant. The average viscosity were obtained 1.21 cp for oats and the differences among varieties were significant.

Tab. 3. Beta glucan, total pentosan, soluble pentosan (% , w/w) and viscosity (cP) of some rye and oats varieties

Cereals	Beta glucan	Total pentosan	Soluble pentosan	Viscosity
Oats varieties				
Faik Bey	3.63 <sup>a</sup>	3.31	0.91	0.73
Seydişehir	2.59 <sup>b</sup>	4.02	1.04	0.87
Çekota	3.12 <sup>ab</sup>	3.78	1.07	0.79
Means	3.11	3.70	1.01	0.79
SEM	0.17	0.19	0.03	0.03
Probability	0.038	0.337	0.246	0.233
Rye varieties				
Aslım	1.96	3.94	0.94	1.19 <sup>b</sup>
Populasyon	1.51	4.03	1.04	1.82 <sup>a</sup>
Means	1.73	3.99	0.99	1.47
SEM	0.14	0.14	0.04	0.15
Probability	0.125	0.764	0.317	0.039

<sup>a,b</sup> Means within a column in each variable with no common superscript differ significantly ( $P < 0.05$ ), SEM Standard error of means (Pooled).

## CONCLUSIONS

In this study, DM, EE, CF, NFE, sugar, starch, NDF, ADF, ME<sub>P</sub>, ME<sub>R</sub>, CA, K, Mg, Ca, P, Na, Zn, Fe, Mn, Cu, beta glucan, total and soluble pentosan contents and viscosity of three oats and two rye varieties grown in Turkey were determined. The differences between the oats varieties in DM, CP, EE, CF, NDF, ADF, ME<sub>R</sub>, CA, K, Mg, Ca, Mn, Cu and beta glucan contents; the rye varieties in CP, NFE, sugar, starch, NDF, ADF, ME<sub>P</sub>, K, Mg, P, Mn, Cu contents and viscosity were significant. Various cereal grains were grown under different environmental conditions but the effects of environmental factors on NFE, sugar, starch, ME<sub>P</sub>, P, Na, Zn, Fe, total pentosan, soluble pentosan content and viscosity of oats varieties and DM, EE, CF, ME<sub>R</sub>, CA, Ca, Na, Zn, Fe, beta glucan, total pentosan and soluble pentosan content of rye varieties were not significant. The study presents an addition to the database of the chemical composition and energy contents of Turkey feed grains. Additional information on these grains, such as amino acid composition, should be obtained in future work.

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## NUTRIENT COMPOSITION OF SOME ORGANIC FEEDSTUFFS FOR POULTRY

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*Preliminary communication*

### Summary

The present study was conducted to determine the nutrient composition and energy value of organic maize, organic wheat, organic barley, organic soybean meal, organic red lentil, organic lucerne hay and organic fresh white clover x rye grass. Dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), total starch (TS) and total sugar (TSU) contents (%) were obtained respectively 86.82, 1.19, 8.04, 3.62, 60.29 and 3.37 for organic maize; 89.07, 2.87, 14.77, 2.02, 49.26 and 4.28 for organic wheat; 89.02, 2.63, 14.87, 2.12, 42.42 and 4.71 for organic barley; 90.64, 5.60, 42.70, 7.45, 4.78 and 10.48 for organic soybean meal; 89.99, 2.76, 27.74, 1.33, 49.46 and 3.04 for organic red lentil; 90.66, 9.20, 13.28, 1.49, 2.72 and 4.87 for organic lucerne hay; 18.36, 1.82, 4.79, 0.55, 0.00 and 3.29 for organic fresh white clover x rye grass. The average invitro metabolisable energy (ME, MJ/kg) values were obtained 11.37 for organic maize; 10.29 for organic wheat; 10.73 for organic barley; 11.11 for organic soybean meal; 13.41 for organic red lentil; 2.49 for organic lucerne hay; 1.36 for organic fresh white clover x rye grass.

Keywords: *organic feedstuffs, nutrient composition, poultry*

### INTRODUCTION

In organic animal production, the feedstuffs must be produced from organic feedstuffs according to EU Council Directive 1804/1999. Until 2012, limited use of conventional feedstuffs is permitted, if there are too few organic feedstuffs available. After December 2011, all feed used in organic animal production should be produced from 100% organic feedstuffs.

The knowledge of chemical and physical features of diets is important in judging their nutritional consequences. However, in some cases, an animal response may correspond to a combination of so many dietary factors that it becomes difficult to distinguish them. In these cases, the prediction of animal responses based only on chemical and physical analyses of diets may be limited, either because some analyses are missing or because there are difficulties in building models that take account of interaction complexities (McNab and Boorman, 2002).

The composition of feedstuffs may vary widely due to differences in climate, soil conditions, maturity, cultivar, management, and processing factors. There is very little published research on metabolisable energy value and the digestibility of organic feedstuffs and diets (Blair, 2008). Commodity-based conventional farming induced applying heavy amounts of mineral and organic fertilizers and stimulated the decline of the animated part of the soil. Integrated, extensive and ecologically sustainable organic agriculture systems started to spread inherently as an alternative to such aggressive conventional farming (Mikulioniene and Balezentiene, 2009).

Maize is the most common energy feedstuff fed to poultry worldwide, although substantial amounts of wheat and barley are also used in poultry diets when price and supply allow for their inclusion. Energy supplements such as maize, wheat and barley constitute ~60–70% of poultry diets. There are many factors, such as genetics, environmental fluctuations, location, irrigation conditions, type of soils, year and fertilizer application that can be effect the nutritional content and nutrient availability in cereal grains. Most of the cereals suitable for use in organic poultry production belong to the grass family (*Poaceae*). Their seeds (grains) are high in carbohydrate and they are generally palatable and well digested (Blair, 2008). Soybean meal is by far the major plant protein concentrate used in poultry diets. The protein content of soybean meal is usually standardized at 44% or 48% CP by dilution with soybean hulls. Its amino acid profile is excellent with regard to feeding most types of poultry and, when combined with maize or sorghum, methionine is typically the only limiting amino acid (Leeson and Summers, 1997). On the other hand organic poultry have access to pasture. Good pasture provided the bulk of vitamins and also supplied a preponderance of protein and minerals. Lucerne hay and fresh clover x grass are used for forage. Moritz *et al.* (2005) reported that organically reared Ross broilers may overcome growth impairments associated with methionine deficiency through foraging.

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The composition of feedstuffs may vary widely due to differences in climate, soil conditions, maturity, cultivar, management, and processing factors. There is very little published research on the nutrient content of organically grown feedstuffs. Therefore, the present study was conducted to determine the chemical composition and energy value of organic maize, organic wheat, organic barley, organic soybean meal, organic red lentil, organic lucerne hay and organic white fresh clover x rye grass.

## MATERIALS AND METHODS

Organic maize, organic wheat, organic barley, organic soybean meal and organic red lentil were obtained from Tiryaki Co. (Gaziantep, Turkey) and organic lucerne hay obtained from Kor Egg Co. (İzmir, Turkey). Organic white fresh clover x grass were grown in Ege University, Ödemiş Vocational School (Ödemiş, İzmir, Turkey). Experimental samples from each feedstuff were ground through a 1 mm screen in preparation for chemical analysis. DM content was determined by oven-drying at 105°C for 16 h. The Kjeldahl method was used for the analysis of total nitrogen content of diets and CP was expressed as nitrogen x 6.25 (AOAC, 1980). EE content was obtained by the Soxhlet extraction using anhydrous diethyl ether. The samples were analyzed for TS, TSU and CA according to the procedures of the AOAC (1980). Three replicated of each feedstuffs samples for nutrient analyzed. Estimates for ME for poultry were based on EE, CP, ether extract, TS and TSU levels determined from the samples using a prediction equation (TSI, 1991):

$$\text{ME, MJ/kg} = 0.3431 (\% \text{ EE}) + 0.1551 (\% \text{ CP}) + 0.1301 (\% \text{ TS}) + 0.1669 (\% \text{ TSU})$$

Data were subjected to ANOVA using General Linear Models (SPSS, 1997).

## RESULTS AND DISCUSSION

Results are presented in Tables 1, 2, and 3. DM, CA, CP, EE, TS and TSU contents (%) were obtained respectively 86.82, 1.19, 8.04, 3.62, 60.29 and 3.37 for organic maize; 89.07, 2.87, 14.77, 2.02, 49.26 and 4.28 for organic wheat; 89.02, 2.63, 14.87, 2.12, 42.42 and 4.71 for organic barley; 90.64, 5.60, 42.70, 7.45, 4.78 and 10.48 for organic soybean meal; 89.99, 2.76, 27.74, 1.33, 49.46 and 3.04 for organic red lentil; 90.66, 9.20, 13.28, 1.49, 2.72 and 4.87 for organic lucerne hay; 18.36, 1.82, 4.79, 0.55, 0.00 and 3.29 for organic fresh white clover x rye grass. The average invitro ME (MJ/kg) values were obtained 11.37 for maize; 10.29 for wheat; 10.73 for barley; 11.11 for soybean meal; 13.41 for organic red lentil; 2.49 for organic lucerne hay; 1.36 for organic white fresh clover x rye grass.

Tab. 1. Nutrient composition of organic maize, wheat and barley, %

Analyzed nutrient composition	Organic maize (n=3)	Organic wheat (n=3)	Organic barley (n=2)
Dry matter	86.82±0.20	89.07±0.27	89.02±0.03
Crude ash	1.19±0.02	2.87±0.45	2.63±0.02
Crude protein	8.04±0.17	14.77±0.52	14.87±0.10
Ether extract	3.62±0.09	2.02±0.06	2.12±0.01
Total starch	60.29±0.48	49.26±1.06	42.42±0.32
Total sugar	3.37±0.59	4.28±0.34	4.71±0.14
ME, MJ/kg	11.37±1.63	10.29±1.49	10.73±0.04

± standart error

The findings about nutrient composition of organic maize, organic wheat, organic barley, organic soybean meal, organic red lentil, organic lucerne hay and organic fresh white clover x rye grass are consistent with reported in the literature. Jacob *et al.* (2008) indicated DM, CP, EE (%) and true ME (kcal/kg) values as respectively, 94.86, 6.14, 3.24 and 3603 for organic maize; 93.91, 16.87, 2.75 and 3592 for organic wheat. Mikulioniene and Balezentiene (2009) determined that organically cropped grain had less protein and fat content, but crude fiber content increased in comparison with those intensively cropped. The researchers explain that decreased protein and fat concentration of cereals under organic farming was caused by insufficient fertilizing. Also, they indicated DM, CP, EE and CA (%) values as respectively, 87.0, 9.6, 2.0 and 2.3 for organic wheat; 88.0, 11.9, 2.2 and 3.2 for organic barley. Kırkpınar *et al.* (2013) determined average *in vitro* ME (MJ/kg) values were obtained 13.31-13.81 for organic maize; 12.42-12.99 for organic wheat; 10.73-11.71 for organic barley; 11.43-12.58 for organic soybean meal.

Tab. 2. Nutrient composition of organic soybean meal and red lentil, %

Analyzed nutrient composition	Organic soybean meal (n=3)	Organic red lentil (n=2)
Dry matter	90.64±0.37	89.99±0.05
Crude ash	5.60±0.06	2.76±0.01
Crude protein	42.70±0.55	27.74±0.10
Ether extract	7.45±0.96	1.33±0.01
Total starch	4.78±0.26	49.46±0.01
Total sugar	10.48±0.49	3.04±0.01
ME, MJ/kg	11.11±0.52	13.41±0.01

± standart error

On the other hand, the results are similar to reported by NRC (1994, 2001). DM, CP, EE (%), apparent ME and true ME (kcal/kg) values as respectively, 89.00, 8.50, 3.80, 3350 and 3470 for maize; 87.00-89.00, 11.50-14.10, 2.50, 2900-3120 and 3167 for wheat; 89.00, 11.00, 1.80, 2640 and 2900 for barley reported by NRC. Also NRC reported DM, CP, EE and CA (%) values as respectively, 89.60, 4.63, 8.10 and 5.50 for soybean meal. Kearl *et al.* (1979) reported CA, CP and EE (g/kg DM) values as respectively, 15, 108 and 48 for maize; 18-32, 143-166 and 22-30 for wheat; 24-27, 111-124 and 19-21 for barley; 77-86, 429-476 and 53-59 for soybean meal. Also the researchers reported that true ME (kcal/kg) values as 3060-3717 and 2356-2614 for wheat and soybean meal respectively. Boldaji *et al.* (1985) determined DM, CP, apparent ME and true ME (kcal/kg) values as respectively, 88.74, 9.31, 3440 and 4040 for maize; 91.90-92.00, 13.14-14.31, 2980 and 3540-3550 for wheat; 91.50-92.00, 10.71-13.59, 2810-2840 and 3370-3440 for barley; 92.25, 52.00, 2510 and 2900 for soybean meal. Lessire (1990), reported that DM, CA, CP, EE (%), apparent ME and true ME (kcal/kg) values as respectively, 89.40, 1.24, 9.50, 3.99, 3324 and 3470 for maize; 88.92, 6.66, 47.70, 16.00, 2366 and 2483 for soybean meal. Nadeem *et al.* (2005) determined DM, CA, CP, EE (%), apparent ME and true ME (kcal/kg) values as respectively, 93.43, 1.52, 13.63, 2.78, 3244 and 3672 for maize; 87.98, 1.19, 13.88, 1.75, 3106 and 3561 for wheat; 91.59, 7.29, 52.34, 0.58, 2544 and 2957 for soybean meal.

Tab. 3. Nutrient composition of organic lucerne hay and fresh white clover x rye grass, %

Analyzed nutrient composition	Organic lucerne hay (n=2)	Organic fresh white clover x rye grass (n=2)
Dry matter	90.66±0.90	18.36±0.19
Crude ash	9.20±0.31	1.82±0.03
Crude protein	13.28±0.26	4.79±0.06
Ether extract	1.49±0.12	0.55±0.06
Total starch	2.72±0.02	0.00
Total sugar	4.87±0.03	3.29±0.04
ME, MJ/kg	2.49±0.66	1.36±0.03

± standart error

Forage composition is influenced by many known and unknown factors such as soil, climate, species, variety, diseases and insects which may affect consumption and digestibility. However, the nutritive value of forages depends primarily on the physiological and morphological development of grasses and legumes (Blaser, 1964). Just as Buchanan *et al.* (2007) determined that poultry may obtain small amounts of energy from pasture forage (285 to 542 kcal/kg). The nutrient contents (% on DM) of clover and grass were determined by Horsted *et al.* (2006) as follows: DM, CA, CP, EE, TS and TSU respectively, 13.13-13.38, 9.74-11.79, 19.26-20.77, 3.01-3.22, 3.67-6.28, 8.31-12.42. The chemical compositions (based on % DM) of lentil were found by Hullar *et al.* (1999) as following: DM, CA, CP and EE respectively, 89.16, 2.80, 26.15 and 1.2. Bhatti *et al.* (1976) determined CA, CP, EE and TS (based on % DM), 2.9, 27.7, 0.8 and 45.0 for pioneer red lentil respectively. Wang (2008) determined CP and TS (based on % DM), 27.59 and 46.96 for four red lentil varieties respectively.

## CONCLUSIONS

Organic farming has in fact developed into one of the most dynamic agricultural sectors in the Europe. Not only organic production but also assessment of production quality is required. Both high productivity and good quality are required for organically feedstuffs. Quality depends on content and ratio of food materials in feedstuffs. Therefore in this study was to establish chemical composition of organic maize, organic wheat, organic barley, organic soybean meal, organic red lentil, organic lucerne hay and organic fresh white clover x rye grass.

In conclusion, the nutrients composition such as amino acids and minerals content and digestibility of organic feedstuffs may need to be considered to improve sustainability of organic production.

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## INVESTIGATION OF POLYMORPHISM AT *STAT5A* GENE BY PCR-RFLP METHOD

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*Original scientific paper*

### Summary

Dramatic decreasing in fertility because of high milk production in dairy cows is one of the major problems in dairy industry. In recent years it has been thought that candidate gene approach may be a powerful toll at the efforts of overcoming infertility problems. Signal transducer and activator 5A (*STAT5A*) is very important in ruminants at establish and maintenance of pregnancy. Due to its critical role at pregnancy, polymorphisms in the gene codes *STAT5A* are thought to be influential in reproductive traits. In this study we investigated the C→G inversion at exon 8 of *STAT5A* gene in 130 Holstein-Friesian dairy heifers from eight dairy farms by using PCR-RFLP technique. We found two alleles (C and G) and three genotypes (CC, CG, and GG). Allele G was found predominant. The chi-square test ( $\chi^2$ ) was used to check whether the population was in Hardy-Weinberg equilibrium and the population was found at Hardy-Weinberg equilibrium for *STAT5A*.

Key words: *Polymorphism, fertility, dairy cows, STAT5A*

### INTRODUCTION

Low fertility and conception rates are one of the major problems of dairy industry. Genetic gain and success of selection are limited in economically important traits, especially reproductive traits due to large generation interval and low heritability. Because of this molecular genetic markers are thought as critical tools to improvement of traditional breeding programs' effect. Molecular genetics techniques and information have advanced very much in recent years. Depending on these advances a lot of genes and gene regions have been suggested as candidate for contribute to breeding program.

Signal Transducer and Activator of Transcription (STAT) Proteins have key role at regulate important hormones and cytokines within target cells (Darnell *et al.*, 1994; Schindler and Darnell 1995) *STAT5A* is also known as mammary gland factor (MGF) and closely related with actions of Prolactin and Growth Hormone (Argetsinger and Carter-Su 1996; Wakao *et al.*, 1994). Due to these functions of the protein, many researchers have been focused on *STAT5A* gene mutations and their associations with different traits for animal production (Flisikowski *et al.*, 2003; Khatib *et al.*, 2008; Khatib *et al.*, 2009; Selvaggi *et al.*, 2009).

Signal Transducer and Activator of Transcription 5A (*STAT5A*) located 19<sup>th</sup> chromosome of bovine genome (Seyfert *et al.*, 2000) is one of the many candidate genes for reproductive and the other economically important traits (Flisikowski *et al.*, 2003; Khatib *et al.*, 2008; Khatib *et al.*, 2009; Selvaggi *et al.*, 2009). The gene is thought to be related with onset and maintenance of pregnancy in cows (Spencer and Bazer 2004; Khatib *et al.*, 2008; 2009). The authors reported that the C allele have favorable effect on embryonic survival while the G allele was associated with low embryonic survival (Khatib *et al.*, 2008).

The aim of the present study was investigate allele and genotype frequencies of C→G transversion at 8<sup>th</sup> exon of *STAT5A* gene effecting on embryonic survival of cattle (Khatib *et al.*, 2008) among Holstein-Friesian heifers.

### MATERIALS AND METHODS

In this study, 130 Holstein-Friesian heifers between 14-28 months of age from different eight commercial dairy farms located Marmara region of the Turkey to investigate C→G inversion at exon 8 of *STAT5A* gene by using PCR-RFLP method (Khatib *et al.*, 2008). Blood samples were taken from coccygeal vein to DNA isolation and total DNA was extracted using a genomic DNA purification kit (K0512, Fermentas, Lithuania) according to the manual instructions.

Spectrophotometric methods were used to determine DNA quality and quantity. PCR reactions were performed as described by Khatib *et al.*, (2008) and PCR products restricted by *BstEII* (NEB #R3162) according to the manual instructions.

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The restriction fragments were directly analyzed by electrophoresis in 2% agarose gels in 1× TBE buffer, stained with SafeView™ Classic (Applied Biological Materials Inc) and visualized under UV light. Direct counting was used to estimate phenotype and allele frequencies of the genetic variants for investigated locus. The chi-square test ( $\chi^2$ ) was used to check whether the population was in Hardy-Weinberg equilibrium. To perform  $\chi^2$  analyses PopGene32 software was used (Yeh *et al.*, 2000).

## RESULTS AND DISCUSSION

While CC genotype reveals 820 bp, GG genotype has 676 bp on agarose gels (Fig. 1.). Electrophoresis analysis revealed two alleles (C and G) and three genotypes (CC, CG, and GG) for investigated locus (Fig. 1.). Allelic frequencies were found as 0.45 and 0.55 for C and G, respectively. Genotype frequencies for CC, CG and GG were found as 0.22, 0.47 and 0.31, respectively. The population investigated was found at Hardy-Weinberg equilibrium for C→G inversion at exon 8 of *STAT5A* locus.

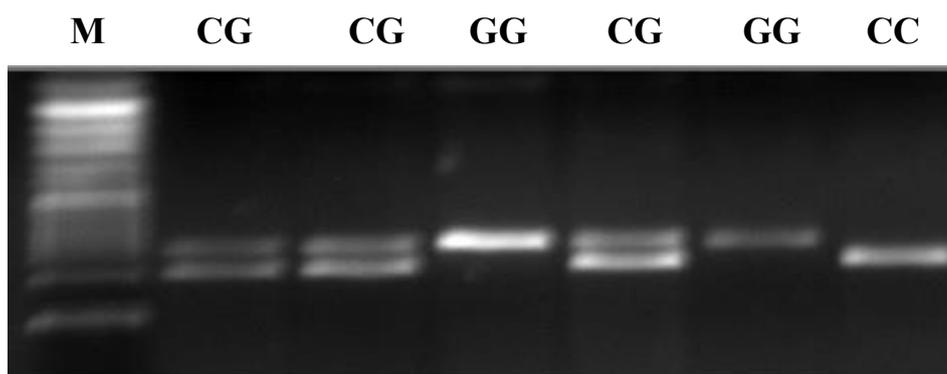


Fig. 1. Electrophoretic illustration of *STAT5A* genotypes

Several polymorphisms have been found in *STAT5A* gene by many researchers (McCracken *et al.*, 1997; Antoniou *et al.*, 1999; Flisikowski and Zwierzchowski, 2002; Brym *et al.*, 2004). Some of them have been found related with meat production (Flisikowski *et al.*, 2003), growth performance (Dario *et al.*, 2009) and milk production traits (Sadeghi *et al.*, 2009; Khatib *et al.* 2008).

In dairy herds infertility problems are increasing and cause to economic loss in dairy industry. Molecular genetics markers are thought to as a tool to improvement success of selection programs. We aimed investigate frequency of C→G inversion at exon 8 of *STAT5A* among Holstein Friesian heifers due to its possible effect on fertility. *STAT5A* gene polymorphism has been investigated by various authors due to its critical role in different pathways (Khatib *et al.*, 2009). The C→G inversion at exon 8 of *STAT5A* has been found important for embryonic survival and fertility (Khatib *et al.*, 2008; Khatib *et al.*, 2008) and previous reports on expression of the gene supported this finding (Nakasato *et al.*, 2006).

## CONCLUSIONS

The results revealed that the unfavorable allele on embryonic survival is predominant in investigated population. Higher number of animals should be scanned to determine frequencies of unfavorable and favorable alleles of not only *STAT5A*, but also the other gene regions seen as candidate for quantitative traits. Further investigations are also needed to exhibit effects of the inversion by using phenotypic data.

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## MANURE MANAGEMENT AND ENVIRONMENTAL POLLUTION IN ANIMAL PRODUCTION

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*Review paper*

### Summary

In animal production, manure management includes the process of the removal of livestock manures from farm field and the appropriate storage of these manures for plant growing. In the case of manure storage in unsuitable conditions and left uncontrolled in environment without processing in appropriate conditions, it causes environmental pollutions especially malodor and visual pollution inside and also outside of farm field. In that respect, there is an important relationship between manure removing system, storage and environmental pollution in farm fields. In case leaving of manure in outside, manure starts to putrefy and deteriorate over time, as a result malodor, harmful gases and dust start to disperse around. These factors that cause environmental pollution also have some negative effects on animal and human health. So, manure removing, storage and evaluation methods in animal production has an importance for protection of environment. In the review, it is aimed to evaluate the manure management in animal production and the possible effects of inaccurate applications in manure management on animal and human health.

Key words: *Manure, environmental pollution, harmful gase, dust, animal health*

### INTRODUCTION

The rapid increase of the World population in recent years has resulted with increasing of demand for nutrients. Accordingly, a rapidly increase has been occurred in plant and food production and especially animal production. Meeting of food demands economically is associated with obtaining maximum yield per unit area or per animal. Therefore, today animal production is made by intensive livestock production worldwide. With the most general definition, intensive livestock production is a production business that is performed by using large capacity farms and advanced production techniques in order to achieve a high level of efficiency from breeding animals. Rapid modernization and technological progressing animal production has precipitated some environmental problems. At this point, disposal of animal waste that is obtained in animal farms or assessing of its in the most appropriate way have gained considerable importance (Ekinçi *et al.*, 2004).

Manure is defined as animal waste that is comprised by mixing of urine, feces and litter material. However, when manure is stored and protected in suitable conditions and used in agricultural areas in certain quantity, it is a material that contributes plant production and protects the soil stability by its organic materials, not like waste (Chadwick *et al.*, 2011). The amount of manure from various animal species is different. A dairy with an average weight of approximately 550 kg produces annually a total of 32 tonnes manure. When this manure is applied to the area of 11 hectares, it provides nutrients plants including 28 kg N, 11.2 kg P<sub>2</sub>O<sub>5</sub> and 13.4 kg K<sub>2</sub>O (Demirkıran, 2004). Average of 100 tonnes manure is obtained weekly from a coop with a capacity of 100000 hens (Demirulus and Aydın, 1996). At this point, manure obtained from animal production is the best material for plant production and manure management and application procedures are key factors that facilitating of using manure (Harris *et al.*, 2001).

Soil and water resources take part among the most important natural wealth of the country. Using and protection of these resources in a reasonable way is too important for socio-development of societies. Despite Turkey has a fresh water reserve as lakes and rivers, but it is not a country rich in water. In fact, if the necessary measures are not be taken, it is likely to have water scarcity (Aküzüm *et al.*, 2003). Deficiency of water is a factor that limiting plant production and water is an indispensable resource for all livings. Therefore, protection of available water resources is important to provide sufficient and safe water for future generations (Çakmak *et al.*, 2005). However, treating of soil with recyclable natural nutrients has great importance for maintainig the efficiency of existing agricultural fields and increasing of the yield potential (Harris *et al.*, 2001).

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Therefore, manure management in animal production is a matter of very crucial for the protection of both of the environment and human and animal health protection. In this article, it is aimed to inform about manure management in animal production and to evaluate the possible effects of incorrect applications in manure management on environmental pollution and human and animal health.

## Manure Management

Manure management includes removing of manure from shelter and processing of store and evaluation of manure for plant production in order to avoid environmental pollution derived from animal husbandry. Manure transport system designed for transporting of manure from shelter is directly related to the intensity of the pollution and malodor insider (Atılgan *et al.*, 2006). After removing of manure from inside, it should be stored under suitable conditions to allow the using for plant production or other purposes (Jacobson *et al.*, 1999). Otherwise, manure that is left in uncontrolled way in external environment losses its structure and characteristics, and is exposed to microbial deterioration, so that it becomes the primary reason for environmental pollution (Atılgan *et al.*, 2006).

Manure is an organic material due to its structure. Under normal conditions, deterioration and putrefaction occur in organic materials over time. When it is not stored in suitable conditions, it provides required substrate for microbial production of ammonia and methane with its nutrients and microorganisms. These gases are produced in all stages of manure management including storage, processing and using in plant production. Environmental factors like as water presence and temperature that is source of microbial process in the production of these gases are effective issues for taking decisions in manure management (Chadwick *et al.*, 2011). In this respect, processing of controlled, programmed and conscious manure storage has great importance. Under normal circumstances, the manure should contain less than 80% moisture to store properly. However manure has moisture approximately 85-87% when it is obtained. Therefore, producers are required to reduce the moisture level of manure by mixing straw and stalk wastes or waste paper materials for a suitable storage processing (Harner *et al.*, 1997).

Another issue related to the storage of manure, it should be stored in area to prevent nutrient loss and protection of nutrition for plants, provide easiness for future process and using in plant production (Jokela, 1992). Storing randomly of solid manure in outdoor causes losing of manure characteristics by absorbing of rain, as a result it becomes sticky and levels of N ve  $K_2O$  decrease. On the other hand, nitrogen leaks from manure that is stored outside by rain, it predisposes to surface and ground water pollution (Camberato *et al.*, 1996).

Manure management is affected by some factors like as animal species, animal capacity, annual manure amount, type of shelter, size of farm. In addition all of these factors, legislation and technical issues should be considered during planning of manure management (Erkan, 2005). In this respect, it should be aimed to both of facilitation of work routine and minimize the negative effects of manure on environment and health.

## Soil and Water Pollution

Soil pollution is a deterioration of physical, chemical, biological and geological structure of soil as a result of human activities. It arises from implementation of wrong agricultural practices, using of wrong and excess fertilizer and agricultural pesticides and accumulation of waste, toxic and hazardous substances in soil. Effect of fertilizer on soil is soil reaction, disrupting of structure, eradication of soil organisms and enrichment of toxic substances in soil. Excessive nitrogen fertilization results in significant accumulation of nitrate and nitrite in plant tissues. Accumulation of these forms of nitrogen in plants could cause serious health problems in human and animals fed with these plants (Namlı, 2013). Manure includes four main pollutants for water as nitrogen, phosphorus, organic matter and pathogens. Therefore, manure is regarded as one of the most important factors causing water pollution (Ogejo, 2010). In recent years, water resources have been exposed to an irreversible pollution, consequently a significant reduction wetland areas and water resources have been occurred. From 1950's until today, 84% of wetland areas in United Kingdom, 57% in Germany and 60% in Spain have been lost (Polat and Olgun, 2009). This loss occurred as a result of uncontrolled and unconscious practices in animal and plant production. Therefore, uncontrolled removal of manure from shelter causes both of soil and surface and ground water resources (Karaman, 2006).

Improper and excess using of fertilizer in agricultural areas leads to increasing of nitrate levels in the soil and ground water. Therefore, soil and water pollution arise from leakages from manure piles created uncontrolled, surface flow from shelter and pastures and manure storage structures (Polat and Olgun, 2009). Phosphorus in

animal manure causes phosphorus pollution in surface water (Gilliam *et al.*, 1999) and it induces water pollution by leaking to water resources as drinking water plants, fishery plants and lakes.

Phosphorus in water with a level higher than 50 mg/l promotes the growth of algae, subsequently the level of oxygen in water reduces and it causes the death of other creatures in water (Zhang *et al.*, 2002). Nitrate pollution in water is a commonly encountered problem in the regions where nitrate uses as output in animal production and as input in plant production and also sustainable intensive agricultural activities (McLay *et al.*, 2001; Polat *et al.*, 2009). Water resources contaminated with excessive levels of nitrate constitutes a major health risk for both human and animal. Consuming of high levels of nitrate in the water causes pain, vomiting, coma and subsequently death in animals (Kaya and Akar, 2002). In humans it causes genetic problems, weakening of immunity and also serious health problems (Weyer, 2001). In Turkey according to drinking water regulations, it is reported that acceptable nitrate level in water should be 45 mg/L for human and 100 ppm for animals (Anonymous, 2004).

Manure includes microorganisms such as virus, bacteria and protozoa that cause infections or diseases. Manure that is not evaluated in a controlled way, also causes contamination of underground and surface water with coliform bacterias (Boyacı *et al.*, 2011). Therefore, maintaining of sufficient distance between manure and water resources has a great importance. Pathogens are often transported to animal drinking water resources by runoff water or erosion. The rivers and lakes that are used for drinking water also intermediate for transporting of pathogens (Ogejo, 2010). When considering of all these negative effects, to minimize water pollution arising manure management, some countries across the World have made certain regulations. In accordance with these regulations, manure storage units have to be away at least 100 m from camp, spor and residential areas, 35 m from river bed, 200 m from swimming area, beaches and fish production areas. In poultry production, semi-liquid manure storage areas should be away at least 500 m from residential areas (Polat and Olgun, 2009).

## **Air Pollution**

In recent years, some problems about manure assessment including getting closer of animal farms to residential areas, absence of standards about manure storage in farms, failure in evaluating of manure, pollutes the environment. Whereas manure causes air pollution by releasing of malodor and harmful gases, it also causes emergence and spread of infectious diseases by accelerating of proliferation of fly, mouse and various pests. Animals release gas to environment by respiration, intestinal or urine and feces. Activities of anaerobic and aerobic microorganisms in manure produce some gases. As a result, water vapor, carbon dioxide and ammonia are always exist inside of shelter, whereas hydrogen sulfide, carbon monoxide and hydrogen sometimes rise (Karaman, 2006). Bacteriological disruption and also gase production begin in manure stored for a long time and excessive amount in shelter. Decomposition of manure by bacterias expose 60 different compounds at various levels release odors to environment (Yağanoğlu, 1987). As a result of anaerobic degradation of manure, volatile fatty acids (acetic acid, butyric acid...), alcohols, aldehydes, esters, phenols and krezeols (mercaptan, ethylmerkeptan...), sulfides (hydrogen sulfide, disulfide...), ammonia and amines (methylamine, ethylamine...), nitrogen heterocycles and odorless gases (carbon dioxide, methane) occur (Karaman, 2006). Depending on releasing of these gases, severe odors starts to spread into the environment. These odors threaten health of staff and also affect negatively animal live quality, welfare and productivity. In a study examined the health of 11 staffs in animal farms, it was found that some serious health problems including cough, chest tightness and respiratory diseases have been recognized more than 50% of staffs (Eliot and Collins, 1982). Besides, installing of animal production areas near settlements is an undesirable case by human (Alagöz *et al.*, 1996).

Increasing of gase concentrations in shelter affects negatively staff health and working performance. As a result of increasing of ammonia concentration, feed consumption decrease and animals begin to take irregular and frequent breath. Ammonia concentration between 10-20 ppm precipitate problems mentioned above and health problems for animals. The concentration between 25-50 ppm induces serious respiratory diseases, exceeding of 50-60 ppm causes a significant decrease in feed consumption and productivity. In poultry houses, ammonia concentration exceeding 500 ppm causes deaths (Alagöz *et al.*, 1996). In a study conducted to determine the effects of ammonia on staff health, it was found that the staff worked in shelter where ammonia concentration was 100 ppm, had keratokonjunctivite at the end of the six week trial (Charles, 1981).

Another undesirable effect of manure in shelter is dust formation. Dust particles have features for carrying of solid particles and absorbing gases and liquids. It was reported that dust particles in shelter carry viruses and bacterias, especially in poultry houses dust particles are effective in carrying and spread of agent of Marek (Lillie, 1970). In addition, dust in shelter creates risks for staff health and causes allergic reactions (Karaman,

2006). Acceptable dust concentration in shelter should be 10 mg/m<sup>3</sup> for animals and 5 mg/m<sup>3</sup> for human (Elliot and Collins, 1982).

## CONCLUSION

As a result, as well as meeting of nutrient requirement of growing population of the world, proceeding of animal production by protection of water, soil and air resources is an important issue. Uncontrolled and irregular assessment of animal waste causes irreversible environmental pollution, some health problems for human and animal, negative effects on animal productivity. However, manure that is accepted a potential factor for environmental pollution, it is actually a potential source for country's economy. Manure could be used in plant production after storage under suitable conditions and processing. Using of manure in agricultural activities helps to reduce environmental pollution, and it also contributes to usage of waste as economic source. In this respect, to eliminate or minimize of existing negative effects, manure management should be practice consciously and controlled, and also legislation and regulation about manure management should be considered by producers.

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## MEAT QUALITY OF AUTOCTHONOUS BOVINE GENOTYPES AND INTENSIVE BEEF BREEDS

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*Review paper*

### Summary

Consumer prefer high quality food, poor in fat but rich in polyunsaturated fatty (Karatosidi, *et al.* 2010). Eichhorn *et al.* (1985) found that cattle breed significantly affected the percentage of most fatty acids in musculus longissimus and subcutaneous adipose. Cifuni *et al.* (2004), Braghieri *et al.* (2005), Plavšić *et al.* (2012) concluded that autochthonous Podolian breed have meat characterised by a higher level of unsaturation than modern intensive beef breeds like an Angus, Charolais, Hereford. This make Podolian meat a valuable food since saturated fatty acids are held responsible for coronary diseases and cancer. Countries of West Balkan have regulation for conservation of some autochthonous cattle breeds, like Podolian and Busha. The concept of sustainable use of animal genetic resources, in addition to economic, environmental and socio-cultural dimensions, contributes to rural development. For conservation of animal genetic resources, there is strong interest, as policy makers, growers and scientists, and consumers themselves. For this reason we have thought interesting to compare some meat's qualitative parameters of the autochthonous cattle breeds of West Balkan countries and intensive beef breeds.

Key words: *cattle, autochthonous breeds, beef breeds, meat quality, fatty acids*

### INTRODUCTION

Healthy meat is largely related to its fat content and fatty acid composition, namely the polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and the saturated fatty acids (SFA) contents. Saturated fatty acids influence cardiovascular diseases in human population. Cattle meat contain high proportion of saturated fatty acids (Plavšić *et al.*, 2012). Ruminants digests feeds by ruminal microorganisms. In that process, majority of dietary unsaturated fatty acid (FA) are hydrogenate to saturated FA. That is the reason why beef has higher concentration of saturated FA than nonruminant meat (Zhang, 2008). However, beef has other quality, such as essential amino acids, B vitamins and minerals. Meat fatty acid composition is influenced by genetic factors, although to a lower extent than dietary factors (De Smet *et al.*, 2004). Same author find out that it is clear that the species are the major source of variation in meat fatty acid composition. Beef and lamb normally have a low P/S (polyunsaturated / saturated fatty acids) ratio compared with pork because of the biohydrogenation of unsaturated fatty acids in the rumen. The aim of this paper is to review some results about fatty acids content variations of autochthonous bovine genotypes and intensive beef breeds and diet influence to beef quality concerning of possibilities to produce healthy cattle meat in rural regions.

Fatty acid composition in muscle and adipose tissue in cattle depends on the amount of fat in muscle and carcass (Wood *et al.*, 2008). Human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids. Western diets (deficient in omega-3 fatty acids) ratio is 15/1-16.7/1 (Simopoulos, 2002). Excessive amounts of omega-6 fatty acids, and a very high omega-6/omega-3 ratio promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. The optimal ratio may vary with the disease under consideration. Therefore, it is quite possible that the therapeutic dose of omega-3 fatty acids will depend on the degree of severity of disease resulting from the genetic predisposition. A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as in the developing countries. Researchers in 2001, at a conference sponsored by the National Institutes of Health concluded that the ratio of omega-6 to omega-3 should be consumed in a 1:1. The Japanese government (2007) recommended a ratio of 4:1, while the Swedish government recommended a ratio of 5:1, and The Institute of Medicine of the National Academy of Science in the United States recommended a ratio of 10:1. (Allport Susan, 2007). Some authors estimates the ratio of

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Omega-6s ( $\omega 6$ ) to Omega-3s ( $\omega 3$ ): is closer to 15:1 (Simopoulos, 2002), or 14 - 25 times more omega-6 than omega-3 fatty acids (<http://umm.edu/health/medical/altmed/supplement/omega6-fatty-acids>)

## DISCUSSION

Barton *et al.* (2007) were used Limousin and Charolais breed to investigate effect of breed and diet (with linseed supplementation) on performance and fatty acids composition of *M. longissimus thoracis* and subcutaneous fat. Linseed supplementation decreased saturated fatty acids and increased polyunsaturated fatty acids in subcutaneous fat. Limousin heifers contained significantly more mono unsaturated fatty acids, polyunsaturated fatty acids, less saturated fatty acids than the lipids of Charolais heifers. Generally, breed have had more effect on fatty acids composition than diet. In his Ph.D. thesis, Zhang (2008) wrote that high dietary intake of saturated fatty acids causes higher plasma cholesterol which lead to cardiovascular disease. There was mentioned too, that myristic acid (14:0) have four times more harmful cardiovascular effect on humans than lauric (12:0) and palmitic (16:0) acids.

The concentration of  $\omega 3$  was found much more higher in Katerinis meat than of Podolian cattle (Karatosidi, *et al.* 2010). Katerini is the Greek Primigenius Steppe breed variety of the Grey Steppe type like Podolian breed.  $\omega 6 / \omega 3$  values for Katerini and Podolian cattle were 1.35 and 3.66 respectively, showing better ratio for Katerini cattle (tab.1). Data from this research indicate that this meat can satisfy the dietetic demands of the modern consumer.

Tab. 1:  $\omega 6 / \omega 3$  ratio of some cattle breeds by different authors

Breed	$\omega 6 / \omega 3$ ratio	Tissue	Authors
Katerini Podolian	1.35 3.66	<i>M. longissimus lumborum</i>	(Karatosidi, <i>et al.</i> 2010)
Limousin (heifers) Charolais	4.40 3.79	<i>M. longissimus thoracis</i>	(Barton <i>et al.</i> 2007)
Limousin (heifers) Charolais	4.39 4.24	Subcutaneous fat	(Barton <i>et al.</i> 2007)
Podolian young bulls Limousine x Podolian	14.00 15.22 <sup>NS</sup>	<i>M. longissimus dorsi</i>	(Braghieri, <i>et al.</i> 2005)
Podolian young bulls DIET Sweet lupin seeds Soybean	5.35 5.55 <sup>NS</sup>	Meat	(Vicenti, <i>et al.</i> 2009)

Lower carcass and muscle fatness of Belgian Blue bulls (De Smet S. *et al.* 2000) improved the polyunsaturated: saturated fatty acid ratio in the intramuscular fat. Angus breed, known as cattle with high content of fat Garmyn, *et al.* (2011) in investigation shows (n = 1,592) that  $\omega 3$  fatty acid (g/100 g) in *M. longissimus dorsi* was 0.63, and  $\omega 6$  fatty acid, 5.16. Concerning on those values n-6 / n-3 ratio is 8.19, what is little bit higher than results of most researchers who explored Podolian breed.

Salughter traits and meat quality of Podolian breed (Plavšić *et al.* 2008) are very similar to the meat of Simmental and beef breeds. Intramuscular fat of *M. longissimus dorsi* was lower and shows that this meat has less calories and can be used for kids and eldes people nutrition.

Two Portugals cattle breeds, the Mirandesa and Barrosa were studied on raw meat fatty acids content of *M. longissimus dorsi* by Dias, *et al.* (2008). The Mirandesa is the native breed of Portugal, used as a draft breed and also for beef production. Barrosa is a domesticated cattle breed from the North/Northwest of Portugal. Beside the production system (traditional and organic farming), authors concluded that the breed had a significant effect on the overall raw meat fatty acids content too. Like a low fat content breeds, in traditional farming,  $\omega 6 / \omega 3$  PUFA ratio was 5.47 for Mirandesa's, and 3.90 for Barrosa's breed. Those results shows that native, extensive breeds, can be utilise for production of healthy cattle meat.

Breed effects (Simmental and Red Angus) on fatty acid composition was aim of research work of Laborde *et al.* (2001). Some data, concerning on fatty acids ratio are shown in table 2.

Tab. 2. Simmental and Red Angus  $\omega_6/\omega_3$  fatty acid ratio Laborde *et al.* (2001)

Characteristics / Breeds	Simmental	Red Angus
Weight at slaughter, kg	659.3	505.2
Intramuscular fat, %	5.73	4.37
$\omega_6/\omega_3$ fatty acid ratio (total lipid fatty acid composition of longissimus muscle)	4.42	3.54

Simmental breed was slaughtered with higher live weight (659.3 kg) than Red Angus (505.2 kg), what can be the reason of higher intramuscular fat. As it can be seen,  $\omega_6/\omega_3$  fatty acid ratio have had better value (3.54) in Red Angus breed than in Simmental. Positive correlation between the amount of fat in muscle and fatty acid composition was aproved by (Wood *et al.*, 2008) too.

Comparing pasture and concentrate feeding of German Simmental bulls, Nuernberg *et al.*, (2002) reported that the  $n-6/n-3$  ratio of pasture grazing was better (1.3) in contrast to 13.7 of concentrate feeding.

Very interesting research was published 3 years later, by same author (Nuernberg *et al.*, 2005) concerning on breed and feed influence on fatty acid composition of *M. longissimus*. Comparing German Simmental and German Holstain cattle, using two different type of ration (grass-based and a concentrate feeding system). Intramuscular fat (%) for Holstein was 2.67 (concentrate) and 2.3 (grass – based) respectively. Results for Simmental bulls shows higher utilisation of grass ration. Muscle fat content was 2.61 for concentrate and 1.51 for grassed based ration.  $\omega_6/\omega_3$  ratio have had singificant influence of breed, feed and interaction of breed and feed respectively. Differences were for Holstein (concentrate : grass) 6.49 and 1.94, and for Simmental 8.34 : 2.04. In conclusion, authors wrote that feeding grass to cattle have positive effects on fatty acid profile and healthier meat.

Similar investigation did Sretenović Ljiljana *et al.* (2010) on Simmental bulls, adding linseed at last 60 days of fattening.  $\omega_6/\omega_3$  ratio for control group was 10.0, and for group which have had 300 grams of linseed in concentrate was 8.23. Authors have had concluded that the linseed improved of beef meat quality by increasing of  $\omega_3$  fatty acid content.

Sevi *et al.* (2002) were investigated hay/concentrate ratio supplementation: group 1 (60/40) and group 2 (70/30) of Podolian bulls, and find out  $\omega_6/\omega_3$  ratio was better in group with higher hay/concentrate ratio (6,37) than in second grup (6,63). Same diet produced better meat fatty acid composition, with higher polyunsaturated /saturated ratio.

In Italy, Chianina beef cattle were slaughtered at two different ages (an age ranging between 18 and 21 months). Authors find out (Giovanna Preziuso, Claudia Russo, 2004) investigated 3 muscles (*triceps brachii*, *longissimus thoracis* and *semitendinosus*) that ether extrat (%) was lowest (1.27) for *semitendinosus* at age 18-19 months and the highest value had *longissimus thoracis* 2.48. They concluded that delay from 18 to 21 months of age had only little differences in meat characteristics, and that the meat had a very low intramuscular fat what is one of the possitive meat quality of Chianina breed.

Chianina crosses bulls were aim of research of Plavšić *et al.* (2014 – personal unpublished results). Crosses of Chianina bulls with Simmental and Holstein Friesina cows were investigated on slaughter traits and meat quality. Some preliminary results ( five male were slaughter – experiment is still on) shows that dressing percent was 60.05 (live weight at slaughter was 580 kg for Simmental crosses), ether extrat (%) of *M. longissimus dorsi* shows low fat content (2.45) and  $\omega_6/\omega_3$  ratio was 4.87. For the first results (22 head are in experiment) it can be expected that Chianina semen can be produce crosses with high quality of beef meat with healthy meat.

Belgian Blue breed is intensive cattle breed for beef production. It is also known as an double – muscling cattle, with high dressing percentage, large proportion of muscle compared to adipose tissue and bone, lower total fatty acid content in comparison with conventional beef breeds, and higher of white muscle fibers, and lower collagen content of muscle. Investigating effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition, Reas *et al.* (2001), found out that the double muscling (mh/mh – muscular hypertrophy) genotypes had lower total fatty acid content and  $n6/n3$  ratio compared with +/+ (normal) genotype. Those results shows that Belgian Blue breed can be used for production of health beef.

Effect of extruded or crushed linseed instead of whole soybeans in diet of Belgian Blue young bulls (Reas *et al.*, 2004) increased  $n-3$  fatty acid content significantly, and consequently decreased the  $n-6/n-3$  ratio.

In review paper, Scollan *et al.*, (2006), concluded that feeding cattle for meat production wiht grass or concentrates containing linseed contributes in a lower  $n 6 / n 3$  ratio. Same authors investigated several aspects

of feeding on cattle meat quality and fatty acids composition. Elimination of ruminal biohydrogenation of dietary polyunsaturated fatty acids (PUFA), contribute better n 6 / n 3 ratio, too. Definitely, grass feeding increases n – 3 PUFA in beef, but also improve of colour shelf life and sensoru attributes of the cattle meat.

## CONCLUSIONS

Concerning on review of research work and mentioned papers, it can be concluded that:

- breed and feed have major influence on meat quality of cattle,
- native breeds (Podolica, Mirandesa, Barrosa, Katerini, Busha) have less intramuscular fat and better  $\omega 6/\omega 3$  ratio than productive beef breeds (Limousin, Angus, Charolais, Hereford),
- forage – based feeding system of cettle gives better and healthier meat quality
- higher hay/concentrate ratio gaves better meat quality concerning on intramuscular fat and  $\omega 6/\omega 3$  ratio
- including linseed in diet contributed lower n-6/n-3 ratio

For the modern consumers, it looks that using native cattle breeds on forage – based diets, can satisfy the dietetic demands and for farmers in rural regions, possibilities to produce more organic (economically viable) meat for market.

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## TURKISH PROPOLIS; BIOLOGICAL ACTIVITIES AND USES AREA

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*Original scientific paper*

### Summary

Propolis is a sticky gum resin collected by worker bees from the young shoots and buds of the certain trees and shrubs for use in and around the hive. Much work has been conducted on the chemistry and properties of propolis. Propolis contains some volatile oils, terpens, and beeswax. Flavonoids are well known plant compounds that have anti-oxidant, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory properties. So these properties of propolis have become a subject of much research in Turkey.

The effectiveness of propolis, formic acid, formic acids + propolis (1:1) and formic acid+propolis (2:1) on the pathogen of chalkbrood disease (*Ascospheera apis*) was studied. Propolis extract, formic acid, formic acid+propolis (1:1) and formic acid+propolis (2:1) were found to be highly effective against to *A.apis* pathogen in vitro conditions (Şahinler and Kurt, 2004).

The chemical composition of propolis from East Mediterranean (Hatay, Adana, Mersin) was studied in order to determine the major compounds by using GC-MS Chemical analysis of propolis extracts and findings indicated that the propolis samples had high concentrations of benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglicine besides the most common compounds such fatty acid, terpenoids, esters, alcohols and hydrocarbons, aromatic acids (Şahinler and Kaftanoğlu, 2005).

Yücel *et al.* (2015) conducted a study to determine the effects of propolis on growth of calves. Five female and five male calves for both control and propolis treatment groups a total of 20 Holstein calves, were used. Calves in both control and treatment groups were fed with same amount of milk one in a day Propolis tincture were given to calves 2cc in a day after milk. Treatment groups were found 312.8 g/day and 392.83 g/day. Daily weight gain of males in both group was found to be 458.31 g/day and 470.50 g/day. Daily weight gain was found to be significant ( $P < 0.05$ ) in females but not in males.

Sönmez *et al.* (2005) carried out a study to investigate the antimicrobial properties of six propolis solutions and evaluate their cytotoxicity on gingival fibroblasts at different dilutions. Two different solutions of powder propolis (Sigma) and Turkish propolis were prepared and propylene glycol (PG) and alcohol were used as solvents for each propolis sample. In addition to the four propolis solutions, two other propolis samples of far away geographic regions (USA and Australia) were included in the study. The antibacterial effects of six solutions on oral pathogen microorganisms were tested and their cytotoxic effects on human gingival fibroblasts were evaluated by MTT assay. The effective dilutions of the six propolis samples on periodontopathogen microorganisms were found to be cytotoxic to gingival fibroblasts. All solutions had strong antifungal activity and the effective dilutions were safe for gingival fibroblasts. As it is strongly antibacterial and non cytotoxic. Propolis could have a promising role in the future medicine, if appropriate solutions can be prepared.

Key words: *Propolis, properties, biological activities, uses area*

### INTRODUCTION

Propolis is a sticky gum resinous substance collected by worker honey bees from the young shoots and buds of the certain trees and shrubs. The plants secrete it to coat the young shoots and buds in order to protect them from the adverse effects of bad weather and from the attacks of bacteria, fungi, moulds and viruses. The bees collect these substances, pack them on their hind legs, and bring them back to the hive to cover the cracks and crevices, reduce the hive entrance and coat the large insects like moths, butterfly, beetles, cicadas etc. (Greenaway *et al.*, 1990; Schmidt, 1997). Bees also use the propolis to cover the inside of the hive and mix it with bees wax while building combs to protect the colony and larvae from the pathogen microorganisms. Propolis has a strong antibacterial, anti fungal and antiviral properties (Ghisalberti, 1979; Bankova *et al.*, 2000). Due to these properties thousands of adult bees and developing bees (larvae, pupae) can be protected from these pathogens (Schmidt, 1997).

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Recently investigations have indicated that the interest for natural preservative have increased. The use of propolis that is non-toxic as alternate preservative agent has been considered by consumers as safe. The composition of propolis varies depending on the origin of the plant species. Bees tend to collect these resins from a large variety of trees and shrubs to take advantage of the antibacterial, anti fungal and antiviral effects. Propolis possesses anti bacterial, anti fungal, antiviral properties and other beneficial biological activities such as anti-inflammatory, anti ulcer, local anaesthetic, anti tumour etc (Bankova *et al*, 2000; Ghisalberti, 1979).

### ANTIFUNGAL ACTIVITY OF PROPOLIS

The chalk brood disease, caused by *Ascosphaera apis* Maasen, was first seen in honey bee (*Apis mellifera*) colonies in Turkey in 1988. It spread all over the country within two years as a result of using contaminated bees wax foundation and migratory beekeeping. It became one of the most serious problems of Turkish beekeepers between 1986 and 1993 and several studies were conducted to control the disease. The disease has also been observed in all Mediterranean countries and it is assumed that it has spread all over the world.

This study was conducted to determine the effects of the propolis and formic acid and formic acid and propolis mixed on chalk brood disease (*Ascosphaera apis*). Propolis, formic acids, formic acids+propolis (1:1) and formic acids+ propolis (2:1) inhibited the growth of *A. apis* significantly ( $P < 0.01$ ). The effectiveness reached to 94% at high doses (50 ppm, 25 ppm) and inhibited the growth of the pathogen. The lower doses had fungistatic effect and lowered the fungus growth. It was also observed that the average effectiveness was higher with the mixture of the formic acids, Formic Acids +Propolis (1:1) Formic Acids+Propolis (2:1) than only propolis (5%) (Şahinler and Kurt, 2004).

### CHEMICAL COMPOSITION OF PROPOLIS

Anatolia is an enormous geographical area, which fits snugly into each other the two most important gene center among the subtropical countries in terms of plant diversity (Şahinler and Kaftanoğlu, 2005). Hatay, Adana and Mersin provinces are located in the East-Mediterranean. Among these provinces, Hatay has an ecology more diversified than the typical Mediterranean ecology. The chemical composition of propolis from East Mediterranean (Hatay, Adana, Mersin) was studied in order to determine the major compounds by using GC-MS. Chemical analysis of propolis extracts and the findings indicated that the propolis samples had high concentrations of benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglycine besides the most common compounds such as fatty acid, terpenoids, esters, alcohols and hydrocarbons, aromatic acids.

The chemical composition of the alcohol extracts of propolis from Hatay, Adana and Mersin, provinces are analysed. There are too many compounds but we will comment briefly on the major groups of compounds present. Aromatic Acids, Terpenoids, Hydrocarbons, Fatty Acids, Alcohols and other compounds. Totally, fourteen fatty acids were determined, seven of them are samples obtained from Hatay, seven are samples obtained from Mersin and three are samples obtained from Adana. Four alcohols were determined, 13-Tetradecanol was found in Hatay samples, Octadecanol was found in Mersin and, 9-Octadecen-1-ol and N-Hentriacontanol-1 were found in Adana samples. In addition to these compounds, three Aldehydes and two Esters were determined in this study.

These aromatic compounds are responsible for the antibacterial, anti fungal, anti viral, anti-inflammatory and anti cancer properties of propolis (Şahinler and Kaftanoğlu, 2005). The propolis samples collected from the Hatay Region had higher concentration of caffeic acid and sesquiterpenes than the propolis collected from Albania, Mongolia, Egypt and Bulgaria. The sesquiterpenes, which has a potential of having high anti-microbial and anti-cancer activities, was identified in eight, four and four varieties of propolis extracts from Mersin, Adana and Hatay, respectively (Table 1). However, propolis samples from Hatay region were quite different than those of the other regions in terms of variety and the rate of the sesquiterpenes.

In conclusion propolis from Hatay region seems to have much higher aromatic acids and sesquiterpenes content than those from Mersin and Adana, having for anti-microbial, anti-fungal and anti cancer activities. The high concentration of ethanol extract of propolis from Hatay could be used as anti-fungal agent against various fungi (Şahinler and Kaftanoğlu, 2005).

Tab. 1. Chemical Composition of Ethanol Extracts of Propolis from Hatay, Mersin and Adana Regions

Substances	Hatay Region (%)	Mersin Region (%)	Adana Region (%)	Substances	Hatay Region (%)	Mersin Region (%)	Adana Region (%)
<b>Aromatic acids</b>				<b>Terpenoids</b>			
Benzyl cinnamate	9.37	-	-	Farnesyl acetone	-	-	-
<b>Terpenoids</b>				Cembre	-	-	13.34
$\alpha$ -Pinene <sup>2</sup>	0.53	1.08	-	<i>trans</i> - $\beta$ -ocimene	-	-	-
Indolin, 2- methylen	1.82	-	-	Germacrene	-	-	-
Cycercene	3.65	-	-	1-Naphthalenepropanol	-	-	-
1S-Cis-Calamenen	0.85	-	-	Totarol	-	-	-
$\alpha$ -Copaene <sup>1</sup>	7.65	0.59	-	Solanone	-	-	1.31
$\beta$ -Maaliene	12.83	-	5.70	Geranyl acetone	-	-	0.55
$\alpha$ -Elemene	3.70	-	-	1,3,3-trimethyl-2-Methyleneidoline	-	-	0.46
$\beta$ -Eudesmol	19.41	2.25	5.52	$\alpha$ -Copaene-11-ol	-	-	1.78
$\alpha$ -Eudesmol	15.39	1.90	8.07	<i>trans</i> -3,4-dimethyl-1-cyclohexenecarbaldehit	-	-	0.46
$\alpha$ -Bisabolol	12.25	-	-	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-Naphthalene	-	-	0.66
L- Limonene	-	0.30	-	$\alpha$ -Cadinol	-	-	4.00
$\beta$ -Pinene	-	0.57	-	(+)Aromadendrene	-	-	2.59
Naphtalene <sup>1</sup>	-	1.49	-	Germacrene A	-	-	3.03
Azulene	-	1.49	-	Aldehydes			
<i>Trans</i> -(+)- carveol	-	1.14	-	Decyl aldehyde	1.05	-	0.26
$\beta$ -Bourbonene	-	0.77	-	Nonanal	-	0.47	-
Junipene	-	0.65	-	$\alpha$ -Campolene	-	0.73	-
$\alpha$ -amorphene	-	0.11	0.72	Hydrocarbons			
$\beta$ -Caryophyllene	-	0.96	7.21	Nonacosane <sup>1</sup>	0.52	-	-
Widdrene	-	0.67	-	Triacotane	0.40	-	-
$\alpha$ -Humulene	-	0.75	0.89	Heneicosane	0.92	-	-
$\beta$ -cubebene	-	0.75	-	Triacosane	1.56	-	-
$\alpha$ -Muurolole	-	0.78	1.16	Nonadecane	-	1.54	-
$\gamma$ -Cadinene	-	0.63	1.82	7,12 - dihydro-7,12-dimethyl - Benzantracene	-	3.21	-
$\delta$ -Cadinene	-	2.16	3.79	7,12-dihidro-7-methyl-Benzantracene	-	1.14	-
$\alpha$ -Bisabolene	-	0.97	-	1,2-dihydro-7,12-Dimethyl-Benzantracene	-	0.61	-
Caryophyllene Oxide	-	2.49	2.51	Dihidroabietan	-	4.79	-
$\beta$ -Selinene	-	2.49	-	<b>Octadecane</b>	-	-	1.55
$\alpha$ -Cedrol	-	32.95	-	Hexacosane <sup>1</sup>	0.49	-	-
Alloaromadendrene	-	3.65	0.43	<b>Ketones</b>			
$\gamma$ -Cadinene	-	1.31	1.82	Pentadecanone	0.48	-	-
<i>trans</i> -Caryophyllene	-	1.12	-	2-Nonadecenone	2.10	-	-
Epimanoyle oxide	-	4.87	-				
Rimune	-	5.02	-				
Substances	Hatay Region (%)	Mersin Region (%)	Adana Region (%)	Substances	Hatay Region (%)	Mersin Region (%)	Adana Region (%)
<b>Fatty acids</b>				<b>Esters</b>			
Hexadecanoic acid <sup>1,3</sup>	-	-	0.56	Borneol acetat	-	0.57	-
9-Octadecen-1-ol	-	-	0.61	1,2-Benzenedicarboxylic acid	-	1.29	-
N-Hentriacontanol(1)	-	-	18.48				
13-Tetradecanol	0.52	-	-				

1 Greenaway at al (1990) 2 Bankova at al (2000) 3 Velikova at al (2000) 4 Ghisalberti (1979)

### THE EFFECT OF PROPOLIS ON ORAL PATHOGENS AND HUMAN GINGIVAL FIBROBLASTS

Propolis is one of the few natural remedies that have maintained its popularity over a long period of time. The aim of this study is to investigate the antimicrobial properties of six propolis solutions and evaluate their

cytotoxicity on gingival fibroblasts at different dilutions. Two different solutions of powder propolis (Sigma) and propolis obtained from pine (*Pinus brutia*) trees of Turkey were prepared and propylene glycol (PG) and alcohol were used as solvents for each propolis sample. In addition to the four propolis solutions, two other propolis samples of far away geographic regions (USA and Australia) were included in the study. The antibacterial effects of six solutions on oral pathogen microorganisms were tested and their cytotoxic effects on human gingival fibroblasts were evaluated by MTT assay. The effective dilutions of the six propolis samples on periodontopathogen microorganisms were found to be cytotoxic to gingival fibroblasts. All solutions had strong antifungal activity and the effective dilutions were safe for gingival fibroblasts. As it is strongly antibacterial and non-cytotoxic. Propolis could have a promising role in the future medicine, if appropriate solutions can be prepared (Sönmez *et al.*, 2005).

#### **EFFECTS OF PROPOLIS ADMINISTRATION ON GROWTH PERFORMANCE AND NEONATAL DIARRHEA OF CALVES**

The purpose of this study was to determine the effects of propolis on growth performance and neonatal diarrhea of calves. Five female and five male calves from both control and propolis treatment groups, a total of 20 Holstein calves, were used. Calves in both control and treatment groups were fed with same amount of milk once in a day Propolis tincture was given to calves 2cc in a day after milk feeding. The research continued for 35 days. Daily weight gain of females in control and propolis treatment groups was found to be 312.8 g/day and 392.83 g/day. Daily weight gain of males in both groups was found to be 458.31 g/day and 470.50 g/day. Daily weight gain was found significant ( $P < 0.05$ ) in females. Significant differences were obtained in both body and shoulder length parameters between control and treatment groups of females ( $P < 0.05$ ). The only statistically important difference was found between two groups of males for withers height. Neonatal diarrhea was not observed in propolis administration group. The results of the research showed that the effect of propolis administration was important in terms of the growth of calves and preventing neonatal diarrhea that cause a serious economic loss (Yücel *et al.*, 2015).

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## EFFECT OF GENOTYPE ON CARCASS COMPOSITION IN MALE LAMBS

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*Original scientific paper*

### Summary

This study was conducted to compare carcass traits of Lori-Bakhtiari (n=50) and Romanov × Lori-Bakhtiari (n=22) crossbred male lambs. Lambs were weaned at the age of  $90 \pm 5$  days. Male lambs were reared in the same condition. Lambs were slaughtered at seven month of age. Data were analyzed using GLM procedure of SAS software. The results showed that live body weight at slaughter in Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs were similar. The dressing percentage for Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs were 50.48 and 46.54, respectively that significantly different. Genotype effect was highly significant on carcass traits. Romanov × Lori-Bakhtiari crossbred lambs for lean percentage (62.72 vs. 49.22%) were significantly ( $P < 0.05$ ) higher than in Lori-Bakhtiari lambs. Percentage of fat-tail and subcutaneous fat in Lori-Bakhtiari lambs were significantly ( $P < 0.05$ ) higher than in Romanov × Lori-Bakhtiari crossbred lambs. While percentage of internal fat in abdominal cavity ( $1.92 \pm 0.11$  vs.  $1.02 \pm 0.07$ ), bone carcass ( $18.38 \pm 0.24$  vs.  $15.06 \pm 0.16$ ) and intermuscular fat ( $5.49 \pm 0.25$  vs.  $3.57 \pm 0.16$ ) in Romanov × Lori-Bakhtiari crossbred percentage of were significantly higher than in Lori-Bakhtiari lambs. In concluded, Romanov × Lori-Bakhtiari crossbred lambs were better than Lori-Bakhtiari lambs for lean and total fat carcass percentage.

Key words: *crossbreeding, carcass, lamb*

### INTRODUCTION

Improving growth performance and carcass characteristics of lambs are very essential for meat production. Profitability of sheep production for meat depends to a great extent on lamb growth and carcass traits, which the later is mainly used to assess muscle development and fatness, as important economic factors. Moreover, in numerous countries, these traits are used in breeding programs for young male (Banks, 1997). Breed diversity is a valuable resource of the sheep industry. Crossbreeding systems use breed diversity to increase productivity relative to pure bred flocks. Crossbreeding systems vary in managerial complexity and in the use of beneficial effects due to crossbred ewes and lambs. Crossbreeding offers two distinct advantages over pure breeding: heterosis and breed complementarity.

Generally, crossbreeding was found to improve slaughter and carcass traits of crossbred lambs from local breeds (Kashan *et al.* 2005; Abdullah *et al.* 2010; Rodríguez *et al.* 2011). Lori-Bakhtiari is a fat-tailed breed of sheep, with a population more than 1.6 million, which is well adapted to hilly and mountain ranges of Bakhtiari region, west of Isfahan stretched out to Southern Zagros Mountain. Relative to other Iranian fat-tailed breeds Lori-Bakhtiari is a large breed, having the largest fat-tail. The Romanov sheep are originally from Russia and are adapted to cold inland climate and local feeding. The Romanov sheep are known for high prolificacy and the mean litter size is 2.2 lambs at birth or even more in some flocks. Because of its high prolificacy, it is used in crossbreeding. These breeds have been widely studied for their reproductive and production traits, but little information is available on their growth at fattening and carcass characteristics in crossing Iranian fat-tailed sheep with Romanov sheep. An evaluation of indigenous genetic resources is essential to assess their potential for crossing systems through the study of fattening performance and carcass characteristics. Therefore, the objectives of present study were to compare carcass traits of Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred male lambs.

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## MATERIAL AND METHODS

In this study, data for body weight at slaughter and carcass traits of Lori-Bakhtiari (n=50) and Romanov × Lori-Bakhtiari (n=22) crossbred male lambs were analyzed. All of the data came from Lori-Bakhtiari sheep breeding station in Chaharmahal and Bakhtiari province, Iran. The station flock was kept generally from December to May inside the barn and the sheep were fed with alfalfa, barley and wheat stubbles, and they were grazed on range and cereal remainder in other months of the year. The breeding season was from late August to late October and ewes were assigned randomly to the rams. About 15 days after parturition, the lambs were creep-fed during the suckling period. The creep-ration consisted of 50% barley, 10% cotton seed meal, 20% wheat bran, 18% dried sugar beet pulp, 1% bone meal, 0.5% salt and 0.5% vitamin, mineral and antibiotic supplement. The ration was ground, mixed and fed *ad libitum*. Lambs had also access to free choice alfalfa hay. The lambs were weaned at 90±5 days of age. After weaning, female lambs were kept on pasture and male lambs remained in drylot. The ration, fed to male lambs, contained 55% alfalfa hay and 45% concentrate. Lambs were slaughtered at seven month of age. After slaughtering and skinning, all the abdominal and thoracic organs were removed. The fatty tissues surrounding kidney (kidney fat), heart (pericardial fat) and those in the abdominal cavity (omental and mesentery fat) were separated and weighted. The warm carcass was weighed immediately after dressing and removal of offal parts. The carcasses were chilled at 3 ± 2°C for approximately 18 h, and then cold carcasses were weighed and separated into right and left sides. The right side was disjointed into the commercial wholesale cuts traditionally offered in Iran. The cuts were leg, shoulder, back (loin + fore-rib), flap (flank + brisket), neck and fat-tail. The method of cutting is described by Farid (1998). All cuts were trimmed, removing subcutaneous fat, intermuscular fat, de-boned and the weight of each component was recorded. Data were analyzed by the GLM procedure of SAS (2010). The statistical model for the analyses of all the carcass traits included genotype fixed effect and weight carcass as covariate.

## RESULTS AND DISCUSSION

The least-square means and standard error of live body weight at slaughter and carcass traits in Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs are presented in Table 1. The live body weight at slaughter in Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs were 46.28 and 44.84, respectively, that purebred and crossbred lambs similar for this trait. The dressing percentage for Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs were 50.48 and 46.54 percent, respectively that significantly different. These results are in agreement with those of Boujenane *et al.* (2003), Kremer *et al.* (2004), Kashan *et al.* (2005) and Abdullah *et al.* (2010), who reported that dressing percentage was affected by the lamb's genotype. However, this disagree with those of Macit *et al.* (2001) who reported that the dressing percentage did not differ significantly between Awassi, Morkaraman and Tushin breeds of Turkey.

Tab. 1. Least-square means (s.e) of live body weight at slaughter and carcass traits in Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs.

Effect	No	Trait							
		body weight at slaughter	cold carcass weight	Dressing percentage (%)	Lean (%)	Bone (%)	Fat-tail (%)	Carcass fat (%)	Total carcass fat (%)
Genotype		ns	***	***	***	***	***	ns	***
Lori-Bakhtiari	50	46.28±0.66 <sup>a</sup>	23.41±0.39 <sup>a</sup>	50.48±0.28 <sup>a</sup>	49.22±0.42 <sup>a</sup>	15.06±0.16 <sup>a</sup>	15.76±0.37 <sup>a</sup>	15.90±0.50 <sup>a</sup>	31.67±0.49 <sup>a</sup>
Romanov × Lori-Bakhtiari	22	44.84±0.99 <sup>a</sup>	20.90±0.59 <sup>b</sup>	46.54±0.42 <sup>b</sup>	62.72±0.66 <sup>b</sup>	18.38±0.24 <sup>b</sup>	1.94±0.58 <sup>b</sup>	17.61±0.77 <sup>a</sup>	19.55±0.77 <sup>b</sup>

\*, \*\*, \*\*\*, Significant at level 0.05, 0.01 and 0.001, respectively.  
ns, non-significant.

Carcass traits were significantly influenced by genotype effect. Romanov × Lori-Bakhtiari crossbred lambs for lean percentage (62.72 vs. 49.22%) were significantly (P<0.05) higher than in Lori-Bakhtiari lambs. Percentage of fat-tail, subcutaneous fat and total carcass fat in Lori-Bakhtiari and Romanov × Lori-Bakhtiari crossbred lambs were 15.76 ± 0.37, 6.85 ± 0.21, 31.67 ± 0.49 and 1.94 ± 0.58, 5.26 ± 0.33 and 19.55 ± 0.77, respectively. Percentage of fat-tail and subcutaneous fat in Lori-Bakhtiari lambs were significantly (P<0.05) higher than in Romanov × Lori-Bakhtiari crossbred lambs. Boujenane *et al.* (2015) reported that meat breeds could be an

interesting option to produce heavier and leaner carcasses. Also, terminal crossbreeding rams could be implemented to improve carcass conformation thus matching market demand for heavy carcasses with limited fat content. While percentage of internal fat in abdominal cavity ( $1.92 \pm 0.11$  vs.  $1.02 \pm 0.07$ ), bone carcass ( $18.38 \pm 0.24$  vs.  $15.06 \pm 0.16$ ) and intermuscular fat ( $5.49 \pm 0.25$  vs.  $3.57 \pm 0.16$ ) in Romanov  $\times$  Lori-Bakhtiari crossbred percentage of were significantly higher than in Lori-Bakhtiari lambs. This is in agreement with results of other authors (Nitter 1975; Boylan *et al.* 1976; Dickerson 1977) who reported that crossbred lambs from prolific breeds had higher internal fat than the most crossbred including local breeds.

## CONCLUSIONS

These results indicate that genotype effect was highly significant on carcass traits. Romanov  $\times$  Lori-Bakhtiari crossbred lambs were better than Lori-Bakhtiari lambs for lean and total fat carcass percentage.

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## THE MIXED HONEY HARVESTING IN TURKEY; EFFECTS ON QUALITY, MARKETING AND STANDARDIZATION

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*Professional paper*

### Summary

Honey is an important bee product in Turkey, economically. In spite of huge potential with 102.000 tons/year honey production, some problems faced on procurement period limited of honey marketing. Honey production period is begin with spring nectar flow in high plateaus and linden, orchard, chestnut, citrus, eucalyptus, buckwheat, chaste, sunflower, oak, pine honeydew and heather honeys trace, sequently. Despite of limited application some beekeepers filtered monofloral honey for marketing. In this case, such kind of honeys represent of dominant characteristics of the nectar source for colour, taste, smell, aroma and marketing by the rendition of it. However, migratory beekeeping is very common in Turkey and most of beekeepers tend to filter mixed honey that handled from different nectar sources for marketing as “flower honey”. Unfortunately, export rate of honey decrease because of demanding “monofloral honey” in external market, gradually. Beside this, quantity of honey production could not be determine in exact rate and standardization problem occurs. It is not only valid for flower honey but also seems in pine honeydew honey. Pine honeydew honey is obtained from South-west coast of Turkey from *Marchalina hellenica* insect lives in *Pinus brutia* trees by honey bees. This geography is unique for handling of 92% of the World pine honeydew honey production, economically. Pine honeydew is very special honey needs to distinctive codex standardization for evaluating of some biochemical characteristics. Otherwise, it accuses adulteration in some criterias are different from flower honeys even of very precious in human nutrition.

Key words: *Honey, harvesting, quality, marketing, standardization*

### INTRODUCTION

Turkey has huge beekeeping potential with over 7 million honey bee colonies and 102.486 tons/year honey production. This enormous capacity provides rank in the 2nd place for hive and 4th place for honey producing in the world. Hence, this status obtained from varies plant species that some of them endemic raised in the country. The most important honey sources are pine, citrus, sunflower, cotton, acacia, oak, linden, eucalyptus and chestnut. Also, there are different honey bee ecotypes adapted to the different ecologic conditions and the rich diversity of plants provide nectar and pollen. In addition to this floral source, there are also very rich honeydew sources of pine trees (*Pinus brutia*) in southwest part of the country.

### QUALITY, MARKETING AND STANDARDIZATION OF HONEY

Even the conspicuous scenery, honey sector faces some problems in the country. One of the most important problem is quality evaluation of honey. Food Codex defines honey as a natural product emerging after honey bees collect plant nectars, secretions excreted by living parts of plants, and secretions excreted by insects which live on and suck living parts of plants, add certain ingredients in them to transform them and lower their water content, store them in honeycombs and let them mature. Honey contains a complex mixture of carbohydrates, amino acids, enzymes, water, minerals, pollens and vitamins. The content of honey depends on nectar source, season and climatic conditions of environment, so that the properties of the nectar and its chemical content are of great importance. In fact, the quality of the honey is directly related to the source of nectar. The taste, aroma and color of the honey are directly affected by the source of plant nectar. Examples include eucalyptus honey which is dark, aromatic and spicy, thyme honey, light and scented, citrus honey, light and aromatic and so on. These examples indicate a direct transfer of components from the nectar to the honey. Flower honey nectars collected by bees from various plants located around their hives. It includes linden honey, clover honey, sunflower honey, cotton honey, eucalyptus honey, and acacia honey whereas honeydew honey excreted by an

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intermediate insect. That insect is a scale insect (*Marchalina hellenica*) and it is needed to produce pine honey. That scale insect feeds by sucking the nectar of pine, and excretes the excess nectar as a sweet substance. That scale insect lives only on *Pinus brutia*. An advantage of pine honey is that it can be stored for a long time without losing its consistency or without crystallizing.

Contents and standards expected from honey is stated in EU Codex for honey. In Turkish Food Codex, honey standards nearly similar as EU Codex. According to legislation honey standards of Turkish Food Codex are given in Table 1.

Tab. 1. Honey standards of Turkish Food Codex (2012)

<b>TURKISH FOOD CODEX for HONEY</b>		
<b>Analyses</b>	<b>Honey Source</b>	<b>Expected Range</b>
HMF ( mg/kg)	All	Max.40
Moisture (%)	All	Max.20
pH	All	3,5 to 5,5
Diastase	All	Min.8
Free acid ( meq/kg )	All	Max.50
C4 – Sugar (%)	Flower	Max.7
	Pine	Max.7
Sucrose(g/100g)	Flower	Max.5
	Pine	Max.10
F/G ratio	Flower	0,9 to 1,4
	Pine	1 to 1,4
Invert sugar (%)	Flower	Min.60
	Pine	Min.45
Prolin (mg/kg)	Flower	Min.120
	Pine	Min.300
Electrical Conductivity(mS/cm)	All	Max.0,8

Even of some physical and biochemical parameters specified in the Turkish Codex, some processing methods such as heating, filtering, straining, storage and preventing crystallization did not clear as well in property for EU Codex. So, this incompatibility affected honey evaluation negatively and demand on decreasing of honey exportation. Especially C4 sugar of pine honeydew honey decreased from 10 to 7 in last year. Indeed, C4 sugar is naturally high in pine honeydew honey, but if considered as it like flower honey it creates risk for evaluation as tricky. It is necessary to evaluate this item again as it natural occurrence in honey and should be changing in Turkish and EU Codex for pine honeydew honey.

Another important point is in Turkey, most of the beekeepers prefer migratory beekeeping and they do not make honey harvest after period of flower honey. So, in nectar period of pine honey, these two kinds of honey (flower and pine) could be mixed up and the physicochemical properties of pine honey could give results similar to “flower honey”. This circumstances cause difficulties in evaluation of honey qualifications and marketing problems. Importer countries want monofloral, specific and certain sources honey, generally.

The mono floral originated honey should assure a better quality of the product, when it guarantees a specific and well-defined flavour and aroma. Because of cultural habits and palate for flower honey, there is a great demand

for it in domestic market. Due to domestic consumers ready to pay serious amount of money for flower honey, export rate is low for international markets. But Turkey demand exportation of pine honeydew honey even could have a better chance realized in. Pine honeydew honey offers such a unique physicochemical parameters that it is not similar to any other honey. It is richly flavoured reminiscent of sweet molasses and valuable honey, "pine honey" with reputed medicinal benefits. It has dark colour, delayed crystallization, more minerals and muddy than flower honey. Because of the high quality and quantity honeydew honey produced in Turkey, it plays important role in the economy of the country. Turkey exports of the total production (approximately 10.000 tons/year) of pine honey to EU countries and earns 7-10 million EURO from this trade. The biggest pine honey importer country is Germany. Besides that, much of the world pine honey production (approximately 92%) realized in southwest coast part (Izmir-Aydin and Mugla) of the country. So, it could be easily said that "Turkey is the unique with its pine honeydew honey production in the world". In summer season (from 15th of August up to 15th of November), *Marchalina hellenica*, which is the most important honeydew honey-producing insect, produces sugar secretion to the honey bees, 50% of the Turkish beekeepers come to these places and produce pine honeydew honey with 3% of world beehives rank. So pine honeydew honey quality, production, marketing and exportation is very important for Turkish honey sector.

Crystallization is another big problem on consumer mind. It is a natural occurrence and physical changing of dextrose in honey. But most of time consumers perceived this as "artificial/faked" or "corrupted" honey. Flower honey could be crystallized faster than pine honeydew honey because of having high glyucose contents.

So, preventing this situation, producers could be prefer blending both flower and pine honey in particular rates because of late crystallization of pine honeydew honey. In this case, it causes difficulties for labelling and marketing honey. Because producing plant sources, harvesting season, region, name of firm, chemical composition of honey should be written on honey labels. Unfortunately, mixed harvesting of honey caused difficulty for giving exact plant origin and composition. So, marketing of such kind of honey creates problem for producers.

In this case, standardization gets importance in quality. Honey standards and certification are an important way for consumers to know what they are getting. With standards it is easier to trust the quality and source by simply checking the label of the honey we buy. It gives least assurance to consumer as the product carried out standard dimensions of quality. In this manner the value of the product (honey) increases and economy is accelerated.

## CONCLUSION

Huge Turkish beekeeping sector should get some precautions for increasing production quality of honey. Traceability from hive to table should be sustained, producers, consumers and traders of honey conscious should be increased. Precautions of monofloral honey production should be emphasized. Turkey is the biggest pine honey producer and exporter in the world. However, special properties of pine honey necessitate a specific consideration of CODEX different from floral honey. The fact that the migratory beekeeping is common in Turkey makes it impossible to produce mono floral originated-honey, in contrary to permanent beekeeping practices in Europe. Therefore, it is greatly likely that pine honey produced in Turkey might be mixed with floral honey in first extraction. This could lead to some mistakes in the assessment of some of the physicochemical parameters. Special properties of pine honeydew honey necessitate a specific consideration of CODEX different from floral honey and honeydew honey. More production, quality research and marketing opportunities should be increased on pine honeydew honey and Turkish pine honeydew honey gets trademark in worldwide as deserved. Exporting facilities of Turkish high quality honey should be increase. Standardization of honey quality should enhanced. If reducing input costs, through pine honey standardization, production controls and packaging should be prioritized in order to increase production and exportation to EU countries. Finally, Turkish beekeeping sector will take a better place in world market, providing governmental financial support and high quality management techniques. Provided that the above-mentioned measures are taken, Turkey is surely ready to share with whole world 'sweet blessing' god gave to this unique geography.

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## **FOOD TECHNOLOGY**

## THE CONTENT OF MACRO AND MICRO ELEMENTS IN PROPOLIS COLLECTED FROM THREE GEOGRAPHIC AREAS IN BOSNIA AND HERZEGOVINA

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*Scientific paper*

### Summary

The chemical composition of propolis, as well as other bee products, varies with nutrients that honey bees collect and feed with. There are numerous compounds identified until today, most of them are flavonoids. We are focused on metals (B, Co, Cu, Fe, Mn, Cr, As, Na, K, Mg, Pb, Cd, Ni, Zn), whose propolis concentration depends on the plant sources, climate, soils, as well as the metabolism of the bee and the function of the propolis in a bee's life.

In total, 18 samples of raw propolis were collected from three different climate zones in Bosnia and Herzegovina (north, center and south of BiH), 6 from each. The samples were taken by scratching the inner side of the hive and manually homogenized. The microwave ashing with mineral acids was used and the content of B, P, Co, Cu, Fe, Mn, Cr, As, Na, K, Mg, Pb, Cd, Ni, Zn were determined by ICP-MS method. The results have confirmed some literature findings that propolis was very heterogeneous with materials of vast ranges of concentrations of analysed elements. Statistical analysis (ANOVA, Tukey test) has shown the existence of statistically significant differences between regions only for the concentrates of sodium.

Key words: *propolis, micro and macro elements, geographic area.*

### INTRODUCTION

Propolis, a valuable bee product with high antimicrobial effect, contains a high number of different substances derived from plant rosin, wax and pollen and from bee secretion. More than 200 different components were identified: various flavonoids, vitamins (B1, B2, B6, C, and E), minerals (Mg, Ca, J, K, Na, Cu, Zn, Mn and Fe). For some of them, the function is unknown and they may be considered as a result of bee food chain (bee pasture and bee metabolism) which includes contamination from air, water or plants.

Only *Apis mellifica* species produce propolis. In Europe, one bee society produces in average between 50 - 150 g yearly and good gathering societies, primarily Caucasian bees, up to 250 - 1000 g (Sobočanec, 2006). Rosin mass is collected by bees from buds as well as leaves, branches and tree bark (Alić, 2009). Depending on the geographical region, material for propolis is gathered from different trees and bushes and less from evergreens.

Chemical properties of propolis are important parameters for characterisation of this bee product because there is correlation between antimicrobial effect and chemical compound (Kujumgiev *et al.*, 1999). The objective of this work is to determine the contents of some macro and micro elements in „raw“ propolis, gathered from three geographical regions in BiH, in order to determine the range of specific elements' concentration in propolis, connection to *in situ* matrix which is shown in contents of microamount of some elements (geographical region identification) and presence and concentration of some elements depending on bee pasture.

### MATERIAL AND METHODS

Propolis samples were gathered from three different regions of BiH: Bosanska krajina (Cazin, Ključ, Sanski Most, Velika Kladuša, Bosanska Krupa and Bihać), central Bosnia (Blažuj, Olovo, Žepče, Jajce, Visoko and Zenica) and Herzegovina (Jablanica, Počitelj, Livno, Konjic, Stolac and Ljubuški). The collecting was conducted in the period from March to June 2014, all of them are a result of bee activity during 2013. The samples were taken by scraping from bee frames within the hive directly on apiaries, and some samples were delivered by beekeepers themselves. The laboratory part was conducted in the laboratories of the Federal Institute of Agriculture in Sarajevo in the period between July through October 2014. The collected samples

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(„raw propolis“) were kept in plastic bottles, and in the first 24 hours after sampling were held on -18°C, in order to dry and grain the propolis, i.e. to get homogenous samples for analysis.

Determining the concentration of macro and micro elements was conducted by inductively coupled plasma with mass spectrometry (ICP-MS) 7700 x, Agilent Technologies, and samples were in microwave digestion oven MDS 8, Sineo.

For statistical data processing, variable analysis (ANOVA) and Tukey test (multiple testing) were used in the case of determining statistical significance.

## RESULTS AND DISCUSSION

As it was stated in the literature (Bankova, 2005), chemical composition of propolis is very complex and it varies a lot. Significant differences in the contents of some compounds (including metals) are present even among some hives within the same apiary. The plants dominating in bee pasture primarily contribute to propolis heterogeneity, as well as all variables affecting the physiological plant status: season, climate, ground, etc. Presence of herbal cells, organelles and different microparticles from the environment was found in propolis (Walker, Crane 1987; Greenaway *et al.*, 1990; Marcucci, 1995; Bankova *et al.*, 1998).

The results of our analyses are presented in Table 2. Within one analysed region, high variations of analysed elements' concentration was found (presented in variation coefficients). Variations are always above 20%, in some cases even 100%, as in Fe, Pb i Cr. These variations within one region do not allow identification of plausible differences between the specified regions in BiH. The concentration of sodium is an exception, for which a statistically significant difference in specified region concentrations was determined. The highest variations (for all regions) was determined for led concentrations, followed by:



It is discernable that the highest variations are in the contents of microelements (herbal nutrients present in microamounts - Zn, Fe, Mn, Cu) and contamination elements (Pb, Cr, Cd, Ni), which eventually indicates specificity of bee posture (region), i.e. air pollution.

Sequence of the amounts in analysed elements from the chosen regions was not equal, as shown in the following order:

Central BiH: K>P>Fe>Mg>Zn>Na>Mn>Pb>Cr>B>Cu>Ni>Co>As>Cd

Herzegovina: K>P>Fe>Mg>Zn>Na>Pb>Mn>B>Cu>Cr>Ni>Co>Cd>As

Bosanska krajina : K>Mg>Fe>P>Zn>Na>Mn>Pb>B>Cu>Cr>Ni>Co>Cd>As

In Herzegovina and central Bosnia, quantitative representation of the first 6 analysed elements is the same (K>P> Fe> Mg> Zn> In), and then there is a difference in the content of microelements, which are indications of geographical origin. The specificity of the representation of some elements is presented already at the second element of Mg, in Krajina.

Tab. 1: The coefficient of variation in individual elements, according to region

Term	Element	Region		
		Northern BiH	Central BiH	Southern BiH
1.	K	20.0	31.0	26.0
2.	P	28.8	46.5	28.8
3.	Fe	112	<b>106.1</b>	48.6
4.	Mg	49.2	69.7	39.6
5.	Zn	<b>135</b>	64.5	<b>101.0</b>
6.	Na	31.0	22.0	42.2
7.	Mn	54.9	64.1	45.7
8.	Pb	63.2	<b>161.9</b>	<b>102</b>
9.	Cr	44.8	142	90.3
10.	B	25.0	55.0	26.5
11.	Cu	38.0	42.7	27.8
12.	Ni	82.0	72.5	56.5
13.	Co	<b>109.0</b>	92.1	97.5
14.	As	50.0	78.6	20.0
15.	Cd	54.5	83.3	<b>107.1</b>

Comparison with literature data for concentrations of the analysed elements, also confirms large variations of metal content in propolis. The following table shows the results obtained and those which were available in the literature:

Tab. 2: The results obtained from the research and those are available in the literature

	Potassium		Phosphorus	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	679.8 – 1312.7	750 – 1090 mg/kg (Eremi and Dabija; 2007) 1270 mg/kg (Finger <i>et al.</i> ; 2014)	16.8 – 66.02	5370 – 5730 mg/kg (Grembecka and Szefer; 2012)
Central BiH	401.9 – 1218.2		117.0 – 618.1	
Bosanska krajina	709.9 – 1216.7		154.6 – 361.5	
Magnesium			Iron	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	177.7 – 528.3	250 – 253 mg/kg (Grembecka and Szefer; 2012) 137 – 823 mg/kg (Formicki <i>et al.</i> ; 2012)	157.6 – 547.9	407.5 – 531.1 mg/kg (Eremia and Dabija; 2007) 28 – 101 mg/kg (Formicki <i>et al.</i> ; 2012)
Central BiH	79.7 – 667.9		103.4 – 1074.7	
Bosanska krajina	120.1 – 580.1		117.6 – 948.4	
Manganese			Copper	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	6.4 – 20.6	0.58 mg/kg (Finger <i>et al.</i> ; 2014) 8.4 – 14.6 mg/kg (Eremi and Dabija; 2007)	1.91 – 4.86	2.18 – 13.7 mg/kg (Eremi and Dabija; 2007)
Central BiH	8.2 – 37.9		0.87 – 3.85	
Bosanska krajina	6.6 – 33.2		1.45 – 3.77	
Zinc			Boron	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	27.4 – 368.7	39.1 – 98.6 mg/kg (Eremia and Dabija; 2007)	3.54 – 7.26	
Central BiH	11.2 – 139.1		0.66 – 7.58	
Bosanska krajina	31.9 – 728.8		2.72 – 5.98	
Cobalt			Nickel	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	0.102 – 1.222	0.05 mg/kg (Eremi and Dabija; 2007)	0.289 – 1.145	1.99 – 9.81 mg/kg (Formicki <i>et al.</i> ; 2012)
Central BiH	0.102 – 1.017		0.388 – 1.963	
Bosanska krajina	0.101 – 0.971		0.166 – 1.699	
Arsenic			Chromium	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	0.065 – 0.115	0.657 mg/kg (Finger <i>et al.</i> ; 2014)	0.803 – 7.558	0.6 – 5.4 mg/kg (Eremi and Dabija; 2007)
Central BiH	0.053 – 0.291		0.215 – 17.333	
Bosanska krajina	0.041 – 0.189		1.084 – 2.997	
Lead			Cadmium	
	Our results, mg/kg	Literature	Our results, mg/kg	Literature
Herzegovina	1.909 – 38.437	9.85 mg/kg (Finger <i>et al.</i> ; 2014)	0.034 – 0.397	0.194 mg/kg (Roman <i>et al.</i> ; 2011)
Central BiH	1.359 – 71.997		0.019 – 0.099	
Bosanska krajina	0.681 – 10.731		0.045 – 0.204	
Sodium				
	Our results, mg/kg	Literature		
Herzegovina	14.45 – 46.12	80 mg/kg (Finger <i>et al.</i> ; 2014); 31.3 – 59.4 mg/kg (Eremi and Dabija; 2007); 92.3 i 38.6 mg/kg (Grembecka and Szefer; 2012)		
Central BiH	23.40 – 46.73			
Bosanska krajina	9.71 – 27.42			

It should be noted that in certain concentration of elements strong or very strong correlation was determined, as it shown in the following table:

Tab. 3: Correlation coefficients (very strong, strong and medium strong) of concentration in the analysed elements in propolis

Elements	B	Na	Mg	P	K	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
<b>B</b>	1														
<b>Na</b>	0.287	1													
<b>Mg</b>	0.813	0.271	1												
<b>P</b>	0.625	0.592	0.649	1											
<b>K</b>	0.793	0.271	0.823	0.777	1										
<b>Cr</b>	0.564	0.326	0.591	0.392	0.368	1									
<b>Mn</b>	0.402	0.190	0.518	0.180	0.263	0.615	1								
<b>Fe</b>	0.648	0.360	0.841	0.431	0.555	0.746	0.447	1							
<b>Co</b>	0.486	0.356	0.552	0.282	0.401	0.684	0.405	0.791	1						
<b>Ni</b>	0.366	0.332	0.382	0.270	0.281	0.621	0.299	0.619	0.559	1					
<b>Cu</b>	0.635	0.424	0.559	0.565	0.579	0.499	0.201	0.641	0.777	0.563	1				
<b>Zn</b>	0.306	-0.014	0.246	0.085	0.444	0.043	-0.073	0.194	0.085	0.151	0.120	1			
<b>As</b>	0.414	0.322	0.335	0.148	0.120	0.708	0.455	0.560	0.449	0.483	0.332	-0.064	1		
<b>Cd</b>	0.026	-0.306	-0.045	-0.022	0.116	-0.114	-0.083	-0.068	0.005	0.097	0.260	0.056	-0.064	1	
<b>Pb</b>	0.527	0.259	0.580	0.347	0.362	0.960	0.567	0.736	0.741	0.626	0.558	0.103	0.578	-0.104	1

It is evident that the type of the element, and similarities in the physic-chemical properties, are affecting the incorporation of certain elements in propolis. Correlation of Pb - Cr ( $r = 0.960$ ) and K - Mg ( $r = 0.823$ ) is a good example of such interaction.

According to these results, it is difficult to make an assessment of possible environmental pollution to the content of contamination elements (Pb, Cr, Cd and Ni) in propolis. Nevertheless, the lowest content of lead and chromium was in the propolis from Bosanska Krajina; in central Bosnia there was the lowest content of cadmium, and nickel in Herzegovina. These results tend to suggest that there is probably no local sources of pollution for these elements in any of the analysed regions, and attention should be paid to the deposition from the atmosphere (air pollution-border transmission).

### CONCLUSION

This was "pioneering" research study and can determine and direct the upcoming ones. It was necessary to determine the existence and concentration range of analysed individual elements in propolis for selected geographic areas in Bosnia and Herzegovina.

Based on these results it can be concluded:

Content of the analysed metals (B, Co, Cu, Fe, Mn, Cr, As, Na, K, Mg, Pb, Cd, Ni, Zn) in propolis varies, not only between different regions in BiH, but also within each of them;

There is no statistically significant differences in the contents of the studied elements, between selected regions of analysis in BiH.

The greatest variations in the concentration of propolis was determined for lead, which can be the result of environmental contamination, and the lowest for sodium, element for which only significantly different concentrations were determined in the studied regions of Bosnia and Herzegovina.

This research study has also pointed to the problems, which should be focused on in the future research:

Studies have shown that there is a significant problem of heterogeneity in bulk sample of propolis. By its nature (different collected material in the making of propolis) propolis is very heterogeneous and it would be good to conduct one study for representative sample preparation.

The relationship between individual plant species of bee pastures of the region and the content of individual elements should be identified. Because the content of trace elements are considered the reflection of autochthonous plants, i.e. the geographical origin, attention should be focused in that direction.

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## DETERMINATION OF TOTAL PROTEINS CONTENT IN BEE HONEY USING BY UV SPECTROPHOTOMETRY

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Esad Tursanović<sup>1</sup>

*Original scientific paper*

### Summary

In this scientific work we were determined the total proteins (TPs) content in different samples of honey from Bosnia and Herzegovina and Croatia. For the determination of TPs, method of UV spectrophotometry was used, where the samples were scanned at wavelengths of 205 and 278 nm, and then calculated to TPs content using the calibration equations from the calibration curve that was constructed using bovine serum albumin (BSA) as a standard. It is important to say that the TPs at 278 nm was obtained by the method of deduction (from baseline absorbance corresponding to the content of the proteins, bioflavonoids, and other compounds with aromatic nucleus present in honey, is subtracted the absorbance value after protein precipitation by centrifugation, so that the resulting difference of absorbance corresponding absorption of proteins). The gathered data showed different contents in different honey samples. The highest content of TPs at 205 nm showed the sample of meadow honey from Kakanj whose content was 3.47 g/100 g, and the lowest value of 0.27 g/100 g showed the sample of acacia honey from Sanski Most (Sasina). At 278 nm, the highest TPs of 4.12 g/100 g showed a sample of mountain honey from Bjelašnica (Rakitnica), and the lowest value of TPs showed a sample of meadow honey from Ključ (0.06 g/100 g), and meadow honey from Sokolac (the proteins were not detected). From the obtained results it can be concluded that the UV spectrophotometric method can be successfully applied for the quantification of TPs in honey.

**Key words:** *Total proteins, bee honey, UV spectrophotometry, Bosnia and Herzegovina.*

### INTRODUCTION

Nowadays the tendency of turning to healthy diet and unpolluted products is higher. People recognize how much can use of healthy food like honey, that is available to everyone, alleviate symptoms, and even some diseases. Honey is natural sweetener, viscous liquid or crystallized product that is produced by honey bee (*Apis mellifera*) from the nectar of flowers honey plants that bees collect add their own specific matter, transform and leave the honeycomb cells to mature (Službeni glasnik of Bosnia and Herzegovina, No. 37).

The composition of honey depends directly on the composition of the nectar from which honey is obtained. Honey contains ≈80 various ingredients (Yilmaz and Kufrevioglu, 2003), while some researchers claim that there are more than 180 ingredients (Al-mammary *et al.*, 2002) or even ≈300 ingredients (Vasquez *et al.*, 2003). Natural honey is sticky and viscous liquid with 80-85% carbohydrates (mainly glucose and fructose), 15-17% water, 0.05-3.10% proteins, 0-2% mineral substances and a small amount of amino acids, phenolic antioxidants (mainly bioflavonoids), enzymes and vitamins (Buba *et al.*, 2013; Nazarian *et al.*, 2010; Won *et al.*, 2009; White and Rudy, 1978). The main constituents of honey are the same for all types of honey, but the precise chemical composition of honey and its physical properties vary according to the type of plants from which the honey is obtained.

Among the abundant range of health effects such as antibacterial, antiviral and antifungal activity, honey helps in healing wounds, gastroenteritis, improves the immune status of the organism, reducing the risks of cardiovascular diseases (Ajibola 1995, Bertoncej *et al.*, 2007, Blasa *et al.*, 2006, Estevinho *et al.*, 2008, Salem 1981). All of its medicinal properties honey owes to complex variety of chemical substances of which is

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assembled. The carbohydrates in honey are an excellent source of energy. Inhibin prevents the growth of bacteria and fungi on the surface of wounds, especially allowing sterility, phenolic compounds and proteins provide antioxidant properties of honey, and allow detoxification of free radicals.

The subject of this work is the determination of total proteins (TPs) in samples of different types of bee honey mainly from Bosnia and Herzegovina and Croatia using by UV spectrophotometric method. This work should also address the comparison of the contents of the TPs in honey obtained by recording at two wavelengths, 205 and 278 nm.

## MATERIALS AND METHODS

The UV spectrophotometry has been used for determination of TP content in different samples of honey. All absorbance measurements were made with a Double beam ultraviolet–visible spectrophotometer (Perkin-Elmer Lambda 25 UV/Vis spectrophotometer) provided with matched 1 cm quartz cells.

The proteins have absorption maxima at wavelengths of  $\approx 280$  nm and 205 nm, wherein the light wavelength of 280 nm corresponds to absorption of the aromatic rings of amino acids (tryptophan, tyrosine and phenylalanine), whereas the absorption at 205 nm corresponds to the peptide bonds of proteins. The absorption spectrum of the reference protein solution (bovine serum albumine, BSA) showed that the maximum absorption corresponds to the aromatic ring at 278 nm, and for the construction of the calibration curve across the absorption of peptide bonds taken is the wavelength of 205 nm, which is (in addition to 210 nm) most often cited in the literature.

For calculating the content of TPs were used the equations of calibration curves representing dependence absorbance of the concentration of BSA.

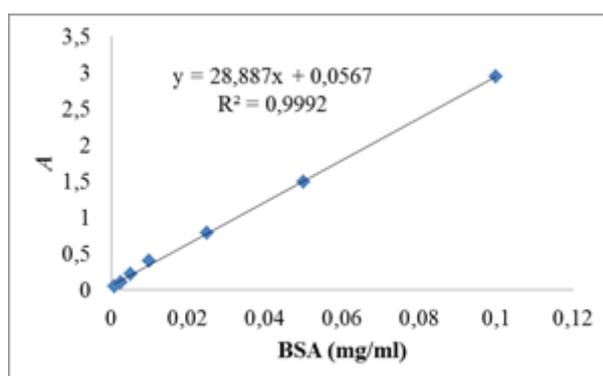
During the experimental work thirty different samples bee honey were used, of which twenty-eight were from Bosnia and Herzegovina, and two samples were from Croatia. Samples of honey from Bosnia and Herzegovina originate from the environment of different cities/regions such as Bihać, Cazin, Sanski Most, Prijedor, Kakanj, Olovo, Ključ (Sanica), Bjelašnica (Rakitnica), Jablanica, Donji Vakuf, Čapljina, Nišićka visoravan etc.

The samples were weighed and transported to the flask of 10.0 ml and diluted with dd water in order to obtain a solution of honey. From these original solution different aliquots were taken which were further diluted, and recorded on a UV/Vis spectrophotometer in the range of 200–400 nm. Since the absorption maximum at a wavelength of 278 nm corresponding to the aromatic nucleus of aromatic amino acids, originally measured protein contents were reasonably too high, because other components in honey also have an aromatic nucleus, for example, flavonoids. Therefore, samples were upon recording on the wavelength of 278 nm subjected to centrifugation for 20 minutes at 15 000 r/min in order to protein precipitation. The supernatant is decanted and re-recorded on the 278 nm. The difference between of this absorbance and the absorbance value of the previous measurement corresponds to the absorption of protein.

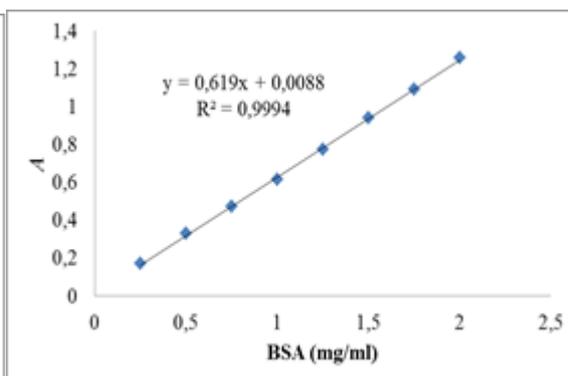
The blank is comprised of dd water.

## RESULTS AND DISCUSSION

For the preparation of calibration curves, it was necessary to prepare a series of solutions of BSA of various concentrations. For this purpose the prepared concentrated solution of BSA (0.0500 g of BSA were dissolved in 10.00 ml of dd water, concentration: 5 mg/mL). By dilution with dd water from this solution were prepared two sets of standard solutions, one with seven concentrations in the range 0.001–0.1 mg/mL for the recording at 205 nm, and the other eight concentrations ranging from 0.25–2 mg/ml for recording at 278 nm.



Graph 1. Calibration curve on 205 nm



Graph 2. Calibration curve on 278 nm

From the equation of the calibration curves protein concentrations are calculated and with the corresponding dilution factor expressed in protein mass (g) by honey weight (100 g). The results of TPs are provided in Table 1.

Tab. 1. Total proteins (TPs) content of different honey samples on 205 and 278 nm

Honey	Location	TPs (278 nm) (g/100 g) ± S.D.	TPs (205 nm) (g/100 g) ± S.D.
Mountain	Bjelašnica (Rakitnica)	4.12 ± 0.41	0.31 ± 0.00
Acacia	Cazin	4.08 ± 0.16	0.34 ± 0.01
Linden	Ključ (Sanica)	3.29 ± 0.09	0.58 ± 0.01
Meadow	Olovo (Olovske luke)	2.88 ± 1.52	0.56 ± 0.01
Sage	Croatia (Medena)	2.57 ± 0.47	0.43 ± 0.01
Meadow	Bosanska Krupa (Unska brina); harvest 2013	2.21 ± 0.18	0.87 ± 0.01
White heather	Čapljina	1.93 ± 0.42	0.31 ± 0.01
Acacia	Sanski Most	1.73 ± 0.27	0.27 ± 0.00
Linden	Rogatica	1.61 ± 0.07	0.83 ± 0.01
Sage	Hercegovina	1.38 ± 0.12	0.86 ± 0.00
Chestnut	Cazin (Stijena)	1.25 ± 0.01	1.20 ± 0.01
Meadow	Visoko	1.15 ± 0.09	0.57 ± 0.00
Blight	-	1.01 ± 0.03	0.69 ± 0.01
Meadow	Hercegovina	0.95 ± 0.18	0.64 ± 0.01
Forest	Donji Vakuf	0.87 ± 0.25	1.05 ± 0.01
Amorpha	Bihać	0.77 ± 0.30	1.24 ± 0.01
Chestnut	Jablanica	0.72 ± 0.32	0.65 ± 0.01
Meadow	Kakanj	0.68 ± 0.14	3.47 ± 0.02
Floral	Bihać	0.60 ± 0.13	0.32 ± 0.01
Forest	Ključ	0.53 ± 0.08	1.07 ± 0.01
Meadow	Bihać (Orašac)	0.52 ± 0.12	0.64 ± 0.01
Meadow	Bihać	0.51 ± 0.20	0.50 ± 0.01
Meadow	Bosanska Krupa (Unska brina); harvest 2011	0.50 ± 0.39	0.59 ± 0.01
Chestnut	Cazin (Brezova Kosa)	0.26 ± 0.11	0.29 ± 0.00
Meadow	Cazin (Pjanići)	0.25 ± 0.26	0.53 ± 0.01
Acacia	Sanski Most (Sasina)	0.24 ± 0.12	0.27 ± 0.01
Meadow	Croatia (Paklina islands-near Hvar)	0.16 ± 0.13	0.81 ± 0.01
Mountain	Nišićka visoravan	0.12 ± 0.01	0.81 ± 0.05
Meadow	Ključ (Sanica)	0.06 ± 0.05	0.79 ± 0.00
Meadow	Sokolac	ND	0.43 ± 0.01

All measurements were performed in triplicate

The results showed different contents of TPs in different honey samples. The highest TPs content at 205 nm showed the sample of meadow honey from Kakanj whose content was 3.47 g/100 g, and the lowest value of 0.27 g/100 g showed the sample of acacia honey from Sanski Most (Sasina). At 278 nm, the highest TPs of 4.12 g/100 g showed a sample of mountain honey from Bjelašnica (Rakitnica), and the lowest TPs showed the sample of meadow honey from Ključ (0.06 g/100 g), and meadow honey from Sokolac (the proteins were not detected). Based on the obtained results and on the calculated mean values for the content of TPs on both wavelengths (205 and 278 nm), it is evident that the measurement at 278 nm gives generally higher values.

In the literature, the TPs content is in interval 0.1-3.3% (Won *et al.*, 2009, White and Rudy, 1978), and 0.05 to 0.2 g/100 g of honey (Bogdanov, 1981; Liberato *et al.*, 2013). Our results of TP content at 278 nm ranged 0.00-4.12%, which is slightly higher compared to the previously published data, while the results of the TPs at 205 nm ranged 0.27-3.47%, which is fully agrees with the used literature.

## CONCLUSIONS

### Quantitative analysis:

#### 1. The TPs content at 205 nm

The highest TPs content at 205 nm showed a sample of meadow honey from Kakanj (3.47 g/100 g), followed by a sample of amorphia honey from Bihać (1.24), chestnut honey from Cazin (Stijena) (1.20), forest honey from Ključ (1.06), forest honey from Donji Vakuf (1.05), meadow honey from Bosanska Krupa (Unska brina, harvest 2013 year) (0.87), sage honey from Herzegovina (0.86), linden honey from Rogatica (0.83), meadow honey from Croatia (Paklina islands-near Hvar) and mountain honey Nišićka visoravan (0.81), meadow honey from Ključ (Sanica) (0.79), a sample of blight (unknown location) (0.69), chestnut honey from Jablanica (0.65), meadow honey from Bihać (Orašac) and meadow honey from Herzegovina (0.64), meadow honey from Bosanska Krupa (Unska brina, harvest 2011 y.) (0.59), linden honey from Ključ (Sanica) (0.58), meadow honey from Visoko (0.57), meadow honey from Olovo (Olovske luke) (0.56), meadow honey from Cazin (Pjanići) (0.53), meadow honey from Bihać (0.50), sage honey from Croatia (Medena) and meadow honey from Sokolac (0.43), acacia honey from Cazin (0.34), flower honey from Bihać (0.32), honey of white heather from Čapljina, mountain honey Bjelašnica (Rakitnica) (0.31), chestnut honey from Cazin (Brezova kosa) (0.29) and the lowest TPs content showed a sample of acacia honey Sanski Most (Sasina) (0.27 g/100 g).

#### 2. The TPs content at 278 nm

At 278 nm, the highest levels of TPs have shown the sample of mountain honey from Bjelašnica (Rakitnica) (4.12 g/100 g) and the sample of acacia honey from Cazin (4.08 g/100 g), followed by: sample of linden honey from Ključ (Sanica) (3.29), meadow honey from Olovo (2.88), sage honey from Croatia (Medena) (2.57), meadow honey from Bosanska Krupa (Unska brina, harvest 2013 y.) (2.21), acacia honey from Sanski Most (1.93), linden honey from Rogatica (1.73), sage honey from Herzegovina (1.61), meadow honey from Kakanj (1.38), meadow honey from Visoko (1.25), blight (unknown location) (1.15), meadow honey from Herzegovina (1.01), forest honey from Donji Vakuf (0.95), amorphia honey from Bihać (0.87), chestnut honey from Jablanica (0.77), flower honey from Bihać (0.72), forest honey from Ključ (0.68), honey of white heather from Čapljina (0.60), meadow honey from Bihać (Orašac) (0.53), meadow honey from Bihać (0.52), meadow honey from Bosanska Krupa (Unska brina, harvest 2011 y.) (0.51), chestnut honey from Cazin (Stijena) (0.50), chestnut honey from Cazin (Brezova kosa) (0.26), meadow honey from Cazin (Pjanići) (0.25), acacia honey from Sanski Most (Sasina) (0.24), meadow honey from Croatia (Paklina islands-near Hvar) (0.16), mountain honey Nišićka visoravan (0.11). The lowest TPs contents have shown the sample of meadow honey from the periphery of Ključ: 0.06 g/100 g, and the sample of meadow honey from Sokolac, in which the proteins at these conditions were not detected.

3. The TPs content in the analyzed samples varies from 0.27 to 3.47% (at 205 nm) and 0.06 to 4.12% (at 278 nm).

4. The results of this study are consistent with the literature, with a better agreement when measured at 205 nm.

5. UV spectrophotometric method has proven to be fast, simple and cost-effective for the determination of the TPs in the samples of bee honey.

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## SPECTROPHOTOMETRIC DETERMINATION OF TOTAL CHLOROPHYLL CONTENT IN FRESH VEGETABLES

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*Original scientific paper*

### Summary

Color of vegetables and fruits is a result of the presence of plant pigments. Chlorophyll is a main porphyrin pigment that converts light energy into chemical energy through photosynthesis process.

The aim of the research was to determinate total chlorophyll content in five different green vegetables (spinach, kale, broccoli, chard and lettuce), collected from 5 different local markets in Sarajevo Canton.

Vegetables samples were prepared with laboratory homogenizer using about 0.5 g of fresh material. 80% acetone was used as extraction solvent. Analytical determination of total chlorophyll (a+b) was performed with spectrophotometer at 662nm and 644nm, respectively.

It can be concluded that content of chlorophyll varies by species. The highest content of total chlorophyll was found in chard (121.34 mg/100 g), since lettuce had the lowest content. In general, obtained result indicated lower values compared with one reported in literature.

**Key words:** *total chlorophyll, vegetables, spectrophotometric method*

### INTRODUCTION

Vegetables are known as good source of vitamins, minerals, fibers, essential oils and phytochemicals with significant physiological effects in human body which have role as antioxidants, stimulating enzymes, destroying bacteria and reducing diseases like cancer and heart diseases (Krishnaswamy and Raghuramulu, 1998).

Chlorophylls are green pigments found in chloroplasts of photosynthetic tissues which are located in the photochemically active biomembranes. These pigments have porphyrin structure. Chlorophyll *a* and *b* are green components, chlorophyll *c* is found with chlorophyll *a* in marine algae (Shwartz and Lorenzo, 1990). Chlorophyll in its structure contains Mg atom in center of porphyrin ring, which is easily removed in acid conditions, forming the pheophytins (Shwartz and Lorenzo, 1990). Due its similarity with haemoglobin structure, chlorophyll is often called green blood of plants, which gives chlorophyll an important role in human health, immune and detoxification system (Kopsell *et al.*, 2005). Content of photosynthetic pigments can be related with internal factors and environmental conditions, as an indicator for response of light shade conditions (Porra, 1991; Vicas *et al.*, 2010) and sensitive biomarker of pollution and environmental stress (Tripathi and Gautam, 2007). It is very important to choose best solvent for extraction or spectrophotometric determination of chlorophyll due to remove spectral interferences (Mantoura *et al.*, 1997). Acetone is found to be best solvent for separation of chlorophyll from sample matrix since gives sharp absorption peaks (Ritchie, 2006).

Green leafy vegetables are good source of phytochemicals. They are used for foods being very rich in beta-carotene, ascorbic acid, minerals and fibers. For this research five different leafy vegetables were used.

Chard (*Beta vulgaris* L. ssp. *vulgaris*) is a leafy vegetable with shiny, green, ribbed leaves with high nutritional values.

Lettuce (*Lactuca sativa* L.) is the most used green vegetable consumed in row form as an importante source of phytochemicals (Chang *et al.*, 2013).

Spinach (*Spinacia oleracea*) contains different phytochemicals with high nutritional levels. Spinach is high in vitamin A, C, E and K, as well in selenium, omega-3 fatty acids and microelements (K, Ca, Mg, Mn, Zn and others).

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Kale (*Brassica oleracea* L. var. *sabauda*) has green or purple leaves, very high in vitamin K, beta-carotene, vitamin C and calcium. Kale, as broccoli (*Brassica oleracea* L. var. *italica*) and other Brassicas contains sulphoraphane, with potent anti-cancer chemical properties (Zhang and Callaway, 2002).

The aim of this study was to determine chlorophyll content in fresh leafy vegetables obtained from local markets in Sarajevo Canton using spectrophotometric method.

## MATERIALS AND METHODS

For experimental purpose five types of vegetables were used (spinach, kale, broccoli, chard and lettuce). Vegetables were purchased at five different local markets in Sarajevo Canton in 200-300 g portions. Each sample was in row form. Fresh samples were washed thoroughly with distilled water, kept to dry at room temperature (20°C) and analysed for the determination of chlorophyll content. UV spectrophotometer (Shimadzu) was used for the absorbance measurements.

Accurately weighted 0.5 g of fresh plant leaf sample was taken, homogenized in a mortar with the addition of quartz sand and 5-10 ml of 80% acetone as a solvent for extraction. After homogenization, extraction and filtration, samples were quantitatively transferred in test tubes for spectrophotometric determination.

Chlorophyll content was analysed by absorption measurements at 662 and 644 nm, respectively and calculated according to the following equations:

$$\text{Chlorophyll } a \text{ (mg/mL)} = 9,784 \times A_{662} - 0,990 \times A_{644}$$

$$\text{Chlorophyll } b \text{ (mg/mL)} = 21,426 \times A_{644} - 4,650 \times A_{662}$$

$$\text{Total chlorophyll (mg/g)} = C_1 \times V \times R / M \times 1000$$

## RESULTS AND DISCUSSION

In the present work content of chlorophyll in fresh leafy vegetables was evaluated and characterized in Table 1.

Tab. 1. Content of chlorophylls in leafy vegetables

Sample	Total chlorophylls mg/100g
Spinach	120.45
Kale	58.88
Broccoli	72.32
Chard	121.37
Lettuce	58.27

Among the vegetables analysed in our study, chard had the highest content of total chlorophylls (121.37 mg/100 g), which was about 2 times higher comparing with kale. Bohn *et al.* (2007) reported that spinach contains 130 mg/100g of chlorophylls; kale, lettuce and chard had content of chlorophyll over 180 mg/100g (Bohn *et al.*, 2007). Korus and Kmeick (2007) characterized green kale leaves and they reported that content of chlorophyll ranged from 118-145 mg/100g. Our research showed lower chlorophyll content in kale (58.88 mg/100g) then reported in literature. Bohn and Walczyk (2014) reported that spinach had 69.1 mg/100g chlorophyll content, and iceberg lettuce had 19 mg/100g of chlorophyll. Duma *et al.* (2014) found 18.8 mg/100g chlorophyll in lettuce and 150.3 mg/100g of chlorophyll in spinach.

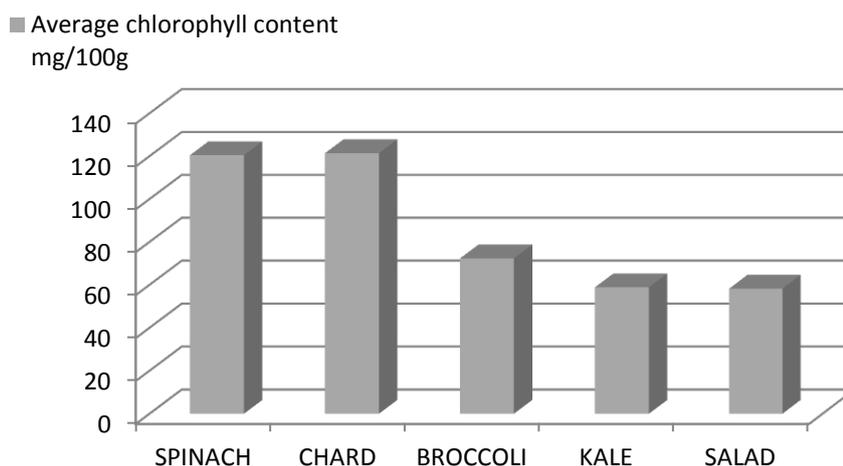


Fig. 1. Average chlorophyll content in fresh vegetables

The obtained experimental results are slightly lower than reported in literature. It is known that chlorophyll content varies between plants. Variation in leaf pigments can be related with internal factors and environmental conditions. Furthermore, sampling can be a factor that affects chlorophyll content because plant material consists of stems, stalks and leafy parts which differ significantly in chlorophyll content. Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts (Nature, 2013), so extraction plays a major role in chlorophyll content determination. Acetone is used because it gives very sharp chlorophyll absorption peaks (Ritchie, 2006), but it is volatile, flammable and narcotic in high concentration. Methanol as an extracting solvent, especially for algae and vascular plants (Porra, 2002) can be used because it is less volatile but it is extremely toxic. Sumanta *et al.* (2014) reported that diethyl ether (DEE) showed as best extractant solvent for chlorophyll *a* and chlorophyll *b* for most of sampled species. Temporal and seasonal changes, as well as geological conditions, can be a reason for variations in chlorophyll concentrations. Reason for lower chlorophyll content could be conditions in laboratory, because chlorophyll is light sensitive and samples must be kept in the dark. Transportation, storage, as well as mechanical damage of plant, could reflect on chlorophyll content. Also, samples were not fully mature and it could be the reason for slightly lower chlorophyll content than reported in literature. Duma *et al.* (2014) found total chlorophyll in leaves of young nettle sprouts to be 10 times higher comparing with lettuce, so consumption of not so traditionally used leafy vegetables should be more promoted and popularized and considered as a good further research topic.

## CONCLUSIONS

The contents of total chlorophyll are as following: chard > spinach > broccoli > kale > lettuce. Chard had 121.37 mg/100g chlorophyll content, and lettuce, as expected, had only 58.27 mg/100g. Significant differences in total chlorophyll content among five analysed vegetables were found. Leafy vegetables from our study basically represent good source of chlorophyll.

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## FATTY ACID COMPOSITION AND ANTIOXIDANTS IN COLD-PRESSED VEGETABLE OILS

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*Original scientific paper*

### Summary

The content of fatty acid and antioxidant were determined in oil of pumpkin, linseed and sunflower. The samples of oils were taken from the areas of Bosnia and Herzegovina. The oils were obtained by cold-pressing process applying screw press. The major fatty acid in pumpkin oil, linseed oil and sunflower oil was linoleic fatty acid, followed by oleic and palmitic fatty acid. Statistical analysis showed differences in fatty acid composition of all oil samples. Significantly higher content of linoleic fatty acid was found in sunflower oil (60.60%) as compared to pumpkin (47.73%) and linseed oil (15.74%). The highest content of oleic acid was found in pumpkin oil (33.72%), as compared to sunflower (27.04%) and linseed oil (22.64%). The linseed oil had a highest content of linolenic acid (50.60%) while it was present only in traces in the two other oils. The highest total phenolic content found in pumpkin oil (4.48 mg/100 g), as compared to sunflower (3.68 mg/100 g) and linseed oil (1.35 mg/100 g). Among the analysed oils, pumpkin oil had significantly higher content of chlorophyll (26.97 mg/100 g) and carotenoids (92.38 mg/ 100 g).

Key words: *vegetable oils, fatty acids, antioxidants*

### INTRODUCTION

Essential fatty acid cannot be synthesized in the body and therefore they have to be taken through a balanced diet. The quality and shelf-life, i.e.oxidative stability of cold-pressed oils depends, amongst several factors, on their fatty acid composition and tocopherol content, as well as other antioxidant properties. Many cold-pressed oils have been reported to contain antioxidants and to possess a remarkable radical scavenging activity and oxygen radical absorption capacity. The nature of antioxidants is not yet known, but due to the mode of preparation (low temperatures), these oils retain tocopherols, phenols, and carotenoids and they may have the potential for applications in the promotion of health and prevention against oxidation damages mediated by free radicals (Radočaj and Dimić, 2013).

According to the presence of fatty acids, pumpkin seed oil is very similar content sunflower oil, sesame and soybean oil can be used as a substitute for mentioned oil. They have a very low content of linolenic acid (0.2%), which contributes to the improved oil stability during its storage (Tsaknis *et al.*, 1997). High quality pumpkin seed oil obtained from pumpkin seeds is dark green in colour or of orange to red fluorescence. The green and red colour oils result from the presence of different tetrapyrrolic components such as protochlorophyll and proto-pheophytin and are located in the inner membrane of seeds tissue.

The aim of this paper was to investigate the composition of fatty acids, phenolic compounds, chlorophylls and carotenoids in the oil obtained from pumpkinseed, linseed and sunflower, which were grown in the territory of Bosnia and Herzegovina. Oils were obtained by cold-pressing on screw press.

### MATERIAL AND METHODS

#### Material

Three different cold-pressed oils, obtained from hull-less pumpkin seed linseed and sunflower seed, were analyzed. The samples of oils were taken from the areas of Bosnia and Herzegovina. For the cultivation of seeds, appropriate agro-ecology conditions for growing seeds were carried out. Oils were obtained by cold-pressing on screw press. Manual harvesting of pumpkin was performed in the month of August, when the oil content in the seeds of the full maturity stage was 40-50%. Time of harvest of sunflower seeds was early

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September and of linseed seeds early August. The harvesting was implemented mechanically, at stage of full technological maturity, when the oil content in sunflower seed was 40-58%, and linseed 35-47%. Upon receipt of raw materials, the seeds were cleaned, weighed (max. 1% of impurity), dried (6-8% of moisture in seeds), ground, heated at 20-25°C, pressed type of Kern Kraft, capacity of 40-50 kg/hour, in dark green glass bottles of 0.25 to 1000 ml, finished product (bottles, labelling, placing in carton and product). All samples of the oil in the original packaging, are kept shortly (up to several days) in the dark and cold room at a temperature of 5 to 8°C, until the time for doing analysis.

## Methods

The fatty acids composition in the investigated oil samples was determined by the standard method. The analysis was done on gas chromatogram GC/MS-6890 II, with use of instrument Hewlett-Packard with selective mass detector (MSD) 689 II: FKKT-UL. Capillary column: SP 2560, 100 m x 0,25 mmID, 0,20 µm; Detector: flame ionization FID, 260°C, separation 100.1; Gas carrier: helium, 20 cm<sup>3</sup>/sec. Temperature of the oven was 140°C/minute, cooling (DGF, C-VI 10a, 1985).

Total phenols content was measured by using spectrophotometer applying method that is based on colour reaction of phenols with Folin-Ciocalteu reagent. 1 ml of the sample was mixed with 15 ml of distilled water, 5 ml of F.C reagent and with 15 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution. After 2 hours of incubation, absorbance of all samples was measured at 765 nm, by using Shimadzu UV visible spectrophotometer UVmini-1240. Total phenol content was quantified by gallic acid calibration curve (Ough and Amerine, 1998).

Chlorophyll content was determined, in undiluted sample of oil by measuring absorbance at 670, 630 and 710 nm, by using spectrophotometer. The results were as the content of pheophytin a (Pokorny *et al.*, 1995).

Carotenoid content was determined, in 10% diluted sample of oil, by measuring absorbance at 445 nm, by using spectrophotometer. The results were as the content of β-carotene (BSM, 1977).

Statistical analysis and comparison of the results were done by t-test (p<0.05, significance level), by using Excel 2007.

## RESULTS AND DISCUSSION

Fatty acid composition in the investigated samples of oil are shown in Table 1. The content of linoleic fatty acid in the analyzed samples of sunflower oil (60.60%) compared with pumpkin oil (47.73%) and linseed oil (15.74%) was being significantly higher. Also, all samples had a high content of MUFA in oil. Pumpkin oil contains a significantly greater amount (33.72 %) of oleic acid compared to linseed (22.64%) and sunflower oil (27.04%).

Tab. 1. Fatty acid composition of cold pressed oils

Fatty acid (%)	Type of oil		
	Pumpkin oil	Linseed oil	Sunflower oil
C12:0	0.88±0.01 <sup>a</sup>	0.18±0.02 <sup>c</sup>	0.60±0.05 <sup>c</sup>
C14:0	0.38 ±0.08 <sup>a</sup>	0.14±0.01 <sup>c</sup>	0.28±0.01 <sup>b</sup>
C16:0	12.27±0.25 <sup>a</sup>	6.16 ±0.10 <sup>c</sup>	7.51±0.10 <sup>b</sup>
C18:0	5.11±0.10 <sup>a</sup>	4.83 ±0.05 <sup>c</sup>	3.91±0.09 <sup>b</sup>
C18:1	33.72±0.07 <sup>b</sup>	22.64 ±0.06 <sup>a</sup>	27.04±0.03 <sup>c</sup>
C18:2	47.73±0.06 <sup>b</sup>	15.74 ±0.16 <sup>c</sup>	60.60±0.10 <sup>a</sup>
C18:3	trace	50.60±0.04 <sup>c</sup>	trace
∑ MUFA	33.72 <sup>b</sup>	22.64 <sup>a</sup>	27.04 <sup>c</sup>
∑ PUFA	47.73 <sup>b</sup>	66.34 <sup>c</sup>	60.60 <sup>a</sup>
∑ SAFA	18.64 <sup>a</sup>	11.31 <sup>c</sup>	13.3 <sup>b</sup>

Different lowercase letters in columns indicate statistically differences between the samples (p<0.05).

Pumpkin oil, has a high content of linoleic (47.73%) and oleic acid (33.72%), and a lower contents of palmitic and stearic acid 12.27% and 5.11% and less than 1% lauric and myristic acid. Linolenic acid was in trace. The highest content of MUFA was found in pumpkin oil (33.72%), sunflower (27.04%) and linseed (22.64%). On the other hand, the highest content of PUFA was detected in linseed (66.34%), sunflower (60.60%) and pumpkin (47.73%) oils. Pumpkin oil had the highest SAFA content (18.64%), followed by sunflower (13.3%) and linseed (11.31%) oil. Compared with other authors (Tsaknis *et al.*, 1997), given total content of

MUFA/PUFA oil pumpkin 81.0%. The dominant were linoleic acid (42.10%), and oleic acid (38.10%). The total content of SAFA was 16.0%. The content of palmitic acid was 12.70% and 6.0% of stearic acid. Other fatty acids were present less than 1.0%. Results by Rafalowski *et al.* (2008), were also similar. They state that pumpkin seed oil contains of linoleic acid from 39 to 42%, oleic acid from 36 to 37%, and a very low of linolenic acid of 0.3%. For pumpkin seed oil Ardabili *et al.* (2011) reported values for linoleic acid of 39.84%, followed by oleic and palmitic of 38.42% and 10.68% respectively. In contrast to the above, Gülay Kirbaslar *et al.* (2012) state results for pumpkin oil with high content of linoleic acid (70.17%), but low content of oleic acid (17.62%), while the content of palmitic acid was consistent with the results of the authors mentioned above (5.62%). The results for the linoleic and oleic acid were higher compared to the results of our research. According to the results by Gülay Kirbaslar *et al.* (2012) content of linoleic acid is high (70.17%) and low content of oleic acid (17.62%), while the content of palmitic acid is in accordance with the above mentioned results (5.62%).

Linseed oil, has a high content of linolenic (50.60%), and oleic acid (22.64%), and a lower contents of linoleic (15.74%), palmitic and stearic acid 6.16% and 4.83% and less than 1% lauric and myristic acid. Linseed oil contains SAFA 11.01% and MUFA/PUFA 88.97% (Popa *et al.*, 2012). According to El-Beltagi *et al.* (2007) in linseed oil linolenic dominates acid as of 30.48%, followed by 22.0% of oleic and 18.3% of linoleic. Among other acids, the present are palmitic (7.1%) and stearic (3.7%). Results by Rusníková *et al.* (2013) were similar for linseed oil with the content of linolenic acid of 38.0%, 27.06% of linoleic and oleic acid of 15.06%. Higher is value of linolenic acid (58.11%), and lower content of linoleic (15.02%) (Hamed and Abo-Elwafa, 2012). According to the results by Radočaj and Dimić (2013), the value was slightly higher and was 53.49%.

Sunflower oil, had a high content of linoleic (60.60%) and oleic acid (27.04%), and a lower contents of palmitic and stearic acid 7.51% and 3.91% and less than 1% lauric and myristic acid. Linolenic acid was in trace. For sunflower oil, Chowdhury *et al.* (2007) give values for oleic acid 45.39%, 46.02% linoleic, palmitic 6.52% and stearic 1.98%. According to Rusníková *et al.* (2013) the linoleic acid in sunflower oil has 50.46%, 28.62% oleic and palmitic 4.78%. Similar results were given by Gülay Kirbaslar *et al.* (2012) for sunflower oil. Content of linoleic was 48.58%, 32.69% oleic and palmitic 11.83%. Sunflower oil had lower value of linoleic acid from 9.34 to 13.52% and palmitic acid from 4.19 to 6.04% (Khoufi *et al.*, 2014). According to Onemli (2012) higher daily temperatures and lower fluctuations in temperature during seed growing contribute to the increase in oleic acid sunflower oil, and have a positive impact on the quality of oil. Turhan *et al.* (2010) mention a significant effect of agro-ecological conditions for sunflowers growing sunflowers on the composition (MUFA/PUFA, SAFA) in oil.

Total phenols, chlorophyll and carotenoids' contents, including t-test results, of different type of oil samples are shown in Table 2.

Tab. 2. Average content of antioxidants in cold-pressed oils

Component	Type of oil		
	Pumpkin	Linseed	Sunflower
<b>Total phenols</b> (mg/kg) or 100 g	4.48 ± 0.85 <sup>a</sup>	1.35 ± 0.09 <sup>c</sup>	3.68 ± 0.90 <sup>b</sup>
<b>Chlorophyll</b> (pheophytin a mg/kg)	26.97 ± 0.18 <sup>a</sup>	1.42 ± 0.55 <sup>c</sup>	3.24 ± 0.75 <sup>b</sup>
<b>Carotenoids</b> (β-carotenoid mg/kg)	92.38 ± 0.69 <sup>a</sup>	12.25 ± 0.28 <sup>b</sup>	1.26 ± 0.13 <sup>c</sup>

Different lowercase letters in columns indicate statistically significant differences in phenolic, chlorophyll and carotenoids between the samples ( $p < 0.05$ ).

Statistical analysis (t-test) showed that the total phenol content, chlorophyll and carotenoids significantly higher in pumpkin oil compared to linseed or sunflower oil.

The research results (Tab.2) show that pumpkin oil (4.48 mg/100 g) contains a significantly greater amount of total phenols in relation to the sunflower (3.68 mg /100 g) and linseed oil (1.35 mg/100 g). The values obtained are in accordance with the results by Seeger *et al.* (2007), who state total phenol content in pumpkin oil of 2.46 mg/100 g, linseed oil of 1.14 mg/100 g and sunflower oil of 1.20 mg/100 g. Pumpkin oil due to its high content of total phenols has a high antioxidant activity. The results of our research for pumpkin seed oil are in accordance with the results of other authors. Total phenol content in virgin pumpkin oil was 0.79 to 2.36 mg/100 g of oil, and cold-pressed at 12.32 to 0.79 mg/100 g of oil (Vujasinović, 2011), while by Ardabili *et al.* (2011) value was 6.62 mg/100 g of oil. The content of total phenols linseed oil is 38.46 mg/100g (Hamed and Abo-Elwafa, 2012). Kostadinović-Veličkowska and Mitrev (2013) reported a much higher total phenol content in unrefined sunflower (19.28 mg/100 g of oil), pumpkin (6.84 mg/100 g of oil), and linseed oil

(2.85 mg/100 g of oil). Rafalowski *et al.* (2008) reported results for cold-pressed oil of pumpkin and linseed for the content of carotenoids 15.01 mg/100 g of oil. In sunflower oil carotenoids were not present. According to the same author, content of carotenoids in pumpkin oil obtained from pumpkin seed „golica“ ranged from 17.0 to 56.5 mg/kg of oil, and chlorophyll content from 1.47 to 3.80 mg/kg of oil.

## CONCLUSIONS

All the analyzed samples of oils had been taken from the same location of Bosnia and Herzegovina had the appropriate content of the examined parameters which ranged in the comparable values. All the samples of oils had a high content of oleic acid which is the most desirable in terms of the nutritive value and provides certain oxidative stability to oils. In linseed oil, the content of oleic acid was considerably higher in comparison to the one in the pumpkin and sunflower oils. The linseed oil had quite a high content of linolenic acid while it was present only in traces in the other two oils. In the sunflower oil, the content of linoleic acid was significantly higher compared to the pumpkin and linseed oils. Although the linoleic acid is an essential fatty acid, it affects the oxidative instability of oils considerably. The content of palmitic acid did not significantly differ in pumpkin and linseed oil. The content of total phenols, chlorophyll and carotenoid was significantly higher in the pumpkin oil compared to the linseed oil and sunflower oil. The content of these components primarily depends on the type of oilseeds.

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## EFFECTS OF USING SOYBEAN OIL AND BEEF TALLOW IN DIET ON THE PROXIMATE COMPOSITION AND CONTENT OF CHOLESTEROL IN FAT TISSUE AND BROILER MEAT

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*Scientific paper*

### Summary

Two groups of broiler chicken Cobb 500 were taken for the experiment. Each group had 60 broiler chickens in total. All broilers were fed a diet rich in beef tallow or soybean oil. The percentage of fat in the diets for broilers was 3% per treatment. After the 12 hours starvation period, the chickens were slaughtered, and the carcasses were cooled at 4°C for 24 hours. Afterwards, they were stored at -18°C until analysis. 60 fat tissue, and dark and white meat samples of the slaughtered broilers were analysed. Total cholesterol content in the fat tissues was measured photo-metrically, and the extraction of fat tissue with the method of Folch.

The results gave insight into the cholesterol content in the fat tissue and broiler meat, after using the mentioned oil and fat. The average values of the total cholesterol content in the fat tissue sample in broilers which were fed a diet containing soybean oil was 64.25mg/100g, and the tissue samples of the broilers fed by a diet containing beef tallow was 87.89 mg/100g. Total cholesterol content in the white meat of broilers fed a diet containing soybean oil was 68.61 mg/100g, and in the broilers which were fed a diet containing beef tallow was 91.67 mg/100g. Total cholesterol content in the dark meat samples of broilers fed by soybean oil was 99.86 mg/100g, and of broilers fed by beef tallow was 119.21 mg/100g.

Student's t-test method determined that there was a very high statistical significance between the treatments ( $p < 0.001$ ) in the total cholesterol content of fat tissue, and the dark and white broiler meat. White meat shows lower cholesterol content levels compared to the content determined in the dark muscle meat.

**Key words:** *beef tallow, broiler meat, fat tissue, soybean oil, chemical composition, cholesterol*

### INTRODUCTION

Using the appropriate types of fat and with the adequate way of adding fat to the broiler diet, the appropriate nutritional components in fats are achieved, which has a direct impact on the quality of fat tissue and broiler meat. Intake of PUFA-rich oils rich can lead to more fatty deposits because more energy for storage is available in the body in the form of triglyceride. There are some studies that show that using polyunsaturated fats causes lower abdominal fat deposition in broilers (Crespo and Esteve-Garcia, 2001).

Cholesterol is present in muscles, as well as fat tissue, because it is important component of the cell membrane and it can be stored as cholesterol ester in lipid droplets (Dessi and Batetta 2003). Cholesterol accumulates in two compartments in a fat cell: in the cellular membrane as functional cholesterol, and in lipid droplets, serving as a reservoir for cholesterol with still an undefined function (Le Lay, 2004). Compared to the fat cells where the most of cholesterol is stored in the form of a droplet, and a smaller amount is found in the cellular membrane as the functional cholesterol, the majority of cholesterol in fibre cells is the main component of the membrane (Hoelscher *et al.*, 1988). The main structural difference of dark and white muscle is the larger quantity of membrane structures, mainly in sarcoplasmic reticuli rich in cholesterol in the dark muscle (Konjufca *et al.* 1997). Furthermore, the total cholesterol content in the dark muscle is larger than in the white one because the total lipid content is higher (Komprda *et al.*, 2003).

### MATERIALS AND METHODS

Experimental research was undertaken on the "Koka-Sana" commercial broiler chicken farm in Sanski Most. Samples of two broiler groups were analysed. The first broiler group of Cobb 500 hybrids (60 broilers) were fed with mixtures containing soybean oil. The second group, also Cobb 500 hybrids (60 broilers) were fed with the identical mixture, but in these beef tallow was added. During the first sixteen days, broilers were fed with a starter mixture, and from the 17th day they were fed by broiler finisher mixtures. Mixtures of food procured by the previously agreed

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recipes of agricultural producers "Mješaona and farm M" from Tešanj, Bosnia and Herzegovina. Starter and finisher mixtures are and formulated the recommendations for broilers, NRC - National Research Council, Nutrient Requirements of Poultry, 9th ed. Washington, DC: National Academy Press; (1994).

Connective tissue, kidney fat tissue, and dark and white broiler meat was taken for the analysis. From 120 Cobb 500 reared broiler hybrids, 60 (from each group at 30) fat tissue and meat samples of slaughtered broilers were analysed. Samples were taken randomly. After processing the bodies' adipose tissue was separately melted in the usual way (heating in the pot) in appropriate laboratory conditions. The samples were analysed in the laboratory of Biotechnical Faculty. Determination of water content in the analyzed samples is done reference to EN ISO 662: 2000 method, the determination of fat content ISO 1443: 1999 method, and protein content (ISO 937: 1999). The test results (mean, variation measures and analysis of variance) were statistically analyzed using the PC software package Microsoft Excel 2003.

Total cholesterol content in the fat tissues was measured spectrophotometry (Huang *et al.*, 1961). The extraction of fat tissue was done with the method of Folch (Folch, J. *et al.*, 1957). PC software package Microsoft Excel 2003 was used for statistical analysis of data obtained on the basis of average values, standard deviation and test the significance of differences.

## RESULTS AND DISCUSSION

Tab. 1. Average chemical analysis values of the fat tissue and broiler meat per treatment

Analysed parameters	Samples					
	MTGL	MTSU	CMGL	CMSU	BMGL	BMSU
<b>Water (%)</b>						
$\bar{X}$	20.34	20.84	75.69	73.54	74.36	74.81
SD	0.929	0.973	1.045	0.974	1.144	1.036
Sx	0.170	0.179	0.191	0.178	0.209	0.189
X min	18.16	19.31	74.13	72.18	72.39	72.99
X max	21.77	22.51	77.99	75.60	76.28	76.97
<b>Statistically significant differences (T- test)</b>	p = 2.033**		p = 8.304****		p = 1.594*	
<b>Fat (%)</b>						
$\bar{X}$	79.28	80.09	3.14	3.66	2.11	2.55
SD	0.43	1.65	0.63	0.68	0.27	0.23
Sx	0.07	0.30	0.11	0.12	0.05	0.04
X min	78.14	76.43	2.19	2.76	1.65	2.15
X max	80.28	84.21	4.43	4.67	2.91	2.97
<b>Statistically significant differences (T- test)</b>	p = - 2.495**		p = - 2.721***		p = - 6.591****	
<b>Protein (%)</b>						
$\bar{X}$	5.52	6.24	20.34	20.84	23.62	26.00
SD	0.50	0.12	0.92	0.97	0.66	0.72
Sx	0.09	0.02	0.17	0.17	0.12	0.13
X min	4.47	5.72	18.16	19.24	22.14	24.56
X max	6.63	7.02	21.77	22.51	24.73	27.03
<b>Statistically significant differences (T- test)</b>	p = -6.685****		p = -2.024**		p = -13.239****	
<b>Energy valuekJ</b>						
$\bar{X}$	3027	3069	461.96	489.70	479.61	536.35

n.- number of samples analysed,\*There is no statistical significance ( $p > 0.05$ ),\*\* There is a statistical significance ...( $p < 0.05$ ),\*\*\* There is a high statistical significance ( $p < 0.01$ ),\*\*\*\*There is a very high statistical significance ( $p < 0.001$ ), MTGL (broiler's fat tissue containing beef tallow), MTSU (broiler's fat tissue containing soybean oil), CMGL (dark broiler meat, diet containing beef tallow),

*CMSU (broiler's dark meat, diet containing soybean oil), BMGL (broiler's white meat, diet containing beef tallow), BMSU (broiler's white meat, diet containing soybean oil)*

A statistical significance ( $p < 0.05$ ) was determined in the water content of fat tissue samples. Testing the effect of fed diets containing soybean oil and beef tallow on fattening broiler chickens (Wongsuthavas *et al.*, 2007), indicate the value of water in fat chickens whose feed added soybean oil in the amount of 4.5%, and for broilers whose feed added beef tallow added 4.9%, which is considerably less than the value of our results. Water content in the dark muscle meat showed a high statistical significance between treatment ( $p < 0.001$ ), and it was determined that there was no statistical significance ( $p > 0.05$ ) in the white meat.

The fat content in the tissue showed a statistical significance between the treatments ( $p < 0.05$ ). Crespo and Esteve-Garcia (2001) added 10% of fat (beef tallow, olive oil, sunflower oil and flaxseed oil) to the fed diet of female broilers between the 21st and the 49th fattening period. Fat tissue lipid content of hybrids fed by diets containing beef tallow was 88.3%, olive oil was 88.5%, sunflower oil 87.4% and flaxseed oil 88.1%. According to our research, higher fat content was determined in fat tissue samples of broilers fed by diets containing soybean oil than those broilers fed by diets containing beef tallow. There was a high statistical significance in the fat content of broilers' dark muscle meat between treatments ( $p < 0.01$ ). Fat content in white muscle meat showed a very high statistical significance ( $p < 0.001$ ). Crespo and Esteve-Garcia (2001) found that the fat content in dark meat was 2.06% in broilers whose diets contained beef tallow, while the fat content in white meat was 1.40%.

Crude protein content in fat tissue and white meat showed a very high statistical significance between treatments ( $p < 0.001$ ). Crude protein content of dark muscle meat showed a statistical significance between treatments ( $p < 0.05$ ). Crespo and Esteve-Garcia (2001) determined that the protein content in dark and white meat of hybrids fed on diets which contained beef tallow was 21.9% and 24.4%, in olive oil was 21.5% and 24.4%; in diets containing sunflower oil was 21.5% and 24.4%, and flaxseed oil 21.4% and 24.3%. The MTSU sample had the highest energy value of 3069, 41 k, followed by MTGL whose value was 3027, 20 kJ. Dark and white meat samples of broilers whose diets contained soybean oil had a bit higher energy value than the samples of dark and white meat of broilers fed by diets containing beef tallow. Slight increase of energy value was due to higher protein and fat content.

Tab. 2. Parameters of total cholesterol content in fat tissue, and dark and white meat of broilers (mg/100g) per treatments analysed

Samples (n= 60)	$\bar{x}$	SD	Sx	Xmin	Xmax	Statistically significant differences (T- test)
MTGL	87.89	8.77	1.60	72.00	104.32	p = 9.421****
MTSU	64.25	10.55	1.92	41.18	83.03	
CMGL	119.21	14.26	2.60	85.46	148.65	p = 6.247****
CMSU	99.86	9.21	1.68	81.27	117.94	
BMGL	91.67	14.51	2.65	69.24	124.66	p = 8.029****
BMSU	68.61	6.16	1.12	59.34	87.35	

According to the results shown in Table 2, it's clearly evident that there was a higher cholesterol content of 87.89 mg/100 g in the MTGL fat tissue samples than in the MTSU samples, which was 64.25 mg/100 g. Beer Ljubić *et al.* (2006) analysed the total cholesterol content in fat tissue of chicken fed by a standard diet (control group) and chicken fed by the same diet containing Sel-Plex organic selenium before and after the starvation period. Total cholesterol content in the control group before starvation period was 244.32 mmol/g of tissue, and after the starvation period 98.73 mmol/g of tissue. In the group whose diet contained organic selenium, total cholesterol content was 118.9 mmol/g in fat tissue, and after starvation it was 112.17 mmol/g. Based on the research done by the same author, the cholesterol concentration in fat tissue of broilers after the 48h starvation period was lower. CMGL samples showed that total cholesterol content was 119.21 mg/100 g, and in the CMSU sample 99.86 mg/100 g. Total cholesterol content in the white meat was higher in the BMGL samples (91.67 mg/100 g), and lower in the BMSU samples, which was 68.61 mg/100 g. A very high statistical significance was determined between the analysed treatments ( $p < 0.001$ ).

Based on the research by Crespo and Esteve-Garcia (2001), total cholesterol content in the dark and white meat, determined that the dark meat had the following values: 81.8 mg – beef tallow, 83.8 mg – olive oil, 77.0 mg – sunflower oil and 76.6 mg – flaxseed oil per 100 g of meat. Total cholesterol content in the white meat

was as follows: 64.7 mg –beef tallow, 61.3 mg - olive oil, 59.8 mg - sunflower oil and 58.8 mg – flaxseed oil per 100 g.

According to the research done by Tangtaweewipat *et al.* (2004), who also added soybean, flaxseed and palm oil with organic minerals (125 and 250 ppm Cu; 200 and 400 ppb Cr) to the broiler diets, cholesterol content was significantly lower in the group fed by palm oil supplemented by 250 ppm Cu, which was 44.1 mg/100g in white meat, and 60.2 mg/100g in the dark meat. Broilers fed with the diet containing soybean oil had 51.1 mg/100g of total cholesterol content in white meat and 71.9 mg/100g in dark meat. These values were significantly lower compared to our results. Broilers fed with the diet containing 2% and 4% of beef tallow had 90.28 mg and 93.14 mg/100g of total cholesterol content in white meat, and 112.67 mg and 117.41 mg/100g in dark meat. Broilers fed with the diet containing 1% of sunflower oil and 1% beef tallow had 112.67 mg and 117.41 mg/100g of total cholesterol content in white meat, and total cholesterol content in dark meat was 95.18 mg/100 g. Using the combination of 2% sunflower oil and 2% beef tallow, the total cholesterol content in white meat was 74.97 mg/100 g, and 94.07 mg/100g in dark meat (Tangtaweewipat *et al.*, 2004).

Duraisamy *et al.* (2013) obtained the data of average cholesterol levels in the control group, which were as follows: 77.33 mg/100g for white meat and 95.43 mg/100g for dark meat.

Adding 2% and 4% of sunflower oil to the broilers' diet, total cholesterol content was 72.80 mg and 69.43 mg /100g in white meat, and 92.45 mg and 90.00 mg /100g in dark meat. Ševčíková *et al.* (2003) analysed the total cholesterol content in white meat in broilers fed with a diet supplemented by copper in the form of Cu sulphate and Bioplex Cu. The average cholesterol content in the control group was 656 mg/kg; in the group where 0.14 g CuSO<sub>4</sub>·5H<sub>2</sub>O/kg was added, cholesterol level was 592 mg/kg, and the cholesterol level where 1.75 g Cu- glycine/kg was added had 493 mg/kg. Adding copper to the diets reduced the cholesterol content up to 24.9%; copper in the form of Bioplex Cu was more efficient than Cu sulphate. Stanačev *et al.* (2011) analysed the amount of cholesterol in the muscle tissue and broiler skin. Four groups were formed: one control group and three experimental groups (the second one's diet was supplemented by 2% garlic, the third one with 2% garlic and 100 ppm copper, and the fourth one with 100 ppm of copper). They concluded that additives significantly reduced cholesterol content in the broilers' tissue. Average cholesterol content levels in red meat per groups were: the first one had 73.24; the second one 12.06; the third one 50.65 and the fourth one had 62.91 mg/100g. White meat per groups: 60.88; 46.17; 41.83 and 52.09 mg/100g, with the average cholesterol content of 384.35; 285.77; 138.76 and 105.59 mg/100g in the broilers' skin per group.

## CONCLUSION

Using the treatment it was determined that there was a high statistical significance ( $p < 0.001$ ) in the water content in the dark meat samples; a statistical ( $p < 0.05$ ) significance in fat tissue samples and it was affirmed that there was no statistical significance between the tested treatments ( $p > 0.05$ ) in the broiler's white meat.

There was a significant effect of using the mentioned treatments on the fat content in white meat. The fat content in the fat tissue showed a statistical significance between the treatments ( $p < 0.05$ ), a high statistical significance ( $p < 0.01$ ) in dark meat, and a very high statistical significance ( $p < 0.001$ ) in the white meat. Higher fat content was determined in the broiler samples fed by the diet containing soybean oil compared to the diets containing beef tallow.

Crude proteins content in the fat tissue and white meat showed a very high statistical significance between the treatments ( $p < 0.001$ ), especially in the white broiler meat which indicates that adding soybean oil in the broiler diets we get foodstuffs with high nutritional value. Comparing the energy value of fat tissue, and dark and white muscle meat of the broilers, we can conclude that the samples whose diets contained soybean oil had a higher energy value than the dark and white muscle meat samples whose food contained beef tallow. The energy value was higher because of the higher protein content and fat in the samples whose diets contained soybean oil.

A very high statistical significance was determined between the treatments ( $p < 0.001$ ) in the total cholesterol content in fat tissue, and dark and white broiler meat. Moreover, the white meat showed lower cholesterol levels than the dark meat. Adding beef tallow to the diets caused higher cholesterol levels than adding soybean oil.

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## PHENOLIC COMPOSITION OF DIFFERENT FRUIT TISSUES OF FIVE AUTOCHTHONOUS APPLE CULTIVARS IN BOSNIA AND HERZEGOVINA

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*Original scientific paper*

### Summary

Apple consumption is related to the prevention of chronic diseases and improved health due to the presence of phytochemicals, especially phenolic compounds. The compositional differences among apple cultivars and fruit tissues can be very significant in respect to the concentration of polyphenolic compounds. In this work, the phenolic composition of the flesh and peel of five autochthonous apple cultivars: 'Bobovec', 'Đulabija', 'Habikuša crvena', 'Samoniklica', and 'Sarija', and two commercial cultivars: 'Idared' and 'Golden Delicious' was investigated using high-performance liquid chromatography (HPLC) coupled with a photodiode array (PDA) detector. All apples originated from the orchard "Srebrenik" (North East Bosnia) and were harvested in 2013. The studied apples were found to contain 14 individual phenolic compound, which belong to the four dominant polyphenolic classes. The eight polyphenolic compounds from peel and flesh of the autochthonous cultivars of apples were quantified and found to contain epicatechin and procyanidin B2 as the most abundant phenolic compounds in the peel, and chlorogenic acid as the most abundant phenolic compound in the flesh. In a comparison of fruit tissue within each cultivar, apple peel had a significantly higher phenolic content than a flesh. In general, it should be stated that according to the amount of polyphenols some autochthonous cultivars do not lag behind the current commercial cultivars.

**Key words:** *autochthonous, apple cultivars, phenolic compounds*

### INTRODUCTION

Apple (*Malus domestica* L.) is one of the most abundant fruit species in fruit production. Epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes. The high number of studies in the field of human nutritionism attribute positive effect of apple to human health to apple's antioxidant characteristics, which are directly correlated with polyphenolic compounds. Polyphenolic compounds are naturally occurring in plant secondary metabolites which determine quality parameters of fruits such as appearance, flavor and health-promoting properties. The main classes of polyphenols in apple are flavonoids, including quercetin, (-)-epicatechin and (+)-catechin, procyanidins, and anthocyanidins; dihydrochalcones such as phloretin and phloridzin; and other polyphenolic compounds such as chlorogenic acid. Many studies show that the concentration of phenolic compounds in apple differ with cultivar, maturity stage, environmental conditions and the part of the fruit (Veberic *et al.*, 2005; Shahidi & Naczki, 2006; Fratianni *et al.*, 2007; Vieira *et al.*, 2009; Duda-Chodak, 2010; Mainla *et al.*, 2011; Schovnakova & Opatova, 2011). Several researches have determined that the some less widespread local apple cultivars contained a higher levels of polyphenolic compounds compared with commercial cultivars (Iacopini *et al.*, 2010; Minnoci *et al.*, 2010; Panzella *et al.*, 2013; Giomaro *et al.*, 2014; Faramarzi *et al.*, 2014). These results suggest the relevance of valorisation of traditional apple in order to increase their productivity and competition in the market. In this work, the peel and flesh of 5 autochthonous apple cultivars from the territory of Bosnia and Herzegovina were screened for their polyphenolic content in comparison with commercial 'Idared' and 'Golden Delicious' cultivars.

### MATERIALS AND METHODS

#### Plant material

Apples were harvested at technological stage of maturity for cultivars from Srebrenik Orchards (Srebrenik Orchards, Tuzla, B&H). In total, 7 cultivars were analyzed: 5 autochthonous and two, commercial cultivars, 'Idared' and 'Golden Delicious'.

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The fruits used in the evaluations were picked randomly and then, placed in polyethylene bags were transported to the laboratory. Experiment comprised 6 apples of each cultivar by trial. All fruit were stored at  $-80^{\circ}\text{C}$  before they were purchased for analyses. The fruit tissues prepared from the apples were: peel (portion of apple fruit without flesh, core and seeds) and flesh (portion of apple fruit without peel, core and seeds).

Peel and flesh were obtained from 6 apples of each cultivars. The fruit was carefully peeled with a ceramic peeler to obtain the peel without pulp, and this was cut into small pieces with ceramic knife. The pulp was obtained from apple without a peel, excluding the core and the seed, and this was cut into small pieces.

The peel and flesh were immediately frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . The frozen samples were lyophilized, using a freeze dryer and ground to a fine powder using mortar. The lyophilized samples then vacuum packed and stored in the dark at  $-20^{\circ}\text{C}$  until required for analysis.

#### Standards and reagents

Solvents: HPLC grade methanol (Chromasolv), acetic acid, p.a. and HPLC grade phosphoric acid (49-51%), and standards: (+)-catechin, (-)-epicatechin, procyanidin B1, quercetin-3-D-galactoside, quercetin-3-glucoside, procyanidin B2, chlorogenic acid and p-coumaric acid were purchased from Sigma–Aldrich Company. Standard phloridzin dihydrate was purchased from Fluka.

#### Chemical analysis

0.5g of freeze-dried apple tissue submitted to direct solvent extraction with with 30 ml of methanol-water-acetic acid (30:69:1, v/v/v) containing 2g of ascorbic acid in an ultrasonic bath during a 10 min. Extractions were performed in three different replicates of each fruit homogenate.

High performance liquid chromatography (HPLC) analyses performed on a Thermo liquid chromatograph combined with PDA detector (Thermo Finnigan PDA detector). The chromatographic separation was performed on a Pursuit XRs 3 C18 (150 x 4.6 mm x 3  $\mu\text{m}$ ) column, at a flow rate of 0.8 mL/min. For separation used mobile phase composed of an aqueous solution of phosphoric acid concentration of 0.01 M (mobile phase A) and methanol (mobile phase B) with a gradient elution as follows: 18% B, 0–25 min; from 18% to 30% B, 25–30 min; 30% B, 30–55 min; from 30% to 45% B, 55–70 min.

Identification of polyphenolic compounds, whose standards are available, was performed by HPLC–DAD by comparison of their spectra and retention times in the samples with those of authentic reference compounds and data reported in the literature. Also, samples were spiked with standard solution for confirmation. In cases where the polyphenolic compounds were commercially unavailable, peaks were ascertained based on the spectrum and elution order, and on data in the literature on the polyphenol forms to be expected in the species analyzed. The purity of peaks was checked by a Diode Array Detector coupled to the HPLC system, comparing the UV spectra of each peak with those of authentic references samples. Quantification of individual constituents was performed using a regression curve, each point in triplicate. Measurements were performed at 280 nm for flavanols and dihydrochalcones, at 320 nm for the chlorogenic acid, at 370 nm for flavonols.

Quantitation was performed by introducing the measured integration areas in the calibration equation of standards more similar to polyphenol quantified. Thus, flavan-3-oles were quantified as (+)-catechin; dihydrochalcones as phloridzin; flavanones as quercitrine; chlorogenic acid with itself.

#### Analysis of data

The results of the tree replicates were pooled and expressed as mean $\pm$ standard deviation. Principal component analysis (PCA) were performed using XLSTAT.

## RESULTS AND DISCUSSION

Using the RP-HPLC method, in the peel and the flesh of the autochthonous varieties of apples were identified eight polyphenolic compounds: (+)-catechin, (-)-epicatechin and procyanidin B2 (flavan-3-ols), chlorogenic acid (hydroxycinnamic acid) and quercetin-3-O-rhamnoside (quercitrin), quercetin-3-O-galactoside (hyperoside) and quercetin-3-O-rutinoside (rutin) from the flavonol class were identified in an apple peel. Four polyphenolic compounds were found to belong to a certain class. Three peaks in chromatograms of apple peel extracts exhibited spectral characteristics identical to those of flavonoles. Their UV spectra exhibited two major absorption peaks in the regions of 355 nm (Band I) and 255–266 nm (Band II). This compounds have been identified as flavonol derivates. The spectrum one compound of apple peel and flesh extracts indicated that the aglycone was phloretin, and it was identified as a phloretin derivate. Its UV spectrum exhibited only one

maximum at 284 nm. The presence of said four polyphenolic compounds in apple peels and pulp has been previously confirmed in other apple varieties.

Findings of other authors confirm the presence of some other polyphenols from the hydroxycinnamic acid class (Šavikin *et al.*, 2014). The hydroxycinnamic acid commonly in nature are present in glycosylated form esters, and, as it is not done hydrolysis extracts in this experimental work, it is impossible to argue that other polyphenols from these classes were not present in extracts of peel and flesh of autochthonous and commercial cultivars of apples.

Informations about the content of individual polyphenolic compounds in apple peel and flesh are given in Table 1 and 2.

Tab. 1. The content of polyphenolic compounds (mg/100 g FW) in the peel of different apple cultivars grown in 2013 at Srebrenik Orchards, Tuzla, B&H

Cultivars	CAT	PB2	EPC	CHLA	KVI	KVII	KVIII	RUT	HYP	QUE	FLO	FLD	TPI
'Bobovec'	1.99	19.04	22.83	14.87	2.27	3.98	9.39	0.86	14.3	4.27	6.22	7.18	107.2
'Đulabija'	2.90	15.08	19.57	17.79	1.39	0.83	2.08	0.24	2.13	1.46	1.90	2.91	68.28
'Habikuša crvena'	7.08	12.12	23.24	17.39	3.87	1.25	0.04	0.74	0.01	3.13	2.40	0.94	72.21
'Samoniklica'	5.57	9.46	14.99	7.85	0.65	0.56	2.16	0.06	1.12	1.11	7.52	7.07	58.12
'Sarija'	2.38	8.79	11.78	10.06	0.57	0.66	1.04	0.07	0.50	0.34	1.39	1.79	39.37
'Idared'	1.30	5.02	6.59	10.23	0.43	1.39	4.03	0.15	4.50	1.71	0.34	3.33	39.02
'Golden Delicious'	1.82	10.62	12.13	10.34	1.33	2.10	5.00	0.14	5.30	2.61	2.11	5.66	59.16

Abbreviations: CAT-catechin; PB2-procyanidin B2; EPC-epicatechin; CHLA-chlorogenic acid; KVI- flavonol derivate I; KVII- flavonol derivate II; KVIII- flavonol derivate III; RUT-rutin; HYP-hyperosid; FLO- phloretin; FLD-phloridzin;

Tab. 2. The content of polyphenolic compounds (mg/100 g FW) in the flesh of different apple cultivars grown in 2013 at Srebrenik Orchards, Tuzla, B&H

Cultivars	CAT	PB2	EPC	CHLA	QUE	FLO	FLD	TPI
'Bobovec'	1.30	6.23	7.36	18.48	2.40	3.56	1.45	40.78
'Đulabija'	0.37	1.79	2.15	6.07	1.17	0.29	0.44	12.28
'Habikuša crvena'	2.39	2.91	5.83	27.52	5.84	0.94	0.89	46.32
'Samoniklica'	2.43	3.01	5.18	11.86	2.02	2.10	1.29	27.89
'Sarija'	0.72	1.31	2.42	6.69	0.20	0.48	0.47	12.29
'Idared'	0.17	0.39	0.63	10.03	0.04	0.1	0.89	12.25
'Golden Delicious'	0.47	5.12	5.30	11.51	1.04	1.08	2.31	26.83

Significant differences in single polyphenol distribution were apparent. According to the declining representation in the peel of the autochthonous apples, the following were identified: epicatechin > chlorogenic acid > procyanidin B2 > phloridzin  $\approx$  phloretin  $\approx$  catechin  $\approx$  hyperin > other derivative flavonols. The following polyphenolic compounds were identified in the flesh of the autochthonous apples, sorted according to the decreasing representation: chlorogenic acid > epicatechin > procyanidin B2 > catechin > quercitrin > phloridzin > phloretin > procyanidin. We have confirmed the literary finds that polyphenols are more represented in the peel than in the flesh of apples.

Total polyphenol index (TPI) was calculated from the sum of the concentration of individual compounds. Apple peel fraction showed a higher significant TPI than pulp. Similar phenomena were observed by Vieira *et al.* (2009) in their research. Except for chlorogenic acid content, there was a higher content of individual polyphenolic compounds in the apple peel, compared with the phenolics in the apple flesh. This unequivocally confirms that the polyphenolic compounds in high concentrations accumulate in the epidermal tissues with respect to their protective role in the fruit (Winkel-Shirley, 2002; Lattanzio *et al.*, 2006). Differences in the biosynthesis of individual polyphenolic compounds in the peel and flesh of apples explained by cellular and subcellular compartment of responsible enzymes, as an important factor that affects the biosynthesis pathway that are dominating and which end-products occur (Shovankova & Abbot, 2011).

All the cultivars examined showed a total phenol content higher than the commercial cultivars 'Idared'. 'Bobovec' and 'Habikuša crvena' were the cultivars with the highest total phenol content in the peel and flesh. Similar results were obtained by Serra (2010), which analyzed 18 traditional Portuguese varieties of apples compared to 8 commercial cultivars. Higher values of total polyphenolic compounds compared to our results have been recorded in the peel and flesh of Fuji, Epagri COOP24 and Epagri F5P283 cultivated in Brazil (Vieira *et al.*, 2009). In a study of autochthonous varieties of apples from the region of Italy, Panzella *et al.* (2013) found that autochthonous cultivars have 2-7 times the total phenol content compared to commercial cultivars. On the other hand, content of individual polyphenolic compounds for each autochthonous apple cultivars indicate significant differences among the 20 autochthonous cultivars.

Principal component analysis (PCA) was used for exploratory analysis allowing for data reduction in the multivariate data set (results of 22 phenolic compounds in 112 samples (12 polyphenols in 7 apple peel samples and 7 polyphenols in 7 apple flesh sample).

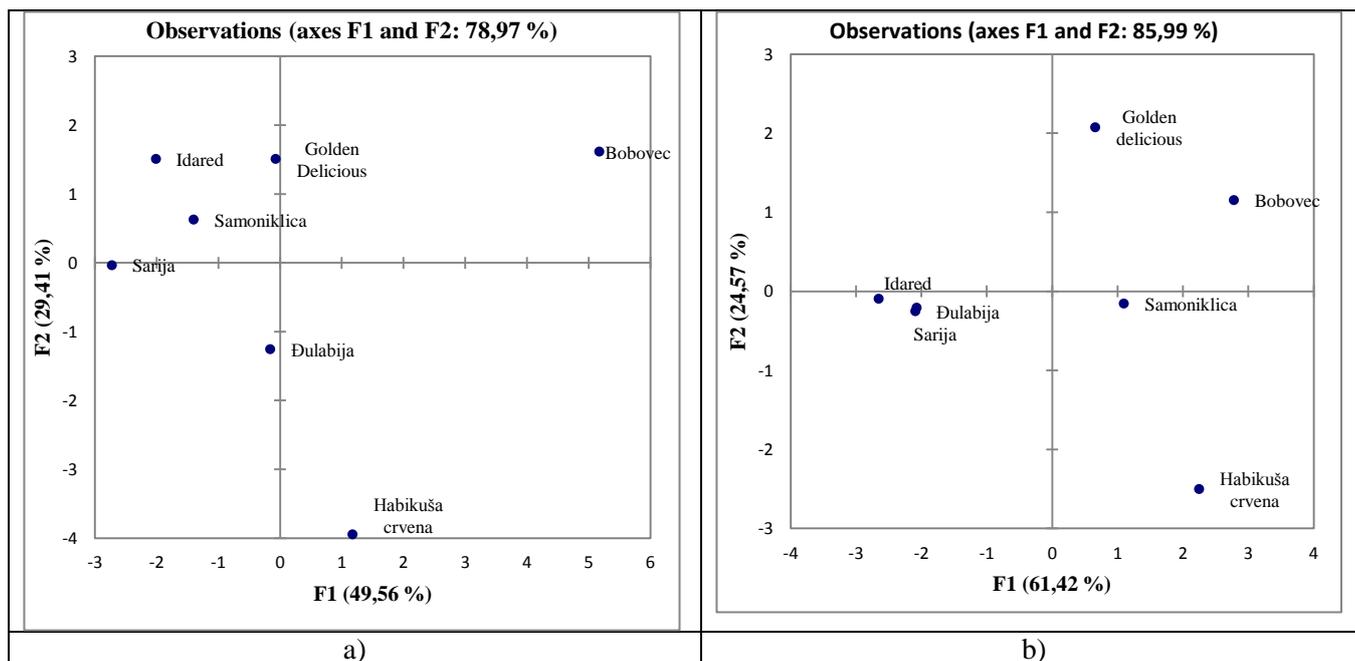


Fig. 1. Principal component analysis (PCA) of the investigated apple cultivar according to the first 2 PCAs obtained by content individual polyphenolic compounds in the (A) peel and (B) flesh

The starting point for the PCA calculations was the matrix of data given in Tab. 1 (Fig. 1a). The first two principal components (PCs) accounted for 78.97% of the total variance. 'Bobovec', 'Habikuša crvena' and 'Đulabija' were clearly separated whereas 'Idared', 'Golden delicious', 'Samoniklica' and 'Sarija' were close. According to the eigenvectors procyanidin B2, epicatechin, rutin, quercitrin and flavonol derivate were highly positively associated with PC1, whereas chlorogenic acid and catechin were negatively associated with PC2. It can be concluded that a high content of quercitrin and flavonol derivate present in the peel of apple samples in the upper half of the plot and high content of procyanidin B2, epicatechin, rutin on the opposite side of the plot. The lower negative value of PC2 showed that cultivars, including 'Đulabija' i 'Habikuša crvena' had higher chlorogenic acid and catechin content in the peel.

Fig. 1b shows the scores plot from PCA carried out on matrix of data given in Tab. 2. PC1, which alone explains 61.42% of the total variance, has high positive loadings for epicatechin and phloretin derivate. The second principal components (PC2) explains 24.57 % of the total variance with a significant parameters quercitrin, catechin, chlorogenic acid and phloridzin. The quercitrin, catechin and chlorogenic acid content in the apple flesh show a strong negative loading, while the phloridzin content show positive loading. 'Bobovec', 'Habikuša crvena', 'Golden delicious' and 'Samoniklica' were clearly separated whereas 'Idared', 'Đulabija' and 'Sarija' were very close. The higher positive value of PC1 showed that 'Bobovec' and 'Golden delicious' had higher epicatechin and phloretin derivate contents in the flesh. The lower negative value of PC2 showed that cultivar 'Samoniklica' had higher quercitrin, catechin, chlorogenic acid and phloridzin content.

The polyphenols have a high antioxidant potential but not particularly desirable in terms of technology as it is associated with proteins and sugars from the cell wall and influence pronounced enzymatic browning, and the final product stability (Duda - Chodak *et al.*, 2010). Cultivars with a higher polyphenolic content could be

recommended for direct consumption, while the cultivars with a lower concentration of phenolic compounds would be more appropriate for apple juice making (Šavikin *et al.*, 2014).

## CONCLUSION

Generally, polyphenols of autochthonous varieties represent a heterogeneous group of compounds. However, it should be noted that by the amount of these substances some autochthonous cultivars do not lag behind the current commercial cultivars. Apple peels possess higher contents of phenolic compounds compared to flesh, and the results finally indicate that the consumption of whole fruit provides phytochemicals in amounts far more abundant than those provided by the (single) tissue. In general, cultivars, 'Bobovec', 'Habikuša crvena' and 'Samoniklica' could be considered as a rich sources of individual polyphenolics compounds according to the results of this study. For this reason those cultivars could be recommended for direct consumption. On the other hand, cultivars 'Đulabija' and 'Sarija' with a lower concentration of phenolic compounds would be more appropriate for apple juice making.

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## THE SMOKE CONSTITUTIONS OF HERZEGOVINIAN TOBACCO TYPES IN DEPENDENCE OF VARIETY TOBACCO AND TEMPERATURE REGIMES DURING FERMENTATION PROCESS

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*Original scientific paper*

### Summary

Cigarettes produce tar, nicotine and carbon monoxide (CO) in measurable and consistent quantities, but smokers of manufactured cigarettes (MC) have known those facts for years. Numbers of hand-made cigarettes (RYO) smokers have no important information about the basic health hazardous components in the product they consume. They believe that RYO are less harmful than MC. Therefore, the aim of this study was to determine tar and nicotine contents in the particulate smoke phase, as well as carbon monoxide content in gas smoke phase of hand rolled cigarettes. There were involved three variety of local Herzegovinian tobacco in the study, those were fermented according different temperature regimes (50°C and 60°C).

Results showed that temperature and variety of tobacco had statistically significant influence on the yield of formed smoke's components. The temperature regime of 60°C caused an increase in the yield of the tar and CO, and reduced the nicotine content in all varieties of tobacco. This study indicated that smoke of hand- filled cigarettes gave higher levels of components like nicotine, tar and CO at both temperature regime, those could have as consequence more addictive smokers.

**Key words:** *type of tobacco, nicotine, TAR, CO, tobacco fermentation, temperature regimes*

### INTRODUCTION

The Herzegovinian type of tobacco has special and different characteristics when compared with the other tobacco types. It has favorable relation of chemical compounds, a specific and pleasant aroma. After being harvested and cured, this tobacco type is not fully stabilized and can not be kept long at this stage. The essential factor which has influence on the properties of Herzegovinian tobacco type is a process of fermentation (Djulančić, 2014).

Although tobacco fermentation has been investigated extensively, the fermentation process is not completely understood. The general opinion is that the transformations which are effected in the leaf, during fermentation process, are purely the result of chemical processes. Fermentation can only take place as stated under suitable conditions of heat and moisture and these are the conditions which favor the development of microbes and enable them to work (Brennan, 2011).

As the plant slowly dies and decomposes special ferments are produced. These ferments set up an oxidization process which splits up the complex organic compounds which still exist in the leaf cells. The starch in the plant is changed into sugar which is slowly consumed. There is a decrease in the fats and gummy substances, also in nicotine and nitrogenous compounds, and there is a formation of certain organic acids such as malic, citric and oxalic which are essential in the production of flavor (Qiu *et al.*, 2003/2004).

The Herzegovinian tobacco is very popular for Roll-your-own (RYO) cigarettes. RYO cigarettes are hand-filled cigarettes made from loose tobacco and rolling papers (i.e. cigarette paper). RYO cigarettes can be hand-rolled by the user or made with a hand-held rolling machine (Mackay *et al.*, 2006). Consuming of RYO tobacco in Bosnia and Herzegovina increased lately and this has been explained by the fact that RYO cigarettes are cheaper than manufactured cigarettes (MC). A common misconception is that RYO cigarettes are more natural and therefore "safer" than manufactured cigarettes; however, both contain the same ingredients. Additionally, in all combustible tobacco products, it is the actual burning of the tobacco that produces many of the toxic chemical components in tobacco smoke (Young *et al.*, 2012).

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With regards to the belief about the harmfulness of the different types of tobacco, O'Connor *et al.* (2007) found that 25% of their survey participants RYO tobacco were less harmful than MC. There were, however, significant differences in this aspect among the countries surveyed. For this reason, the main objective of the present work was to determine tar and nicotine contents in the particulate smoke phase, as well as carbon monoxide content in gas smoke phase of hand rolled cigarettes, originating from Herzegovinian tobacco type.

## MATERIALS AND METHODS

Cured leaf tobacco of local Herzegovinian type, produced during year 2014, was used as research material. Table 1 shows chemical properties of three varieties of tobacco, which were used in this study. Tobacco samples were fermented according to two different temperatures (50°C and 60°C), and relative humidity at 65%.

Tab. 1. Chemical properties of experimental varieties of Herzegovinian tobacco

Variety of tobacco	Nicotine (%)	Total nitrogen (%)	Proteins (%)	Reducing sugars (%)
Veliki Hercegovac	2.20±0.038 <sup>a</sup>	1.83±0.066 <sup>a</sup>	11.84±0.35 <sup>c</sup>	8.07±0.13 <sup>c</sup>
Šeginovac	1.85±0.065 <sup>b</sup>	1.46±0.101 <sup>b</sup>	13.69±0.56 <sup>b</sup>	12.73±0.66 <sup>b</sup>
Ravnjak	1.52±0.075 <sup>c</sup>	1.72±0.095 <sup>a</sup>	15.06±0.58 <sup>c</sup>	15.02±0.46 <sup>a</sup>

Mean values with different letters in a column for each group are significantly from one another ( $p < 0.05$ ).

The samples of cured tobacco whole leaves (lamina and stem), after fermentation process were cut by semi-automatic laboratory cutter "Comas", which was set to the width of cut of 0.8 mm. The cigarettes for the smoking experiments were prepared by weighing the empty tube (include the filter plus the paper cylinder) and the tube completely refilled, by hand, with the corresponding tobacco. These tubes have no ventilation holes. The procedure used for filling the tubes was the same in all the cases. The results obtained for the composition of the mainstream smoke have been expressed in terms of mass of component per gram of smoked tobacco.

All the cigarettes, after preparation, were conditioned for at least 48 h at 22°C and a relative humidity of 60%, based on the ISO 3402 standard [2]. Cigarettes were smoked in a smoking machine under smoking conditions based on the ISO 3308 [1]. Puff volume was 35 mL, taken during 2.0 s, with a puff frequency of 60 s. Total smoke condensate TPM and NFDPM (tar) were collected on a 92-mm glass-fiber filter pad that was weighed in the holder (ISO 4387) before and after smoking [3]. The nicotine content was determined according to ISO 3400. [4]. In the gas phase of the smoke, the content of carbon monoxide (CO) was determined according to ISO 8454 [5]. For the purposes of the analysis, 20 cigarettes were taken from each sample. All the analyses were conducted in triplicate.

Results were reported as mean ± standard deviation (SD). The statistical analysis was performed by the SPSS 17 software. ANOVA with post hoc test analyses based on Turkey was used to compare differences between samples (De Coster, 2004).  $p$ -value of 0.05 was considered statistically significant. Also, the relationship between the components in the smoke and the tobacco components in the cigarettes was estimated by using the test of correlation. To determine the strength of correlation, the classification by Maletić (2005) was used.

## RESULTS AND DISCUSSION

Components that are fully deposited on the Cambridge filter are marked as particulate phase of smoke or total smoke condensate (TPM). Major components of TPM are nicotine and tar. Tar is defined as the aggregate of substances extracted from TPM, after deducting the content of nicotine and water (Jenkins *et al.*, 1996). Results of investigations of TPM and tar yield in smoke of Herzegovinian tobaccos are shown in Table 2.

According to results shown in Table 2, it can be concluded that temperature conditions at 60°C during fermentation process, resulted in an increase yield of TPM, in all three varieties of Herzegovinian tobacco. The smoke from cigarette made from variety Veliki Hercegovac had statistically ( $P \leq 0.05$ ) significant highest amount TPM-a (31.36 mg/g smoked tobacco), while the smoke from cigarette made from variety Ravnjak contained the lowest yield of TPM (26.58 mg/g smoked tobacco). Analysis of variance showed that the factors temperature, variety of tobacco, and their interactions, had a significant effect on the yield of TPM.

Tab. 2. Yield of the TPM and tar in smoke of Herzegovinian tobaccos

Variety of tobacco	TPM		TAR	
	(mg/g of smoked tobacco)		(mg/g of smoked tobacco)	
	Temperature regime 50°C	Temperature regime 60°C	Temperature regime 50°C	Temperature regime 60°C
Veliki Hercegovac	27.97±0.22 <sup>a</sup>	31.36±0.18 <sup>a</sup>	15.29±0.40 <sup>a</sup>	17.69±0.15 <sup>a</sup>
Šeginovac	27.18±0.24 <sup>b</sup>	30.53±0.50 <sup>b</sup>	14.94±0.19 <sup>a</sup>	18.21±0.50 <sup>a</sup>
Ravnjak	24.67±0.20 <sup>c</sup>	26.58±0.03 <sup>c</sup>	11.82±0.30 <sup>b</sup>	15.16±0.57 <sup>b</sup>

The highest average yield of tar (table 2) was achieved in smoke of variety Šeginovac (18.21 mg/g smoked tobacco). There were no significant differences among tar content in smoke of varieties Veliki Hercegovac and Šeginovac. However, in tobacco smoke, of variety Ravnjak was observed the statistically significant lowest amount of tar.

A strong negative correlation was observed (Table 4) between tar yield in the smoke, and proteins ( $r = -0.84$ ) as well as reducing sugars ( $r = -0.80$ ) content in tobacco. The results showed that there was a very strong positive correlation between the yield of tar and the nicotine content ( $r = 0.88$ ) in TPM. Also, it is important to emphasize that there was a statistically very strong positive correlation ( $r = 0.99$ ) between amount of tar and the yield of TPM. These data are in agreement to those previously reported (Djulančić, 2007).

Our research results showed that the variety of tobacco had the main influence on tar yield (table 2). Factor temperature has also had a significantly effect on the tar yield. Therefore, it can be stated that increased temperature during fermentation process, contributed to increase yield of tar in smoke all three varieties of Herzegovinian tobacco.

Different authors have studied the mainstream smoke from RYO cigarettes. Marcilla *et al.* (2014) found that the yields of tar in the mainstream smoke from RYO cigarettes produced by 57% of the smokers were above the current maximum of 15 mg/cigarette for manufactured cigarettes. In this study, varieties Šeginovac and Ravnjak, which fermented at 50°C, deliver yield of tar under 15 mg/smoked tobacco.

Other, very important compound to be considered is nicotine, because this is the compound responsible for the addictive characteristics of tobacco. Table 3 indicates that different temperature conditions have a significant impact on nicotine yield. The significant highest nicotine yield (2.41 mg/g smoked tobacco) was obtained in smoke of variety Veliki Hercegovac (Table 3), which was fermented at a temperature 50°C. There were no statistically different between nicotine yield in the smoke of cigarettes made from varieties Veliki Hercegovac and Šeginovac, at both temperature regimes of fermentation. In the cigarette smoke, made from variety Ravnjak, fermented at 60°C, was observed the lowest (1.49 mg/g) nicotine yield, which may have been related to the action of enzymes such as proteinases. According to Tomic (1973) temperature at 60°C accelerate the activity of enzymes by 10%, compared to a temperature at 50°C, leading to a greater nicotine degradation.

Tab. 3. Yield of the nicotine and CO in smoke of Herzegovinian tobaccos

Variety of tobacco	Nicotine		CO	
	(mg/g of smoked tobacco)		(mg/g of smoked tobacco)	
	Temperature regime 50°C	Temperature regime 60°C	Temperature regime 50°C	Temperature regime 60°C
Veliki Hercegovac	2.41±0.19 <sup>a</sup>	2.29±0.17 <sup>a</sup>	25.10±0.12 <sup>c</sup>	26.86±0.32 <sup>b</sup>
Šeginovac	2.39±0.40 <sup>a</sup>	2.13±0.02 <sup>a</sup>	26.85±0.14 <sup>b</sup>	28.70±0.59 <sup>ab</sup>
Ravnjak	1.73±0.02 <sup>b</sup>	1.49±0.04 <sup>b</sup>	29.60±0.55 <sup>a</sup>	30.22±0.87 <sup>a</sup>

Although, the 60°C temperature significantly reduced nicotine yield in tobacco smoke, the main significant impact was made by the factor variety of tobacco ( $r=0.93$ ). Strong positive correlation (Table 4) between nicotine yield in smoke and nicotine content in variety of tobacco ( $r=0.93$ ), confirms the obtained results. Similar results were reported in other studies (King and Borland, 2004; Djulancic, 2007).

This study of mainstream tobacco smoke involves the analysis of its carbon monoxide content (Table 3). CO is under specific regulations in many countries due its known high toxicity. The level of CO per MC in the Bosnia and Herzegovina is declared to be approximately 10 mg/cig.

As seen (table 3), the yields of CO have, in both cases, a very high value. Comparison of temperatures (50°C and 60°C), during fermentation process tobacco, shows that as temperature increases, the yield of CO in smoke also increases.

The main significant effect on yield of CO in tobacco smoke had factor variety of tobacco. It can be explained with very strong positive correlation between CO yield and protein content ( $r=0.90$ ), as well as reducing sugars contents ( $r=0.94$ ) in tobacco samples. Very strong negative correlation was observed between CO yield and nicotine content ( $r=-0.94$ ) in tobacco samples, which is consistent with the literature data (Djulančić, 2007). For these reasons, variety Ravnjak, which contained a higher content of those compounds, produced a larger amount of CO.

Based on the data presented in Table 4, it can be concluded that there is a very strong negative correlation ( $r= -0.94$ ) between the CO yield in the smoke and the content of nicotine in the tobacco. On the other hand, very strong positive correlation was noted between CO and proteins ( $r=0.90$ ) and reducing sugars ( $r=0.94$ ) in the tobacco samples. For these reasons, variety Ravnjak, which contained a higher content of those compounds, produced a larger amount of CO.

Yield of CO was significantly higher in cigarette smoke from variety Ravnjak (30.22 mg/g), which is fermented at 60°C, compared to other varieties of tobacco samples. However, there was no significant difference between yield of CO, from variety Šeginovac and Veliki Hercegovac. The lowest yield of CO had the smoke originating from variety V. Hercegovac (25.10 mg/g) which was fermented at temperature 50°C.

It is important also to point out on antagonism between yield of CO (Table 4) and tar ( $r= -0.94$ ), the same as amount of CO and TPM content ( $r=-0.90$ ).

Tab. 4. Pearson's Correlation between components of the smoke and components of tobacco

	TPM	TAR	Nicotine (smoke)	CO	Nicotine (tobacco)	Total nitrogen	Proteins	Reducing sugars
TPM	1							
TAR	,989**	1						
Nicotine in smoke	,959**	,984**	1					
CO	-,976**	-,943**	-,899**	1				
Nicotine in tobacco	,926**	,876**	,816**	-,943**	1			
Total nitrogen	-0,009	-0,14	-0,2	-0,108	0,293	1		
Proteins	-,899**	-,841**	-,791*	,903**	-,967**	-0,312	1	
Reducing sugars	-,880**	-,803**	-,741*	,944**	-,949**	-0,385	,944**	1

\*\*Correlation is significant at the 0.01

\*Correlation is significant at the 0.05.

Results indicated that variety of tobacco had significant effect on generation of those compounds. Concentrations of CO in smoke Herzegovinian tobacco samples were higher to those reported in the literature. Castaño Calduch *et al.* (2012) analyzed up to 70% of the fine-cut tobacco market in Spain, and showed that the yields of CO was in the range of 13.5–18.4 mg/cigarette.

According to Djulančić *et al.* (2013) and Chen *et al.* (2014) the yield of nicotine, tar and CO in tobacco smoke was greatly influenced by many variables including the type of tobacco. Results of research showed that Ravnjak delivered the smallest tar and CO yields in smoke, those were highly influenced by temperature of fermentation. Future examinations of tobacco smoke could be organized with the usage of ventilated filter tubes, that would probably influence the generation of smaller tar, nicotine and CO yields in smoke.

## CONCLUSIONS

The smoke from Herzegovinian tobaccos are not less hazardous than the smoke from manufactured cigarettes, which may be contrary to the popular belief. Based on the results presented in this paper, hand-rolled cigarettes made from variety of Ravnjak fermented at 50°C delivered tar yield under 15 mg/gr of smoked tobacco.

All examined varieties of tobacco fermented on lower temperatures caused reduction of tar and CO yield, and increase in nicotine content in smoke.

Through smoking these cigarettes, a greater quantity of nicotine, tar and CO are inhaled than with MCs, which makes them more addictive and harmful to one's health.

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## EFFECTS OF SPELT (*Triticum spelta* L.) FLOUR INCORPORATION AND FLOUR EXTRACTION RATE ON BISCUIT QUALITY

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### Summary

In a recent study we explored the possibilities of using spelt (*Triticum spelta* L.) flour (SF) in different concentrations in wheat biscuit formulations and determined the effects of flour extraction rate on biscuit quality. Five different mixtures of common wheat (WF) and spelt flours (SF) were used for biscuit formulations: WF/SF in combinations 100/0 (control sample), 75/25, 50/50, 25/75 and 0/100, according to procedure described in AACC method 10-52. All samples were prepared with 2 different flours: whole meal WF and SF (A flours); and flours of extraction rates of 87% - WF and 84% - SF (B flours). A total of 10 samples were prepared in two replicates. Physical analysis included diameter (D) and thickness (T) before and after baking, diameter (DI) and thickness increase (TI), spread ratio (SR) and specific volume (SV) determination. In addition, chemical analysis included pH, antioxidant activity (AO) and total phenol content (TPC) determination. Sensory evaluation by QDA was conducted using a scale of 1-5 scores on 4 selected properties: taste, aroma, melting and overall acceptability. Statistical analysis (Two-way analysis of variance) showed significant influence ( $P < 0.05$ ) of SF incorporation and flour extraction rate on all physical properties. SF incorporation at lower levels (to 50%) decreased SR. Results showed that the incorporation of SF, A and B flours, increased the antioxidant activity of the biscuits compared to the control samples S0A and S0B. Sensory properties weren't affected with SF incorporation, while flour extraction rate affected all properties excepting aroma. Sample obtained from SFB 100% was characterized as the best according to sensory evaluation. In addition, according to sensory evaluation B flour samples were more acceptable compared to A flour samples based on significantly higher scores for all sensory properties, especially for melting.

**Key words:** *spelt wheat flour, common wheat flour, biscuit quality, sensory properties*

### INTRODUCTION

For many years, cultivation of spelt (*Triticum spelta* L.) declined, but recent interest in use of spelt for ecologically grown foods has led to resurgence in its cultivation (Bonafaccia *et al.*, 2000; Zieliński *et al.* 2008). Spelt wheat is a low-input crop, suitable for growing in harsh ecological conditions and in marginal areas of cultivation, without the use of pesticides (Bonafaccia *et al.*, 2000). Even with low fertilizing spelt wheat gives a good harvest and has a better mineral uptake in comparison with *Triticum aestivum* L. (Bojňanská & Frančáková, 2002). Contrary to common wheat, spelt is harvested as a hulled grain and must be dehulled prior to milling or grinding. According to Zieliński *et al.* (2008) spelt flour is characterised by the yield of dehulled grains of about 65%. It is caused by a tough spelt hull, which makes it more difficult to process than modern wheat. The hull protects the grain from pollutants and insects and enhances the retention of nutrients in the kernel and improves freshness (Abdel-Aal *et al.*, 1997).

The nutritive value of spelt wheat is high and it contains all the basic components which are necessary for human beings such as starch and nonstarch carbohydrates, proteins, lipids, vitamins and minerals (Bojňanská & Frančáková, 2002). It is believed that spelt possesses valuable nutritional potential due to its protein content and composition as well as its lipids, crude fibre, vitamin and mineral content (Wieser 2001; Abdel-Aal & Hucl 2002; Pruska-Kędzior *et al.*, 2008). Even at low fertilization level, spelt grains are reported to have higher contents of protein (16–17%) thanks to higher portion of aleuron layer, lipids and minerals compared to common wheat (Ruibal-Mendieta *et al.*, 2002; Wiwart *et al.*, 2004; Kohajdová & Karovičová, 2008).

The nutritional qualities and technological properties of spelt are being evaluated in various European projects. Most of the investigated spelt varieties demonstrated good milling properties and high baking quality and many researchers claim that the technological potential of spelt for milling, bread making, and pasta production is very promising (Bonafaccia *et al.*, 2000; Zanetti *et al.*, 2001; Bojňanská & Frančáková, 2002; Ceglińska, 2003; Kohajdová & Karovičová, 2007; Oručević *et al.*, 2011). The comparison of the bioactive compounds content between spelt breads and wheat showed a similar level of these compounds (Zieliński *et al.*, 2008). Oručević *et*

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*al.* (2013) reported significant increase of total phenols and antioxidant activity with increasing the share of spelt wheat in the bread formula.

However, at present, little information is available on the using of spelt flour for biscuit production, and also there is lack of information in respect to the antioxidant properties and antioxidants content of biscuits containing spelt wheat. Therefore, the incorporation of spelt flour at various levels to wheat biscuit formulations was investigated in order to assess the optimal formulation, characterise the improvement in the nutritional value and antioxidant properties and evaluate physical and sensory properties of the products. Another objective of the study was to investigate the effects of flour extraction rate on biscuit quality.

## MATERIALS AND METHODS

Flour samples from organic growing spelt and conventionally growing wheat grains from growing region Nišići (season 2012) were manufactured by Company „Bionatura“ Breza. Manufactured flours were with two different extraction rates: wholemeal wheat (WF) and spelt (SF) flours (A flours); and flours of extraction rates of 87% - WF and 84% - SF (B flours). Starting with a basic biscuit making procedure - AACC method 10-52 with modification according to Morales *et al.* (2009), the biscuits were prepared with different ratios of WF and SF, as shown in Table 1. Five different mixtures were used for biscuit formulations: WF/SF in combinations 100/0 (control sample), 75/25, 50/50, 25/75 and 0/100. Biscuit dough was sheeted to a thickness of 8 mm and cut into circular shapes (of 50 mm diameter) using circular mould. All samples were prepared with A and B flours. Total of 10 samples were prepared in two replicates.

Tab.1. Content of WF and SF used to prepare the A and B biscuit samples

Formulations	WF content %	SF content %
S0	100	0
S25	75	25
S50	50	50
S75	25	75
S100	0	100

Physical analysis included diameter (D) and thickness (T) before and after baking (Kaur *et al.*, 2012), diameter (DI) and thickness increase (TI), spread ratio (SR) and specific volume (SV) determination. Diameter and thickness increase were calculated as ratio of biscuits diameter and thickness before and after baking, and expressed in %. Spread ratio was calculated by dividing the average value of diameter to average value of thickness for 10 biscuits at a time (Ajila *et al.*, 2008). Specific volume was calculated as volume (determined by „seed replacement“ method) divided by mass of biscuits.

The homogenized biscuit samples (particle size <1 mm) obtained by milling biscuits on an electrical mill were used for chemical analysis. Total proteins (Kjeldahl) and ash contents were estimated using the standard methods of analysis (AOAC, 2000). pH was measured using a pH meter. Total phenolics content (TPC) of biscuits was determined colorimetrically ( $\lambda=765$  nm) using Folin-Ciocalteu reagent according to the method described by Vranac *et al.* (2013). The antioxidant activity of biscuits was performed using FRAP (Ferric Reducing Antioxidant Power) method (Benzie & Strain, 1996).

Sensory evaluation by Quantitative Descriptive Analysis (QDA) was conducted on 4 sensory properties: taste, aroma, melting and overall acceptability using scale 1-5 scores by 10 well trained panel members.

All results are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis included a descriptive analysis, followed by two-way analysis of variance (MANOVA), used to determine the effect of spelt flour incorporation and flour extraction rate on the physical, chemical and sensory properties of biscuits. Determined differences were tested by the Tukey test at a significance level of 0.05.

## RESULTS AND DISCUSSION

The physical and chemical properties of biscuit samples are presented in Table 2.

Physical characteristics of biscuits, such as D, T, DI, TI, SR and SV were affected significantly ( $P < 0.05$ ) with the incorporation of SF as well as with flour extraction rate.

The changes in D and T are reflected in SR, considered as one of the most important quality parameter of biscuits because it correlates with texture, grain finesse, bite and overall mouth feel of the biscuits (Bose & Shams-ud-Din, 2010). According to Eissa *et al.* (2007) cookies having higher SR are considered most desirable. SR in both, A and B biscuits, significantly ( $P < 0.05$ ) decreased with SF incorporation to 50% level, and with further increase in

the level of SF, SR significantly ( $P < 0.05$ ) increased at level of 75% SF and then again decreased at level of 100% SF. So, incorporation of 75% SF improved spreading of biscuits, and samples S75A and S75B were significantly ( $P < 0.05$ ) different from the other samples, excepting control samples S0A and S0B. On the other hand, SF incorporation at lower levels (to 50%) decreased SR. Other researchers also reported decrease in SR when barley (Gupta *et al.*, 2011) buckwheat (Baljeet *et al.*, 2010) and funugreek (Sharma & Chauhan, 2002; Hooda & Jood, 2005) flours and fibre from different cereals (Sudha *et al.*, 2007) were incorporated in wheat biscuit formulation in levels 0-40%. Sample with 75% SFB had highest value (11.50) for SR, and was significantly ( $P < 0.05$ ) different from samples with 25.50 and 100% SFB. But it wasn't different from control sample, S0B. The smallest value of SR had sample with 50% SFA, which significantly ( $P < 0.05$ ) differed from samples S0A and S75A.

Tab.2. Physical and chemical properties of biscuits

	F.E.R.	A					B				
	M	S0	S25	S50	S75	S100	S0	S25	S50	S75	S100
Physical properties	D (mm)*	80.70 ± 3.55 <sup>b</sup>	79.75 ± 2.34 <sup>b</sup>	74.61 ± 5.13 <sup>c</sup>	85.62 ± 2.06 <sup>a</sup>	78.18 ± 3.11 <sup>b,c</sup>	83.69 ± 3.02 <sup>a</sup>	82.37 ± 3.91 <sup>a,b</sup>	84.89 ± 3.31 <sup>a</sup>	85.35 ± 2.05 <sup>a</sup>	78.56 ± 3.84 <sup>b</sup>
	T (mm)*	7.60 ± 0.19 <sup>c</sup>	8.91 ± 0.59 <sup>a</sup>	9.19 ± 0.73 <sup>a</sup>	8.18 ± 0.18 <sup>b</sup>	8.80 ± 0.35 <sup>a</sup>	7.65 ± 0.47 <sup>b,c</sup>	8.26 ± 0.34 <sup>a</sup>	8.13 ± 0.40 <sup>a,b</sup>	7.43 ± 0.32 <sup>c</sup>	8.57 ± 0.29 <sup>a</sup>
	DI (%)*	61.39 ± 7.09 <sup>b</sup>	59.50 ± 4.68 <sup>b</sup>	49.22 ± 10.25 <sup>c</sup>	71.25 ± 4.12 <sup>a</sup>	56.36 ± 6.22 <sup>b,c</sup>	64.39 ± 6.04 <sup>a</sup>	62.12 ± 7.81 <sup>a</sup>	59.50 ± 6.61 <sup>b</sup>	70.97 ± 4.11 <sup>b</sup>	56.74 ± 7.68 <sup>b</sup>
	TI (%)*	-4.95 ± 2.32 <sup>c</sup>	11.38 ± 7.34 <sup>a</sup>	14.90 ± 9.11 <sup>a</sup>	2.25 ± 2.20 <sup>b</sup>	9.94 ± 4.40 <sup>a</sup>	-4.44 ± 5.83 <sup>a,b</sup>	3.24 ± 4.24 <sup>a</sup>	1.66 ± 5.03 <sup>a</sup>	-7.08 ± 3.96 <sup>b</sup>	7.18 ± 3.58 <sup>a</sup>
	SR*	10.61 ± 0.44 <sup>a</sup>	8.99 ± 0.69 <sup>b</sup>	8.17 ± 0.91 <sup>b</sup>	10.48 ± 0.33 <sup>a</sup>	8.90 ± 0.36 <sup>b</sup>	10.99 ± 0.89 <sup>a,b</sup>	9.99 ± 0.65 <sup>b,c</sup>	10.47 ± 0.85 <sup>b</sup>	11.50 ± 0.58 <sup>a</sup>	9.17 ± 0.50 <sup>c</sup>
	SV(cm <sup>3</sup> /g)*	1.36 ± 0.47 <sup>a</sup>	0.99 ± 0.23 <sup>a</sup>	1.04 ± 0.47 <sup>a</sup>	1.32 ± 0.13 <sup>a</sup>	1.16 ± 0.10 <sup>a</sup>	2.39 ± 0.60 <sup>a</sup>	1.32 ± 0.36 <sup>b</sup>	1.47 ± 0.13 <sup>b</sup>	1.09 ± 0.38 <sup>b</sup>	1.17 ± 0.20 <sup>b</sup>
Chemical properties	pH***	8.79 ± 0.04 <sup>a</sup>	8.10 ± 0.40 <sup>b,c</sup>	7.78 ± 0.49 <sup>c</sup>	8.59 ± 0.06 <sup>a</sup>	8.47 ± 0.03 <sup>a,b</sup>	8.73 ± 0.11 <sup>a</sup>	8.43 ± 0.02 <sup>a</sup>	8.19 ± 0.04 <sup>b</sup>	8.66 ± 0.00 <sup>a</sup>	8.54 ± 0.01 <sup>a</sup>
	Proteins (%)**	5.20 ± 0.85 <sup>d</sup>	6.10 ± 0.16 <sup>c</sup>	6.38 ± 0.74 <sup>c</sup>	6.91 ± 0.14 <sup>b</sup>	7.44 ± 0.09 <sup>a</sup>	5.01 ± 0.12 <sup>d</sup>	5.69 ± 0.31 <sup>c</sup>	6.12 ± 0.22 <sup>c</sup>	6.65 ± 0.41 <sup>b</sup>	7.31 ± 0.29 <sup>a</sup>
	Ash (%)**	1.49 ± 0.16 <sup>a</sup>	1.62 ± 0.58 <sup>a</sup>	1.68 ± 0.34 <sup>a</sup>	1.69 ± 0.23 <sup>a</sup>	1.70 ± 0.64 <sup>a</sup>	1.46 ± 0.74 <sup>a</sup>	1.49 ± 0.49 <sup>a</sup>	1.54 ± 0.32 <sup>a</sup>	1.55 ± 0.16 <sup>a</sup>	1.58 ± 0.56 <sup>a</sup>
	TPC (mg GA/L extract)**	38.10 ± 1.04 <sup>a,b</sup>	27.97 ± 0.16 <sup>c</sup>	34.98 ± 0.61 <sup>b</sup>	40.00 ± 0.27 <sup>a</sup>	39.13 ± 0.37 <sup>a,b</sup>	37.31 ± 1.46 <sup>a</sup>	25.22 ± 0.11 <sup>b</sup>	27.61 ± 0.03 <sup>b</sup>	36.11 ± 3.54 <sup>a</sup>	36.29 ± 0.29 <sup>a</sup>
	AO (μmol Fe2+/L of extract)**	528.47 ± 1.64 <sup>c</sup>	546.95 ± 1.05 <sup>d</sup>	571.15 ± 0.89 <sup>c</sup>	589.38 ± 1.24 <sup>b</sup>	604.51 ± 0.74 <sup>a</sup>	465.89 ± 0.45 <sup>d</sup>	490.47 ± 0.29 <sup>c</sup>	509.52 ± 0.98 <sup>b</sup>	530.64 ± 0.68 <sup>a</sup>	542.35 ± 0.62 <sup>a</sup>

Different letters in rows from a to e for each parameter indicate significantly different values among mixture at  $P < 0.05$

F.E.R. – flour extraction rate, M – mixture; \* $n=10$ , \*\* $n=8$ , \*\*\* $n=4$

SV varied from 0.99 to 2.39 cm<sup>3</sup>/g. Sample S0B had the highest SV, similar to SV reported by Osella *et al.* (2008), and was significantly ( $P < 0.05$ ) different from the other B samples, while sample S25A had lowest SV. No definite trend in increase or decrease in SV contents was observed.

Statistical analysis showed that flour extraction rate had significant ( $P < 0.05$ ) influence on all chemical properties, while SF incorporation didn't show significant influence only on ash contents.

Proteins ranged from 5.01 (S0B) to 7.44% (S100A). Results showed significantly ( $P < 0.05$ ) increase in protein contents of A and B biscuits with increasing the share of SF in biscuit formulation. The same trend was also observed by other researchers with incorporation some plant sources (Hassan *et al.*, 2012) and buckwheat flour (Baljeet *et al.*, 2010; Filipčev *et al.*, 2011) in wheat biscuit formulation. Since flour extraction rate had significant ( $P < 0.05$ ) influence of proteins content, biscuits with A flour had significantly ( $P < 0.05$ ) higher contents of proteins compared to those one with B flours. The protein contents of control samples S0A (5.20%) and S0B (5.01%) were similar as one reported by Kaur *et al.* (2012).

Ash content varied from 1,46 (S0B) to 1.70% (S100A) and it increased with incorporation of SF in both, A and B biscuits, but the increasing was not significant ( $P < 0.05$ ). This increase in ash content may be due to the high mineral content in spelt flour. The same trend was reported by Baljeet *et al.* (2010), and Hassan *et al.* (2012). On the other

hand flour extraction rate had significant ( $P < 0.05$ ) influence on ash contents, and biscuits with A flours had significantly ( $P < 0.05$ ) higher ash contents compared to biscuits with B flours.

pH value of biscuit samples vary from 7.78 (S50A) to 8.79 (S0A). In both, A and B biscuits, pH decreased significantly ( $P < 0.05$ ) with incorporation of SF to 50% level, and with further increase in the level of SF, the decrease in pH observed was marginal. Obtained pH values for control samples, S0A and S0B were higher than those reported by Niaba *et al.* (2013).

TPC was in range from 25.22 (S25B) to 39.13 (S100A). The highest AO had biscuit sample S100A, and was significantly ( $P < 0.05$ ) different from the other samples, while the smallest AO had sample S0B. As the level of SF in wheat biscuit formulation was increased, AO was also significantly ( $P < 0.05$ ) increased.



Fig. 1. Spider chart of the sensory evaluation of A (a) and B (b) biscuit samples

Comparing mean scores for taste, aroma and melting sample with 100% of SFB (Fig 1.) appeared to be better than the other samples. On the other hand sample with 25% SFA had lowest scores for all evaluated properties. The analysis of variance didn't show statistically significant ( $P < 0.05$ ) influence of SF incorporation on sensory properties, while the flour extraction rate had significant ( $P < 0.05$ ) influence on all sensory properties, excepting aroma. Therefore, all samples with B flours showed significantly ( $P < 0.05$ ) higher scores for sensory properties especially for melting, compared to samples with A flours.

## CONCLUSIONS

SF incorporation in wheat biscuit formulation improved nutritional value of biscuits in terms of proteins and ash. Results showed significant increase of AO with increasing the shear of spelt wheat in the biscuit formulation. The best quality according sensory evaluation showed sample produced from 100% SFB. Due to significantly higher scores for sensory properties especially for melting, samples with B flour were more sensory acceptable compared to samples with A flours.

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## COMPARISON OF SENSORY PARAMETERS OF SIX LOCALLY GROWN TOMATO VARIETIES

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### Summary

The tomato fruit has a unique flavor profile which is generated by a diverse set of chemicals including sugars (glucose and fructose), acids (citrate, malate and glutamate) and 16 volatiles that contribute to the tomato flavor. The flavor quality of many fresh fruits, including tomato, is generally believed to have deteriorated due to intensive breeding with the prime aim of increasing crop yields. In order to analyze possible variations in chemical composition between fruits of six tomato varieties (5 modern and one traditional cultivar – ‘Jabucar’) a sensory evaluation was conducted. The evaluation panel, consisting of six trained evaluators, analyzed 13 attributes of taste and flavor and 4 attributes of aftertaste. Principal component analysis (PCA) showed that the proportion of variance retained in the first two principal components was 65.0% for taste and flavor attributes and 80.0% for the attributes of aftertaste. PCA plots, based on the first two principal components, displayed a certain level of variability among the analyzed material. The traditional cultivar ‘Jabucar’ clearly differentiated from all other varieties based on all analyzed sensory parameters.

Key words: *sensory analysis, tomato varieties, ‘Jabucar’, PCA*

### INTRODUCTION

Quality of fresh tomato is determined by nutritional value and sensory properties (appearance, texture and flavor) (Kader *et al.*, 1978). Research regarding the tomato sensory quality are mostly focused on tomato flavor (Berna *et al.*, 2005; Krumbein *et al.*, 2004; Tandon *et al.*, 2003; Maul *et al.*, 2000). The unique tomato fruit flavor profile is primarily generated by a diverse set of chemicals including sugars, acids and several volatiles. Flavor quality of tomato fruits is generally believed to have deteriorated due to intensive breeding with the prime aim of increasing crop yields. Furthermore, based on the market demand for fresh tomato fruits, genetic research has focused on developing products that have specific agronomic and qualitative properties (disease resistance, response to fertilization, longer shelf-life and higher nutrient content). Nonetheless, such characteristics do not necessary guarantee quality in relation to the aroma, flavor and texture of the fruit. Molecular studies indicate that there is a relatively low rate of DNA sequence diversity within the modern tomato varieties, *Solanum lycopersicum*, consistent with a genetic bottleneck due to two periods of domestication in Central America and Europe (Jiménez-Gómez and Maloof, 2009). In contrast, the variation observed in the volatile content of heirloom tomato varieties were as much as 3,000-fold across 152 cultivars (Tieman *et al.*, 2012).

Therefore, the objectives of this study are: 1) to create a sensory profile of the fruits of five modern tomato varieties and one traditional cultivar (‘Jabucar’) using the lexicon for sensory analysis of fresh tomato fruits; 2) to group the tomato varieties in relation to the main source of variability using a multivariate statistical approach.

### MATERIALS AND METHODS

#### Samples

All analyzed modern (‘Big Beef’, ‘Volovsko srce (pink)’, ‘Viva’, ‘Medeno srce’, ‘Volovsko srce (red)’) and the traditional (‘Jabucar’) tomato variety were grown in a greenhouse in the Dobojski Region (northern part of Bosnia and Herzegovina; average daily temperature in the period June-August is 18,13°C), in a completely randomized block design with three replications and 10 plants per plot. Tomato varieties included in this study have indeterminate growth, almost the same fruiting period (‘Volovsko srce (pink)’ starts fruiting 4-7 days earlier than other varieties) and are suited for greenhouse production. Fruits from the 6 varieties were sampled and

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transported to the laboratory of the Faculty of Agriculture and Food Sciences in Sarajevo for the sensory evaluation.

### Sensory evaluation

The evaluation panel consisted of 6 trained evaluators, 4 females and 2 males, aged between 26 and 50 years. Panelists have used the modified lexicon for sensory analysis of fresh tomato fruits (Baldwin *et al.*, 1998; Hongsoongnern and Chambers, 2008; Belović *et al.*, 2012). A five point scale was used for each of the analyzed descriptors, where 5 was considered high and 1 low for detection of taste, flavor and aftertaste intensity (ISO 4121, 1987). For sensory analysis purposes, fruits were washed and cut into wedges. Each sample received a three digit code and was presented to the panelists in a random order. The sensory evaluation was conducted twice by the same evaluation panel.

### Data analysis

Principal component analysis was (PCA) was used as it summarizes patterns of correlations among observed variables and reduces a large number of observed characters to a smaller number of derived variables or components (Hotelling, 1936). Computations were performed on the correlation matrix for the 13 attributes of taste and flavor and 4 attributes of aftertaste of six analyzed tomato varieties. PCA was performed using StatBox 6.7 (GrimmerSoft, Paris, France).

## RESULTS AND DISCUSSION

### Taste and flavor attributes

A summary of the 13 measure attributes of taste and flavor which were observed and analyzed are presented in Table 1. The first two principal components accounted for 65.00% of the total variance observed, which is slightly lower than the values published by Belović *et al.* (2012) who analyzed the 25 sensory parameters on fresh tomato fruits (70.41%) and Gilsenan *et al.* (2007) how analyzed the flavor of organic and conventional grown tomatoes (77.00%).

Tab. 1. Average values for the 13 attributes of taste and flavor on 6 tomato varieties.

Taste and flavor attributes	Tomato variety					
	Big Beef	Volovsko srce (pink)	Viva	Medeno srce	Volovsko srce (red)	Jabucar
salty	2.0	2.6	2.2	2.5	1.5	1.6
sweetness	1.5	2.0	3.4	2.1	2.0	2.5
sourness	2.0	2.0	2.4	1.9	1.0	2.2
bitterness	1.0	1.2	1.0	1.0	1.3	2.3
astringent	1.0	2.0	1.8	1.5	1.2	2.0
ripeness	3.9	4.6	3.9	4.9	4.8	4.4
umami	4.5	2.8	2.3	4.7	5.0	3.3
odour	4.0	4.5	4.0	3.0	4.0	3.6
aroma intensity	3.5	4.2	3.9	3.0	2.8	3.5
flavor typicality	4.5	4.3	5.0	3.4	3.5	4.4
grassiness	3.2	3.3	2.7	3.1	3.0	3.4
moldiness	1.0	1.6	1.3	1.0	1.0	1.0
fruity flavor	1.1	1.0	1.8	1.3	1.1	2.0

The first principal component (PC1) accounted for 44.00% of the total variance, and had high contributing factor loadings from aroma intensity, moldiness and saltiness. The second principal component (PC2) had high contributing factor loadings from the analyzed attributes fruity flavor, umami and bitterness, and contributed 21.00% to the total variation.

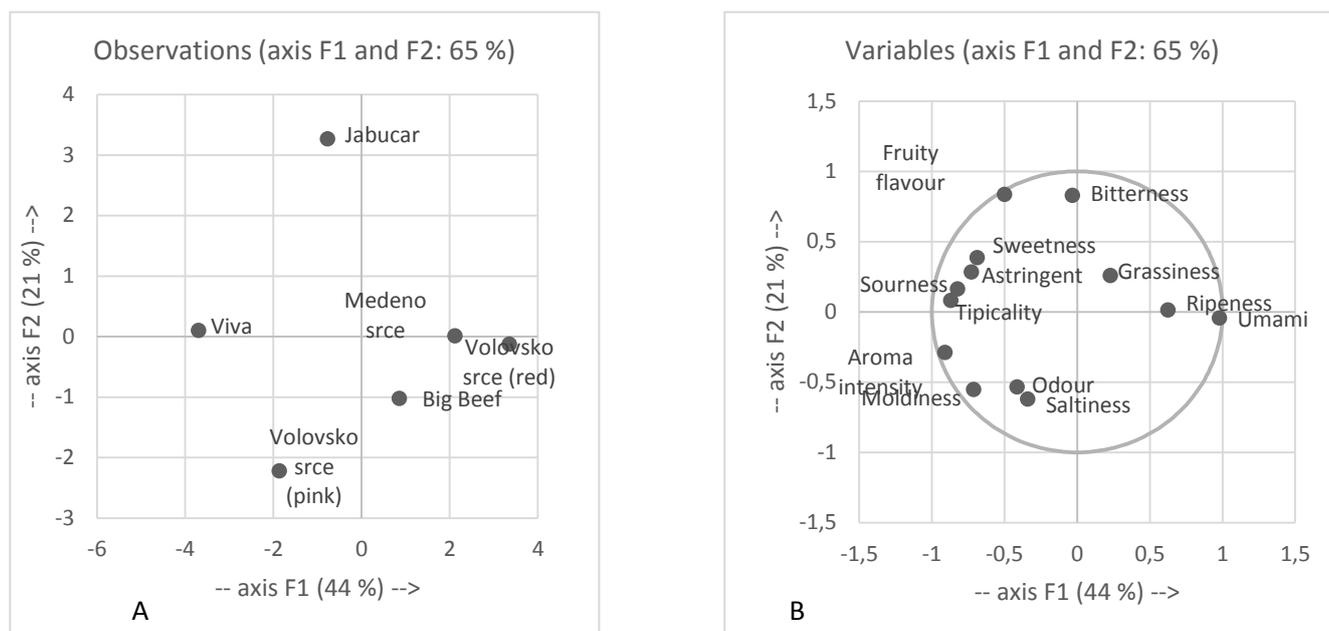


Fig. 1. Multivariate analysis of analytical data. Biplot showing relationship between tomato varieties (score plot, left) and sensory attribute of taste and flavor (loading plot, right).

Distribution of the varieties in relations to the first two principal components shows the separation of the 6 tomato varieties studied based on the main sources of variance (Fig. 1 - right). The first two principal components grouped the varieties into four distinct groups (Fig. 1 - left). The first group (‘Medeno srce’, ‘Volovsko srce (red)’ and ‘Big Beef’) was separated due to high values for umami and ripeness. Varieties contained in the second (‘Volovsko srce (pink)’), third (‘Viva’) and the most distinct fourth (‘Jabucar’) group were separated due to high values for odour and saltiness, typicality and sourness and fruity flavor and bitterness respectively.

**Attributes of aftertaste**

The list of four descriptors of aftertaste is presented in Table 2. PCA results showed that 81.00% of the total variance was contained in the first two principal components. The first component accounted for 55.00% and the second for 26.00% of the total variance (Fig. 2). The variables with highest eigenvectors, and thus most discriminating power, were after-umami for PC1 and after-sour for PC2.

Tab. 2. Average values for the 4 attributes of aftertaste on 6 tomato varieties.

Aftertaste attributes	Tomato variety					
	Big Beef	Volovsko srce (pink)	Viva	Medeno srce	Volovsko srce (red)	Jabucar
overall after taste intensity	2.5	3.8	3.0	3.2	3.4	3.0
After-bitter	1.0	1.1	1.0	1.0	1.2	1.5
After-sour	1.5	2.1	1.4	1.5	1.6	1.7
After-umami	3.3	2.3	3.5	3.8	4.4	2.8

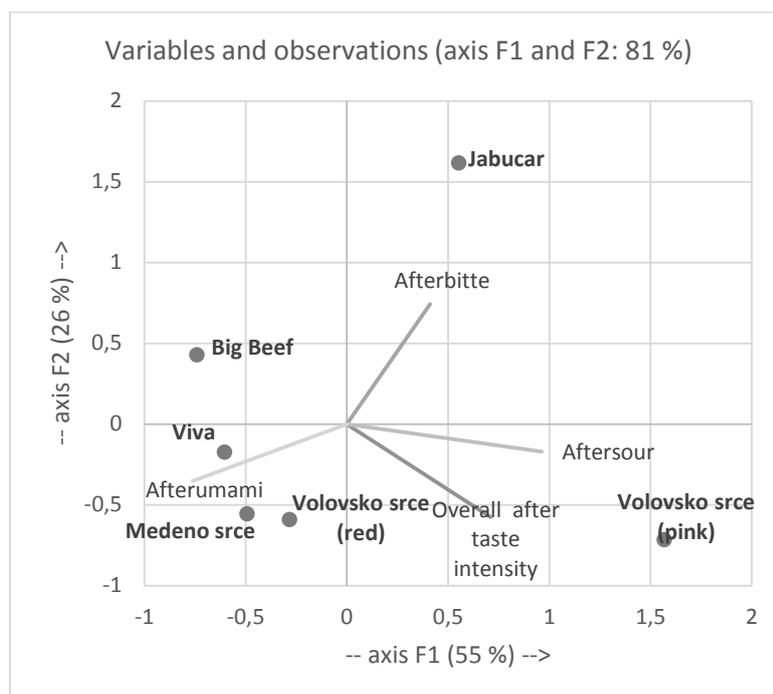


Fig. 2. Multivariate analysis of analytical data. Biplot showing relationship between tomato varieties and sensory attribute of aftertaste.

The grouping of the tomato varieties on the PCA plot, determined by the first two principal components (Fig. 2), is somewhat similar to the grouping of PCA on the attributes of taste and flavor. The main difference is that in this case the variety ‘Viva’ was grouped together with ‘Medeno srce’ and ‘Volovsko srce (red)’, and the variety ‘Big Beef’ formed a distinct group. Again, the traditional variety ‘Jabucar’ proved to have the most divergent fruits of all analyzed tomatoes.

## CONCLUSIONS

Bosnia and Herzegovina’s producers have access to a very diverse assortment of tomato varieties regarding the taste, flavor and aftertaste properties of the fruit. Nevertheless, the traditional cultivar ‘Jabucar’ proved to be the most divergent one according to all analyzed sensory parameters. This cultivar’s germplasm should be further investigated in order to utilize it future breeding programs.

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## SPECTROPHOTOMETRIC QUANTIFICATION OF NITRITE IN DRIED MEAT

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*Professional paper*

### Summary

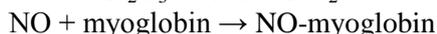
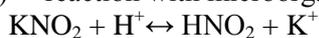
Meat and meat products represent the main source of proteins in human nourishment. The effects of dried meat on health depend on the manner of consumption, the size of the portions and on the sort of dried meat. In the processing of meat and meat products, as a chemical reaction product between additional nitrites and meat proteins, N-nitrosamines can occur. They are considered as harmful and carcinogenic compounds. Nitrites are growth inhibitors of many bacteria, especially pathogenic species *C. botulinum*. In 1990, Scientific Committee on Food established that 50-150 mg of nitrites as NaNO<sub>2</sub>/kg meat products is enough for most products in order to inhibit growth of *C. botulinum*. In Bosnia and Herzegovina, such control is not implemented, as well as in the previous legislation where a maximum allowable concentration was not prescribed. Quantification of nitrites was done by spectrophotometry from 5 samples domestic and 5 samples industrial dried meat because of comparison. The obtained results for dried meat were ranging from 1.46 mg/kg to 5.62 mg/kg for domestic production and 4.86 mg/kg to 44.75 mg/kg for industrial production.

Key words: *nitrites, dried meat, spectrophotometry*

### INTRODUCTION

Dried meat is normally meat which has been treated with nitrate and/or nitrite and immersed in brine. The nitrite (either added or produced from nitrate-reducing organisms) reacts with myoglobin to produce the red colour of pickled meats. Nitrate can act as a reservoir for nitrite but as a preservative it is similar in effectiveness to sodium chloride, the difference being that nitrate prevents the growth of *Clostridium botulinum*. Nitrate may react with amines to form carcinogenic nitrosamines and consequently it has become increasingly important to monitor nitrate and nitrite levels in meat products (Eggers & Cattle, 1986). In addition nitrate can be reduced to nitrite in the oral cavity and in the stomach (Duncun *et al.*, 1997). Once in the stomach, nitrite can react with amines and amides, which are organics containing nitrogen such as amino acids, to form a group of carcinogens known as N-nitroso compounds (Acher, 1989). Stomach is mostly at risk from endogenous N-nitroso compound synthesis since stomach acid catalyses nitrosation reactions. High nitrate intake was associated with gastric cancer in England, Colombia, Chile, Japan, Denmark, Hungary and Italy (Forman & Shuker, 1997). Exposure to endogenously formed N-nitroso compounds had been associated with increased risks of cancer of the stomach, oesophagus and bladder (Bartsch *et al.*, 1990). Hoagland (Honikel, 2008) suggested the following series of chemical reactions during processing (finishing) of meat:

(KNO<sub>3</sub>) salpetre (nitrate) → reaction with microorganisms → nitrite (KNO<sub>2</sub>)



Nitrites in blood oxidise hemoglobin to methemoglobin, which is not carrying oxygen to tissues, which is manifested as cyanosis, and in higher concentrations as asphyxia (cessation of breathing). Symptoms include shortness of breath and blueness of the skin.

The dietary intake of nitrates and nitrites in foods can vary greatly from region to region depending on factors such as farming practices, climate, soil quality, manufacturing processes and legislation.

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The aim of this study is to quantify nitrite in dried meat and develop an accurate, simple and cost-effective method for quantifying the nitrite contents in commonly consumed dried meat produced in Bosnia and Herzegovina.

## MATERIALS AND METHODS

### Reagents

In experiment below mentioned solutions were used:

- saturated solution of sodium tetraborate
- solution of Carrez II

Solution is prepared by dissolving 30 g zinc sulphate ( $ZnSO_4$ ) in 100 ml of redistilled water.

- N-(1-Naphthyl)ethylenediamine dihydrochloride, [ $C_{10}H_7NH(CH_2)_2NH_2 \cdot 2HCl$ ], concentration was 0,5 g/l (NED)

Solution is prepared by dissolving 0,5 g of substance in 1000 ml of redistilled water. Solution must be kept in dark bottle because of the influence of light on the quality of solution.

- Solution for colour development

Solution for colour development is prepared when 5,0 g of sulfanilic acid ( $C_6H_4SO_3HNH_2$ ) is dissolved in 600 ml of redistilled water and 50 ml of glacial acetic acid is added ( $CH_3COOH$ ). Solution is gently warmed. After cooled to room temperature. 100 ml of NED solution is added, and filled with redistilled water to final volume of 1000 ml. Solution also must be kept in dark bottle.

### Dried meat samples

Five dried meat samples of beef were purchased at supermarkets and kept at refrigerator and analysed within 24 h. Five other dried meat samples of beef were purchased at local market place and stored and analysed as above mentioned samples.

### Spectrophotometric methods of analysis

#### Standards

Sodium nitrite ( $NaNO_2$ ) was mixed in redistilled water in volumetric flasks to give a range between 0,025 and 1 mg/l for nitrite ions. Prepared solutions were left for 15 minutes because of full color development. Absorbance of the solution at a wavelength of 545 nm was measured on a UV-Vis spectrophotometer (LKB Ultrospec II).

#### Samples of dried meat

Nitrites present in sample of dried meat product were extracted from exact mass of sample (10g) using warm water (50 ml) in presence of saturated solution of borax ( $Na_2B_4O_7 \cdot 7H_2O$ ) 5 ml for 30 minutes on 60-70°C. Solution is made clear by adding Carrez II solution (1ml) while hot. After clearing the solution add water to bring volume to 100 ml. Solution is then filtered (white line filter paper). In 10 ml of filtered solution 10 ml of sulfanilic acid +NED solution was added to develop a colour, which occurs because of the nitrites presence. Colour occurs when nitrites present in sample react with sulfanilic acid, forming diazonium salt, who then reacts with phenyl substituted derivatives and are making red to pink colour.

#### Calculation of $NO_2^-$ in standards and dried meat samples

$$c(\text{readed value}) \text{ mg/l} \rightarrow 50 \text{ ml}$$

$$x \rightarrow 10 \text{ ml}$$

$$X = c(\text{readed value}) \text{ mg/l} \cdot 50/10$$

$$m(NO_2^-) \text{ mg} = c(\text{readed value}) \text{ mg/l} \cdot 10/50 \cdot 0,1 (100 \text{ ml})$$

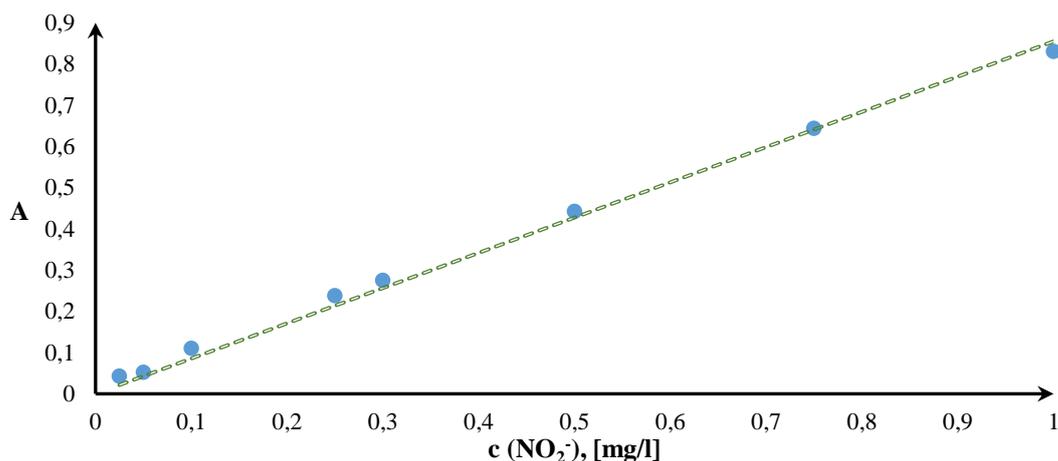
$$w(NO_2^-) = m(NO_2^-)/m(\text{sample}) \cdot 100 \Rightarrow w(NO_2^-) = \frac{c(\text{readed value}) \text{ mg} \cdot \frac{50}{10} \cdot 0,1}{m(\text{sample}) \text{ g}} \cdot 100$$

$$w(NO_2^-) = c(\text{readed value}) \text{ mg/l} \cdot 0,5/m(\text{sample}) \text{ g} \cdot 100$$

$$w_{(NO_2^-) \text{ in } \% NO_2^-} = [c(\text{readed value}) \text{ mg/l} \cdot 50]/m(\text{sample}) \text{ g}$$

## RESULTS AND DISCUSSION

Standards were analysed for nitrite and the results are showed in Graph 1. Samples of dried meat were extracted and analysed for nitrite and the results are showed in Table 1.

Graph 1. Calibration curve of standards ( $y = 0,8547x$ ,  $R^2 = 0,995$ )

Tab. 1. Nitrite in samples of extracted of dried meat

Sample	$A_x$	$c_x\text{-NO}_2^-$	$X$ (mg/l)	$m[\text{NO}_2^-]$ mg	$\omega[\text{NO}_2^-]$	mg/kg
1	0.096	0.112	0.562	0.056	0,56	5.62
2	0.064	0.075	0.374	0.037	0.37	3.74
3	0.025	0.029	0.146	0.015	0.15	1.46
4	0.064	0.075	0.374	0.037	0.37	3.74
5	0.076	0.089	0.445	0.044	0.44	4.45
6	0.765	0.895	4.475	0.448	4.48	44.75
7	0.452	0.529	2.644	0.264	2.64	26.44
8	0.107	0.125	0.626	0.063	0.63	6.26
9	0.083	0.097	0.486	0.049	0.49	4.86
10	0.577	0.675	3.375	0.338	3.38	33.75

Samples of dried meat bought in the market (samples 6-10) showed higher concentrations of  $\text{NO}_2^-$  comparing to domestic production samples (samples 1-5).

In addition of forming carcinogens in the stomach, nitrite is also genotoxic and can readily induce methaemoglobinaemia especially in babies (Gangolli *et al.*, 1994). Furthermore, the lethal dose for nitrite in adults was estimated to be between 2 and 9 g  $\text{NaNO}_2$  per day, or 33-250 mg/kg body weight, whereas the lethal dose for nitrate ions was estimated at 20 g per day, or 330 mg nitrate ions/kg body weight (Gangolli *et al.*, 1994). Although it is unlikely to reach these toxic levels from dietary intake alone, the long-term effects may be harmful, based on epidemiological and clinical studies.

## CONCLUSIONS

Without the addition of nitrite is very difficult to ensure microbiologically safe dried meat, however, as a result of the addition of nitrite a potential danger of the toxic N-nitrosamines occurring because of the reaction of free-residual nitrite that remain available during the processing of meat is possible. So far, no better substance is available to replace nitrites. Therefore, it is important to find the one "ideal" amount which will inhibit the growth and development of *C. botulinum*.

Samples of dried meat bought in the market showed higher concentrations of  $\text{NO}_2^-$  comparing to domestic production samples.

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## STUDY OF CHEMICAL AND SENSORY PROPERTIES OF BEEF DRIED MEAT PRODUCED IN A TRADITIONAL WAY

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*Professional paper*

### Summary

This study describes the chemical and sensory properties of the samples of beef dried meat that were produced in the traditional way by four individual producers from different locations at Una-Sana Canton. The results of chemical analysis show a highly statistically significant difference ( $p < 0.01$ ) for the fat content and the sodium chloride content among surveyed producers. For the contents of water, ash and protein there is not found a statistically significant difference ( $p > 0.05$ ) between the samples. The water content in the samples was in the range of 53.10% to 59.00%; ash content of 5.63% to 6.98%; a protein content of 30.50% to 35.39%, a fat content of 0.86% to 5.11%, and the sodium chloride content of 4.35% to 5.73%. Commission for sensory evaluation of beef dried meat consisted of the five assessors, who evaluated the following sets of characteristics: appearance and color, texture, smell and taste. The results of sensory evaluation show a highly statistically significant difference ( $p < 0.01$ ) for the sensory properties of appearance and color, and a statistically significant difference ( $p < 0.05$ ) for the texture, smell and taste.

Keywords: *beef dried meat, chemical and sensory properties*

### INTRODUCTION

Traditional cured meat products are a special group of food products. Their originality is connected with the geographical area in which they are produced, and they have characteristic sensory properties, which contribute to their very high quality. Production of cured meat products in Bosnia and Herzegovina has a long tradition, especially when it comes to the Bosnian beef dried meat, one of the local products. However, one of the biggest problems in the traditional production of beef dried meat is uneven quality of meat parts, which with unstandardized processing technology, result in high variability of beef dried meat's quality. On the properties of traditional dried meat products may affect different factors, which affect the quality of fresh meat, such as genotype, mode of holding, feeding, procedures before slaughter and conditions after slaughter. A number of other factors, such as: the selection of fresh meat, the fat, the addition of salt and spices, hygiene and environmental conditions (eg. temperature, brine, smoking, drying, etc.) may also contribute to the diversity of the properties of the final product (Pleadin *et al.*, 2013). Among other things, a significant impact on the characteristics and quality of these products have the general characteristics of the area, in particular, specific climatic conditions, typical for a specific geographical area (Ganić *et al.* 2012).

Beef dried meat is one of the most famous indigenous dry-cured products according to the traditional procedure of products in the Una-Sana Canton, and all phases of the production process are carried out without professional supervision, according to specific family experience and tradition. So, aim of this study was to find the best technological solutions in the traditional production of beef dried meat with local manufacturers, which would result in a sufficient quality in terms of chemical, microbiological and sensory characteristics of the product, which are regulated by the current Regulations of the quality of meat and meat products in Bosnia and Herzegovina.

### MATERIALS AND METHODS

The research began in December 2013. and the first task was to conduct the selection of the producers of beef dried meat in the municipalities of Cazin and Bosanska Krupa. Out of the ten potential producers of beef dried meat, we selected four producers as four representative ways of producing beef dried meat: two producers

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belonging to the municipality of Cazin (localities Stijena and Glogovac), and two producers belonging to the municipality of Bosanska Krupa (localities Veliki Badić and Pištaline). In the preparation of beef dried meat, these producers used the shoulder's meat of young Simmental calf whose average weight was about 500kg before slaughter. Other potential producers, whose production processes we have discussed, in preparation of beef dried meat were using other beef breeds taken from different regions of the bodies of animals, which this research doesn't include. During the preparation of the meat's pieces, producers removed manually more or less fat and connective tissue, and cut pieces of meat in the form of blocks. Producers who belonged to the localities of the municipality of Cazin: Stijena and Glogovac were marked as: producer I and producer II, and producers who belonged to the localities of the municipality of Bosanska Krupa: Veliki Badić and Pištaline were marked as: producer III and producer IV. Each producer prepared beef dried meat to their family recipes (Table 1) and procedures (Table 2) that are transmitted from generation to generation.

Tab. 1. Brine recipes and spices used by the selected producers

INGREDIENTS	PRODUCER			
	I	II	III	IV
Salt %	3.62	3.29	1.93	2.52
Sugar %	0.73	-	-	0.61
Garlic %	4.34	6.16	5.02	4.97
Ground pepper %	0.14	0.20	0.31	0.15
Peppercorns %	-	-	-	0.08

Tab. 2. Periods of salting/brining and smoking/drying in the production of beef dried meat

Producer	Period of salting and brining	Period of smoking and drying	Wood used for smoking
I	08/12/2013 – 24/12/2013 17 days	24/12/2013 – 03/01/2014 12 days	beech and spruce
II	15/12/2013 – 06/01/2014 23 days	06/01/2014 – 17/01/2014 12 days	beech
III	15/12/2013 – 06/01/2014 24 days	06/01/2014 – 17/01/2014 12 days	beech
IV	26/12/2013 – 24/01/2014 30 days	25/01/2014 – 30/01/2014 6 days	beech and pine

We took three samples of beef dried meat from each producer, two samples for chemical analysis and one sample for sensory analysis.

We performed the next chemical analysis (four repetitions according to one sample):

Determination of water content - the method of drying to constant mass, BAS ISO 1442;

Determination of total fat content - the method by Soxhlet, BAS ISO 1443;

Determination of total ash - the method of BAS ISO 936;

Determination of protein content –the method by Kjeldahl, determining nitrogen and multiplying by a factor of (N $\times$ 6,25), BAS ISO 937;

Determination of sodium chloride content - volumetric method (AOAC 2000).

We performed the sensory tests by the method ISO 4121:1987-E (two repetitions according to sample) and evaluated the following characteristics: appearance of beef dried meat, colour of appearance, cross-sectional appearance (marbling), cross-sectional colour, toughness/softness at chewing, suitability for grinding in the mouth, juiciness of chewing, smell, flavour (retronasal olfaction), salinity, sour taste.

All data obtained by the chemical and sensory analysis we have processed using the relevant statistical - mathematical methods. We performed the analysis of sensory and chemical properties of beef dried meat using the Kruskal-Wallis test at the significance level of  $\alpha \leq 0.05$ . The significance of differences between the treatments we determined using Mann-Whitney test at the significance level of 0.05. The full set of obtained data we have processed using the computer statistical program Past (ver. 2.17).

## RESULTS AND DISCUSSION

The chemical composition of the samples of beef dried meat are shown in Table 3 and the results of sensory evaluation are shown in Table 4.

Tab. 3. The average values of compounds in beef dried meat

Parameter	Producer				P
	I	II	III	IV	
Water, %	56.68 ± 2.97	59.00 ± 4.93	54.46 ± 1.67	53.10 ± 5.09	P>0.05
Fat, %	0.86 ± 0.08	1.23 ± 0.34	2.77 ± 0.30	5.11 ± 0.29	P<0.01
Ash, %	6.98 ± 1.51	5.63 ± 0.28	6.11 ± 0.17	5.80 ± 0.37	P>0.05
Proteins, %	30.50 ± 7.04	34.38 ± 2.27	35.39 ± 1.68	35.37 ± 1.32	P>0.05
Sodium chloride, %	5.73 ± 0.26	4.62 ± 0.27	4.54 ± 0.39	4.35 ± 0.19	P<0.01

Tab. 4. Statistical variations of parameters of sensory evaluation according to the group of evaluated properties

No.	Appearance and color	p	No.	Texture	p	No.	Taste and smell	p	TOTAL
I	$\bar{X} = 1.42$	p < 0.01	I	$\bar{X} = 1.50$	p < 0.05	I	$\bar{X} = 1.80$	p < 0.05	<b>28.00</b>
	$\Sigma = 8.50$			$\Sigma = 10.50$			$\Sigma = 9.00$		
II	$\bar{X} = 0.93$		II	$\bar{X} = 1.44$		II	$\bar{X} = 1.96$		<b>25.50</b>
	$\Sigma = 5.60$			$\Sigma = 10.10$			$\Sigma = 9.80$		
III	$\bar{X} = 1.13$		III	$\bar{X} = 1.48$		III	$\bar{X} = 1.98$		<b>27.10</b>
	$\Sigma = 6.80$			$\Sigma = 10.40$			$\Sigma = 9.90$		
IV	$\bar{X} = 1.25$		IV	$\bar{X} = 1.33$		IV	$\bar{X} = 1.70$		<b>25.30</b>
	$\Sigma = 7.50$			$\Sigma = 9.30$			$\Sigma = 8.50$		

$\bar{X}$  - mean,  $\Sigma$  - the sum of all assessors.

**Appearance and color:** appearance of beef dried meat, colour of appearance, cross-sectional appearance (marbling), cross-sectional colour, cross-sectional colour uniformity, cross-sectional colour intensity.

**Texture:** structure, freshness (shine) of sectional surface, dryness of sectional surface, hardness/softness to the pressure of the teeth, plasticity to the pressure of the teeth, suitability for grinding in the mouth, juiciness of chewing.

**Taste and smell:** smell, flavour, salinity, sour taste, bitterness.

The water content (Table 3) was in range from 59.00% (producer II) to 53.10% (producer IV). Ganić *et al.* (2012) obtained significantly lower results of water contents in the samples of beef dried meat, that were ranged from 41.34% to 44.00%. Radovanović *et al.* (2003) obtained the average water content 54.19%; they observed significantly large fluctuations in the average water contents, that were in range from 44.36% to 60.41%. The lowest fat content (Table 3) was determined by the producer I (0.86%), and the highest fat content by the producer IV (5.11%). Stamenković *et al.* (2003) found that the fat content, under an intense fuming process of beef ham, was 1.00%, while under the mild, occasional smoking process the fat content was 1.50%. Radovanović *et al.* (2003) examined the chemical indicators of two groups of beef prshuta. In the first group, where the beef prshuta can be cut (water content 60.41%), fat content was 3.80, and in the second group, where the beef prshuta is very dry (water 44.36%), fat content was 5.79%. The composition and quality of fats significantly depends on the composition of the feedings of livestock, especially in the last phase of fattening. Ash content (Table 3) was ranged from 5.63% to 6.98%. Radovanović *et al.* (2003) report that the beef ham's samples, which can be cut, contained only 5.74% ash, while very dry samples contained 9.52. The highest protein content was determined by the producer III (35.39%), and the lowest by producer I (30.50%). Stamenković *et al.* (2003) determined the chemical composition of the beef dried meat and obtained the protein content in the range from 39.80% to 40.90%; Ganić *et al.* (2012) obtained that protein content in the samples of "Visočka pečnica" was in the range from 27.85% to 28.56%. Radovanović *et al.* (2003) obtained the protein content in the samples of beef dried meat was in the range from 30.13% to 39.49%. The content of sodium chloride (Table 3) was in the range from 4.35% to 5.73%. Radovanović *et al.* (2003) obtained lower content of sodium chloride (4.30% to 4.42%); Ganić *et al.* (2012) obtained significantly higher content of sodium chloride in the samples of beef dried meat, that was average 7.70%. The addition of sodium chloride is an initial barrier to the growth of undesirable microorganisms in the case of traditional production of cured meat products, when there is not nitrite added. The relatively high salt concentrations are required to inhibit microorganisms (Lilić *et al.*, 2013). Based on the results of sensory analysis all samples had acceptable quality provided that the pattern of producer I (Cazin locality Stijena) scored 28 of a maximum 35 points. In the group of sensory properties Appearance and color producer I had the highest number of points (70.83% of the maximum points) as well as for a group of sensory properties Texture (87.50% of the maximum number of points). In the group of sensory properties Taste and smell, producer III (municipality of Bosanska Krupa locality the Veliki Badić) won the highest overall rating (90% of a maximum 35 points). Radovanović and Stamenković (2004) found that the beef prshuta can be cut has a profile of a desirable sensory characteristics;

very dry beef prshuta has a black colour of cross-section with expressed salinity. Ganić *et al.* (2012) have performed sensory evaluation of dried meat produced by traditional crafts and industrial way of making, where they found that the samples from traditional production are better evaluated than industrial samples. The color of traditional process and the smell of industrial samples are best rated from sensory indicators. Appearance section in the traditional process and consistency for samples of industrial preparing are the worst-rated sensory parameters. During the examination of the sensory properties of beef ham, Robović *et al.* (2003) found that the external appearance is best rated with 77.33% of a maximum 35 points and the taste is the worst rated with 58.40% of the maximum points.

## CONCLUSIONS

Generally speaking, all producers are meeting the quality parameters in terms of chemical analysis and sensory evaluation of beef dried meat. Analyzing derived values results of chemical analysis, it can be concluded that the beef dried meat has plenty unbalanced chemical composition. Although the samples come from the same batch of meat troops, the technological process of standardization contributes to imbalances of chemical composition. Analyzing the results of sensory evaluation, the best overall score achieved is a producer I (Cazin, the locality of Stijena) with 28.00 points, followed by a producer III (municipality of Bosanska Krupa, the locality of Veliki Badić) with 27.10 points, while slightly less overall assessment are established for a producer II (Cazin, the locality of Glogovac) with 25.50 points and a producer IV (municipality of Bosanska Krupa, the locality of Pištaline) with 25.30 points. Taking into account the results of the chemical and sensory analysis of samples of beef dried meat, with a view to recommend a technological process (that would allow the most suitable quality of product) to local producers, we have decided for the manufacturing process of producer III (municipality of Bosanska Krupa, the locality of Veliki Badić). Although the pattern of producer III, by total number of points in the sensory evaluation, is ranked at the second place, high protein content of 35.39%, moderate fat content of 2.77% and water of 54.46% lead us to isolate this producer in relation to others. The recipe for salting and curing of producer III contained evidently lower salt (1.93%) in comparison to other producers, which was reflected in salt content in the final product (4.54%). Also, in a group of sensory properties Taste and smell, producer III had the highest number of points (90% of the maximum number of points). Results from this study may help in the standardization of quality beef dried meat as traditional cured meat products produced in the Una-Sana Canton.

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## ENCAPSULATION OF BIOACTIVE COMPOUNDS FROM BLACK SOYBEAN SEED COATS BY INTERNAL GELATION

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*Original scientific paper*

### Summary

Black soybean is widely used as a medicinal plant and healthy food especially in the Oriental medicine. In recent years, interest in bioactive compounds of the black soybean seed coats, mainly anthocyanins and proanthocyanidins, has been increased. These components have strong antioxidant characteristics which contribute to a positive effect on health and reduce the risk of chronic diseases. For food product is very important to preserve the stability of bioactive compounds. Encapsulation is a process that increases stability and preservation of bioactive compounds and their wide application.

The aim of this study was to preserve the bioactive compounds of black soybean seed coat by using internal gelation as encapsulation technique and to test the effect of different concentrations of carrier materials and active components on the forming of particles. In this research, 1.5 and 4 mass% solutions of alginate and pectin as carrier materials were compared. The effectiveness of forming particles was examined at mass concentration of the black soybean seed coats powder 10, 15, 20, 25 and 30 % w/w per weight of carrier material. The obtained particles were analyzed by optical microscope. Release study was determined at different pH value conditions by using pH differential method.

The best results were achieved with the portion of 20% of encapsulated powders of alginate and pectin. For those samples, the release profiles in water, simulated gastric fluid and simulated intestine fluid were monitored for 120 min. The results showed that the difference between carrier materials was not statistically significant. This indicates that the internal gelation technique is suitable for encapsulation of bioactive compounds from the black soybean seed coats and for achieving high portion of encapsulated components.

Encapsulation of bioactive components of black soybean seed coat can contribute to preserving the stability of bioactive compounds during processing and storage and to the development of new, functional products.

**Key words:** *black soybean seed coat, internal gelation, alginate, pectin, encapsulation*

### INTRODUCTION

Black soybean (*Glycine max* (L.) Merr) is a widely used in food industry and medicine of the Orient. Nutritive values and beneficial health effects of bioactive components concentrated in black soybean have become very interesting to researches. For distinct of yellow soybean, black soybean seed coat has a higher content of polyphenols, with anthocyanins and proanthocyanidins as predominant (Ito *et al.*, 2013).

Anthocyanins are natural pigments which give colour to the soybean seed coats and show beneficial properties such as anti-oxidative, anti-carcinogenesis and anti-inflammatory effects (Kim *et al.*, 2015). During storage, chemical and physical factors can cause changes in the quality of black soybeans such as decreasing of anthocyanins content and lower antioxidant capacity (Lee & Cho, 2012). The additions of anthocyanins into food products can significantly increase the value of products, but the instability of these bioactive compounds is limiting factor. So, it is very important to find solution to preserve stability and bioactivity and protect these compounds from external influences. For this purpose encapsulation can be used as useful tool (Nedović *et al.*, 2011).

Generally, encapsulation is a process to entrap bioactive compounds within carrier materials. In this way, active agents are completely covered and physical barrier is formed between active and environment (Nedović *et al.*, 2013). Encapsulation can be used in order to protect actives of undesirable environmental conditions (e.g. oxygen, moisture, or light) or interactions (e.g. with food matrix), preserve its stability during processing,

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prevent degradation, increase shelf-life, and achieve controlled delivery of encapsulated components. In shortly, encapsulation contributes to the stabilization and increase bioavailability of bioactive compounds, thus on this way compounds remain fully functional (Fang & Bhandari, 2010; Nedović *et al.*, 2013). Internal gelation also referred to as “internal setting” or “in situ gelation” can be used for the preparation of alginate and pectin gels cross linked with calcium ions. For the preparation of gels it is necessary to mix the carrier material solution with a water insoluble calcium salt, such as calcium carbonate. Principle of this process is cross linking which starts after dissolution of calcium carbonate and release of calcium ions by acidification. In that purpose,  $\delta$  - glucono - lactone can be used as a chemical compound able to lower the pH of carrier material. The internal gelation is suitable for the preparation of matrices for active agent release and it produces homogeneous gels (Marison *et al.*, 2004; Moreira *et al.*, 2014; Paques *et al.*, 2014). Anthocyanins from black soybean seed coats were encapsulated into alginate and pectin by electrostatic extrusion (Kalušević *et al.*, 2013) and wax particles (Salević *et al.*, 2015). According to our knowledge, there is no study about encapsulation of these soybean coats compounds by internal gelation.

In this study, the process of internal gelation was used for encapsulation of black soybean seed coats powder. Different concentration of carrier materials and black soybean seed coat were tested in order to establishing optimal formulation of encapsulate that provides protection and stabilization of bioactive compounds such as anthocyanins. The aim was to achieve high portion of encapsulated black soybean seed coats powder and to compare carriers' efficiency.

## MATERIAL AND METHODS

### MATERIAL

In this study seed coats of *Black Tokyo* variety were used (from genbank of Maize Research Institute, Zemun Polje, Serbia). Sodium-alginate and pectin were generously obtained from Palco (Šabac, Serbia). Calcium carbonate was provided from Zorka (Šabac, Serbia).  $\delta$  - gluconolactone was purchased from LG hemija (Belgrade, Serbia). Potassium chloride was procured from Centrohem (Belgrade, Serbia). Citric acid and sodium hydrogen phosphate were purchased from Hemos (Belgrade, Serbia). Potassium dihydrogen phosphate was provided by Fisher Chemical (Loughborough, UK), ammonium hydroxide, hydrochloric acid, and sodium carbonate were purchased from Merck (Darmstadt, Germany).

### DETERMINATION OF TOTAL ANTHOCYANINS

Anthocyanins of seed coat were extracted according to the method described by Žilić *et al.* (2013). The content of total monomeric anthocyanins was determined by pH differential method that is based on the structural transformation of anthocyanins in change in pH (Lee *et al.*, 2005). The monomeric anthocyanins are subject to structural transformation of the reversible as a function of pH, wherein at pH = 1 are in the form of intensely stained flavylium cation, and at a pH = 4.5 taking the form of a colorless hemiketal. The content of anthocyanins is determined by measuring the change in absorbance at two different pH values (pH = 1 and pH = 4.5), whereby the difference in absorbance of a pigment at a wavelength of 520 nm is proportional to the concentration of the pigment. The concentration of the monomer is expressed in anthocyanin cyanidin-3-glucoside according to the equivalents of the following formula:

$$\text{anthocyanin content (cyanidin-3-glucoside equivalents, mg/L)} = A \times MW \times DF \times 10^3 / \varepsilon \times l,$$

where  $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5}$ ; MW = molecular weight of cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in D; l = pathlength (1 cm);  $\varepsilon$  = molar extinction coefficient (26900), and  $10^3$  = conversion factor.

### ENCAPSULATION OF BLACK SOYBEAN SEED COATS ANTHOCYANINS

Previous to encapsulation, the seed coats were separated of the endosperm and obtained seed coats were ground on a lab mill (Perten120, Sweden) to fine powder.

Black soybean seed coats powder was homogenised with calcium carbonate in different ratios (10:1, 15:1, 20:1, 25:1, 30:1) and added into 1.5% w/w and 4% w/w solutions of sodium-alginate or pectin on magnetic stirrer (Velp, Scientifica, Italy).  $\delta$  - glucono - lactone was added with purpose of forming gels. The effectiveness and homogeneity of forming gels was examined at concentration of the black soybean seed coats powder: 10, 15, 20, 25 and 30% w/w. Only gels with highest concentration of homogeneously distributed powder (without sedimentation) were further characterized. The obtained gels were cooled at temperature of 5°C. After cooling,

gels were dried in dryer (Memmert, GmbH + Co. KG, Schwabach, Germany) at a temperature of 45°C for 2h and ground to powder. Morphological characteristics of the particles were observed using optical microscope Leica DC 300 (Leica microsystem, Weitzlar, Germany).

#### RELEASE KINETICS OF ANTHOCYANINS AT DIFFERENT pH VALUES

The experiment was carried out in distilled water and in the buffer solutions with pH values 1.2 and 6.8 (corresponding to the pH of the gastrointestinal tract). 15 mL of each buffer solution heated on 37°C (corresponding to the organism temperature) and 50 mg of encapsulated powder was added in the tubes. A change in colour of buffer solutions was observed in order to determine whether there is diffusion of anthocyanins in specified pH values during 2h.

The release of anthocyanins from particles was performed using a DFZ 60 flow-through cell dissolution tester (Erweka, Heusenstamm, Germany) under a constant medium flow rate at 1 mL/min. The release studies were done in distilled water (2h) and in pH conditions of gastrointestinal tract: 0.1M HCl solution (artificial gastric juice, 2h, pH 1.2); USP phosphate buffers (artificial small intestinal fluid, 2h, pH 6.8) (Čalija *et al.*, 2013). At scheduled time intervals, samples were withdrawn and assayed spectrophotometrically (UV/VIS double beam HALO-DB/2S, Dynamica, Switzerland) on 520 nm by above described pH differential method. All the assays were carried out at 37°C. The percent of anthocyanins released was plotted versus time.

### RESULTS AND DISCUSSION

#### DETERMINATION OF TOTAL ANTHOCYANINS

The average yield of the separated seed coat was 10% w/w in regard to whole black soybean seed. The content of total anthocyanins in black soybean seed coat powder was 1.18g CGE/100g<sub>dw</sub>.

The pH differential method is simple; therefore it had been used by many scientists to determine anthocyanin content in various samples, like black bean, purple wheat, and black soybeans. Astadi *et al.* (2009) investigated *Cikuray* and *Mallika* varieties of black soybeans. In their research insignificantly different content of anthocyanins in seed coats has been found 1.45g/100g and 1.36g/100g, respectively. Žilić *et al.* (2013), in their investigations have found high content of anthocyanins in *Black Tokyo* variety, 1.65g CGE/100g. In recent years, increased attention has focused on the possible health effects of naturally occurring phenolics and anthocyanins.

#### ENCAPSULATION OF BLACK SOYBEAN SEED COATS ANTHOCYANINS

Powders containing bioactive compounds of black soybean seed coats were completely encapsulated into alginate and pectin gels by internal gelation (Figure 1). The highest concentration of black soybean seed coats powder encapsulated into the homogenous gels that was achieved, in case of both carrier materials, was 20% w/w. Higher concentration have given inhomogeneous gels with precipitated powder. The lowest concentration of black soybean seed coats powder encapsulated and homogeneously distributed in alginate and pectin gels was 10% w/w.

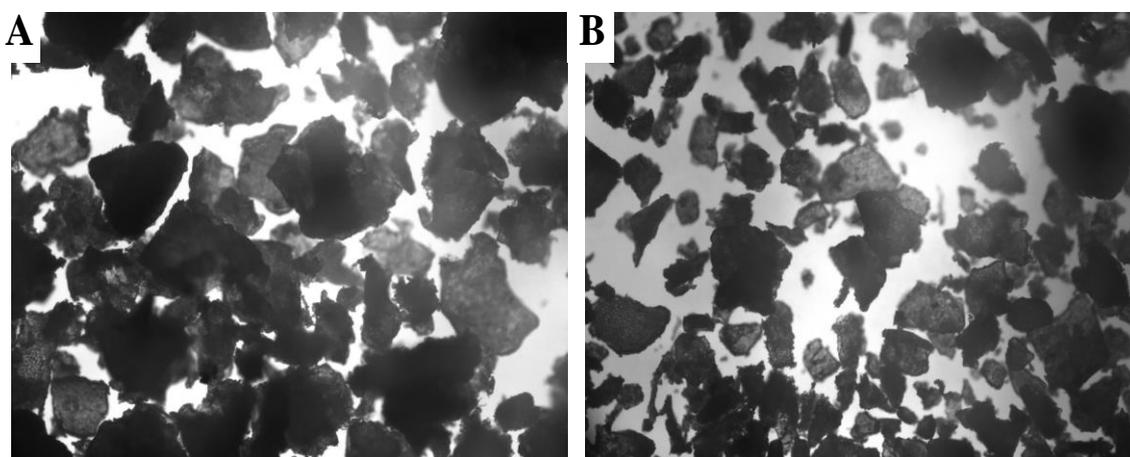


Fig. 1. Dry particles obtained by internal gelation using alginate (A) and pectin (B) as carrier material

Also, different concentrations of carrier materials (1.5 and 4 % w/w) did not have impact on particles formation. Related to these results, release studies with the alginate and pectin (1.5% w/w solutions) with 20% w/w of black soybean seed coats powder were done in order to compare carrier materials.

#### RELEASE KINETICS OF ANTHOCYANINS AT DIFFERENT pH VALUES

Release kinetics showed no statistically significant difference between the alginate and pectin particles during two hours of monitoring. Results given at a Figure 2 indicated prolonged release of dried alginate and pectin encapsulates of anthocyanins. As can be seen, the slowest release is achieved in water which is very important for application in different food products.

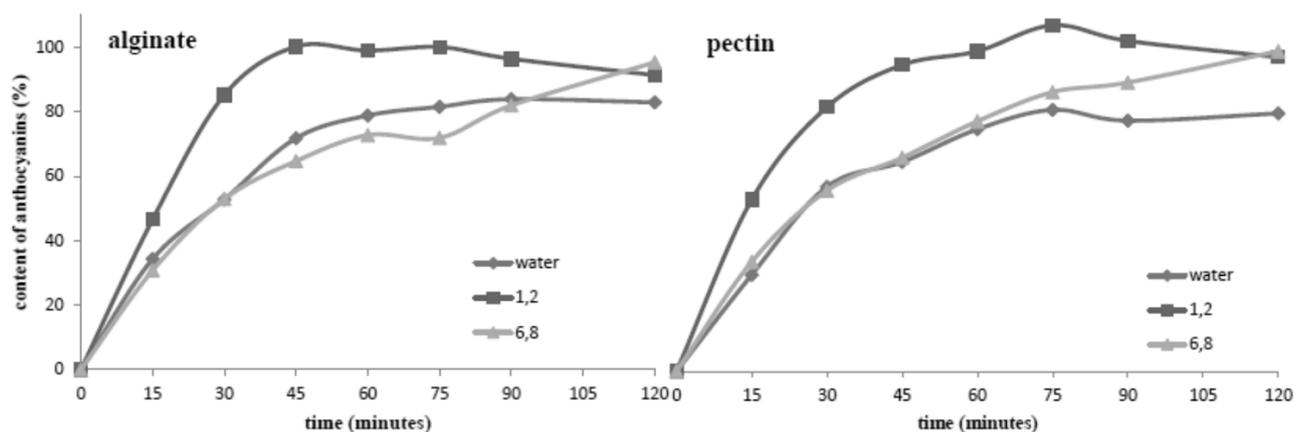


Fig. 2. Release of anthocyanins from alginate and pectin particles at different pH conditions

There are no similar studies with this technique and release of anthocyanins. Still, some authors encapsulated anthocyanins (of blackberry) by thermal gelation (Ferreira *et al.*, 2009). The release profiles of their gels with anthocyanins showed much stronger burst effect than in our study. To specify, the anthocyanins were completely released (100%), after 20 minutes, while in our research after same period of time about 35% (in pH 6.8 and water) and 50% (in pH 1.2) of total anthocyanins were released (Figure 2).

On the other hand, Oidtmann *et al.* (2012) found that bilberries anthocyanins released from pectin capsules within 10-20min in SGF conditions, in comparison with SIF where release reached 64% after 20 minutes. Monitoring of the release of black soybean coats anthocyanins at pH values 6.8 showed that efficiency was acceptable high by this technique and these carrier materials.

However, results of the release at pH 1.2 have given reasons for the improvement in terms of used material for protection of active components in stomach conditions.

#### CONCLUSIONS

Black soybean seed coat is a rich source of bioactive compounds and its bioactivity can be demonstrate and maintain thanks to encapsulation.

In this study, convenient way of internal gelation with using alginate or pectin as carrier material to achieve high portion of encapsulated black soybean seed coats powder was confirmed. Maximum achieved concentration of encapsulated black soybean seed coats powder was 20% w/w. The same results were achieved in terms of portion of encapsulated black soybean seed coats powder regardless whether alginate or pectin was used as carrier material. This approach can contribute to protection and preservation of valuable and sensitive bioactive compounds such as anthocyanins, i.e. provide their more complete action.

Encapsulated black soybean seed coats powder is partially protected from influences of different pH values which could contribute to a development of new, functional products enriched with these bioactive colourants. Since the investigated systems do not appear to be able to fully prevent early release into the SGF, further optimization is required. Nevertheless, the results clearly show the potential utility of encapsulating anthocyanins as colourants, as well as bioactive compounds in food technology.

## ACKNOWLEDGEMENTS

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## MICROENCAPSULATION OF HERBAL EXTRACT BY SPRAY DRYING

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*Original scientific paper*

### Summary

The polyphenolic compounds naturally present or integrated in food staff have great importance due to their bioactive and antioxidant characteristics. On the other hand, those compounds are very unstable and under certain conditions could lose their active properties. Microencapsulation is one of the approaches to protect and improve stability of these compounds.

In this study, the herbal extract were obtained by aqueous extraction of 12 medicinal and aromatic plants. These plants have very pleasant sensory characteristics and represent a source of many bioactive compounds.

The aim of this study was to produce microcapsules preserving polyphenolic compounds of medicinal herbal extract using spray drying technique and maltodextrin as carrier material. The influence of three different inlet air temperatures (140°C, 160°C, 180°C) on the content of bioactive compounds of extract was observed in order to optimize the process of spray drying. The content of total polyphenolic compounds was determined by the Folin-Ciocalteu method and FRAP method is used to determine antioxidant capacity of extract and the obtained powders. The water activity of microencapsulates was determined by  $a_w$  meter.

The results of this study showed that using spray drying as microencapsulation technique and maltodextrin as carrier material could provide significant preservation of polyphenolic content as well as antioxidant capacity of the plant extracts. The inlet temperature that provides optimal encapsulation was found to be 140°C while  $a_w$  values in the case of all encapsulates were less than 0.32.

The obtained extract may be used for the development of natural soft drinks with improved bioactive properties.

**Key words:** *medicinal herbs, extract, spray drying, microencapsulates, polyphenols, antioxidant capacity*

### INTRODUCTION

Medicinal and aromatic herbs contain a wide range of bioactive compounds such as alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones, oils, flavours, and pigments. Recently, investigations on herbal food supplements medicinal products, and phytochemicals, have been intensified (Chen *et al.*, 2007). In addition, infrequent compounds like furanocoumarins, hydroxycoumarins, naphthoquinones, acylphloroglucinols and sterols are distributed among these herbs, as well (Saroya, 2011). Medicinal herbs are a superb source of phenolic compounds which determine their considerable role in the prevention of numerous degenerative diseases (Capecka *et al.*, 2005). Apart from a physiological role, phenolic compounds have a significant influence to sensorial properties of herbal products, such as appearance, taste, mouth-feel, fragrance, astringency and bitterness. Phenolic compounds are generally considered as one of the main antioxidant sources in herbs, and antioxidant capacity is highly correlated with the total phenolic content (Jaganath *et al.*, 2010).

Medicinal and aromatic plants are widely used in the food, pharmaceutical and cosmetic industries in different forms. However, the most common and the most convenient form are powders (Wang and Weller, 2006).

There are several encapsulation techniques that can improve stability of herbal extracts – freeze-, spray-, vacuum-drying, electrostatic extrusion, etc (Nedović *et al.*, 2011; Kalušević *et al.*, 2012a; Isailović *et al.*, 2012; Kalušević *et al.*, 2012b).

Nevertheless, spray drying is the most widely used method of microencapsulation. This technology offers a high production rate, lower operating costs in comparison with others, stable and easily applicable powders (Nedović *et al.*, 2013). Spray drying microencapsulation of herbal extracts has been providing higher powder and polyphenolic yield by employing certain carrier materials, in comparison to drying plain herbal extract (Belščak *et al.*, 2014). Maltodextrin has been widely used as carrier for spray drying. Maltodextrin, a

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hydrolyzed starch, is a popular material in encapsulation by this technique. It offers advantages such as low cost, neutral aroma, and taste, low viscosity; protection against oxidation etc (Đorđević *et al.*, 2014). The aim of this study was to compare different conditions of spray drying process using maltodextrin as a carrier material in term of antioxidative capacity and content of polyphenols of obtained herbal extract powders.

## MATERIAL AND METHODS

### PREPARATION OF HERBAL EXTRACT

The aqueous extraction of following mixture of 12 medicinal and aromatic plants was performed: peppermint (*Mentha piperita*), chamomile (*Matricia chamomilla*), wild thyme or creeping thyme (*Thymus serpyllum*), mountain germander (*Teucrium montanum*), winter savory (*Satureja montana*), common yarrow (*Achillea millefolium*), common sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*), centaurea (*Erythraea centaureum* Pers.), wall germander (*Teucrium chamaedrys*), stinging nettle (*Urtica dioica*) and wormwood (*Artemisia absinthium*). The extraction of herbs was carried out in glass bottles which were placed on shaker in dark place at room temperature. Air-dried plants or its parts were cut into small pieces and placed into bottles. After that, the water was poured over the plants. The plant materials-water ratio was 1:8. Temperature of water was 80°C, while extraction time was 24 hours.

### PREPARATION OF FEED MIXTURE

The feed mixture was prepared by mixing the maltodextrin into herbal extract. The preparations were carried out under continuous magnetic stirring at ambient temperature until the final content was adjusted to 10% w/v. In this study, maltodextrin in concentrations of 10% in all samples was used in order to avoid stickiness and low powder yield. This is confirmed by Sansone and the coauthors who observed that during the spray drying of different extracts of aromatic plants, using maltodextrin in concentrations lower than 10%, the stickiness of the most material to the drying chamber wall occurs and results in a low production yield (Sansone *et al.*, 2011).

### ENCAPSULATION BY SPRAY DRYING

The feed mixture was fed into a mini spray drier BUCHI B-290. The flow rate was maintained at 8 mL/min. The inlet air temperature of the spray dryer was 140, 160 and 180°C.

### DETERMINATION OF TOTAL POLYPHENOLS

The amounts of total polyphenols content (TPC) in extracts were determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.25 mL of diluted extract powders were mixed with 1.25 mL of 10-fold diluted Folin-Ciocalteu's phenol reagent and allowed to react for 5 minutes. One milliliter of sodium carbonate solution (75 g/L) was added to the mixture and then shaken. After 2 h of reaction at room temperature, the absorbance at 760 nm was measured. The calibration curve was prepared with gallic acid solution, and the results were expressed as milligrams of gallic acid equivalents per liter of sample (mg GAE/L).

### FRAP ASSAY

The FRAP assay was performed according to the procedure previously described by Benzie and Strain, with some modification (Benzie and Strain, 1996). The FRAP reagent solution was made by mixing acetate buffering agent (pH=3.6), TPTZ (10 mM TPTZ solution in 40 mM HCl) and FeCl<sub>3</sub> x 6H<sub>2</sub>O in volume ratio 10:1:1, respectively). All samples, standards and reagents were pre-incubated at 37°C. An aliquot of each diluted extract powder sample (0.1 mL) was mixed with distilled water (0.3 mL) and FRAP reagent (3 mL). After the reaction at 37°C for 40 min, the absorbance at 593 nm was measured. The calibration curve was prepared with Trolox solution and the results were expressed as mol of Trolox equivalents per liter of sample (mmol TE/L).

## RESULTS AND DISCUSSION

### ENCAPSULATION BY SPRAY DRYING

When the inlet air temperature increased from 140 to 160°C, the powder yield decreased by about 6% (Table 1). This can be attributed to partially sticking of the insufficiently dry particles on the dryer walls at higher temperatures e.g. 160°C. Very similar effect is noticed by Nadeem and coauthors during research about spray drying of mountain tea at different temperatures (Nadeem *et al.*, 2011). In the production of *S. montana* dry powder by adding maltodextrin in amount of 10%, production yield was about 66% (Vidović *et al.*, 2014)

Decreased product yield by increasing the air inlet temperature was also reported by Chegini and Ghobadian (2007) in case of spray drying of orange juice.

The water activity of the samples decreased insignificantly with higher inlet air temperatures (all samples had  $a_w$  values about 0.30). By comparison, the water activities of the instant mountain teas spray dried in temperature range 145-165°C were in the range of 0.258-0.402 for the control and the carrier added samples, respectively.

Tab. 1. Parameters of spray drying process and obtained powders

Inlet T (°C)	Outlet T (°C)	Yield (%)	Water activity
140	~70	81.1	0.32
160	~82	75.2	0.31
180	~92	76.7	0.30

#### DETERMINATION OF TOTAL POLYPHENOLS

The major aim in the design of a spray drying of herbal extract is to set up process conditions which will lead to the production of a high quality and effective powder, with the highest concentration of polyphenol compounds.

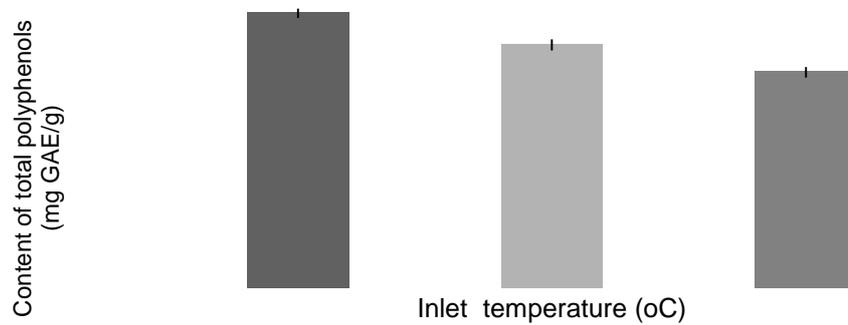


Fig. 1. Content of total polyphenols in powders obtained by drying at different temperature

Therefore, the efficiency of the spray drying of the herbal extract using different temperatures was investigated. Regarding the content of beneficial health compounds it can be concluded that the content of total polyphenols decreases with the increase of inlet temperature (Figure 1.). Same conclusion has been confirmed by Nadeem and coauthors (2011). They reported that increase in the inlet air temperature to 165°C decreased phenolic content slightly. Also, has been reported a slight decrease from 11.75 mg/g to 10.96 mg/g in the total polyphenol content of the spray-dried soybean extract by increasing the inlet air temperature.

#### FRAP ASSAY

Investigation of the influence of inlet temperature on antioxidant properties showed that by increasing of temperature decreases antioxidant potential of the powders. The same powder showed the highest total polyphenol content had the highest antioxidant activity (Figure 2.).

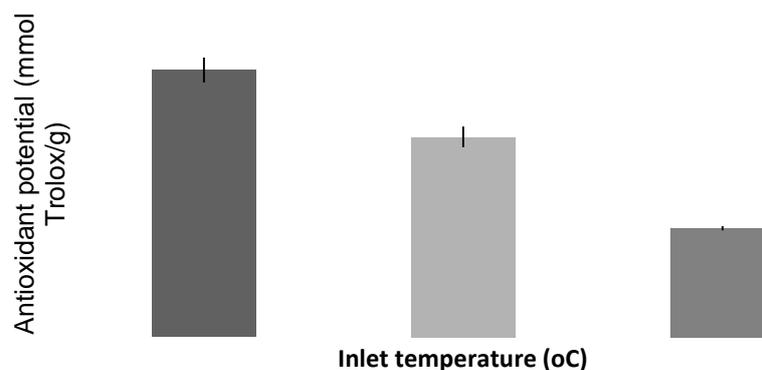


Fig. 2. Antioxidant potential of herbal extracts spray dried at different temperature

Between the results of the total polyphenol content and antioxidant potential of the samples there was a significant positive correlation (0.996).

## CONCLUSIONS

Maximum production capacity is aspiration on an industrial processing level. Therefore, the analysis of production yield and efficiency, as well as the analysis of the appearance of phenomena such as wall depositions or stickiness have great importance. The obtained results suggest that the yield, TPC and antioxidant activity was the highest for sample of the extract powder produced by drying at 140°C. There were statistically insignificant differences between yields of samples dried at 160 and 180°C. Powders, especially those produced at 140°C, are very rich source of phenolic compounds with considerable antioxidant activity. Therefore, these powders of extracts are a very good ingredient for development of different types of food or drinks with increased functional value.

## ACKNOWLEDGMENTS

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## **SPELT (*Triticum spelta* L.) - HEALTHY FOOD**

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*Professional paper*

### **Summary**

Spelt (*Triticum spelta* L.), also known as dinkel wheat or hulled wheat, is one of the oldest known grains and it originates from Asia. It was known even to the Egyptians, the oldest findings of this type of wheat were found in the Nile valley and they date to the fourth millennium BC. This edible, chaff-like, type of wheat was used for food by the ancient Romans who grew it throughout the empire. This cereal was very important from the Bronze Age to the Middle Ages in Europe. However, in recent times it has largely been replaced by other types of wheat (*Triticum aestivum* and *Triticum durum*). This grain has again started attracting attention in the past two decades because of the possibility of organic breeding, since it requires lesser use of fertilizers and pesticides compared to the common wheat. Due to the increasing awareness of consumers who pay more attention every day to the variety and quality of food, spelt is increasingly gaining importance with the tendency of multiplying sown areas so the goal of this study was to determine the nutritional characteristics of spelt shelled beans of studied varieties Bc Vigor and Ostro (water content, ash, starch, protein and crude fat) for nutritional use.

Key words: *spelt, nutritional characteristics, nutrition*

### **INTRODUCTION**

According to the systematic affiliation spelt belongs to the family *Poaceae* (grass), subfamily *Pooideae* (*Class grass*), genus *Triticum* (wheat). As a botanical form spelt belongs to annual crops, has a typical autogamous pollination and is mostly a winter crop. It contains three genomes A, B and D each of seven different chromosomes of phylogenetic origin. Unlike the common wheat spelt belongs to the chaff-like-form with fragile class spindle. The product of harvests are spikelets that usually contain two grains tightly close in glumes, to a lesser extent, as with the common wheat, fully harvested grain (caryopsis) is also included. Solid glumes (*glumae*) or chaff protect the floral organs and grains that are being developed within the thinner, softer and bright flowering glumes (*Palea inferior*) and palea (*Palea superior*). The base of harvested spikelets is made of class spindle joint segment. The break of class spindle is caused by its structure. The upper, broad, part of a class spindle joint has conducting bundles only in peripheral tissue, while the interior is filled with white loose parenchyma, which in ripe condition gradually rots. Due to thinning of tissue class spindle joints in the phase of full ripening break and separate easily. The break of class spindle causes attrition and therefore the spelt harvest procedure must conform to the basic requirements of plants (Mlinar and Ikić, 2012). Due to the difficult harvests and additional process of peeling, in which chaff and glumes are removed and thus grain is prepared for grinding, already in medieval Europe, farmers have abandoned production of spelt in favor of Rodney *Triticum aestivum* wheat. But today such act showed a good side, because the chaff and grain glumes protect against negative influences, disease and pest attacks, especially in unfavorable conditions for germination.

It is also not necessary to treat the grains against diseases (Kling, 1989). If glumes are removed immediately before processing, the preservation of nutrients is provided, while the grain remains fresh. Spelt has modest demands when it comes to climate, soil conditions, and the agro-technology, and since it is pest and disease resistant, it is also suitable for organic production. Organic production of cereal crops is based on the use of crop rotation with greater part of legumes, crop remains, the green fertilization, the use of microbiological preparations, mechanical cultivation and biological diseases control, pests and weeds (Kovačević, 2010). Greater choices and acceptability of cultivation system based on the lower external investment imposes as the perfect solution for improving the economic and environmental problems and human health who have arisen from the conventional system, as suggested by Liebman and Davis (2000).

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By creating high-yielding varieties of common wheat this very important species has almost perished and was maintained only in gene banks worldwide. However, it was started grown again in the seventies of the last century thanks to the environmental awareness development in Switzerland, then in Austria, and later in other developed countries of Western Europe and North America (Dolijanović *et al.*, 2012). The reason is growing demand for food products made by technology applicable to sustainable and organic agriculture. Nutritional and medicinal properties of dehulled spelt grain have proven almost ideal to fulfill this goal (Lacko - Bartošova *et al.*, 2010; Reisen *et al.*, 1986). Spelt nutritional value is similar to wheat nutritional value. It contains proteins, unsaturated fats, vitamins (A, C, B group), minerals (calcium, cobalt, iron, phosphorus, magnesium, manganese, potassium, copper, selenium, sodium) and fibers (Dolijanović *et al.*, 2012). It also retains a high antioxidant properties (selenium prevents the formation of free radicals) and therefore is especially suitable for people exposed to stress, for menopausal women and the elderly. Spelt is of great use in diets for weight loss (it quickly gives a feeling of satiety and facilitates the work of the intestine) since it is rich in fiber, and because of the richness in carbohydrates, iron and calcium it is recommended to athletes, anemic people and those suffering from osteoporosis. The unique nutritional and rheological properties of these grains tend to increase the nutritional value of bakery products. Spelt is a very resistant crop suitable for organic farming. It is possible to increase the diversity of grains in the human diet by including spelt in organic production (Mlinar and Ikić, 2012). Today you may find more products on the market containing spelt flour to a greater or lesser extent, and these are primarily bread, pasta and biscuits, cakes, pretzels and cereal. Except for the production of flour, spelt is used for the production of malt to make beer, as a coffee substitute or as fried or dried "Grunkern" grain in the milk ripening. It is also used as an addition to soups, stews and other dishes. Although it contains gluten, sometimes people allergic to products of common wheat tolerate the use of it. In order to increase its beneficial effects, it is recommended to be used in the germinating state (Dolijanović *et al.*, 2012).

The aim of this paper is to determine the nutritional characteristics of spelt grain of two studied varieties in natural patterns.

## MATERIALS AND METHODS

The research was conducted in 2014 at the Laboratory of the Department for Agricultural Technology, Storage and Transport, University of Zagreb, Faculty of Agriculture on the shelled grain, chaff and glumes and stems of two varieties of spelt (Bc Vigor and Ostrao). These varieties of spelt were grown on the experimental station of Department for Special Plant Production, University of Zagreb Faculty of Agriculture. The study used two varieties of spelt: Bc Vigor and Ostro. The grain is separated from the chaff and glumes and samples of hulled grain, chaff and glumes, and stems of each variety separately are prepared. Samples were homogenized, i.e. crushed in a laboratory mill.

According to standard methods these factors were determined: the content of water in a laboratory dryer (INCO ST - 40, Croatia) (ISO 6540: 2002), the ashes in the muflon oven Nabertherm B170 (Lilienthal, Germany) (CEN / TS 14775: 2004), starch-polarimeter (KRÜSS, P3001, Germany) (ISO 6493: 2001), the crude protein on Kjeldahl digestion unit K 12 (Behr Labortechnik GmbH, Germany) (ISO 1871: 1999) and crude fat in a Soxhlet extractor R 304 (Behr Labortechnik GmbH, Germany) (ISO 6492: 2001).

## RESULTS AND DISCUSSION

Table 1 shows the nutritional characteristics of the spelt grain: initial moisture in natural patterns of peeled grain Bc Vigor variety and Ostro variety, ash content, starch, fat and protein.

Tab. 1. Nutritional characteristics of spelt grain of two studied varieties in natural patterns

Variety	Water (%)	Ash (%)	Starch (%)	Fat (%)	Protein (%)
Bc Vigor	13.783	2.336	50.346	2.301	24.879
Ostro	13.217	2.462	50.368	3.986	20.950

The water content of the examined spelt varieties ranged from 13.217% (Ostro) to 13.783% (Bc Vigor), which is greater than the value obtained in the study of Bojnanska and Francakova (2002) where the average water content in spelt was 9.03%, but grain moisture depends on the year.

Ash content is determined by burning the samples of analyzed material of known mass and measuring the remains where organic substances in the sample burn out, and mineral matter falls behind, i.e. ash that is actually an inorganic residue after biomass burning. Ash content in the grain of Bc Vigor variety was 2.336%, it was slightly higher in the grain of Ostro variety (2.462%). Similar results were obtained by Bojnanska and Francakova (2002), in whose study the ash content in spelt grain was 2.10%.

Starch is a polysaccharide of vegetable origin, and in the body is decomposed to monosaccharides. It is the most important of the carbohydrates in the diet, and is found in fiber (cellulose and pectin) (Carlson, 1993). Spelt grain, unlike other grains such as rice, rye, oats, corn, wheat, millet, buckwheat, containing less starch. In the investigated sample, starch content among varieties Bc Vigor and Ostro did not differ as the Bc Vigor variety's starch content was 50.346%, and Ostro variety's was 50.368%. The results obtained in both cultivars show that spelt buckwheat contains a small amount of starch as opposed to, for example, wheat in which, according to Pospišil (2010), the amount of starch in the grain can vary from 49 to 73% depending on the variety and growing conditions.

The proportion of fat in dry matter for two studied spelt varieties was also measured in the lab. According to Bavec and Bavec (2007) spelt grain contains 1.5 to 4% fat, which corresponds to the values obtained in this investigation. Fat content calculated in dry matter for the Bc Vigor variety was 2.301%, while the fat content in Ostro variety was higher, 3.986%, which also depends on the year. If the year is more humid, the percentage of oil is lower and vice versa.

The crude protein content calculated in dry matter in Ostro variety was 20.950%, while the crude protein content in Bc Vigor variety grain is higher and amounts to 24.879%. Bojnanska and Francakova (2002) report that spelt contains more protein (16 - 17%) in comparison to wheat. According to Bavec and Bavec (2007) spelt seed contains: 13.9 to 16.7% protein, while the newer varieties of spelt have a very high protein content, which for some may be higher than 20% (Dolijanović *et al.*, 2012).

## CONCLUSION

Spelt is an old and almost forgotten culture, to which a growing number of producers and consumers are coming back because there is a great potential to expand the cultivation of this crop because of its nutritional and energy value, and because of favorable climatic conditions for growing throughout Croatia. By including spelt in organic production it is possible to increase the variety of grains in the human diet. Because of increasing awareness of consumers who pay more attention to the variety and quality food every day, spelt is increasingly gaining in importance and there is a tendency to increase sowing areas.

Based on the research, from the results obtained from the peeled grain, chaff and glumes and stems of two different spelt varieties: Bc Vigor and Ostro, following can be concluded:

The content of starch in the spelt grain spelled of Bc Vigor variety was 50.346%, and of Ostro variety was 50.368%. The results obtained in both cultivars show that spelt grain contains a very small amount of starch, unlike other grains such as rice, rye, oats, corn, wheat, millet, buckwheat.

Spelt grain also contains a high proportion of protein. The crude protein content of Ostro variety was 20.950%, while in the grain of Bc Vigor variety was higher and amounts to 24.879%.

The content of fat in the spelt grain of Bc Vigor variety was 2.301%, while the fat content of Ostro variety was higher and amounted to 3.986%.

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## EFFECT OF RIPENING PERIOD AND DIFFERENT MEAT QUALITY ON THE PHYSICO-CHEMICAL PROPERTIES OF „BARANJA KULEN“

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*Original scientific paper*

### Summary

The aim of this study was to determine the effect of ripening length, different recipes (traditional and industrial) and different meat quality on the physico-chemical properties of the Baranja kulen. Fat content (after 3 months 8.93% and at 6 months 12.61%) and protein content (after 3 months 35.83% to 41.16% after 6 months of ripening) increased. The water content after 6 months of ripening was 37.57% and decreased in comparison to kulen after 3 months of ripening (46.35%). Different meat quality influenced the color of kulen. Kulen with meat labeled B (pale meat) had the highest L\* value and kulen meat labeled A (normal colour) had the highest a\* value. The b\* value was the highest in kulen with meat labeled B. Traditional kulen had a slightly higher average pH 5.67 than industrial kulen whose pH value was 5.27.

Keywords: *Baranja kulen, ripening, meat colour, physico-chemical properties*

### INTRODUCTION

Kulen is a dry fermented salami produced in the winter in Croatia (Slavonia and Baranja), northern Serbia (Srem, Backa) and Hungary. It is produced according to traditional procedure from minced pork meat and fat with the addition of salt, red sweet paprika, red hot paprika and garlic. Production process involves fermentation, ripening and drying with or without smoke. Final product contains a maximum of 40% water and at least 16% of protein (Benčević and Petričević, 1999). The aim of this study was to determine the effect of ripening length (3 and 6 months), different recipes (traditional and industrial) and different meat quality on the physico-chemical properties of the Baranja kulen. Content of water, protein, fat, salt and ash, as well as water activity ( $a_w$ ), pH and color of kulen were determined.

### MATERIALS AND METHODS

24 samples of Baranja kulen were analysed for physico-chemical properties: 12 samples with different recipes (traditional and industrial) at 3 months of ripening and 12 samples with different recipes (traditional and industrial) at 6 months of ripening. In table 1 recipe for traditional and industrial production of Baranja kulen are shown. Meat was classified according to the colour of the meat (L\* values) and labelled with letters A (L\* 33.90-46.49), B (L\* 47.26-56.30) and AB. Meat labelled A was normal meat colour and meat labelled B was pale. Meat labelled with AB was a mixture of meat A and B in ratio 50:50. For the production of Baranja kulen partially defrosted meat from pig's *biceps femoris* ( $\approx$  54 kg) and fat ( $\approx$  6 kg) was used. Colour was measured before defrosting. Total primary and secondary raw materials and other materials required for the production of kulen were obtained from a local meat processing industry. Meat and fat were minced and homogenized for 10 minutes with spices and additives. Then the mixture was stuffed in pig's appendix, which was then hand-tied around a rope, hung on rods and transported to the chamber for smoking and drying. Time of ripening was 3 and 6 months.

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Tab. 1. Recipes for traditional and industrial production of Baranja kulen

Traditional kulen		Industrial kulen	
NaCl	20 g	NaNO <sub>3</sub> + NaNO <sub>2</sub>	max 1.5 g
Red sweet paprika	80 g	NaCl	20 g
Red hot paprika	60 g	Dextrose	30 g
White pepper	10 g	Sodium isoascorbate	max 15 g
Garlic powder	15 g	Starter - Culture	1.25 g
Saccharose	≈10 g	Red sweet paprika	80 g
NaCl	20 g	Red hot paprika	60 g
		White pepper	10 g
		Garlic powder	g

Fat, protein and ash contents were estimated according to methods recommended by the AOAC (AOAC, 1999). Water content and sodium chloride were determined according to AOAC methods (AOAC, 1984). Two replicates of each sample were analysed and the mean value was used in the data analyses. Water activity ( $a_w$ ) was determined with a precision multi-function measuring instrument, Testo 650 (Testo Inc., New York, USA). Two replicates of each sample were analysed and the mean value was used in the data analyses. Colour measurements were carried out with a Minolta CM-3500d (Osaka, Japan) spectrophotometer in the CIE L\*a\*b\* space: lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) (CIE, Commission Internationale de l'Eclairage, 1976). The pH was determined by 704 pH Meter, Metrohm, glass electrode 6.0236.100. Samples of kulen were homogenized and mixed with water in ratio 1:4. pH was directly measured by inserting electrode in the mixture.

## RESULTS AND DISCUSSION

In table 2 physico-chemical properties of Baranja kulen with different meat quality are shown. Meat quality had no effect on fat, protein and water content. Protein content of kulen labeled A was 37.70%, B 38.75% and AB 39.03%. Kulen labelled with B had the highest  $L^*$  value (lighter) while kulen labelled with A the highest  $a^*$  value.  $b^*$  value was the highest in kulen labelled B (18.70) while kulen labelled with A (15.12) and AB (16.52) had lower values. Regarding pH value, kulen labelled A had a pH value 5.69 B pH 5.14 and AB 5.51. Different meat quality did not affect  $a_w$  value and was 0.85.

Tab. 2. Physico-chemical properties of Baranja kulen with different meat quality

Parameter	Meat quality		
	A	B	AB
Fat	10.16±2.26	11.20±2.06	10.95±2.09
Protein	37.70±2.85	38.75±3.45	39.03±3.38
Ash	7.12±1.00	6.92±0.77	7.15±0.69
Water	41.93±4.66	41.87±4.51	42.08±5.74
NaCl	5.63±0.95	5.45±0.69	5.45±0.60
$a_w$	0.85±0.03	0.85±0.03	0.85±0.03
$L^*$	34.60±3.42	38.16±3.56	36.03±3.53
$a^*$	21.51±1.27	19.20±3.11	17.85±2.05
$b^*$	15.12±3.76	18.70±3.06	16.52±3.33
pH	5.69±0.36	5.14±0.20	5.51±0.28

In table 3 effect of different recipes and ripening period on chemical composition of Baranja kulen are shown. The average water content of kulen with traditional recipe was 42.93%, and for industrial 40.99%. Protein content for traditional recipe was 37.52% and industrial 39.47%. The water content after 6 months decreased (37.57%) with respect to a portion after 3 months (46.35%). The protein content (after 3 months 35.83% and

after 6 months 41.16%) and fat content increased (after 3 months 8.93%, and after 6 months 12.61%). Different recipes and ripening period did not have an effect on ash and NaCl content.

Tab. 3. Effect of different recipes and ripening period on chemical composition of Baranja kulen

	Fat	Protein	Ash	Water	NaCl
Recipes					
Traditional	11.01±2.04	37.52±2.98	6.99±0.88	42.93±4.89	5.38±0.86
Industrial	10.53±2.25	39.47±3.21	7.13±0.77	40.99±4.79	5.64±0.60
Ripening period					
3 months	8.93±1.18	35.83±1.82	6.38±0.44	46.35±2.25	5.04±0.45
6 months	12.61±0.96	41.16±1.75	7.75±0.44	37.57±1.90	5.98±0.69

Similar results were obtained in other types of sausages. The average value of water content in a traditional Chorizo de cebolla sausage was 32.20% and 29.76% in industrially produced (Salgado *et al.*, 2006). In the study of Moretti *et al.* (2004) on the typical Sicilian sausages water content in traditional sausage was 26.8%, and industrial 29.8%. The result are linked with higher temperatures and lower RH value in traditional ripening compared to the ripening in controlled atmosphere. Fat content of pork meat most varies and depends on the sex of the animal, the proportion of body fat, slaughtering weight, anatomic location, et al. (Inmaculada *et al.*, 2002). The high fat content of fermented sausages at the end of ripening is necessary for sensory properties such as hardness, juiciness and flavor (Olivares *et al.*, 2011).

In table 4 effect of different recipes and ripening period on color, pH and  $a_w$  of Baranja kulen is shown. Different recipes did not had effect on  $L^*$   $a^*$   $b^*$  value.  $L^*$  value after 3 months of ripening was 39.46 and after 6 months decreased at 33.06.  $a^*$  valued also decreased and after 3 months of ripening (20.52) and after 6 months of ripening (18.52).  $b^*$  value decreased with the length of ripening. After 3 months was 19.16 and decreases to 14.39 after 6 months of ripening. Kulen with traditional recipe had a slightly higher pH value (5.67) than that industrial (5.27). Different recipes had no significant effect on  $a_w$  value and was around 0.85. The average pH after 3 months of ripening was 5.27 while after 6 months 5.66. Water activity after 3 months of ripening was 0.87 and 0.82 after 6 months.

$L^*$  value of the Turkish fermented sausages soudjouk was 38 at the beginning of ripening, and has decreased during the ripening process at the 28. Decrease of  $L^*$  value represents a darker color due to browning reactions. The  $a^*$  value increased from 8.5 to 11 during the first 5 days of maturation, and by the end of the production process decreased to 7.9. During the first days of ripening nitrogen compounds are combined with myoglobin to produce a desirable red color of the product. After this period begins denaturation of formed pigment and results in lower  $a^*$  value.  $b^*$  value is also reduced from 13.1 to 7.7 during production process (Bozkurt and Bayram, 2006). Decrease of the  $b^*$  value is a result of microbial activity (Perez-Alvarez *et al.*, 1999).

Water activity is the most important for controlling spoilage because of its reduced value prevents the growth of most bacteria ( $a_w < 0.91$ ) and moulds ( $a_w < 0.80$ ) (Leistner, 1991). Due to the low water activity Baranja kulen can be kept at room temperature and after 3 months of ripening becomes bacteriological stable product. The average  $a_w$  value of traditional Spanish Chorizo de cebolla sausage did not differ from industrial and was 0,84; while the pH value of the traditional sausage was 4.74 and industrial slightly lower (4.48) (Salgado *et al.*, 2006). This is in accordance with the results in this study where traditional kulen also had a slightly higher pH value in relation to the industrial and  $a_w$  value did not differ.

Tab. 4. Effect of different recipes and ripening period on color, pH and  $a_w$  of Baranja kulen

	$a_w$	$L^*$	$a^*$	$b^*$	pH
Recipes					
Traditional	0.85±0.03	36.42±4.11	19.32±3.04	17.58±4.01	5.67±0.37
Industrial	0.84±0.02	36.10±3.39	19.71±2.36	15.98±3.11	5.27±0.22
Ripening period					
3 months	0.87±0.01	39.46±1.90	20.52±1.55	19.16±2.77	5.27±0.25
6 months	0.82±0.01	33.06±1.86	18.52±3.22	14.39±2.75	5.66±0.35

The pH value of the Turkish fermented sausages soudjouk in the first 3 days of ripening decreased from 5.7 to 4.8. After that slowly increased and remained almost constant (Bozkurt and Bayram, 2006). From the research of Di Cagno *et al.* (2008) on three types of Italian fermented sausages changes in pH are similar to the changes in this study- pH value increases during ripening. After fermentation, the pH was 5.30 in the sausage Varzi, 4.90 in Brianza and 5.34 in Piacentino. The pH at the end of ripening was significantly lower in Brianza sausages (5.99) than in Varzi (6.62) and Piacentino (6.57).  $a_w$  values were 0.87-0.89 for all three types of sausages.

## CONCLUSIONS

Fat content (after 3 months 8.93% and at 6 months 12.61%) and protein content (after 3 months 35.83% to 41.16% after 6 months of ripening) increased. The water content after 6 months of ripening was 37.57% and decreased in comparison to kulen after 3 month of ripening (46.35%). Different meat quality influenced the color of kulen. Kulen with meat labeled B (pale meat) had the highest  $L^*$  value and kulen meat labeled A (normal colour) had the highest  $a^*$  value. The  $b^*$  value was the highest in kulen with meat labeled B. Traditional kulen had a slightly higher average pH 5.67 than industrial kulen whose pH value was 5.27.

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## EVALUATION OF ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS FROM NETTLE LEAF (*Urtica dioica* L.) USING BRIGGS-RAUSCHER REACTION

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*Original scientific paper*

### Summary

The Briggs-Rauscher oscillating reaction can be used as test for antioxidant activity of pure compounds or food extracts consumed on a daily basis, as like fruits, vegetables, juices, etc. Antioxidants are components that have ability to neutralize free radicals, which are harmful to human health. Adding the substances with antioxidant ability to the reaction mixture, oscillations temporarily stops, and after a certain time the oscillating reaction starts again. This time of no oscillations is known as a time of inhibition, and it's proportional to the quantity of antioxidant species in reactive mixture. In this study the Briggs-Rauscher reaction was used to determine antioxidant activity of aqueous extracts of nettle-leaves (*Urtica dioica* L.). Samples were collected at two different altitudes (800 and 900 m) in Presjenica and Barice (Bosnia and Herzegovina). Changes in Briggs-Rauscher reaction mixture were measured potentiometrically at room temperature. The manner and time of thermal treatment had an influence on antioxidant activity of aqueous extracts. The highest antioxidant activity showed nettle extract from Presjenica area prepared by leaving sample in boiling water for 15 minutes. Standard used for this mesurment was aqueous solutions of gallic acid in concentration range between 100 mg/l and 2000 mg/l, and the results were expressed as its equivalents. The obtained results indicate that the aqueous extracts of nettle leaf are a natural source of antioxidants.

**Key words:** *Urtica dioica* L., antioxidant activity, Briggs-Rauscher reaction

### INTRODUCTION

Antioxidants are substances with ability of donating electrons or hydrogen atom to free radicals (Marković and Talić, 2013). Free-radical reactions naturally occurs in human organism. Overmuch production of these reactive species during oxidative stress can cause damage of biomolecules, which can induce development of chronic, cardiovascular diseases, aging, and cancer. A harmful influence of free radicals can be inhibited by substances with antioxidant ability. Antioxidants neutralize free radicals, and in that way detoxify human organism (Lagouri and Nisteropoulou, 2009; Moskovitz, Yim, and Choke, 2002). Today there is a large trend to use natural antioxidants (Lagouri and Nisteropoulou, 2009). Plant extracts, which are used today as food additives, have great ability of donating hydrogen, which makes them extremely efficient antioxidants. Their antioxidant activity is mostly caused by presence of phenolic acids (gallic acid, caffeic acid, rosmarinic acid), phenolic diterpens, flavonoids, essential oils. Some plant extracts showed like good metal-helating agents, good proton-donors (Brewer, 2011).

Nettle (*Urtica dioica* L.) is an annual or perennial plant, with prominent hairs on the leaves and stem. Steroids, terpenoids, polypropanoids and coumarines were isolated from the root of this plant, and seven flavonol glycosides were isolated from the flowers. Most of the isolated glycosides showed anticancer, antiinflammatory, antioxidant and antiallergic activity (Yener, Celik, Ilhan and Bal, 2009). Shicimic acid is precursor of caffeic acid, different esters, like chlorogenic acid, that were shown as very good antioxidants (Upton, 2013). Besides mentioned compounds, which are the main source of antioxidants of nettle-extracts, the extracts contain also flavonoides and vitamins (Joshi, Mukhija, and Kalia, 2014).

One of the methods for determination of total phenols is Briggs-Rauscher reaction, oscillating reaction. This method of determination of total phenolic compounds is based on inhibition of Briggs-Rauscher reaction by free radicals. Generated hydroxyl radicals are main intermediates of Briggs-Rauscher system. After addition of antioxidants to Briggs-Rauscher mixture, there is an immediate quenching of the oscillations. This time of no-oscillations represents inhibition of oscillating reaction, which is linearly dependent on the antioxidant

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concentration. When the inhibition is finished, oscillations continue, and the time of no-oscillation represents inhibition time (Höner and Cervellati, 2002).

The aim of this paper is evaluation of antioxidant activity of aqueous extracts from nettle leaf using Briggs-Rauscher reaction. This method is very suitable for determination of antioxidant activity of natural polyphenolic compounds and plant extracts. Nettle, which grows at Balcan Peninsula as danel, was used as traditional medicine for ages in this area, and was tested for this very reason.

## MATERIALS AND METHODS

All used chemicals and reagents were of analytical grade: potassium iodate, Pliva; sulfuric acid, 96%, Merck; hydrogen peroxide, 30%, Semikem; malonic acid, Merck; manganese(II)sulphate, Merck, starch, Merck and gallic acid, Semikem. All stock solutions were made using double distilled water.

### Preparation of the solutions for Briggs-Rauscher reaction

Three solutions (A, B and C) were prepared. A: Solution of potassium iodate ( $0.2 \text{ mol/dm}^3$ ) in sulfuric acid (0.43%); B: Solution of hydrogen peroxide (15%); C: Malonic acid ( $0.15 \text{ mol/dm}^3$ ), manganese(II)sulphate ( $0.02 \text{ mol/dm}^3$ ), starch (0.03%). Mixture of equal volumes of the solutions A, B and C represents Briggs-Rauscher reaction mixture, which is used for determination of antioxidative activity.

### Preparation of the aqueous extracts

Nettle (*Urtica dioica* L.), whose extracts were used for determination of antioxidative activity, was taken from two locations: Barice (900 m altitude) and Presjenica (800 m altitude). Nettle-leaves were dried, finely powdered and extracted. Finely powdered nettle samples (2 g) were topped in 50 ml of double distilled water, and extractions were made with reflux condenser for 10 and 20 minutes. The other way of extraction was spilling 50 ml of boiling double distilled water over finely powdered nettle leaves. Samples spilled over with boiling water were left for 5, 10 and 15 minutes. After extraction, solutions of nettle samples were filtered, and prepared extracts were diluted (1 ml of extract was brought up to final volume of 25 ml with double distilled water). Diluted extracts (1ml) were added to 30 ml of active, well-stirred Briggs-Rauscher reaction mixture after third oscillation.

### Evaluation of antioxidant activity using Briggs-Rauscher reaction

Oscillations in the Briggs-Rauscher mixture were followed potentiometrically by recording the potential of a platinum wire electrode and Ag/AgCl/KCl<sub>(sat)</sub> reference electrode (+197 mV vs. SHE). The electrode was connected to a pH multimeter (Phywe). All measurements were conducted at room temperature,  $25 \pm 0,5^\circ\text{C}$ . The mixture was stirred by a magnetic stirrer (600 r.p.m.). A non-inhibited Briggs-Rauscher reaction has cca 15 oscillations in approximately 5-10 minutes that can be observed by colour changes from colourless over yellow to dark blue repeatedly. Addition of a diluted extracts, that contains antioxidants, causes an immediate effect of quenching of oscillations. The oscillations stop and start again after a period because the reaction produces hydroperoxyl radicals that are quenched by antioxidants. The quenching of oscillations is measured as an inhibition time which is correlated with contents of the added antioxidant.

## RESULTS AND DISCUSSION

The results obtained for antioxidant activity were represented as equivalents of gallic acid. The calibration curve, for different concentrations of gallic acid, was recorded (figure 2).

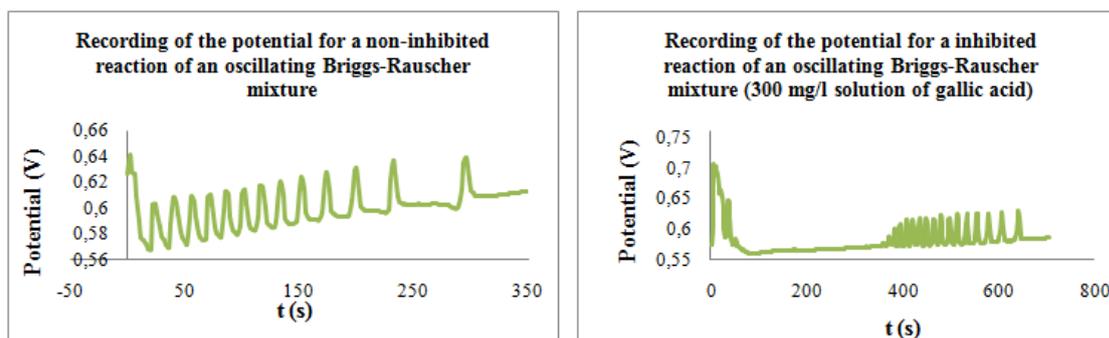


Fig. 1. Recording of the potential for a non-inhibited and inhibited reaction of an oscillating Briggs-Rauscher mixture

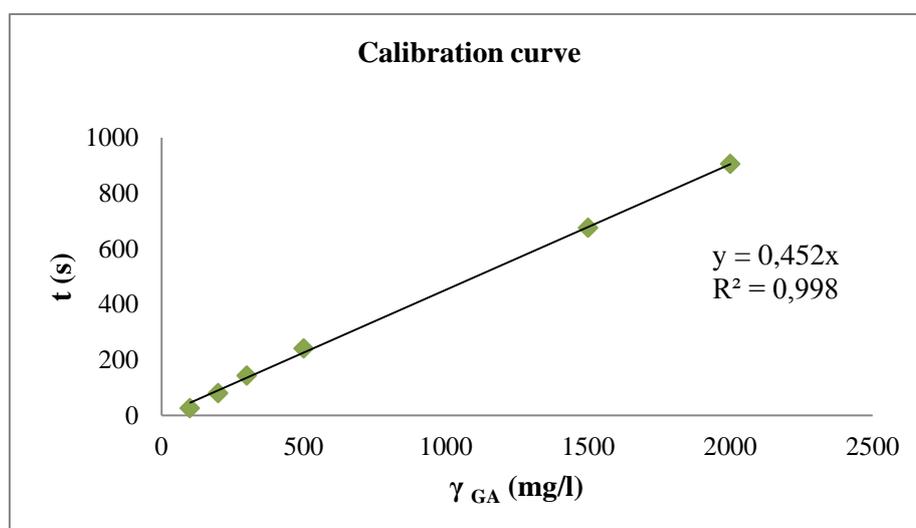


Fig. 2. The calibration curve between inhibition time and concentration of gallic acid

Tab. 1. Results for nettle-extracts antioxidant activity calculated from calibration curve and expressed as milligrams of gallic acid in gram nettle-sample

<i>Barice</i>	<b>Dilution</b>	<b>mg<sub>GA</sub>/g<sub>sample</sub></b>
Extraction 10 min	1ml/25ml	5.25
Extraction 20 min	1ml/25ml	7.61
Boiling water 5 min	1ml/25ml	8.30
Boiling water 10 min	1ml/25ml	15.49
Boiling water 15 min	1ml/25ml	22.68
<i>Presjenica</i>		
Extraction 10 min	1ml/25ml	5.25
Extraction 20 min	1ml/25ml	23.78
Boiling water 5 min	1ml/25ml	21.02
Boiling water 10 min	1ml/25ml	27.65
Boiling water 15 min	1ml/25ml	53.65

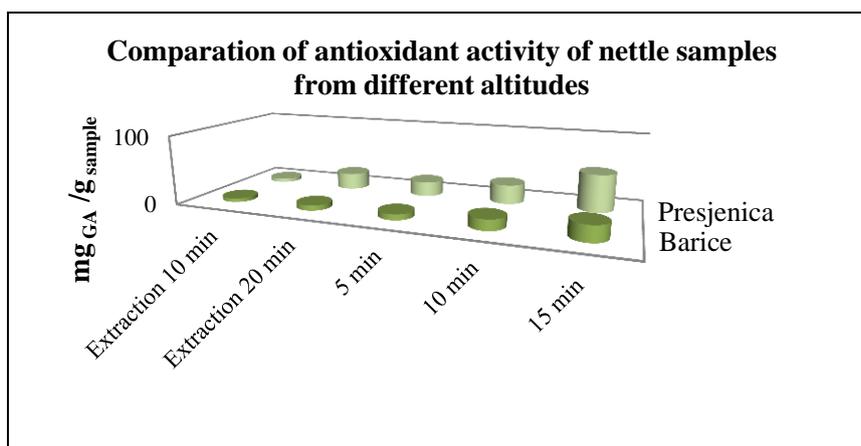


Fig. 3. Comparison of antioxidant activity of nettle samples from different altitudes

From results, presented in Table 1 and Figures 1-3, nettle extracts, showed certain antioxidant ability. Nettle-leaves showed different time of inhibition Briggs-Rauscher reaction. The longest time of inhibition showed nettle extract from the Presjenica area, left in boiling water for 15 minutes. Antioxidative activity is expressed as equivalents of gallic acid and the maximum value is 53.65 mg gallic acid by the gram of sample. The smallest, equal, antioxidant activity showed nettle samples from the Barice and Presjenica area, whose extracts were prepared with reflux condenser for 10 minutes. The results obtained showed that nettle-extracts from Presjenica area showed greater capacity of inhibition, respectively greater content of antioxidants, than nettle-extracts from Barice area. It is assumed that explanation for those results lays in the fact that location of Presjenica is far from the traffic, polluted air, soil and water. Extracts prepared by extraction with reflux condenser showed lower antioxidant activity. It is assumed that those results are the consequence of thermal degradation of antioxidants present in nettle extracts.

## CONCLUSIONS

Nettle extracts, from both areas, showed inhibition of Briggs-Rauscher reaction, respectively presence of the species with certain antioxidative ability. Content of the antioxidants is greater in nettle extracts from Presjenica area. Longer inhibition time showed nettle extracts which are prepared by spilling boiling water over nettle samples. Inhibition effect increases with standing time of the nettle sample in boiling water. Therefore, nettle leaves (*Urtica dioica* L.) traditional tea in Southeast Europe is a natural source of the antioxidants, which is greatly used today in pharmaceutical and food industry.

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## COMPARISON OF DIETARY HABITS AMONG ADOLESCENT BOYS AND GIRLS ACCORDING TO THE PLACE OF RESIDENCE IN CANTON SARAJEVO

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*Original scientific paper*

### Summary

Adolescence is a particularly challenging period of life with respect to the physical and cognitive growth and development, and perception of events within and around you. Adolescence is also considered as a deadline for the correction of all the bad habits acquired. Objective of the study was comparison of dietary habits among adolescent boys and girls. Study involved 630 adolescents aged 13-15 years living in Canton Sarajevo, Bosnia & Herzegovina, regarded as urban and rural area. Participants filled in specially designed Food Frequency Questionnaire. Some of the results show that: breakfast cereals are not consumed by rural adolescent boys; carbonated and non-carbonated drinks are more present among male adolescents in both areas; the diversity of food is the most prominent within rural female adolescents; the number of meals during the day is higher in the rural area; breakfast is mandatory among the male adolescents in both areas; milk is more consumed by urban adolescent boys and rural adolescent girls; tea is more common among the adolescents of both sexes in the rural area; the protein diet is prevalent among the adolescent boys, and the carbohydrate diet among the adolescent girls; a higher percentage of consumption of milk/yogurt is present among the adolescent boys from the urban and the adolescent girls from the rural areas; the weekly consumption of milk/yogurt is more present among rural adolescent boys and urban adolescent girls and the fish consumption is more common in the adolescent girls from both areas. It is impossible to draw a single conclusion about the existence of (non) regular dietary habits in relation to sex and the place of residence. Dietary patterns are too complex, but it is noticeable that there is an overall prevalence of the irregular form.

**Key words:** *Adolescents, dietary habits, sex, place of residence.*

### INTRODUCTION

Adolescence is a period of intense growth and development and thus is sensitive towards growing conditions. Non regular eating pattern (inadequate, insufficient or excessive eating) can lead to serious growing and health issues. Therefore, adolescents are interesting and complex population for the study, which made and makes an additional motivation to study their habits and attitudes.

Place of residence as a factor of influence on dietary habits is one of studied parameters in the last few years (Davis *et al.*, 2008; Tognarelli *et al.*, 2004), and with development of technology, infrastructure and business in today's world, the boundaries between rural and urban are not stable nor clearly delineated and presented also, one of the challenges in defining and developing this research. Results related to the dietary habits of adolescents are generally consistent everywhere in the world while the factors that influence dietary habits give diverse results also related to the place of residence.

### SUBJECTS AND METHODS

The study was conducted in Canton Sarajevo which was defined for the purpose of this study as urban and rural upon current Urbanistic plan (Zavod za planiranje razvoja Kantona Sarajevo, 2006). Participants were adolescent boys and girls aged 13-15 years (60 boys and 73 girls from rural area and 264 boys and 233 girls from urban area). The study was conducted in twelve elementary schools and has been approved by Ministry of Education and Sciences of Canton Sarajevo. Parents and participants were introduced to the study protocol. Dietary habits were monitored with specially designed Food Frequency Questionnaire (FFQ) which was used as a method and it included twenty one food items. Quantities were described as units of servings and participants were asked to be sincere when answering how often and in which quantities they eat listed food. Adolescents

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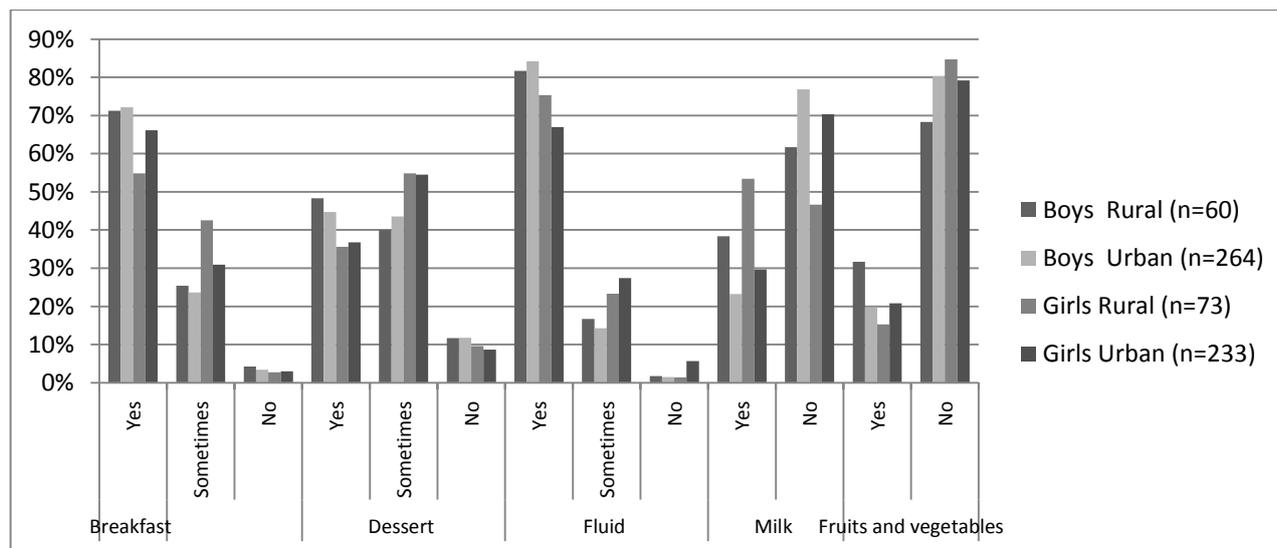
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participated voluntarily. Statistical analysis was performed using the statistical software package SPSS 19.0. (SPSS Inc, Chicago, Illinois, USA). For descriptive purposes were used: arithmetic mean, frequencies and percentages. Test of Normality was done with Kolmogorov-Smirnov and since most of the variables did not satisfy the conditions of normality, non-parametric U Mann-Whitney test was used and it showed statistically significant differences between the groups at  $p < 0.05$ . Dependence was tested by Chi-square test and the bond strenght by Spearman's rank correlation coefficient ( $\rho$ ) and t-test for independent samples.

## RESULTS AND DISCUSSION

Results are presented in percentages by the place of residence and by the sex. They are given in following graphs and were sorted by consumption frequency and/or in servings.

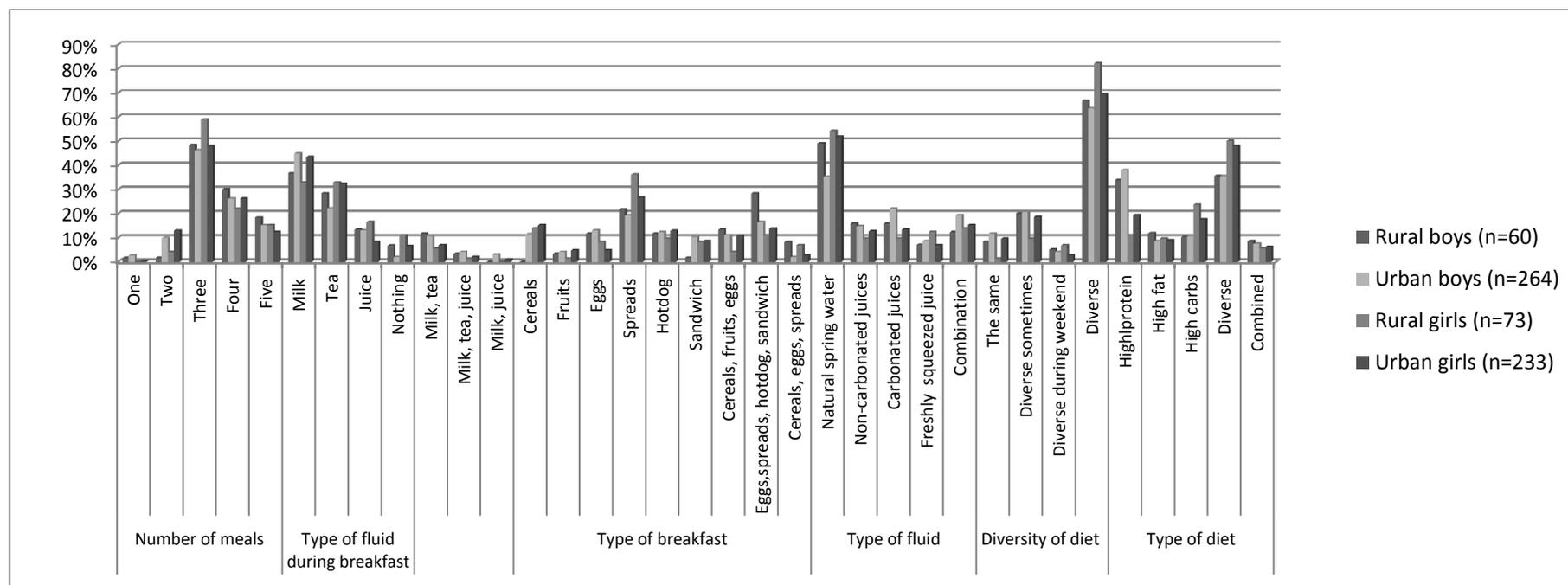


Graph 1. Distribution of participants based on food frequency consumption

Statistical significance of the place of residence regarding boys was demonstrated in the daily consumption of milk/yogurt ( $p=0.014$ ,  $\chi^2=5.819$ ) and daily consumption of fruits and vegetables ( $p=0.043$ ,  $\chi^2=4.079$ ). Statistical significance regarding girls was demonstrated in daily consumption of milk/yogurt ( $p<0.005$ ,  $\rho=0.211$ ). A significantly higher percentage of adolescents (rural vs. urban: 61.67%: 76.81%) do not regularly consume milk/ yogurt. Large percentage of adolescent girls in urban areas do not consume milk/yogurt every day (rural vs. urban: 46.60%: 70.30%) which might be an indication of negative eating habits of urban adolescents.

The habit of eating breakfast has almost the same percentage of adolescent boys in both areas (cca. 70.00%). A slightly higher percentage of adolescent girls in urban areas has the habit of eating breakfast (urban vs. rural: 66.10%: 54.80%). This data is similar to that obtained in a previous study among adolescents in Sarajevo (Hodžić and Smajić, 2012) which states that habit of eating breakfast has developed the largest number of students (68.00%). 56.00% of students (aged 11, 13 and 15) in Croatia have breakfast on weekdays (Ministarstvo zdravlja R Hrvatske, 2013) while Bertić (2013) states that the habit of eating breakfast every day has 39.00% of respondents. According to Tognarelli *et al.* (2004) 67.00% of children eat breakfast regularly. Results of daily consumption of fruits and vegetables shows that more adolescent boys in urban areas (80.31%) do not consume fruits and vegetables every day against 68.33% of adolescents from rural areas. Extremely high percentage of adolescent girls do not consume fruits and vegetables every day (rural vs. urban: 84.70%: 79.20%). Davis *et al.* (2008) point out that urban children consume a little more servings of fruits from rural but rural eat a little more servings of vegetables than urban. When analyzed together, portions of fruit and vegetables are higher in urban children than in rural ( $t(130)=0.424$ ,  $p=0.672$ ). Daily habit of consuming fruits twice a day developed 29.40% and three times a day 28.70% of adolescents (Hodžić and Smajić, 2012). Eating vegetables is very similar in relation to the residence and toward sex. Also, in relation to the habit of eating fruits, a small percent of adolescent boys and girls in both areas consume vegetables regularly and occasionally, and it is recommended to consume one serving of vegetables more than the fruit. In Croatia, 66.00% of students do not eat fruit every day, and as much as 76.00% do not eat vegetables every day

(Ministarstvo zdravlja R Hrvatske, 2013). In Canton Sarajevo (cca. 37.00%) adolescents consume vegetables once and twice a day (Hodžić and Smajić, 2012). Fluid consumption shows that most of adolescents consume recommended amount of liquid during the day. It's lower percent among girls. Half of both groups consume dessert sometimes.

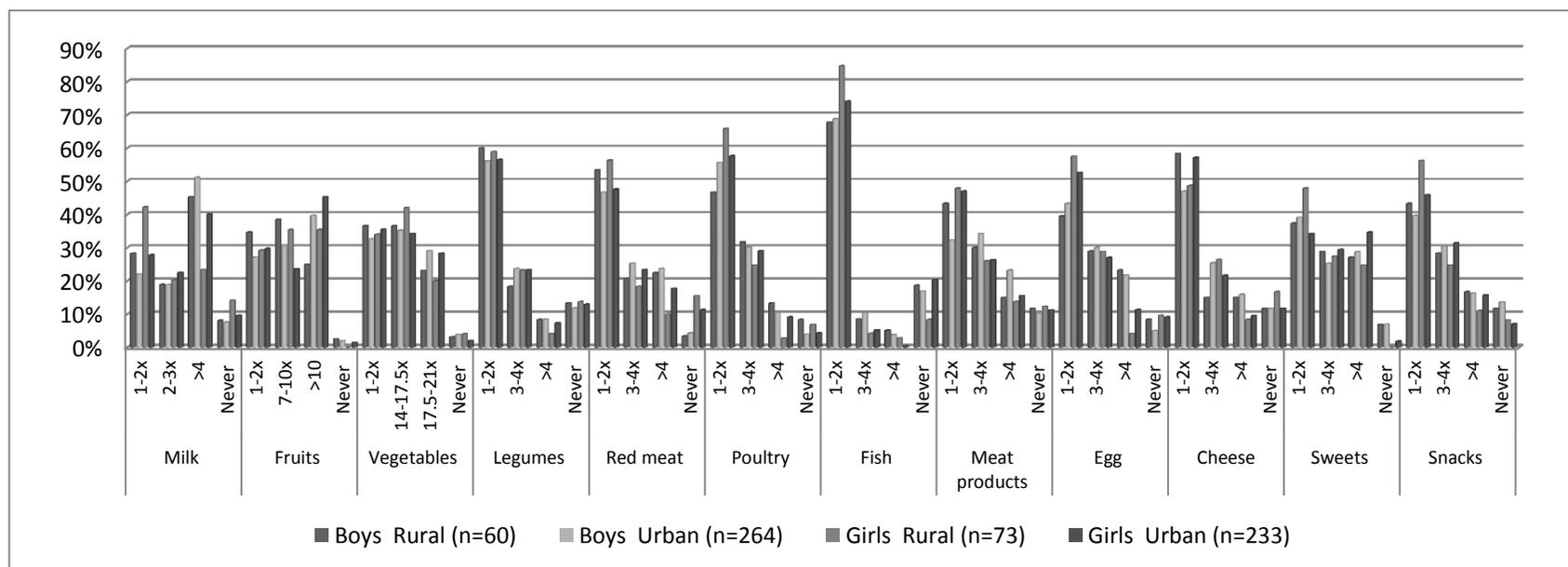


Graph 2. Frequency and type of diet (food/fluid) consumed

The impact of adolescent boys' place of residence in their dietary habits showed statistical significance in relation to the type of breakfast consumed ( $p=0.002$ ,  $\rho=0.172$ ) and weak correlation with respect to the type of fluid consumed ( $p=0.036$ ,  $\rho=0.118$ ). Cereals are not consumed by boys in the rural areas, while 11.5% of urban adolescents consume it. Cereals are highly recommended foods for breakfast, and are consumed by girls in both areas (rural vs urban: 13.9%: 15.1%). Different combinations are present in breakfast in both areas and by both sexes. Adolescents mostly consume natural spring water (boys: rural vs. urban: 49.12%: 35.27%; girls: rural vs. urban: 54.20%: 51.90%). Carbonated drinks are consumed more by adolescent boys than girls (rural vs. urban: 15.79%: 22.09%). According to Dinarević *et al.* (2011) in Canton Sarajevo half of the students the same age as in this study (54.72%) consumed spring water, followed by non-carbonated drinks (21.50%). According to the study made in Croatia by Bertić (2013) the majority of students (43.00%) consume soft drinks more times a week, every day 18.00%, rarely 36.00%. He points out that the consumption of carbonated drinks is very popular.

Impact of residence on dietary habits of adolescent girls are statistically significant in relation to the diversity of diet ( $p=0.012$ ,  $\rho=-0.144$ ). Diet of girls in both areas is diverse every day (rural vs. urban: 82.20%: 69.30%) which satisfies one of the basic principles of proper nutrition. Diet is diverse also in majority of boys (rural vs. urban: 66.70%: 63.50%). Boys' diet in relation to the representation of macronutrients shows that almost equally are present high protein (rural vs. urban:

33.90%: 37.89%) and various/diverse diet (rural vs. urban: 35.59%: 35.55%). Girls show it differently. Half of girls have diverse diet (rural vs. urbano: 50.00%: 48.00%), and the other half highly carbohydrate diet (rural vs. urbano: 23.60%: 17.60%). Carbs are more present in rural girls' diet indicating non regular pattern. Three meals per day dominate in both groups (boys: rural vs. urban: 48.30%: 46.20%; girls: rural vs. urban: 58.90%: 48.10%) and it's a bit more positive habit among rural adolescents. According to a survey conducted in Sarajevo (Hodžić and Smajić, 2012), 49.60% of adolescents consumed three meals a day, while four meals consumed 31.00% of respondents. The most commonly consumed fluids during breakfast are milk and tea in both areas and by both sexes. A slightly higher percentage of adolescent boys and girls in urban areas consume milk and in rural drink more tea. According to Dapi *et al.* (2005) there is a difference between urban and rural milk consumption. In an urban area, dairy products are affordable and are consumed during breakfast as a beverage with sugar and bread, while in rural area breakfast consists of the remains of traditional food. Contrary to an urban area, dairy products are expensive, less abundant and not considered "food" in rural areas.



Graph 3. Food consumption per week

Statistical significance regarding girls was demonstrated in weekly consumption of fish ( $p=0.047$ ,  $\rho=0.114$ ) and weekly consumption of sweets ( $p=0.021$ ,  $\rho=0.133$ ).

Recommendation by USDA (2010) for the studied population is five ounces per day of red meat, poultry and fish. One ounce is 28.34 grams, so daily intake of meat, poultry and fish should be 141.7 grams, a small steak or a small fish. On a weekly basis, the recommended amount of this protein food group would have been seven such steaks or smaller fish. It is known that restaurant' portion of meat is 250 grams, so it is taken into account when designing FFQ and the recommended amount would be 4 times a week combined red meat, poultry and fish. Consumption of fish is represented mostly 1-2 times a week and more in girls' diet (boys: rural vs. urbano: 67.80%: 68.80%; girls: rural vs. urban: 84.70%: 74.10%). Approximately 20.00% of the total number of respondents do not consume fish. Most of adolescents consume red meat 1-2 times a week (boys: rural vs. urban: 53.40%: 46.70%; girls: rural vs. urban: 56.30%: 47.60%).

Regarding poultry, half of adolescent boys (rural vs. urban: 46.70%: 55.60%) consume it 1-2 times a week as well as girls (rural vs. urban: 65.80%: 57.60%). Hodžić and Smajić (2012) point out that the poultry is consumed by 66.50% of adolescents, while it's considerably lower consumption of red meat (17.30%) and fish (15.60%). Meat products are mostly consumed 1-2 times per week (boys: rural vs. urban: 43.30%: 32.30%; girls: rural vs. urban: 47.90%: 47.00%). Eggs are also in protein food group and are consumed mostly 1-2 times per week (boys: rural vs. urban: 43.30%: 39.50%; girls: rural vs. urban: 57.50%: 52.60%). According Dapi *et al.* (2005) meat / fish / eggs are present and affordable in urban areas while in rural are eaten in special situations due to high prices and less presence. Cheese is also consumed mostly 1-2 times per week (boys: rural vs. urban: 58.30%: 47.00%; girls: rural vs. urban: 48.65%: 57.10%). In the study by Tognarelli *et al.* (2004) the most interesting data were obtained with the response "never eat" and those are yogurt (36.00%), poultry (21.00%), fish (15.00%), eggs (15.00%), vegetables (14.00%), milk (9.00%), fruit (8.00%), red meat (1.00%) and bread (1.0%). It is interesting that there is no difference in the consumption of any food (every day of the week, few days in the week and never) between urban and rural children ( $p>0.05$  for all tests). Almost an equal number of adolescent boys in both areas consume sweets in large quantities, 1-2 times per week or 3-4 times per week. Adolescent girls from rural areas in the highest percentage consume sweets 1-2 times a week while girls in urban areas consume sweets in the highest percentage, more than 4 times a week. Dinarević *et al.* (2011) states that 65.50% adolescents eat sweets every day, and the rest of the students from time to time. Bertić (2013) points out that 31.00% eat sweets every day, 28.00% few times per week, 27.00% up to two times per week and 14.00% rarely. Snacks are consumed in the highest percentage 1-2 times a week (boys: rural vs. urban: 43.30%: 39.70%; girls: rural vs. urban: 56.20%: 45.90%). Snacks are very popular among adolescents and are more popular in girls' diet. Similar data is shown in Bertić (2013) study: 46.00% few times a week.

## CONCLUSIONS

According to all above mentioned, it is not possible to draw a single conclusion about the existence of (non) regular dietary habits in relation to sex and place of residence. Dietary patterns are too complex, but it is noticeable overall prevalence of irregular form of diet among adolescents in Canton Sarajevo. It is therefore necessary to conduct more studies like this and to promote adoption of proper dietary habits at an earlier age than adolescence, nurture it and encouraged during adolescence to enabled this population a better transition into a mature, adult life.

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## CHEMICAL AND FATTY ACID COMPOSITION OF FAT IN THE COW'S CHEESE "BASA" PRODUCED IN THE UNA-SANA CANTON

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*Original scientific paper*

### Summary

The aim of this study was to evaluate chemical composition and contents of fatty acid of fat in the cow's cheese "Basa" produced in the Una-Sana Canton. The fatty acid contents of cheese was determined by gas chromatography. Contents of water, dry matter, fat, crude protein were respectively: 65.68%, 34.32%, 17.10%, 12.70%. The content of total n-3 fatty acids expressed as mg/100 g of the cheese was 324.90, and content of total n-6 fatty acids was 451.44 mg/100g of the cheese. Content of CLA (conjugated linoleic acid) was 336.87 mg/100g of the cheese. The value of 1.39 obtained for the ratio n-6/n-3 fatty acids is recommended guideline for the human diet.

Keywords: *cow's cheese, fatty acids contents*

### INTRODUCTION

Since prehistoric time, milk and milk products have been one of the most essential segments in human nutrition. Those products are not important only in terms of their nutritional value, but also as representatives of the cultural and historical identity of the people and countries. Una – Sana Canton has wide range of autochthonous milk products that take primary place in traditional meal. They are characterized by high nutritional value, but their production technology is not standardized.

"Basa" belongs to a group of autochthonous fresh soft cheese (Havranek, 1995) and is produced in the region of Lika, Krajina, Bihać and beyond from sheep's or cow's milk. It is soured with sour milk or whey (Sarić i Bijeljic, 2003). "Basa" has a long tradition of production, but there is no information about when its production has started and origin of the name. It was first mentioned in Zdanovski's book "Sheep's dairy" (Zdanovski, 1947). "Basa" is usually produced from the milk of two milkings: evening and morning. Milk, that is milked in the evening, is strained through a double cheesecloth and left until the morning in the cold room. Fresh, strained milk from the morning milking is mixed with the milk from the evening milking and put to cook on low heat (15 to 30 minutes). Boiled milk is usually put at the end of the stove for an hour to separate skin (skorup). Further separation of skorup takes place in a cold room (3 to 5°C). Separated skorup is stored in a refrigerator; the semi - skimmed milk is poured into the pot, and usually acidified and heated to temperatures of about 43 to 45°C (milk temperature is determined on the basis of experience). Then, the whey is added to the heated milk (2 liters of whey per 10 liters of milk) and the curd is obtained for about 3 hours. White soft cheese, that is obtained after straining, is mixed with the skorup and the salt (according to the desired quantity) and mixed well to obtain a homogeneous mixture. Kept in a refrigerator "Basa" can be consumed within 10 days.

In spite of the high levels of saturated fatty acid in milk fat, milk and cheese are known to play an important role in human nutrition and, more recently, are also recognized as a source of biologically-active substances (Collomb *et al.*, 2006; Prates and Mateus, 2002). Recent numerous research activities and advanced compositional identification of a large number of bioactive compounds in milk and dairy products have led to the discovery of specific biochemical, physiological and nutritional functionalities and characteristics that have strong potential for beneficial effects on human health. Four major areas of bioactivity of milk components have been categorized: 1) gastrointestinal development, activity and function; 2) infant development; 3) immunological development and function; and 4) microbial activity, including antibiotic and probiotic action (Gobbetti *et al.* 2007).

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## MATERIALS AND METHODS

Cow's cheese was made by local producer by traditional techniques, all based on the utilization of fresh raw milk, from animals reared in traditional farming systems based on grazing. Eight cheese samples were collected by local producers and transported under refrigerated conditions. All samples were kept frozen at  $-20^{\circ}\text{C}$  until they were analyzed. Chemical analyses (water, dry matter, fat, crude protein) were performed by AOAC methods (2000) in the Laboratory of Biotechnical faculty, University of Bihać. Specialist analysis of the fatty acid's profile was carried out in the Food Institute "Emona", Ljubljana (Slovenia). For analysis of fatty acids composition we used in situ preparation of fatty acid methyl esters in food (Park and Goins, 1994). The content of fatty acids was determined by using gas chromatography (Agilent Technologies model 6890 N with a FID detector and Supelco Omegawax 320 column, 30 m x 0.32 mm x 25  $\mu\text{m}$ ). All data was analyzed by the software package Statistica. The results are given as mean and standard deviation.

## RESULTS AND DISCUSSION

The chemical composition of the cow's cheese samples is shown in the Table 1.

Tab. 1. Chemical composition of the cow's cheese (n = 8)

Composition	Moisture %	Dry matter, %	Fat, %	Crude protein, %
Mean	65.68	34.32	17.10	12.70
Standard deviation	2.88906	0.76291	0.80197	1.033482

In the Tables 2 and 3 are shown the contents of fatty acids in the samples of cheese.

Tab. 2. Contents of fatty acids in the cheese (n = 8)

Fatty acid	mg/100g fat (mean)	mg/100 g of cheese (mean)	Standard deviation
C 10:0	3.04	519.84	4.35623
C 11:0	0.08	13.68	0.34092
C 12:0	3.43	586.53	3.45421
C 12:1	0.16	27.36	0.59057
C 13:0	0.09	15.39	0.40722
C 13:1	0.20	34.20	2.22983
C 14:0	13.06	2233.26	11.64456
C 14:1	0.69	117.99	1.72812
C 15:0	1.36	232.56	3.74175
C 16:0	29.31	5012.01	1.92937
C 16:1, <i>c+t</i>	1.39	237.69	13.74898
C 17:0	1.00	171.00	5.34522
C 18:0	12.97	2217.87	8.75638
C 18:1, <i>c+t</i>	25.37	4338.27	10.21961
C 18:2, <i>c+t</i> , n-6	2.36	403.56	4.21746
C 19:0	0.12	20.52	1.97664
C 19:1, <i>c+t</i>	0.15	25.65	3.05224
C 18:3 <i>c</i> , n-3	1.55	265.05	4.05928
C 18:2 <i>c</i> , n-7	1.97	336.87	2.37728
C 20:0	0.26	44.46	1.14372
C 20:1, <i>c+t</i>	0.29	49.59	1.25604
C 20:2, n-6	0.06	10.26	0.37340
C 20:3, n-6	0.09	15.39	0.60354
C 21:0	0.08	13.68	0.43595
C 20:4, n-6	0.11	18.81	0.55319
C 20:3, n-3	0.04	6.84	0.71023
C 20:5, n-3	0.13	22.23	1.76219
C 22:0	0.14	23.94	1.05950
C 22:1, <i>c+t</i>	-	-	-

C 22:2, n-6	0.02	3.42	0.88967
C 23:0	0.09	15.39	1.21227
C 22:5, n-3	0.18	30.78	2.16496
C 24:0	0.10	17.10	1.18551
C 22:6, n-3	-	-	-
C 24:1	0.03	5.13	0.90639
C 25:0	0.02	3.42	0.92487
C 26:0	0.06	10.26	1.22690

Tab. 3. Contents of fatty acids (mg/100 g of cheese)

Total n-3 fatty acids	324.90
Total n-6 fatty acids	451.44
n-6/n-3	1.39
Monounsaturated fatty acids	4834.17
Polyunsaturated fatty acids	114.92
Saturated fatty acids	11150.91
Unsaturated fatty acids	5949.09
Unsaturated/Saturated	0.53
Polyunsaturated/Saturated	0,01

The average water content was 65.68% (Table 1). Kršev (2015) obtained 75.00% water in fresh "Basa" cheese, and 63.00% water in five days old "Basa" cheese. Ljubojević and Tratnik (1975) obtained 71.50% water in Lička "Basa" cheese. Considering the fact that dry matter content of cheese indicates its nutritive value, cow's cheese from Una-Sana Canton is a valuable, nutritious cheese type. The average fat content of cheese samples was 17.10% (Table 1); Kršev (1974) established 18.10% fat in fresh "Basa" cheese and 21.30% fat in five days old "Basa" cheese. Ljubojević and Tratnik (1975) obtained 15% fat in Lička "Basa" cheese. The average crude protein content of cheese samples was 12.70% (Table 1). The CLA content (Table 2) was 1.97 mg/100g of fat; Kinik *et al.* (2005) in white pickled cheese established the CLA content in range from 0.13 to 2.20 mg/100 g of fat. Among the fatty acids classes (Table 3), the saturated ones were predominating (11150.91 mg/100 g of cheese), followed unsaturated fatty acids (5949.09 mg/100 g of cheese) and monounsaturated fatty acids (4834.17 mg/100 g of cheese). The fatty acid profile (Table 2) shows that palmitic acid was predominant (29.31 mg/100 g of fat), where as oleic was the major monounsaturated fatty acid (25.37 mg/100 g of cheese). Recent recommendations suggest a polyunsaturated/saturated ratio to be about 1 for fatty foods (British Department of Health, 1994). In our study, the values of 0.01, obtained for the relation polyunsaturated/saturated fatty acids, were consistently below the recommended guidelines.

## CONCLUSIONS

As a final conclusion it can be said that "Basa" cheese is nutritionally valuable food. Also, because of its characteristic taste and aroma, this cheese may be of interest for broader commercialization. Market expansion for "Basa" cheese can give opportunity to households to develop and improve its production which can increase economic prosperity of those households. Because the dairy products have a low polyunsaturated/saturated fatty acid ratio and unbalanced ratios of saturated/monounsaturated/polyunsaturated fatty acids, there is a need of dietary changes for the populations who consume much cheese and other dairy products in their diet.

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## COMPARATIVE STUDY OF THE QUALITY CHARACTERISTICS OF COLD-PRESSED AND VIRGIN PUMPKIN SEED OILS

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*Original scientific paper*

### Summary

This study presents a comparison of sensory and nutritive quality, as well as of oxidative stability of cold-pressed and virgin pumpkin seed oils obtained by regular production process. The samples of cold-pressed oil were produced from the seeds that are not thermally treated, while the virgin oils were obtained using roasted seeds. Six commercially available samples were investigated, as follows: 3 cold-pressed and 3 virgin oils. The results have pointed to considerable differences between sensory quality and the most important bioactive components of oils. Unlike virgin oils which have prominently specific and very pleasant aroma and flavour, cold-pressed oils made of unroasted seed have significantly less intensive odor and milder taste. With both of the oil types dominates the reddish-green color with recognizable fluorescence. The total carotenoid content was 7.43-8.40 mg/kg and 14.19-15.36 mg/kg in cold-pressed and virgin oil samples, respectively. Virgin oils are to a large extent richer in biologically active components, especially in phospholipids and total phenolic compounds. Due to this, virgin oils are of much better oxidative stability at 100°C, where induction period amounts to 22.8-31.2 h. Induction period of cold-pressed oils ranges between 16.9 to 18.3h. Cold-pressed oils, due to absence of roasting process of seed before pressing, have far more lower content of primary and secondary oxidative products. Totox value ranges from 1.08 to 2.48, whereas in virgin oils it ranges from 8.16 to 9.68.

Key words: *pumpkin oil, sensory characteristics, nutritive quality, oxidative stability*

### INTRODUCTION

The production of edible nonrefined, so-called virgin and cold-pressed oils involves neither heat nor chemical treatments, and it is becoming an interesting substitute for conventional practices because of consumers' desire for natural and safe food products. Over the last years many vegetable oils from different seeds that are produced by mechanical extraction, *e. g.* pressing, without use of any solvent emerged and are now available for the consumers, resulting in increased competition for market shares (Beardsell *et al.*, 2002, Dimić, 2005, Matthäus and Spener, 2008, Raß *et al.*, 2008). Among them the pumpkin seed oil is an appreciated salad oil. It is produced in the southern part of Austria (Styria, Carinthia), in the north of Croatia, in Slovenia, Hungary, Germany, and over the last years in Voivodina region, northern province of Serbia.

Pumpkin seed oil (*Cucurbita pepo* L.) belongs to the group of oils of high nutritive value due to favourable fatty acid profile and different bioactive components. Positive therapeutic properties of a pumpkin oil, such as plasma cholesterol-lowering effect and beneficial effects in the treatment of benign prostatic hyperplasia (BPH), due to minor bioactive constituents (sterols, squalene, phenolic compounds, tocopherols) are also well known (Fruhvirt and Hermetter, 2007, Fruhwirt and Hermetter, 2008, Siger *et al.*, 2008).

According to traditional technology, pumpkin seed oil is obtained from roasted seed by pressing on hydraulic press. At the market this oils is present as **virgin pumpkin seed oil**. In this case to the dried ground kernels, fresh water and table salt is added to form a soft pulp. The pulp is roasted for up to 60 min at temperatures around 100-120°C, even to 150°C, which results in coagulation of the protein fraction and permits convenient separation of the oil by pressing (Vujasinovic *et al.*, 2012). The pressing process is performed under isothermal conditions at pressures between 300 and 600 bar (Murkovic *et al.*, 2004, Fruhwirth and Hermetter, 2008).

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**Cold-pressed pumpkin seed oil** is a relatively new product in Republic of Serbia and closer surrounding. The technological process of cold-pressed pumpkin seed oil includes the following: the seeds are harvested in mid autumn and immediately dried to a residual water content of around 7-8% and stored.

Before the processing the seed passes through the magnetic cleaner, followed by complete removal of organic impurities on the selector. The clean dry seed is fed to the screw press, which grinds and presses the material, squeezing the oil which is collected in vessels. Turbid matter resulting from ruptured plant material of the seeds during pressing is removed by sedimentation or filtration at room temperature. Filtered oil is filled into dark glass bottles of 250 mL. Cold-pressed pumpkin seed oil produced in this way is specific for our region, and the consumption of this oil is increasing (Vujasinovic *et al.*, 2010).

The aim of this study was the investigation of sensory characteristics, nutritive quality, oxidative state and shelf life of oil produced by cold pressing on screw press of dried-raw naked pumpkin seed and of virgin oil that is produced by pressing of roasted seed on hydraulic press.

## MATERIALS AND METHODS

Three oil samples (1, 2 and 3) of cold-pressed and three samples (4, 5 and 6) of virgin pumpkin oils were purchased from different local small facilities. All oil samples were freshly prepared from naked pumpkin seed *Cucurbita pepo* L. according to described technology procedures involved in regular production process.

**Sensory evaluation.** The analysis was carried out by three-member panel of experienced assessors. Sensory characteristics of oil, smell, taste and color are given descriptively.

**Analytical methods.** For investigation of certain chemical parameters, the following standard methods were applied: peroxide value (PV) - SRPS EN ISO 3960: 2011, *p*-anisidine value (AnV) - SRPS EN ISO 6885: 2011, total phosphatides content AOCS Ca 12-55, the content of total carotenoids was determined by British standard method (1977).

Content of total tocopherols was determined by spectrofotometric method according to Emmerie-Engel (Pacqout *et al.*, 1967).

The Folin-Ciocalteu reagent was used to determine the total phenolic compounds following a procedure previously described by Haiyan *et al.* (2007).

Oxidative stability at elevated temperature was evaluated by the ISO 6886: 1996 method. Stability was expressed as the induction period-IP, measured with the Rancimat 617 apparatus (Methrom Co, Basel, Switzerland) using an oil sample of 2.5 g at 100°C and an air flow of 18 l/h.

## RESULTS AND DISCUSSION

### Color characterization

Sensory analysis of the samples (Table 1) has pointed out significant differences in characteristics of cold-pressed and virgin pumpkin seed oils.

Tab. 1. Descriptive sensory characteristics of cold-pressed and virgin pumpkin seed oils

Characteristic	Cold-pressed oils			Virgin oils		
	1	2	3	4	5	6
Odor	The odor of all samples is of low intensity, it can be hardly defined, it reminds of the smell of raw naked pumpkin seed			The odor is intensive, aromatic, pronounced and rather specific to roasted kernel		
Taste/flavour	The taste also reminded of dried/raw pumpkin seed, although it is of low intensity. The flavour is pleasant, mild fruity			Very typical strong nutty and roasty flavour. Pleasant, intensive aroma		
Color	The color of all samples is visually similar, red with orange-green shades in pass-by daylight			The color of oils is visually completely the same, dark red-brown with concomitantly fluorescence		

The smell and aroma of cold pressed pumpkin seed oil are specific and „unique“. The oil flavour, taste in the first place, is mild fruity like raw pumpkin seed, slightly greeny, without any „roasty“ taste. The odour of cold pressed oils is also specific, but rather mild, slightly expressed. Literature data on odour components of cold pressed pumpkin seed oil are lacking, however, oils obtained from roasted seed were analyzed in details. Matsui *et al.* (1997) identified 24 active odoriferous components, attributing the characteristic flavour of virgin

pumpkin seed oil to pyrazines. The furans, 2-pentylfuran, generally contribute to „roasty“ odor (caramel like). Siegmund and Murkovic (2004), and Haiyan *et al.* (2007) also analyzed the volatile compounds profile of roasted pumpkin seed oil, and the obtained results are different. Virgin pumpkin oil has a very characteristic roasted nut-like aroma that is tightly connected to its production process, which involves a roasting step of the ground seed before pressing.

Color is generally considered as an important sensory property of nonrefined edible oils, because it is immediately perceived and strongly influences most customer preferences. In addition, color is related to other chemical and physical properties of oils. Consequently, a colorimetric characterization of virgin oils would be useful in the quality control of this product. Certain differences in color of pumpkin seed oil, depending on the production technology, can be visually determined, as well. It is important to note that the visual perception of color with both kind of oils is highly dependant on the thickness of oil layer. Oils spread to a thin layer have prominent green color, which is lighter green with cold-pressed oils and dark green with virgin oils.

#### Nutritive quality

The cold-pressed edible oils are preferred by consumers because the cold-pressing procedure may increase the retention of beneficial phytochemicals. The content of tocopherols, phenolic compounds and phospholipids, which are recognised for their potential health benefits, are shown in Table 2.

Tab. 2. Bioactive components of cold-pressed and virgin pumpkin seed oils

Total content of	Cold-pressed oils			Virgin oils		
	1	2	3	4	5	6
Phospholipids (mg/kg)	45.75±3.22*	34.25±2.25	44.28±4.21	2933±33	1527±48	2288±89
Tocopherols (mg/kg)	883.5±89.2	731.8±52.5	665.9±66.1	1236.3±98.6	832.8±55.2	932.9±76.4
Phenolic compounds (mg/kg)	9.45±1.22	10.64±0.89	12.24±1.05	24.22±2.45	23.88±3.00	25.86±1.42
Carotenoids (mg/kg)	7.43±1.00	8.40±1.11	8.11±0.67	15.36±3.23	14.19±2.00	15.08±1.55

\*Values are means ± SD; n=3

As it is shown by results presented in Table 2, the roasting process of the seed prior to pressing has a strong influence on the content of bioactive components. Virgin oils have significantly higher content of total phospholipids, from 1527±48 to 2933±33 mg/kg in virgin oils, whereas their content in cold-pressed oil samples ranged from 34.25±2.25 to 45.75±3.22 mg/kg. Schuster and co-workers (1983) analysed several components in the seed from an international variety trial. According to their results average phosphatide contents were about 0.82% and were significantly influenced by variety, location as well as interaction location/year. In this trial the total phospholipid concentration in Styrian pumpkin seed oil showed considerable variation (0.5-1.04%), whereas the average content was 0.84%.

Tocopherols are widespread natural antioxidants, and their amount in oils depends on the kind of oil and is probably governed by the content of unsaturated fatty acids (Radočaj and Dimić, 2013). The content of total tocopherols (Table 2) in cold-pressed oils ranges between 665.9±66.1 to 883.5±89.2 mg/kg, whereas their content in virgin oils was higher, up to 1236.3±98.6 mg/kg in sample no. 4. Murkovic *et al.* (2004) noticed changes of the tocopherol content as a result of seed roasting. By Nedjeral *et al.* (2006) the mean values of total tocopherols in roasted pumpkin oils of husk and naked seeds were 651 and 454 kg/kg, respectively. According to the results of Vujasinovic *et al.* (2012), the tocopherol content in cold-pressed oil was 266 mg/kg, while in virgin oils ranged between 279 and 351 mg/kg (HPLC-method).

Cold-pressed oils usually contain phenolic compounds and they may have the potential for applications in the promotion of health and prevention of oxidative damages caused by radicals. As it can be seen from Table 2 the total phenolic compounds in virgin oils is twice higher compared to cold-pressed oils. The highest content of phenols of 25.86±1.42 mg/kg was found in virgin oil sample no. 6, and the lowest of 9.45±1.22 mg/kg in cold-pressed oil sample no. 1. Literature data of total phenols content in pumpkin oil mainly refer to the oil obtained from roasted seed and they range from 9.6 to 24.6 mg/kg (Haiyan *et al.*, 2007; Parry *et al.*, 2006; Sieger *et al.*, 2008).

*Oxidative stability*

The results presented in Table 3 show that roasting of seed, before pressing, affects the oxidative status and oil stability. Due to seed roasting, the primary and secondary oxidation products accumulated in oil, resulting in significant increase of peroxide and anisidine value in virgin oils. So, totox value in virgin oil is about 8-9, but in cold-pressed oil is much lower, about 1-2.5.

Tab. 3. Oxidative stability and shelf-life of cold-pressed and virgin pumpkin seed oils

Characteristic	Cold-pressed oils			Virgin oils		
	1	2	3	4	5	6
PV (mmol/kg)	0.69±0.02	0.56±0.01	0.28±0.00	2.90±0.05	2.61±0.05	2.52±0.08
AnV (100A <sup>1%</sup> <sub>350nm</sub> )	0.61±0.05	1.36±0.06	0.50±0.03	2.07±0.09	3.27±0.08	4.05±0.11
Totox value (2xPV + AnV)	1.99	2.48	1.06	7.87	8.49	9.09
Induction period (h)	17.20±0.21	16.90±0.22	18.30±0.68	31.20±0.42	22.80±0.36	30.33±0.55

\*Values are means ± SD; n=3

Oxidative stability of virgin oils at temperature of 100°C is markedly better, IP ranges from 22.80±0.36 to 31.20±0.42h. At the same time, the longest IP in cold-pressed oil is only 18.30±0.68h. Parry *et al.* (2006) reported the induction period of roasted pumpkin seed oil at 80°C to be 61.7 hours (with an air flow of 7 L/h), and according to Murkovic and Pfannhauser (2000) the mean value of induction period at 120°C was 6.83 hrs. According to the literature data (Sigmund and Murkovic, 2004), during the roasting process of pumpkin seed the concentration of Strecker degradation products, as well as products of lipid peroxidation and Maillard's reaction change to a high extent. Maillard's reaction products can have a synergy effect with tocopherols and thus contribute to better stability (Kamal-Eldin, 2006). Compounds that contributes to better stability of oils are certainly tocopherols, phenolic compounds and phospholipids which are present in higher amounts in virgin oils than in cold-pressed oils (Table 2).

## CONCLUSIONS

The results showed that sensory characteristics, nutritive quality and shelf life of cold-pressed and virgin pumpkin seed oils are very different. The odor and aroma of cold pressed oil are specific, mild fruity and slightly greeny, resembling raw pumpkin seed, while virgin pumpkin oil has a very characteristic roasted nut-like aroma. Virgin oils are to a large extent richer in bioactive components, especially in phospholipids and total tocopherols, as well as phenolic compounds. The content of total phospholipids in virgin oils amounts 1527±48 to 2933±33 mg/kg, whereas their content in cold-pressed oil samples ranges from 34.25±2.25 to 45.75±3.22 mg/kg. The content of total tocopherols in cold-pressed oils ranges between 665.9±66.1 and 883.5±89.2 mg/kg, whereas their content in virgin oils is much higher, up to 1236.3±98.6 mg/kg. The total phenolic compounds in virgin oils, from 23.88±3.00 to 25.86±1.42 mg/kg, is twice higher compared to cold-pressed oils, from 9.45±1.22 to 12.24±1.05 mg/kg. Oxidative stability of virgin oils at temperature of 100°C is markedly better, IP ranges from 22.80±0.36 to 31.20±0.42h. At the same time, the longest IP in cold-pressed oil is only 18.30±0.68h. However, cold-pressed oils, due to absence of roasting process of seed before pressing, have far more lower content of primary and secondary oxidative products. Totox value ranges from 1.08 to 2.48, whereas in virgin oils it ranges from 8.16 to 9.68.

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## **PLANT PRODUCTION**

## A STUDY ON THE DIFFERENT SOIL TILLAGE SYSTEMS IN SECOND CROP MAIZE PRODUCTION IN SOUTH AEGEAN CONDITIONS

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*Original scientific paper*

### Summary

This study was carried out in conditions of South Aegean Region; at the Dalaman Agricultural Enterprises in 2013 and 2014 sowing seasons. In this study, the best suitable soil tillage and sowing methods that can be used in second crop maize were investigated. Four different tillage methods including conventional tillage (Y1), reduced tillage 1 (Y2), reduced tillage 2 (Y3) and direct planting (Y4), applied in second crop maize has been compared in term of plant growing and yield. In the study, fuel consumption and average labour success of machines, growing parameters such as average outflow time of the plant, seedling emergence, plant height, thousand-kernel weight and grain yield were determined. The effects of tillage methods on soil particle size distribution, soil salinity and organic matter content were investigated. The study was conducted according to the completely randomized design with three replicates. As a result of the evaluation the effect of tillage methods on yield of maize were significantly ( $p < 0.05$ ) different between treatments. The highest and lowest yield for maize was detected in conventional (1295.4 kg/da) and direct planting (1102.3 kg/da) method respectively. The highest fuel consumption was observed in conventional method (Y1) whereas the lowest value was found in direct planting method. The highest labour success was detected in direct planting method, while the lowest values were determined in the Y2 method.

*Key words: Soil tillage, second crop, Maize, direct planting, yield*

### INTRODUCTION

Maize (*Zea mays* L.), having considerably an important role due to its total production and market amount in the world trade; is an important nutritional source due to its nutrients. Maize in Turkey is most commonly produced grain as main and second crop after wheat and barley. Maize cultivation area in Turkey is around 600 thousand hectares (Anonymous, 2012). Seedbed preparations and sowing with appropriate tools and machines are important in the agricultural mechanization processes which are necessary in order to improve the effectiveness; affordability and working conditions of the agricultural technology used in Turkey. To reduce the field traffic, to decrease the production cost to minimum, conventional tillage systems for various purposes such as controlling soil erosion, leaves its place to newer soil tillage systems in recent years. 70 percent of the fuel in the traditional grain production is used in the primary and secondary soil tillage. The increase of soil moisture content loss and erosion in parallel with the amount and frequency of the soil mixture causes to gradually increase the trend in this direction. The purpose of soil tillage is to maintain soil fertility, reduce soil erosion and soil compaction, to ensure the protection of diversity and protection of flora and fauna in the soil (Aykas and Önal, 1999). It is possible to bring the soil to suitable condition and to maintain the status by cultivating, hence the soil tillage comes as a major agro-technical precaution in increasing of production. A large part of the energy used in crop production is used in soil tillage. As in all businesses to get the most revenue with minimal input is a priority objective in the agricultural enterprises (Karaağaç and Barut, 2009). Dealing with the subject in terms of the total fuel consumed for the preparation of seedbed and the time required, reduced soil tillage methods have given better results. The differences between the results obtained in studies on this subject have been found economically important (Yalçın *et al.*, 1997). The advantages of direct planting methods according to the conventional and reduced soil tillage methods have been reported as provider of more water storage, more organic matter accumulation in soil and at least 60% savings in fuel costs and reducing of erosion (Gray *et al.*, 1997). Çakır *et al.*, (2006) have researched for the effects of conservation tillage and the effects of direct planting in second crop maize production made in the Menemen region. According to the obtained results, they have indicated that direct planting is applicable. Barut and Çelik (2010) have studied the effects

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of different soil tillage methods on seedling emerge, homogeneous distribution and yield in the second crop maize.

The effect of tillage methods on seedling emerge, homogenous distribution and yield have been found statistically significant. The yield values obtained by soil tillage tools used for maize production, varies from region to region, climate to climate. Therefore, it is important to determine the appropriate soil tillage system in that area. In addition to these approaches, subjects like environment and soil erosion that frequently mentioned, minimum soil tillage and direct planting methods that are widely used, can be suggested as a solution to these subjects.

As for the above-mentioned goals and objectives in this study alternative soil tillage methods that can be used in the second crop maize production after the main crop wheat are investigated.

## MATERIALS AND METHODS

### Material

Chemical analyses were done on the different parts of the soil samples of trial field taken from 0-30 cm depth. Trial field soil where 21% of clay, 40% silt and 38% sand rates within the territory were found out. Analysis of soil samples that are in a close proximity to each other and having found the traces of loamy soil examples in every sample has shown that the structure is relatively homogenous. It is determined that the Soil pH value slightly alkaline, poor in humus and has no salinity hazard.

In trials the tractors already present in operation and have different engine power are used as a power source. For direct planting, 6 Sequential Gaspardo precision planter and in other systems 4-row Gaspardo pneumatic precision planter used. Agricultural tools and technical specifications used in the experiments are given in Table 1.

Tab. 1. The specification of the tools used in experiment

Tool	Working Width (cm)	Working Depth (cm)	Power Supply
Moldboard Plow	180	20	T7040 Newholland
Disc harrow	220	15	T7040 Newholland
Chisel	290	25	T7040 Newholland
Seedbed Combination Implement	270	8-10	Valtra T-173
float	220	-	Massey Ferguson 185
Direct Drilling Machine	420	5-15	T7040 Newholland
Precision Drilling Machine	280	5-15	TD 900 Newholland

### Method

The experiment was set up by a random block design with three replications on a total of 12 plots. Each trial plot is set approximately 140 meters long and 20 meters wide. While approximately one meter distance is left among methods, no distance is given among the plots in the methods. Four different tillage methods including conventional tillage (Y1), reduced tillage 1 (Y2), reduced tillage 2 (Y3) and direct planting (Y4), applied in second crop maize has been compared in term of plant growing parameters and yield. The methods were;

**Y1:** Moldboard Plow+ Disc Harrow + Seedbed Combination Implement + Planting + Float,

**Y2:** Chisel + Disc Harrow + Seedbed Combination Implement + Planting + Float,

**Y3:** Disc Harrow + Seedbed Combination Implement + Planting + Float,

**Y4:** Direct Planting.

Before starting soil tillage and planting, field has been irrigated by means of flooding irrigation method. After the soil reaches the appropriate condition (field capacity), soil tillage and planting methods have been carried out. After all sowing, sprinkler system is used as an irrigation system. The second product in the whole parcel for the maize planting is set as depth of 6 cm, 70 cm row spacing, 18 cm plant spacing in rows and kept sowing in about 8000 plants per decare.

Soil temperature, soil physical properties such as moisture content, bulk density and penetration resistance, measured before and after soil tillage and planting methods. Fertilizing process is carried out together with the direct planting method whereas in the other methods before soil tillage and planting methods it is performed with disc spreaders. This compound fertilizer (NPK 15-15-15) is given in the norm of 30 kg.da<sup>-1</sup>.

In the second crop maize production in conventional and reduced tillage methods, inter-row cultivating applications are performed for weed control with rider. For this purpose, about 5 weeks later when planting and the plant height reaches about 25-30 cm, the process of inter-row cultivating and throat filling is being performed.

To determine the effects of soil tillage methods on soil particle size, after tillage on each plot soil samples taken around 3 kg each from the effective working depth (0-15 cm) were left to dry for 1.5 months in laboratory conditions. Anon (1974), mentioned pulley diameter of 200 mm and (0.5, 1, 2, 3.3, 4.47, 8, 16) mm square holes, soil samples were divided into eight separate fractions (Eghball *et al.*, 1993). In the analysis result, by weighing separately of 8 fractions, % weight values of each fraction according to their total sample was determined relatively.

After harvest to determine the effects of different soil tillage and planting methods on soil organic matter and salinity, soil samples were taken from each plot and different depths (10-10 cm, 10-20 cm and 20-30 cm), carbon and total salinity contents were determined by means of analysis. With the establishment of trial of fuel consumption each different soil tillage and planting methods used in trial, tank of the tractor being simultaneously is filled to capacity before soil tillage and planting. The fuel balance used also after work was found by completing with a replicated measured beaker. Total fuel consumption was calculated as L/ha (Göktürk, 1999).

Soil tillage in every plot of trial, preparation of seed bed and total time measured to do sowing was calculated in field. Thanks to this, labor success (da/h) was calculated.

With the purpose of determining germination ability of the seed after planting, seedling emerge have been counted. To determine mean germination time (MGT) and outflow percentage (OP) value, four methods in every two years and randomly three rows of 15 meters each in every plot were selected. The sprouted plants to the soil surface within specific time intervals were counted until they are fixed germination and with the help of equation 1 and 2 AGT and OP values were calculated. (Erbach, 1982)

$$MGT = \frac{G_1B_1 + G_2B_2 + \dots + G_nB_n}{B_1 + B_2 + \dots + B_n} \quad (1)$$

$$OP = \frac{N_b}{N} \quad (2)$$

B: Number of seeds germinated in the n<sup>th</sup> time

G: Time from the start of the experiment to the n<sup>th</sup> observation

N<sub>b</sub>: Number of seeds germinated in length unit (seed/meter)

N: Number of seeds drilling in length unit (seed/meter)

Total ears harvested from the middle and including three rows in each parcel were discussed and field efficiency was determined. Moisture content of the obtained samples were measured and the previously recorded field data based on 15% moisture grain yield per unit area to be converted into grain yield per hectare (kg/ha) were calculated.

## RESULTS AND DISCUSSION

To determine the effect of different soil tillage methods in the study on soil particle size distribution, soil samples taken from each parcel after soil tillage and planting were analyzed by sieve analysis and the result was given as %weight (Figure 1). As we look at the obtained results the highest value 38.51% that ranges 1-8 mm. particle size distribution (Adam and Erbach, 1992) that is the optimum particle size distribution ratio measured in the conventional soil tillage method. In this fraction the lowest value as 29.20% was obtained in the direct planting method. Samples taken in direct planting method were taken from planting rows and form the soil under the effect of drilling machine.

With the aim to determine the effects of different soil tillage methods used in the study to the plant growing parameters, counting had been done in the way mentioned in the method. Results were given on Table 2.

The highest outflow was measured as 92.1% in Y3 method. The lowest value is obtained as 89.2% in direct planting method. In the mean germination time measurements of the fastest outflows were measured results in Y2 method as 4.33 days. The latest outflows were determined as 4.8 days of average in Y4 (direct planting) and Y1 methods. When we look at the obtained yields the highest grain yield is 1295.4 kg/da in Y1 (conventional) method whereas the lowest yield was detected as 1102.3 kg/da in Y4 (direct planting) method. As a result of variance analysis, the effect of soil tillage methods on MGT, OP, ear height and ear length on the level of (P<0.05) wasn't found statistically significant. Besides, plant height, ear diameter, grain/ear ratio, thousand kernel weight and effect of soil tillage methods on yield on the level of (P<0.005) was found statistically significant (Table 2).

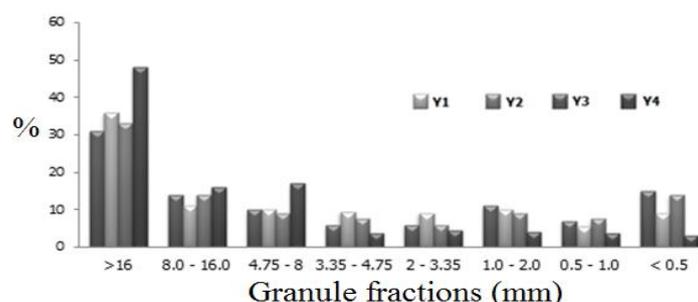


Fig. 1. Soil particle size distribution in different soil tillage methods

Tab. 2. Effects of different soil tillage methods on the plant growing parameters

Method	MGT	OP (%)	Plant Height (cm)	Ear Height (cm)	Ear Dia. (mm)	Ears Length (cm)	Grain/Ear Ratio	1000 Kernel Weight (g)	Yield (kg/da)
<b>Y 1</b>	4.46	91.7	232.13	61.22	50.10	21.05	86.8	342.2	<b>1295.4</b>
<b>Y 2</b>	4.33	90.2	230.32	63.65	50.25	20.20	86.4	338.4	<b>1225.8</b>
<b>Y 3</b>	4.80	92.1	219.75	64.36	48.63	19.10	85.5	333.8	<b>1165.1</b>
<b>Y 4</b>	4.80	89.2	215.20	65.55	44.55	18.95	83.9	329.1	<b>1102.3</b>
P value	<b>0.31</b>	<b>0.11</b>	<b>0.00*</b>	<b>0.17</b>	<b>0.00*</b>	<b>0.07</b>	<b>0.00*</b>	<b>0.00*</b>	<b>0.00*</b>

(\*)=P5% ; statistically significant

In the study operation speed (km/h), slip (%), labour efficiency (da/h) and fuel consumption (L/ha) of tools and machinery used in different soil tillage and planting methods were measured. The results were given in Table 3. As a result of measurements in terms of labour efficiency and fuel consumption, direct planting method was found more superior in comparison with other methods.

Tab. 3. Values of field efficiencies and fuel consumption of tillage methods measured in study

Method	Labour Efficiency (da/h)	Fuel Consumption (L/ha)
<b>Y 1</b>	4.71	49.3
<b>Y 2</b>	4.57	29.8
<b>Y 3</b>	6.11	20.8
<b>Y 4</b>	24.79	6.1

To determine effect of soil tillage methods on organic matter and salt content, soil samples were taken from different depths were analyzed. Soil organic matter analysis results showed that Y4 (direct planting) method had a significant rise. Y3, Y2, Y1 methods also followed after this method (Table 4). Among the methods in terms of salinity content, the most variation (decrease) occurred in direct planting method. However salinity hazard hadn't been detected in all the methods (Table 5).

Tab. 4. Soil OM content (% C)

Method	0-10 cm		10-20 cm		20-30 cm	
	2013	2014	2013	2014	2013	2014
Y1	1.97	2.00	2.02	2.01	2.02	2.07
Y2	2.03	2.09	2.04	2.10	2.03	2.02
Y3	2.05	2.04	2.11	2.16	2.03	2.13
Y4	2.17	2.27	2.13	2.26	2.07	2.18

Tab. 5. Soil salinity (µs/cm)

Method	0-10 cm		10-20 cm		20-30 cm	
	2013	2014	2013	2014	2013	2014
Y1	588	629	661	603	585	618
Y2	592	561	615	628	609	612
Y3	595	584	600	619	638	582
Y4	611	573	680	562	601	581

## CONCLUSIONS

Soil particle size distribution that is formed by different soil tillage methods is important especially in terms of seed bed properties introduction. Well-prepared seedbed acts directly on seedling emergence rates. Conventional tillage methods that create the ideal environment for seeds in the optimum particle size distribution and particle size distribution in the accepted range 1-8 mm yielded the best results in the first and second year with 37.6% and 38.51%. One of the most important parameters affecting the yield, ear diameter, grain /ear ratio and thousand kernel weight methods' effects were statistically found significant. The effect of tillage method on maize yield was significant. The highest yield was found in the plots applied to conventional soil tillage, on the other hand lowest grain yield method was found in direct sowing method. Fuel consumption for tillage and planting methods had been collected and the total fuel consumption value required for production per unit area was found. Among the methods the highest total fuel consumption was obtained in conventional tillage methods; whereas the lowest total consumption value was determined in direct planting methods. When total fuel consumptions had comparison among the methods in conventional tillage methods about 90% more fuel was consumed than the direct planting method. Likewise, in terms of labour efficiency direct planting method had been shown to be superior to other methods. When the effect of methods to organic matter content in the soil was examined, at all depths of direct planting methods that had no soil tillage and on the field surfaces where stubbles were left had the highest increase. The effect of salinity content in soil at all depths of soil tillage methods were found insignificant in general. Decrease in salinity in direct planting method has been more significant than other methods. Values of rise or fall remained under the threat of salinity in soil. In the light of all these results where trial had taken place and where first crop wheat and second crop grain maize production will take place, conventional soil tillage method had revealed better results in terms of product yield. However, direct planting method when compared to others saves time and labour productivity and in large areas of production sowing and harvest is foreseen to be completed without delay.

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## EFFECT OF PLANT GROWTH REGULATORS IN ANTHHER CULTURES OF DIFFERENT PEPPER (*Capsicum annuum* L.) VARIETIES

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*Scientific paper*

### Summary

Anther culture is a modern breeding technique which is time saving and useful way to obtain 100% homozygous lines. Genotype is the most significant parameter in anther culture success. In this research, the responses of five different pepper varieties ('Bell pepper', 'Capia pepper', 'Charliston pepper', 'Thin-long pepper' and 'Long pepper') were investigated in MS (Murashige and Skoog, 1962) culture media including different plant growth regulators in order to get haploid plants through indirect embryogenesis from anthers. While 2,4-D (0.1 mg/L) and KIN (0.1 mg/L) were used with or without MgCl<sub>2</sub>.6H<sub>2</sub>O (1.6 mg/L), NAA (0.1, 1, 5 and 10 mg/L) and BAP (0.1, 1, 5 and 10 mg/L) were used in combination. MgCl<sub>2</sub>.6H<sub>2</sub>O were used for inhibition of darkening. After callus regeneration, all callus tissues were transferred to MS basal medium (embryo regeneration medium) supplemented with 4 mg/l NAA, 1 mg/L BAP, 30 g/L sucrose, 0.25% activated charcoal and 15 mg/L AgNO<sub>3</sub> for providing embryo regeneration. There were differences between responses among all varieties. Best callus regeneration (100%) was obtained from 'Charliston pepper' cultured on MS media which including 0.1mg/L 2,4-D and 0.1 mg/L KIN and MS media supplemented with 0.1 mg/L NAA, 1 mg/L BAP. The media which include MgCl<sub>2</sub>.6H<sub>2</sub>O enhanced darkening problems. Darkening occurred in all anthers, but after a short period light green or cream callus having compact structure were regenerated from those darkened anthers. Best embryo regeneration (48.39%) on embryo regeneration medium, were obtained from 'Capia pepper' calli regenerated from MS medium including 0.1 mg/L 2,4-D, 0.1 mg/L KIN. All embryos stayed in globular stage of embryogenesis.

Key words: *pepper, Capsicum annuum* L., *in vitro*, *anther culture*, *plant growth regulators*

### INTRODUCTION

Pepper (*Capsicum annuum* L.) which is the most basic ingredients in a several cuisines, is a crop plant which can be used as a vegetable, an ingredient of sauces, a colouring and pungent agent of foods, or in pharmaceutical applications (Lantos *et al.*, 2012). Due to the all this factors, pepper is an important vegetable crop for human being (Irikova *et al.*, 2011).

The main aim of the different breeding programs in pepper is to develop new commercially important cultivars (Lantos *et al.*, 2012). The creation of pepper varieties via conventional breeding techniques is a long-term and labour-consuming process (Koleva-Gudeva *et al.*, 2007). Using biotechnological methods, breeding programs of these new cultivars can be sped up (Lantos *et al.*, 2012). Especially *in vitro* production of haploid regenerants from anther culture and genome diploidization, are very useful techniques for overcoming all difficulties in conventional breeding programs (Koleva-Gudeva *et al.*, 2007).

Androgenesis is one of the most widely studied mechanisms. The thousands of microspores are located in each anther so using anther culture technique numerous haploid plants can be obtained from a single anther. The main principles of anther culture is the obstruction of the pollen cells development and the induction of embryos similar to somatic cells from immature pollen cells directly or indirectly. The most important step of these techniques is production of haploid plants (Taşkın *et al.*, 2011). Haploid plants are important for rapid production of homozygous dihaploid lines in cross-pollinated plant species. Using conventional technique, this process takes 10 to 12 years in open pollinated plants, and 6 to 7 years in self-pollinated plants. However, with haploidy techniques this period takes 1 to 2 years (Akyol, 2015).

Many factors such as genotype, donor plant growing conditions, pretreatment of buds or anthers, microspore development stage and incubation conditions are significant in anther culture success but especially medium, plant growth regulator compositions and genotype are the most important factors (Ercan *et al.*, 2006; Alremi

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*et al.*, 2014). Therefore the aim of this present study was determination of the effects of genotype and plant growth regulator compositions on anther culture responses of five pepper varieties.

## MATERIALS AND METHODS

All plant materials were grown in greenhouse conditions in Antalya. For determination of relationship between flower bud size and microspore developmental stage, different size of flower buds were collected and transferred to Izmir packed with aluminum foil in cold bag. All flower buds were grouped according to their sizes. Anther isolated from buds were transferred to the glass slide. Aceto-orcein staining solution dropped on anther and covered with cover glass. Using the back of matchstick the slide was hit with intermediate strength. Slides were observed under a light microscope to identify the flower bud size containing the highest percentage of tetrad microspores (7 mm). This finding is similar with Çiner and Tıprıdamaz (2002).

Flower buds were surface-sterilized by immersion in 0.1% (v/v) HgCl<sub>2</sub> for 5 min and rinsed three times by sterile distilled water. Anthers were carefully dissected and transferred to the culture vessels. For determination of best medium composition, MS basal media (Murashige and Skoog, 1962) including different plant growth regulators in different concentrations as shown Table 1 were used. As a carbon source, 30 g/L sucrose was added in all media and sterilization was established with autoclave at 121°C, 15 min. All callus tissues for providing embryo regeneration were subcultured to MS basal media supplemented with 30 g/L sucrose, 0.25% activated charcoal, 15 mg/L AgNO<sub>3</sub>, 4 mg/l NAA, 1 mg/L BAP. Contamination ratio, callus and embryo regeneration ratio, and callus characteristics were determined.

Tab. 1. The list of culture medium composition used for callus regeneration.

Culture medium	2,4-D (mg/L)	KIN (mg/L)	NAA (mg/L)	BAP (mg/L)	MgCl <sub>2</sub> .6H <sub>2</sub> O (g/L)
1	0.1	0.1			
2	0.1	0.1			1.6
3			0.1	1	
4			1	1	
5			5	1	
6			10	1	
7			1	0.1	

**3 g/L gelrite were used as a gelling agent and pH regulated to 5.8.**

## RESULTS AND DISCUSSION

Overall 1050 anthers were taken from five varieties and contamination ratio was found as 17.24%. After all anthers were transferred to different culture media, darkening was occurred in short time. As shown Table 2 below, depending on medium composition and genotype these varieties gave different responses. All anthers cultured medium 2 had shown darkening completely. MgCl<sub>2</sub> can be a reason for darkening problem. Most darkening occurred at 'Charlston pepper' on medium 2 (58.82%). It can be said that MgCl<sub>2</sub> is unnecessary for regeneration in pepper anther culture.

All darkened anthers gave cream or greenish calli. Callus tissues were generally too compact. Best callus regeneration ratio was obtained from 'Charlston pepper' on medium 5 and medium 1 (100%). Thin-long pepper just gave response to the medium 6. Plant growth regulators showed different effects on callus regeneration depending on genotype. For all genotypes, increasing of NAA concentration reduced callus regeneration. MS including 1 mg/L NAA and 1 mg/L BAP gave the best callus regeneration ratio (57.69%) for 'Long pepper'. MS media supplemented with 10 mg/L NAA and 1 mg/L BAP for 'Thin-long pepper', 0.1 mg/L NAA and 1 mg/L BAP for 'Capia' and 'Bell pepper', 5 mg/L NAA and 1 mg/L BAP for 'Charlston pepper' were determined as the best callus regeneration medium compositions.

All embryos were developed from callus tissue with indirect organogenesis and all embryos stayed at globular stage. Best result for embryo formation (48.39%) was taken from 'Capia pepper' calli on MS medium including

0.1 mg/L 2,4-D and 0.1 mg/L KIN. 2,4-D and KIN generally gave better response for embryo formation. Embryo regenerations occurred in ‘Thin-long pepper’ calli on MS with 10 mg/L NAA and 1 mg/L BAP, in ‘Capia’ on MS with 0.1 mg/L NAA and 1 mg/L BAP, in ‘Charliston pepper’ on MS with 1 mg/L NAA and 0.1 mg/L BAP. These results are similar with the callus regeneration ratio and it shows that embryo regeneration depends on callus regeneration capacity (Table 2; Figure 1, 2 and 3).

Tab. 2. Darkening, callus and embryo regeneration ratios

Medium	DARKENING (%) <i>Genotype</i>					CALLUS REGENERATION (%) <i>Genotype</i>					EMBRYO REGENERATION (%) <i>Genotype</i>				
	<i>L</i>	<i>Tl</i>	<i>Cp</i>	<i>B</i>	<i>Ch</i>	<i>L</i>	<i>Tl</i>	<i>Cp</i>	<i>B</i>	<i>Ch</i>	<i>L</i>	<i>Tl</i>	<i>Cp</i>	<i>B</i>	<i>Ch</i>
1	11.54	0	0	33.33	0	50	0	61.29	41.67	100	0	0	48.39	2.78	0
2	15.15	12.50	35.71	18.75	58.82	39.39	0	0	62.50	0	0	0	0	0	0
3	3.23	0	0	19.44	0	48.39	0	94.12	80.56	0	3.23	0	5.88	16.67	0
4	5.26	0	11.54	37.50	6.25	42.11	0	86.54	12.50	43.75	0	0	1.92	10.42	0
5	0	10	7.14	25	0	57.69	0	67.86	25.00	100	0	0	7.14	0	0
6	30	10	11.11	13.64	41.18	35.00	40	37.04	4.55	35.29	0	10	3.70	4.55	0
7	30	0	3.45	50	0	43.33	0	79.31	37.50	83.33	3.33	0	0	0	16.67

\**Genotype. L: Long pepper, Tl: Thin-long pepper, Cp: Capia pepper, B: Bell pepper, Ch: Charliston pepper*

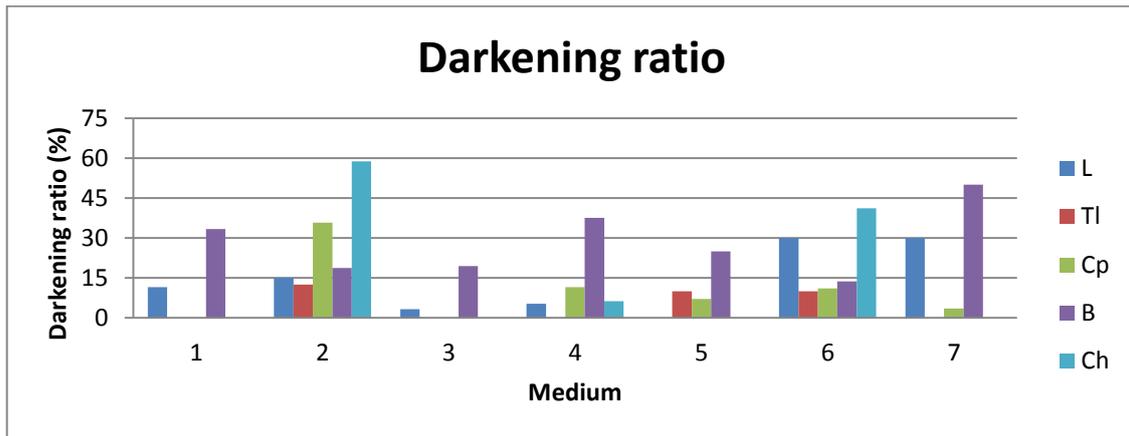


Fig. 1. Darkening ratio in anthers.

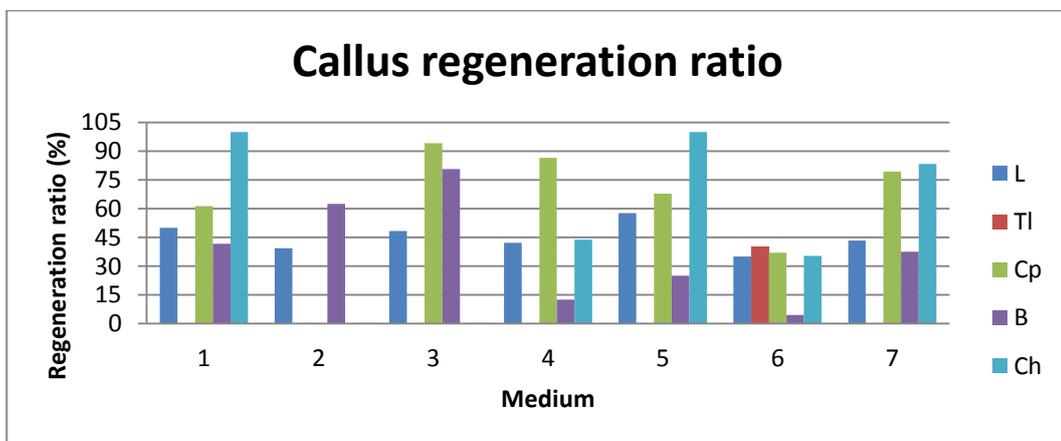


Fig. 2. Callus regeneration ratio

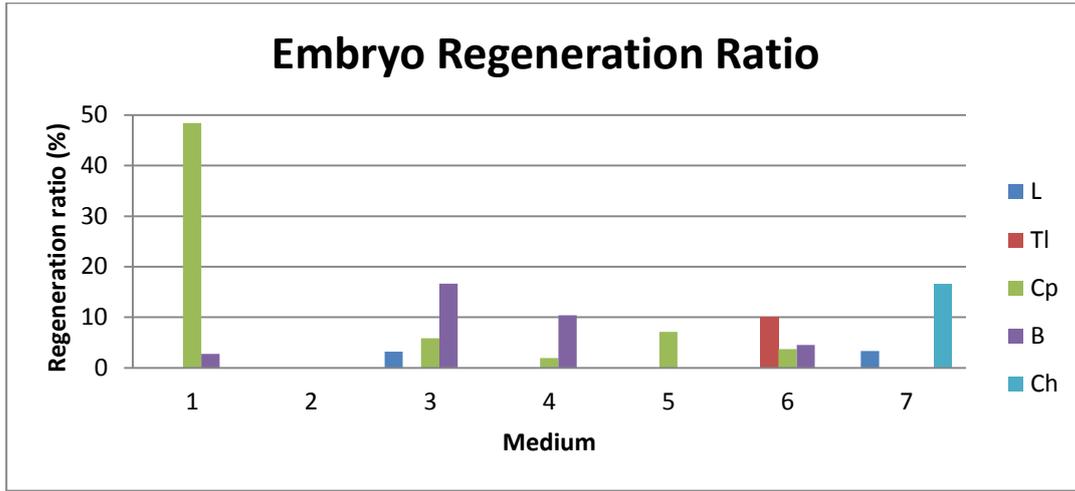


Fig. 3. Embryo regeneration ratio.

### CONCLUSION

Biotechnological techniques such as anther culture mainly are very important for improvement of food and agriculture industry. With the result of this study, we can say that genotype and plant growth regulators are important for embryo production. After determining the suitable medium composition, providing embryo germination and whole plantlet development for each genotype, anther culture can be used as a fast technique for production of homozygous dihaploid lines.

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## EFFECT OF DIFFERENT AGROTECHNICAL MEASURES ON FORAGE YIELD, BOTANICAL COMPOSITION AND YIELD QUALITY ON GRASSLAND

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*Scientific paper*

### Summary

Permanent grasslands occupy more than 50% of agricultural area in Bosnia and Herzegovina having important role in ecology and especially in forage production. However, forage yield and its quality can be very different, from quite small to rather high. This is due to grasslands complexity, which is connected to soil and weather conditions and grassland management. As forage yield and forage quality depend on soil fertility, soil acidity, water availability as well as grassland management, it is of essential importance to know all these elements. Having in mind that botanical composition (crucial for forage quality) changes with time and depends on soil characteristics and grassland management, there is a need to continue putting new efforts in looking for the best solution that will give the best yield and best quality from this area.

With this purpose in mind, a field experiment was set up in which influence of mineral nitrogen, organic manure, nature zeolite and mixture of organic manure and zeolite on forage yield, botanical composition and forage quality were investigated. Results of this investigation indicate different effect of examined factors on botanical composition, especially on portion of legumes, grasses and other species, as well as forage yield and forage quality. Except pure zeolite, all other treatments had constantly positive influence on forage yield. On the other hand, protein content and protein yield were positively influenced by all experimental factors compared to the control variant (without treatment). Significant changes in botanical composition of the sward between botanical composition before and after the experimental period were found. It is important to mention that portion of legumes was gradually higher at the end of the experimental period, which contributed to higher protein content and protein yield.

**Key words:** *Grassland, agrotechnical measures, botanical composition, forage yield, forage quality*

### INTRODUCTION

Grasslands are very important in providing habitat for wildlife, plant genetic resources, plant diversity, recreation, high quality water and air, and other environmental services. Natural and semi-natural grasslands occupy more than 50% of agricultural area in Bosnia and Herzegovina, and have an extremely important role in forage production and animal farming. However, forage yield quality on Bosnian grasslands can be very variable, from quite small (usually) to considerably high. This is due to the complexity of grasslands, which is related to soil and weather conditions, grassland management, but also to the situation of animal farming in Bosnia, agricultural production and economy.

Current grassland management is more or less extensive, i.e. permanently grazed, mown late and periodically fertilized with organic manure and to a lesser extent with mineral fertilizer. Cattle are the main source of manure in Bosnia due to a significantly high number and relatively high daily excretion rates. As cattle manure is mainly handled as a solid mixture of feces, urine, bedding materials and spoiled feed, significant losses of nitrogen (N) can occur throughout the manure management chain, i.e. animal housing – manure storage–manure application.

These N losses can pollute the air, groundwater and surface waters. Moreover, these losses reduce the N fertilizer value of the manure. Jarvis *et al.* (1987) noted that NH<sub>3</sub> volatilization can result in losses of up to 60-80% of the urinary N applied to pastures. NH<sub>3</sub> losses from applied slurry are variable, but are always the greatest immediately after the application, and are reduced by injection. Reducing negative impacts on the environment

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through N- losses and maximizing the fertilizing value of the manure are important for sustainable use of solid manure. Shah (2013) found that the application of bedding additives like zeolite inside the barn reduced NH<sub>3</sub> emission rates by 85% compared with the control (no additives applied during animal housing, manure storage and after manure application to grassland).

As forage yield and forage quality depend on soil fertility, soil acidity, water availability, as well as grassland management (i.e. fertilizing – organic or mineral manure, grazing – rotational or permanent, mowing – early or late), it is of essential importance to find the best solution for improving or sustaining grassland production. Having in mind that botanical composition (crucial for forage quality) changes with time and depends on soil characteristic and grassland management, it must be underlined that constant efforts for maintaining and/or improving botanical composition to get the best yield and best quality from this area should be invested.

With this purpose, a field experiment was set up in which the influence of mineral N, organic manure, nature zeolite and mixture of organic manure and zeolite (cattle manure treated with zeolite) on forage yield, botanical composition and forage quality was investigated.

## MATERIAL AND METHODS

The field experiment was established in Roščevine near Kakanj (440 asl), close to the river Bosna, on a semi-natural grassland, with an average annual precipitation of 905 mm and an average annual temperature 12.6°C. Soil characteristics of this experimental field are very diverse. Accordingly, the pH (H<sub>2</sub>O) of the soil is slightly alkaline (7.39 to 7.62). The soil is very poor on phosphorus. The concentration of P-Al is variable, ranging from 6.11 mg kg<sup>-1</sup> to 38.44 mg kg<sup>-1</sup>. The K, Ca and Mg concentration in the soil of the experimental field is relatively high. Potassium content in soil was very high (192 to 508 mg kg<sup>-1</sup>). The Ca content was also relatively high (35 to 59 mg kg<sup>-1</sup>) while the Mg concentration ranged between 351 and 726 mg kg<sup>-1</sup>. We used the randomized complete blocks design with four replications and plot size of 10 m<sup>2</sup>. The experiment was set up during the summer of 2012 with following variants: control (no treatment), zeolite (3 t ha<sup>-1</sup>), organic manure (30 t ha<sup>-1</sup>), mixture of organic manure and zeolite (30 t ha<sup>-1</sup> + 3 t ha<sup>-1</sup>) in which zeolite was added on matured manure and mineral N was applied. Organic manure, zeolite and mixture of organic manure and zeolite were applied in the autumn of 2013. Mineral N (63 kg ha<sup>-1</sup> and 38.7 kg ha<sup>-1</sup>) was applied in the spring of 2013 and 2014 respectively. Mowing was performed in the heading stage of grasses and the beginning of flowering of legumes. In 2013 four cuts were obtained, while in 2014 three cuts were conducted. Botanical composition was determined through the Braun-Blanquet method. Dry matter yield (DM) was calculated on the basis of the green matter yield and drying according to the drying factor. Protein yield was calculated on the basis of the DM yield and protein content (N × 6.25). Total N was determined by using the Kjeldahl method. The results were subjected by the SPSS 22 programme and tested with the LSD test.

## RESULTS AND DISCUSSION

Before the research results are shown, it is necessary to underline the fact that the soil analysis (conducted after the field experiment was set up, but before the application of experiment variants) indicates on significant differences in organic matter and concentration of all macro elements over the experimental field. This could be the explanation to the fact that no significant differences were found due to big variation of repetitions and between plots.

### Dry matter yield

Figures for DM yield (tab. 1) indicate that the best result in total (19.41 t ha<sup>-1</sup>) was given after application mineral N, and also in the first experimental year. Similar results were noted by Alibegović-Grbić and Čivić (2003). This could be explained by the quick reaction of plants on available mineral N and especially because different grass species prevailed in the sward. However, in the second year, the DM yield was considerably smaller (8.21 vs. 11.20 t ha<sup>-1</sup>) due to lower rate of applied N, as well as the obtainment of only three cuts.

The second best yield (18.43 t ha<sup>-1</sup>) was achieved by application of organic manure treated with zeolite. In both years this sample was higher than an average DM yield for all variants. These findings are in line with researches done by Jarvis *et al.* (1987) and Shah (2013). However, lower effect of this variant on DM yield could be explained due to fact that the zeolite was not applied to the cattle manure directly in the barn, but rather after ammonia had in some extent been volatilized. The least DM yield (16.77 t ha<sup>-1</sup>) was acquired by applying pure zeolite. It was lower even when the amounts were manually controlled. This could perhaps be explained by the capability of available N in the soil to easily bind with zeolite.

Tab. 1. Dry matter yield (t ha<sup>-1</sup>)

Variants	Year		Total
	2013	2014	
Control	9.86	8.00	17.86
Zeolite (3 t ha <sup>-1</sup> )	9.19	7.58	16.77
Organic manure (30 t ha <sup>-1</sup> )	8.84	8.68	17.52
Organic manure + Zeolite (30 t ha <sup>-1</sup> ), mixed	9.58	8.85	18.43
Mineral N (63 <sup>1</sup> and 38.7 kg ha <sup>-1</sup> )	11.20	8.21	19.41
Organic manure (20 t ha <sup>-1</sup> )	9.24	9.21	18.36
<b>Average</b>	<b>9.65</b>	<b>8.41</b>	<b>18.06</b>

The means presented in the table are not statistically significant (P>0.05).

### Protein Yield

Forage quality is a reflection of, first of all, botanical composition and stage of plant growth, but also depends on whether condition and soil fertility (Alibegović-Grbić *et al.*, 2014). Protein yield is directly connected to N concentration, protein content in the forage, and DM yield. Results of this study showed significant differences in protein content among cuts (9.37 to 18.31 %), but in some lesser extent among applied treatments (9.37 to 12.25; 15.00 to 17.56; 13.63 to 15.63 and 15.75 to 18.31 in the first year and first, second, third and fourth cut, respectively).

Tab. 2. Protein yield (kg ha<sup>-1</sup>)

Variants	Year		Total
	2013	2014	
Control	1201.74	1089.90	2291.64
Zeolite (3 t ha <sup>-1</sup> )	1233.66	1076.70	2310.36
Organic manure (30 t ha <sup>-1</sup> )	1141.91	1116.28	2258.19
Organic manure + Zeolite (30 t ha <sup>-1</sup> ), mixed	1266.00	1139.68	2405.68
Mineral N (63 <sup>1</sup> and 38.7 kg ha <sup>-1</sup> )	1531.09	1032.59	2563.68
Organic manure (20 t ha <sup>-1</sup> )	1237.73	1068.80	2306.53
<b>Average</b>	<b>1268.68</b>	<b>1087.32</b>	<b>2356.01</b>

The means presented in the table are not statistically significant (P>0.05).

The highest protein yield in total (2563.68 kg ha<sup>-1</sup>), was achieved through the application of mineral N, however as shown in table 2, it was the lowest in 2014 (1032.59 kg ha<sup>-1</sup>). Corresponding with DM yield, second best protein yield in total and per year, respectively (2405.68, 1266.00 and 1139.68 kg ha<sup>-1</sup>) was obtained by applying organic manure treated with zeolite, which indicates that some ammonia was preserved from volatilization. The least protein yield (2258.19 kg ha<sup>-1</sup>) was found by application of 30 t ha<sup>-1</sup> organic manure, which could be explained by volatilization of ammonia during manure management (Shah *et al.*, 2013) and by the fact that the manure was applied over the surface of the sward. However, significant differences among treatments were not found.

### BOTANICAL COMPOSITION

Due to different grassland management, the botanical composition of the trial field was changed from 2012 to 2014 (table 3), which was also found by Alibegović-Grbić and Čustović (2002). In 2014, generally more legumes in grass sward were noted, but the highest portion (32.3%) was found in treatment with 30 t ha<sup>-1</sup> organic manure, while the least (7.0%) by N application. From legumes, a considerable increase of *Lotus corniculatus* and in some extent of *Trifolium pratense* was noted, both considered very valuable forage species. To a lesser abundance, *Trifolium repens* and *Medicago sativa* were also found, as well as some species from *Vicia* and *Lathyrus* genera.

Tab. 3. Botanical composition (%)

Variants	Grasses (%)		Legumes (%)		Other (%)	
	2013	2014	2013	2014	2013	2014
Control	62.00	58.00	6.00	23.00	32.00	22.00
Zeolite	73.00	57.00	26.00	7.00	20.00	17.00
Manure + zeolite	79.30	66.00	2.70	11.60	18.40	22.40
Manure (30 t ha <sup>-1</sup> )	74.00	48.00	6.60	32.30	19.40	22.50
N (63 <sup>l</sup> and 38.7 kg ha <sup>-1</sup> )	85.00	73.00	2.00	7.00	13.00	13.00

This had a positive influence on protein content in DM in 2014. Portion of grass species were decreased, while *Holcus lanatus*, considered to be less valuable for forage quality, disappeared after the first year. *Holcus lanatus* is perennial, but in some cases behaves as annual. It should usually be reaped early, and if cut late, it can seed and reseed by itself. This could explain its disappearance from the grass sward, while experimental field was cut in the early stage of plant growth and could therefore not be reseeded. Contrary to these, *Dactylis glomerata* and *Arrhenatherum elatius* increased their abundance. There was an increase of other grasses such as *Festuca pratensis*, *Lolium multiflorum*, *Lolium perenne*, as more valuable forage grasses that have positive effect on forage quality.

### CONCLUSION

Research results showed that grassland management has an important impact on forage and protein yield, botanical composition and forage quality. Some differences in DM and protein yield among different treatments were found, however their significance is of minor importance primarily due to large differences in concentrations of elements within the experimental field (this was not known when field was chosen). However, organic manure treated with zeolite increased DM and protein yield compared to pure organic manure. This justifies the treatment of organic manure with zeolite before grassland manuring. It is also of ecological interest, however economic advantage has yet to be proved.

Botanical composition was changed from 2012 to 2014. In 2014 generally more legumes in grass sward were noted. The highest portion (32.3%) was found in treatment with 30 t ha<sup>-1</sup> organic manure and the least (7%) by N application. Changes in botanical composition had some positive effect on forage quality, especially in the 2<sup>nd</sup> and the 3<sup>rd</sup> cut.

Anyway, it would be reasonable to repeat the same research on grassland with more unique soil characteristic.

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## THE USE OF TREATED WASTEWATER FOR AGRICULTURAL IRRIGATION AND POTENTIAL RISKS

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*Review paper*

### Summary

Most important consumer of fresh water on the world through irrigation is agriculture. Irrigation of agricultural lands accounted for 70-80% of the water used worldwide while water shortage is increasing in the world for many years. For that matter finding alternative sources for irrigation in agriculture has become an important issue. In recent years use of wastewater for irrigation as an alternative water source for agricultural purpose is a worldwide practice. Thus, wastewater use in agriculture can have substantial benefits for agriculture and water resources management. On the other hand wastewater can include chemical risks to plant health, and risks to the environment in the form of soil and groundwater pollution. The risks are primarily to public health from the microbial pathogens contained in wastewater including bacteria, viruses, protozoa, helminth, chemical load and heavy metals. For to avoid from all these risks that may occur after using wastewater for irrigation, Turkey and many other countries were set some regulations to ensure quality criteria of wastewater use for irrigation.

**Key words:** *irrigation, regulation, soil, wastewater, heavy metals.*

### INTRODUCTION

Agriculture is the largest consumer of freshwater reserves in the world, using about 65-75% (Bennett, 2000). Currently, agricultural activities consumes about 75% of the total fresh water resources of Turkey. The growing demand for water by its rapidly increasing population is reducing the amount of water available for use in agriculture. This situation emphasizes the need for optimal water resource management and the economic use of water (Cakmak and Apaydin, 2010). As irrigated agriculture is more productive and profitable, the use of irrigation has grown significantly with increasing global water scarcity. Irrigation with wastewater can increase the available water supply or release better quality supplies for alternative uses because of the water scarcity. Based on information from the countries providing data on irrigated areas, it is estimated that more than 4-6 million hectares are irrigated with wastewater or polluted water (Jiménez and Asano, 2008; Keraita *et al.*, 2008; UNHSP, 2008). A separate estimate indicates that 20 million hectares land are irrigated with polluted water globally. This figures out approximately 7% of the total irrigated land in the world. On the other hand, the area irrigated with treated wastewater reported as 10% of the total irrigated land in the world (WHO, 2006).

Wastewater irrigation effects supply of macro and micronutrients for plant growth, soil pH, soil buffer capacity, and soil cation exchange capacity (CEC) (Rusan *et al.*, 2007). Soil microorganisms have been observed to have increase metabolic activity when sewage effluent is used for irrigation (Meli *et al.*, 2002). These can be seen as a positive effect but irrigation with wastewater should focus on two important points; which are short-term and long-term effect of soil fertility parameters. On the other hand, wastewater may contain undesirable chemical constituents and pathogens that pose negative environmental and health impacts with possible accumulation of heavy metals and other hazardous pathogens such as viruses, bacteria, helminth, and fecal coliforms in the soil-plant system (Papadopoulos, 1995). And this situation composes an important risk for soil and the environment.

There is 460 active wastewater treatment facility in Turkey and these facilities process the waste of approximately 78% of the country's population. The wastewater treatment effluent is mostly discharged to the sea (44.9%) and rivers (44.3). It is also being used for irrigation purposes and in forestry. Wastewater reuse for municipal and industrial applications is currently under consideration in Turkey. According to Turkish Statistical Institute data for the year 2012, only 0.9% of the total wastewater has discharged to fields after treatment. However, 74.8% of the total wastewater amount is given to the land without any treatment. In addition, there is no information whether the remaining 24.3% is used for irrigation (TUIK, 2012).

Some countries, especially the developed ones, have national guidelines addressing wastewater to use in agriculture. The best-known international guidelines are those produced by the UN and in particular the WHO.

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This paper was prepared as a review for describing the use of treated wastewater for irrigation. The potential risks of wastewater uses in agriculture was compiled. This paper also presents the Turkish water quality criteria for irrigation which is used in an on-going research project.

### Potential Risks of Wastewater Use in Agriculture

Potential benefits and risks arising from the use of wastewater in agriculture. Selected potential impacts are summarized by Hussain *et al.* (2001). Generally, contents of wastewater and their potential influences through agricultural uses are shown in Tab 1.

Tab 1. Contents of wastewater and the potential influences of wastewater for agricultural use (Hussain *et al.*, 2002; Qadir and Scott, 2010).

Contents	Influences	
	Positive	Negative
Macronutrients: N, P, K	No or minimal need for chemical N, P and K fertilizers N supplied through wastewater helps in crop establishment in early growth stages by mitigating the negative effects of excess salts if added through wastewater irrigation or present in pre-irrigation soil - P added to the wastewater-irrigated soil helps in crop establishment throughout the growth period - Optimal level of K helps in crop maturity and quality, and in mitigating the negative effects of excess salts (particularly sodium) applied through wastewater irrigation or present in pre-irrigation soil	Excess N applied through wastewater may lead to excessive vegetative growth (green biomass), delay in crop maturity, lodging and low economic yield - Excess N and P in wastewater can cause eutrophication of natural water bodies and in irrigation systems, undesirable growth of algae, periphyton attached algae and weeds - Leaching of N can cause groundwater pollution and methaemoglobinemia (generally in infants) in case of drinking N-rich groundwater (particularly high levels of nitrates, NO <sub>3</sub> ) - P can accumulate in the soil where it is immobile
Total dissolved solids (TDS) and major ionic elements: Na, Ca, Mg, Cl, B	Ca supplied through wastewater improves soil structure and counterbalances the negative effects of accompanying high concentrations of Na and Mg - High electrolyte concentration, particularly resulting from Ca salts, improves hydraulic properties of low permeability soils	Excess Na and Mg can cause deterioration of soil structure and undesirable effects on hydraulic properties such as infiltration rate and hydraulic conductivity - Excess salts impact plant growth through osmotic effects - Specific ion effects from Cl, B and Na possible, including phytotoxicity - Deterioration of water quality of natural surface-water bodies receiving wastewater or drainage from wastewater-irrigated land - Salt leaching into groundwater
Metals and metalloids: Cd, Cr, Ni, Zn, Pb, As, Se, Hg, Cu, Mn	No or minimal need for micronutrient fertilizers supplying essential metals ions such as Cu, Zn, Fe, and Mn	- Excess levels in irrigated soils and the environment may reach phytotoxic levels - Systemic uptake by crops, particularly those consumed by humans and animals - Possible toxicity in humans and animals - Possible contamination of groundwater under highly permeable and shallow water table conditions
High organic matter content, suspended solids and algal particles	Organic matter added through wastewater improves soil structure; can enhance cation exchange capacity and bind, and gradually releases essential nutrients for crop growth - Organic matter may also hold some undesirable metal ions rendering them in less available form for plants - Can contain nutrients	Plugging of micro irrigation systems such as drippers and sprinklers - Hypoxic conditions due to depletion of dissolved oxygen in water - Possible occurrence of septic conditions - Possibility of increased mortality in fish and other aquatic species
Emerging contaminants (residual pharmaceuticals, Endocrine disruptor compounds, active residues of personal care products)	Only limited evidence of possible uptake by crops and the food chain, especially in developing countries where use of pharmaceuticals and personal care products is lower than in developed countries	Possible contamination of groundwater with emerging contaminants and other contaminants, particularly under highly permeable and shallow water table conditions
Pathogens: viruses, bacteria, protozoa, helminth eggs, faecal coliforms	None	Can cause a range of communicable diseases for farmers, traders and food consumers, such as diarrhoea, typhoid, dysentery, cholera, gastroenteritis, ascariasis, hepatitis, ulcer, food- poisoning

## Regulations

There is not a common regulation of wastewater reuse in the world due to various climatic, geological and geographical conditions, water resources, type of crops and soils, economic and social aspects, and country/state policies towards using wastewater influents for irrigation purposes.

The most important criteria for evaluating the suitability of treated wastewater for irrigation use are: public health aspects, salinity (especially significant in arid regions), heavy metals and harmful organic substances. In addition to standards, regulations can include best practices for wastewater treatment and irrigation techniques as well as regarding crops and areas to be irrigated. Concerning the microbiological standards, the Turkish regulation consists of only faecal coliform parameter and, it seems to be insufficient and needs to be revised in terms of health aspects. Turkey and other countries have developed guidelines that give quality criteria and advice on how effluents should be reused for irrigation purposes. Regulations developed for Turkey are shown in Tab 2-7 (TCRG, 1991). In Turkey, the WHO standards have been adopted. Irrigation waters are classified in five water quality classes, ranging from perfect quality to improper harmful, on the basis of a range of parameters.

Tab 2. Turkish water quality criteria for irrigation.

Quality Criteria	Class I (Perfect)	Class II (Satisfactory)	Class III (Usable)	Class IV (Usable with care)	Class V (Improper harmful)
EC <sub>25</sub> (micromhos at 25 °C) × 10 <sup>6</sup>	0-250	250-750	750-2,000	2,000-3,000	>3,000
Sodium (Na, %)	<20	20-40	40-60	60-80	>80
Sodium Adsorption Ratio (SAR)	<10	10-18	18-26	>26	
Residual Sodium Carbonate (RSC) in meq/l or mg/l	>1.25 <66	1.25-2.5 66-133	>2.5 >133		
Chloride (Cl) in meq/l or mg/l	0-4 0-142	4-7 142-249	7-12 249-426	12-20 426-710	>20 >710
Sulphate (SO <sub>4</sub> <sup>-</sup> ) in meq/l or mg/l	0-4 0-192	4-7 192-336	7-12 336-575	12-20 575-960	>20 >960
Total Salt Concentration (mg/l)	0-175	175-525	525-1,400	1,400-2,100	>2,000
Boron Concentration (mg/l)	0-0.5	0.5-1.12	1.12-2.0	>2.0	-
Class of irrigation Water *	C <sub>1</sub> S <sub>1</sub>	C <sub>1</sub> S <sub>2</sub> , C <sub>2</sub> S <sub>2</sub> , C <sub>2</sub> S <sub>1</sub>	C <sub>1</sub> S <sub>3</sub> , C <sub>2</sub> S <sub>3</sub> , C <sub>3</sub> S <sub>3</sub> , C <sub>3</sub> S <sub>2</sub> C <sub>3</sub> S <sub>1</sub>	C <sub>1</sub> S <sub>4</sub> , C <sub>2</sub> S <sub>4</sub> , C <sub>3</sub> S <sub>4</sub> , C <sub>4</sub> S <sub>4</sub> , C <sub>4</sub> S <sub>3</sub> , C <sub>4</sub> S <sub>2</sub> C <sub>4</sub> S <sub>1</sub>	-
NO <sub>3</sub> <sup>-</sup> -N or NH <sub>4</sub> <sup>+</sup> -N (mg/l)	0-5	5-10	10-30	30-50	>50
Faecal Coliform** 1/100 ml (CFU in 100 ml)	0-2	2-20	20-100	100-1,000	>1,000
BOD <sub>5</sub> (mg/l)	0-25	25-50	50-100	100-200	>200
TSS (mg/l)	20	30	45	60	>100
pH	6.5-8.5	6.5-8.5	6.5-8.5	6.5-9	<6 or >9
Temperature (°C)	30	30	35	40	>40

Below, Tab 3 states the technical limitations and related on reuse of water in irrigation and Tab 4 indicates the suitability of treated domestic wastewater in irrigation without disinfection. Maximum allowable concentration of heavy metals and toxic elements in irrigation water is given in Tab 5 and is adopted from EPA.

Tab 3. The technical limitations and related basis on reuse of water in irrigation.

Type of crops	Technical limitations
Orchard and vineyards	-No spray irrigation -Fruits falling on ground cannot be eaten -Faecal coliform <1,000/100 ml
Fibrous and seed crops	-Surface or spray irrigation -Disinfection and biological treatment are required for spray irrigation -Faecal coliform <1000/100 ml
Feed crops, flowers, vegetables which are not eaten raw	-Surface irrigation -Minimum mechanical treatment

Tab 4. Suitability of treated domestic wastewater in irrigation without disinfection.

	Arable land	Meadow and pasture	Vegetables	Feed crop	Fruit production	Forestry or woodland
Effluent of biological treatment plant or pre-treatment effluent (with 2 hours detention time sedimentation tank)	(+) for both NP & P	(+) for both NP & P	(-) for both NP & P	(+) for NP (-) for P	(-) for both NP & P	(+)
Effluent of aerobic stabilization ponds and lagoons	(+) for NP (-) for P	(+) for NP (-) for P	(-) for both NP & P	(+) for NP (-) for P	(-) for both NP & P	(+)

NP= no plantation, P= plantation (with or without fruits)

Tab 5. Maximum allowable concentration of heavy metals and toxic elements in irrigation water.

Elements	Max. total amount to be given to unit area of land (kg/ha)	Maximum allowable concentration in every type of soil and under continuous irrigation (mg/l)	Maximum allowable concentration in clayey soil (pH: 6.0-8.5) irrigation less than 20 years (mg/l)
Aluminium	4,600	5.0	20.0
Arsenic	90	0.1	2.0
Beryllium	90	0.1	0.5
Boron	680	****	2.0
Cadmium	9	0.01	0.05
Chromium	90	0.1	1.0
Cobalt	45	0.05	5.0
Copper	180	0.2	5.0
Fluoride	920	1.0	15.0
Iron	4,600	5.0	20.0
Lead	4,600	5.0	10.0
Lithium *	-	2.5	2.5
Manganese	920	0.2	10.0
Molybdenum	9	0.01	0.05**/** <sup>1</sup>
Nickel	920	0.2	2.0
Selenium	18	0.02	0.02
Vanadium	-	0.1	1.0
Zinc	1,840	2.0	10.0

\* 0.075 mg/l is recommended for irrigation of citrus fruits  
 \*\*/\*\* allowable concentration in only acidic clay soil with high iron content  
 \*\*\*\* specified in Table 6 of the regulation

Boron concentrations are known to be important for our conditions Boron is an essential element for plant growth (Chloride is also essential but in such small quantities that it is frequently classed non-essential.) Boron is needed in relatively small amounts; however, if present in amounts appreciably greater than needed, it becomes toxic. For some crops, if 0.2 mg/l boron in water is essential, 1 to 2 mg/l may become toxic. Classification of irrigation water with respect to resistance of plants to boron is given in in Tab 6.

Tab 6. Classification of irrigation water with respect to resistance of plants to boron.

Classification of irrigation water	Boron concentration (mg/l) sensitive plants <sup>1</sup>	Boron concentration (mg/l) semi-sensitive plants <sup>2</sup>	Boron concentration (mg/l) tolerable plants <sup>3</sup>
<b>I</b>	< 0.33	< 0.67	< 1.0
<b>II</b>	0.33-0.67	0.67-1.33	1.00-2.00
<b>III</b>	0.67-1.00	1.33-2.00	2.00-3.00
<b>IV</b>	1.00-1.25	2.00-2.50	3.00-3.75
<b>V</b>	> 1.25	> 2.50	> 3.75
	walnut, lemon, fig, apple, grape and bean	barley, wheat, maize, oats, olive and cotton	sugar beet, clover, horse bean, onion, lettuce and carrot

The suitability of wastewater for irrigation is still discussing in the world because soil is a natural non-renewable resource. By using not suitable water (or wastewater that contains toxic elements) for irrigation causes many undesirable problems in the soil. Some information on suitability of wastewater for irrigation from different types of industries is given in Tab 7.

Tab 7. Suitability of wastewater for irrigation from different types of industries.

I	II	II
If suitable land nearby, used as irrigation water	Suitable for use as irrigation water in certain circumstances *	Not suitable for use as irrigation water
Beer, malt, wine, potatoes, canned vegetables, jams, canned fruits, milk, potato starch factories	Yeast, sugar, rice and cereal starch, leather glue, bone glue factories, slaughterhouses, plants meat boilers, plate-digits, margarine plant, paper plant, carton factory, textile industry (bleaching, mercerizing, dyeing, printing-houses, etc.) wool washing, fish, canned fish, mining.	Paint and varnish factories, soap factory, heavy inorganic chemical industry; pharmaceutical factory, metal factory, sulphite pulp factory, viscose rayon factory, pyrolysis plant, gas plants gas turbine generators, hardware, oil industry, coal washing, dynamite industrial, wood coking plant.

\* This industrial wastewater treatment should be up to the value of the Tables 3 and 5

## CONCLUSION

Numerous parameters are used to define irrigation water quality, to assess salinity hazards, and to determine appropriate management strategies. A complete water quality analysis will include the determination of total concentration of soluble salts, the relative proportion of sodium to the other cations, the bicarbonate concentration as related to the concentration of calcium and magnesium, the concentrations of specific elements and compounds, total solids, the amount of floating substances such as oil and grease, and organic loads and pathogenic organisms. In addition, after determining a suitable irrigation method depends on soil characteristics, climate of the region and plant requests, treated wastewaters in accordance with regulations can be used for irrigation.

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## THE DERMINATION OF NEW PEANUT VARIETIES AND THEIR YIELD AND QUALITY IN OSMANIYE REGION

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*Professional paper*

### Summary

This study was conducted in 2014 as a main crop, between April and September at the research farm of the Oil Seeds Research Institute in the Osmaniye region, in Turkey. In this study, we were compare the yield and product quality of the most cultivated NC-7 peanut variety in Osmaniye region and other 12 different peanut varieties alternatives (Halisbey, Arıoğlu-2003, Sultan, Ç-1, Osmaniye-2005, Bradley, Wilson, Batem-5025, Batem Cihangir, Georgiya Green, Florispan ve NC-V 11).

The experimental design was Randomized Complete Blok with three replications. Pod number per plant, pod yield per plant, pod yield per ha, 100 pod weight, 100 seed weight, seed/pod ratio, first quality pod weight ratio, first quality pod number ratio, second quality pod weight ratio, second quality pod number ratio, oil content, protein content and fatty acid were analyzed.

In this study, while the lowest pod yield was obtained from Çin-1 varieties (3952.3 kg ha<sup>-1</sup>) the highest pod yield per hectare was obtained from Halisbey (6293.6 kg ha<sup>-1</sup>).

Key words: *Peanut, variety, yield and yield components.*

### INTRODUCTION

Peanut (*Arachis hypogaea* L.) also known as peanut, is an important oil seed, food and feed crop grown. Peanut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production area of the total oil seed in country. Peanut (*Arachis hypogaea* L.) is cultivated in the semi-arid tropical and sub-tropical regions of nearly 100 countries in six continents between 40° N and S of the equator. It is an important legume grown and consumed globally and in particular in sub-Saharan African countries (Okello *et al.*, 2010a). According to years 2013, peanut which is 25.5 million hectares of harvest area is production 45.3 million tons and average yield 179.0 da in the world. China, India, USA, Nigeria and Indonesia are the first row peanut production in the World.

For years 2014, harvest area for peanut is 33 thousand hectares in Turkey. Production is 123 thousand tons. Adana is provided the highest yield in Turkey. This city is followed with production 3.4 tons by Osmaniye. In many countries, peanut cake and haulms (foliage, straw/stems) are used as livestock feed. Peanut is also a significant source of cash income in developing countries that contributes significantly to livelihoods and food security. There has been a substantial increase in peanut production as both a food and cash crop because of increased awareness of their value as a source of protein (23-25% content), fat (40-50%), oil (40-52% content) and (10-20%) carbohydrate depending on the variety (Savage and Keenan, 1994).

With the costs of animal protein ever increasing, peanut is becoming an even more important source of protein. A kilogram of peanuts is high in food energy and provides approximately the same energy value as 2 kilograms of beef, 4 liters of milk, or 36 medium-size eggs. Peanut seeds are also a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Peanut is consumed raw, roasted, blanched, as peanut butter, crushed and mixed with a cooked paste. Peanut production, marketing and trade provide sources of employment, income and foreign exchange.

### MATERIALS AND METHODS

This research was conducted in 2014 to determined growing possibilities of some Virginia, Spanish and Runner market type peanut varieties as a main crop, between April and September at the Oil Seeds Research Institute research farm in the Osmaniye region, in Turkey. Thirteen peanut varieties were used as a material. Certified

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seeds of 13 peanut varieties: Nc-7, Halisbey, Arioğlu-2003, Sultan, Ç-1, Osmaniye-2005, Bradley, Wilson, Batem-5025, Batem Cihangir, Georgiya Green, Florispan and NC-V 11 were procured from Cukurova University, Agricultural Faculty, Adana. The soil test in 2014 indicated pH of 7.9 with enough concentrations of K<sub>2</sub>O and low concentrations of P<sub>2</sub>O<sub>5</sub>. In addition, the organic matter and nitrogen content of the soil was very low. The lime content was 5.11% in the enough layers.

In the Osmaniye province of Turkey, winters are mild and rainy, whereas summers are dry and warm, which is a typical of a Mediterranean climate. The average monthly air temperature was 24.1 to 29.0°C range in 2014. The total rainfall was 78.6 mm during the growing periods in 2014, respectively (Anonymous, 2015).

The experimental design was a Randomized Block Design with three replications. The experimental site was cultivated deeply by the moldboard following the harvest of the previous crop in the autumn. And then the soil was prepared by using disked-harrowed the day of planting in 2014. Before planting, 200 kg ha<sup>-1</sup> of DAP fertilizers were applied. Traflen was applied at 2.0 l ha<sup>-1</sup> before planting for weed control. The plots size was 14.0 m<sup>2</sup> (2.8 x 5.0 m) and each plot had four rows. Plots were planted by hand in the first week of April in 2014, with 70 cm between-row distance. Seeds pretreated with fungicide were used for sowing. Uniform and undamaged 300 seeds for were used one parcels. On day 15 after emergence, seedlings were thinned. 1. and 3. in front of irrigation were given 33% nitrate fertilizer into the soil. After this, standard cultural practices (weed cleaning, insecticide, hoeing, fertilization, sprinkler irrigation vs.) were followed during the growing season.

At the end of maturity, 20 plants from the middle two rows were selected randomly and harvested for seed yield. After, pods of twenty plants were dried in the sun (until the fall moisture content approximately 9%). Total dry weight (pod only) was determined. Flowering days, maturity days, pod number per plant, pod yield per plant, pod yield per ha, 100 pod weight, 100 seed weight, seed/pod ratio, first class pod weight ratio, first class pod number ratio, second class pod weight ratio, second class pod number ratio, oil ratio, oil yield, protein ratio and fatty acid analysis were taken and analyzed. All the readings are mean of 20 different plants. The data were statistically analyzed by using JUMP package program with Randomized Complete Blok design. The least Significant Differences (LSD) test was used to compare the treatments at 0.05 level.

Tab. 1. The used cultivars in the experiment and some of their growth characteristics

Varieties	Characteristic
<b>Halisbey</b>	Virginia market type, dark green leaves color, large-seeded, seeds are light pink, semi-vertical, maturity period 140-160, high yield.
<b>Nc-7</b>	Virginia market type, large-seeded, highly branched, semi-recumbent, maturity period 140-160, high yield.
<b>Sultan</b>	Virginia market type, dark green leaves color, large-seeded, seeds are light pink, semi-vertical, maturity period 140-160, high yield.
<b>Çin-1</b>	Virginia market type, semi-recumbent, very early (20-25 days more early according to Nc-7), it has not yet been registered.
<b>Osmaniye-2005</b>	Virginia market type, large-seeded, semi-upright, dark green leaves, grains of dark red, and smooth.
<b>Brantley</b>	Virginia market type, dark green leaves, semi-recumbent, light pink color of seed and like NC-7.
<b>Wilson</b>	Virginia market type, medium-seeded, seeds are a light pink color.
<b>Batem-5025</b>	Virginia market type, semi-recumbent, large-seeded, light pink color, seed characteristics is similar to NC-7.
<b>Arioğlu-2003</b>	Virginia market type, large-seeded, seeds are light pink, leaf is light color, according to Sultan and Halisbey varieties are more upright.
<b>G. Green</b>	Runner market type, small-seeded, the main stem and the side branches are fully recumbent.
<b>Florispan</b>	Spanish market type, small-seeded, little branched, upright, grain round and light skin color, maturity period 120-130, mechanized arable.
<b>Nc V 11</b>	Virginia market type, large-seeded, semi-recumbent, susceptible to iron deficiency.

## RESULTS

The palmitic acid percentage of peanut varieties varied between 6.65-13.37% in main cropped. The differences the between the varieties were statistically significant. The highest palmitic acid percentage was obtained from Florispan (13.37%) variety and the lowest from Brantley (6.65%) variety. The differences between the varieties for the palmitic acid do to their genotypes differences. The significant differences for palmitic acid among

peanut varieties were attributed to the genetic make-up and place of origin of particular varieties. Weiss (2000) has been reported a range of 6.3–8.2% of palmitic acid in Australian varieties. Data revealed statistically significant differences among varieties for stearic acid (Table 2). The stearic acid percentage of peanut varieties varied between 2.47-3.99%. The varieties Brantley accumulated the highest (3.99%) stearic acid which significantly differed from rest of the varieties. Georgia Green accumulated the lowest (2.47%) stearic acid. Anderson *et al.*, 1998 reported that significant differences among the varieties were due to genetic makeup of the cultivars and environmental changes. Stearic acid range of 4.9-6.2% in Australian Peanut which is higher than those observed in present study (Weiss, 2000). Data presented revealed statistically significant differences among varieties for oleic acid content in Table 2. The highest (81.56%) value reported in Bradley which was statistically significantly different from rest of the cultivars. The lowest (39.69%) oleic acid was reported in Florispan. Weiss (2000) was observed a range of 52.3-60.1% of oleic acid in different cultivars commonly grown in Australia. The differences between the varieties for the oleic acid do to their genotype differences. Linoleic acid percentage in different varieties showed statistically significant variations (Table 2). The highest (39.72%) linoleic acid was reported in Florispan which was significantly different from rest of the cultivars. The lowest linoleic acid (3.39%) was reported in Brantley which was statistically. The significant differences among varieties may be concerned to their genetic makeup. A range of linoleic acid is 20-40% in different cultivars (Weiss, 2000). The type of groundnut of the varieties is also considered responsible for variation of linoleic acid. Sunflower and Brassica linoleic acid showed inverse relationship with oleic acid. The differences between the varieties were statistically significant. The linolenic acid percentage of peanut varieties varied between 1.31-1.73% in main cropped. The highest linolenic acid percentage was obtained from Sultan (1.73%) variety and the lowest from Georgia Green (1.31%) variety. The differences between the varieties for the linolenic acid do to their genotypes differences. Statistically significant is differences among varieties for arachidic acid (Table 2). The highest arachidic acid percentage was obtained from Brantley (1.20%) variety and the lowest from Florispan (0.79%) variety. The highest behenic acid percentage (0.07%) in peanut varieties was reported in Georgia Green whereas the lowest from Çin-1 (0.02%) variety.

Changes in protein content and oil content in the developing seeds of groundnut are shown in Table 3. The protein content of the varieties varied between 25.08-30.91%. The highest protein content was obtained from Florispan (30.91%) variety and the lowest from Georgia Green (25.08%) variety. Bhardwaj and Hamama 2003 reported that protein content of the peanut varieties increase with seed development while maximum value at physiological maturity.

In present study, Peanut cultivars differed statistically for oil content (Table 3). The maximum oil content (52.09%) observed in Georgia Green which remained significantly higher and different from rest of the tested varieties. The variety Wilson had the minimum oil content (46.72%). The significant differences among different varieties were attributed to the genetic makeup and the environmental conditions. Qadir *et al.* 2006 reported that higher oil accumulation with increase in temperature is similar to other oilseed crops. Oil content is increase with increase in the temperature during flowering to maturity in Sunflower and Maize Oil content is increase when 1°C rise temperature increased %1 in sunflower (Demurin *et al.*, 2000).

The 100 pod weight, 100 seed weight and kernel percentage of the twelve groundnut varieties were given in Table 4. The number of pegs and pods are the most important yield components that affect the yield potential of groundnut (Awal and Ikeda, 2003). There were statistically significant differences among the varieties in terms of 100 pods weight and 100 seeds weight. 100 pod weight is highest in the varieties Sultan (355.00 g) and lowest in the variety (146.33 g) Florispan (Table 4). The variety, Florispan that had the lowest 100 pods weight and 100 seed weight, has highest protein content (Table 3 & 4). Arıoğlu *et al.* 2001 reported that the 100-seed weight was affected by environmental conditions. Kernel percentage was statistically different among the different varieties studied it ranged from 61.38% to 75.33%. The higher 100 seed weights recorded in Osmaniye-2005 (138.14 g) and NC-7 (138.15 g) varieties (Table 4). 100 seed weight had positive relationship with 100 pod weight. Although, Florispan which has the lowest 100 seed weight, gave the highest oil content (Table 3 & 4).

Statistically significant differences were observed among peanut varieties in Table 5. The economically important part of groundnut plant is the pod which encloses the seeds. The pod and the number of seeds per pod are important criteria. The pod weight of peanut varieties varied between 50.03-88.20 g plant<sup>-1</sup> in main cropped (Table 5). The highest pod weight was obtained from Osmaniye-2005 (88.20 g plant<sup>-1</sup>) variety and the lowest from Florispan (50.03 g plant<sup>-1</sup>) variety. But Florispan variety has the highest protein content. Table 5 showed the highest pod number, first quality pod number and first quality pod weight were obtained from Georgia Green variety. Oil content is the highest in Georgia Green variety but seeds are small. The differences between the varieties were their genotypic differences. The highest second quality pod number, second quality pod

weight and pod yield were obtained from Çin-1 variety. Among the varieties the highest pod yield was observed from Halisbey (6293.60 kg ha<sup>-1</sup>) variety (Table 5).

Tab. 2. Fatty acid content of peanut varieties

Varieties	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)	Arachidic (%)	Behenic (%)
Halisbey	10.52 ef	3.11 ef	54.49 ef	27.76 cd	1.57 cd	1.03 cd	0.04 cd
NC-7	9.21 g	3.48 cd	63.35 cd	19.01 f	1.60 cd	0.98 de	0.04 cd
Sultan	10.81 de	3.74 abc	55.78 e	25.47 d	1.73 a	0.85 fg	0.03 de
Çin-1	12.24 b	3.95 ab	48.01 g	32.16 b	1.54 cde	0.63 h	0.02 e
Osmaniye-2005	10.53 ef	3.12 ef	54.59 ef	27.31 cd	1.58 cd	0.98 de	0.04 cd
Brantley	6.65 ı	3.99 a	81.56 a	3.39 h	1.70 a	1.20 a	0.06 ab
Wilson	8.02 h	3.68 bc	71.42 b	11.79 g	1.61 bc	1.09 bc	0.05 bc
Batem-5025	9.17 g	3.69 bc	64.59 c	18.29 f	1.68 ab	0.98 ef	0.04 cd
Arioğlu-2003	11.26 cd	2.94 f	52.25 ef	29.19 c	1.49 e	0.91 ef	0.04 cd
G. Green	11.57 c	2.47 g	51.52 fg	29.89 bc	1.31 f	1.17 ab	0.07 a
Florispan	13.37 a	3.25 de	39.69 h	39.72 a	1.52 de	0.79 g	0.03 de
NC V 11	10.01 f	3.27 de	60.40 d	22.21 e	1.57 cd	0.97 de	0.04 cd
<b>Average</b>	<b>10.28</b>	<b>23.85</b>	<b>58.14</b>	<b>23.85</b>	<b>1.58</b>	<b>0.97</b>	<b>0.04</b>
<b>LSD (%5)</b>	<b>0.545</b>	<b>0.286</b>	<b>3.735</b>	<b>2.934</b>	<b>0.077</b>	<b>0.076</b>	<b>0.013</b>

Tab. 3. Oil content and protein content of peanut varieties

Varieties	Protein content (%)	Oil content (%)
Halisbey	27.17 e	48.47 cde
NC-7	29.13 bc	47.68 defg
Sultan	27.16 e	48.33 def
Çin-1	29.27 bc	47.43 fgh
Osmaniye-2005	27.45 e	48.19 def
Brantley	28.44 cd	49.24 bc
Wilson	29.06 bc	46.72 h
Batem 5025	27.80 de	46.87 gh
Arioğlu-2003	29.54 b	49.80 b
G. Green	25.08 f	52.09 a
Florispan	30.91 a	48.55 cd
NC V 11	28.92 bc	47.62 efgh
<b>Average</b>	<b>28.33</b>	<b>48.42</b>
<b>LSD (%5)</b>	<b>0.897</b>	<b>0.908</b>

Tab. 4. 100 pod weight, kernel percentage and 100 seed weight of peanut varieties

Varieties	100 Pod weight (g)	Kernel percentage (%)	100 Seed weight (g)
Halisbey	345.31 ab	61.38 f	134.42 abc
NC-7	345.33 ab	71.04 cd	138.15 a
Sultan	355.00 a	62.78 ef	137.63 ab
Çin-1	281.67 d	63.75 ef	125.25 cd
Osmaniye-2005	341.67 ab	64.59 e	138.14 a
Brantley	354.67 a	71.86 c	134.56 abc
Wilson	335.00 ab	70.64 cd	127.55 bcd
Batem 5025	316.33 bc	71.67 cd	132.91 abc
Arioğlu-2003	295.33 cd	69.42 d	118.50 d
G. Green	176.33 e	77.79 a	77.43 e
Florispan	146.33 e	75.33 b	59.42 f
NC V 11	341.33 ab	69.65 cd	137.45 ab
<b>Average</b>	<b>302.86</b>	<b>69.16</b>	<b>121.78</b>
<b>LSD (%5)</b>	<b>33.853</b>	<b>2.380</b>	<b>10.221</b>

Tab. 5. The important characteristics of peanut varieties

Varieties	Pod Weight (g plant <sup>-1</sup> )	Pod Number (No. plant <sup>-1</sup> )	First quality pod number (%)	First quality pod weight (g)	Second quality pod number (%)	Second quality pod weight (g)	Pod yield (kg ha <sup>-1</sup> )
Halisbey	75.78 ab	27.77 de	73.42 abc	84.04 abcd	26.58 bcd	15.96 abcd	6293.60 a
Nc-7	73.60 abc	31.87 cd	70.98 bcd	82.61 bcd	29.02 abc	17.46 abc	5530.41 abc
Sultan	76.63 ab	28.10 de	67.83 cd	81.23 bcd	32.28 ab	18.89 abc	5544.93 ab
Çin-1	65.75 bcd	37.23 bc	64.10 d	78.72 d	35.90 a	21.28 a	3952.32 e
Osmaniye-2005	88.20 a	28.97 de	67.48 cd	79.55 cd	32.52 ab	20.46 ab	5569.67 ab
Brantley	70.70 bc	25.93 de	73.69 abc	85.52 abc	26.31 bcd	14.36 bcd	5228.75 bc
Wilson	71.87 bc	28.43 de	70.80 bcd	83.43 abcd	29.20 abc	16.45 abcd	4589.47 cde
Batem-5025	57.93 cd	23.27 e	67.92 cd	80.40 cd	32.08 ab	19.52 ab	5018.51 bcd
Arioğlu-2003	68.20 bc	29.60 cde	69.94 bcd	81.15 bcd	30.06 abc	18.75 abc	5027.56 bcd
G. Green	74.60 ab	59.80 a	79.77 a	88.92 a	20.23 d	11.02 d	5699.94 ab
Florispan	50.03 d	40.73 b	77.72 ab	86.71 ab	22.28 cd	12.96 cd	4231.13 de
Nc V 11	66.07 bcd	25.40 de	68.39 cd	81.37 bcd	31.61 ab	18.55 abc	5395.73 abc
<b>Average</b>	<b>71.13</b>	<b>29.92</b>	<b>71.00</b>	<b>82.80</b>	<b>24.08</b>	<b>15.14</b>	<b>5173.50</b>
<b>LSD (%5)</b>	<b>16.066</b>	<b>7.997</b>	<b>8.349</b>	<b>6.207</b>	<b>8.316</b>	<b>6.154</b>	<b>95.214</b>

## CONCLUSIONS AND SUGGESTIONS

The seed yield was significant in peanut yield. In present study, while the lowest pod yield was obtained from Çin-1 varieties (3952.3 kg ha<sup>-1</sup>) the highest pod yield per hectare was obtained from Halisbey (6293.6 kg ha<sup>-1</sup>). Based on these results, the suitable planting pattern could be suggested as Halisbey in main crop peanut farming in Osmaniye, Turkey. In peanut, large seeds have consumer and market preference, for confectionery and value addition, in domestic and international markets (Nadaf *et al.*, 2009).

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## ROSES (*Rosa spp.*) IN PUBLIC GREEN SPACES OF SARAJEVO

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*Original scientific paper*

### Summary

With over a hundred species and thousands of cultivars and hybrids, roses (*Rosa spp.*) are among most popular ornamental plants, grown for their beautiful, showy and often fragrant flowers. Rose shrubs range in size and shape, from compact, miniature roses to large-flowered tea roses and climbers which can reach seven meters in high. In public green areas roses are used as landscape plants, due to extremely long flowering period. This paper aims to give the first detailed overview of rose species and cultivars in public green spaces of four municipalities of Sarajevo, and discuss and propose their use in order to improve the overall appeal of the city.

Key words: *Rosa spp.*, public green spaces, Sarajevo

### INTRODUCTION

The rose (*Rosa spp.*) is an deciduous shrub native to Northern Hemisphere, cultivated for its attractive, sweet-scented flowers. The cultivation of the rose is as old as any branch of horticulture, as Horace (20 BC) mentions growing roses in beds, and Pliny (AD 50) advises deep digging of the soil for their better cultivation (Henslow, 1922). There are over 100 species and thousands of cultivars of roses today, prized for their showy flowers, long blooming period and pest resistance. There are also several classification methods, but one of the most popular divides roses into hybrid teas, floribundas, climbers, miniature roses and species roses (Ljujić-Mijatović & Mrdović, 1998). Hybrid teas are the most popular type of roses today, with large, fragrant flowers, borne singly on long, strong stems, mostly grown for cutting. Hybrid teas are vigorous and generally bloom throughout the growing season. Floribundas are compact bushy, hardy shrubs which produce flowers in clusters and have very long blooming period, which makes them excellent landscape roses. Old Damask roses are often included in this group. Climbers have long, stiff branches, with flowers borne in numerous clusters along the stems. Miniature roses reach 20-50 cm in height and are popular indoor plants. Species roses occur naturally have simple flowers which bloom once a year, but they have numerous ornamental hips and are extremely resistant to pests and diseases, which makes them useful in spots where more delicate roses might not be a good choice (Rogin, 1989; Bhattacharjee, 2010). Roses are very important and prized garden elements in private gardens but also in public green areas of Sarajevo, due to attractive scented flowers, extremely long flowering period and overall ornamental value (Bečić *et al.*, 2013), but unlike many cities, Sarajevo doesn't have specialized rose garden, and only data on roses grown in public green spaces in Sarajevo are found in paper by Janjić (1998).

### MATERIALS AND METHODS

In order to give the first overview of rose species and cultivars in public green spaces, the research was conducted in four municipalities of city of Sarajevo: Novi Grad, Novo Sarajevo, Centar and Stari Grad. The investigated sites were visited from May to September 2013. The identification of cultivars was conducted using the available literary sources (Hessayon, 1996; Janjić 1999; Quest-Ritson & Quest-Ritson, 2003, Bhattacharjee, 2010), by comparing characteristics of roses (number of petals, color, scent, height, blooming time) and the information of where some new cultivars were plated was obtained by personal communication with employees of KJKP "Park", responsible for maintenance of public green areas in Sarajevo. The overall condition of rose shrub plantations was determined visually, based on their aesthetical appearance.

### RESULTS AND DISCUSSION

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During this research, a total of 47 roses, of which one species rose (2,13%), one climber (2,13%), 22 floribundas (46,81%) and 23 tea hybrids (48,94%), were recorded in public green spaces of Sarajevo (Table 1.).

Tab. 1. The overview of *Rosa* cultivars and species recorded in public green spaces of city of Sarajevo

Type	Species/Cultivar	Novi Grad	Novo Sarajevo	Centar	Stari Grad
Species	<i>Rosa rugosa</i> Thunb.		*		*
Climbers	<i>Rosa</i> 'Fairy Rose'			*	
Floribundas	<i>Rosa</i> 'Alain' Meilland, 1964	*			
	<i>Rosa</i> 'Allgold' Le Grice, 1956	*			
	<i>Rosa</i> 'Anny Duprey' Meilland, 2006		*		
	<i>Rosa</i> 'Carte d'Or' Meilland, 2001			*	
	<i>Rosa</i> 'Dortmund' Kordes, 1955	*	*		
	<i>Rosa</i> 'Elmshorn' Kordes, 1951				*
	<i>Rosa</i> 'Friesia' Kordes, 1973				*
	<i>Rosa</i> 'La Minuette' Lammers, 1969	*	*	*	*
	<i>Rosa</i> 'Lavaglut' Kordes, 1978	*			*
	<i>Rosa</i> 'Lichtkönigin Lucia' Kordes, 1966		*	*	
	<i>Rosa</i> 'Lilli Marleen' Kordes, 1959	*	*		*
	<i>Rosa</i> 'Lydia' Kordes, 1973	*		*	*
	<i>Rosa</i> 'Niccolo Paganini' Meilland, 1990		*		
	<i>Rosa</i> 'Masquerade' Boerner, 1949	*		*	
	<i>Rosa</i> 'Mein Munchen' Cocker, 1987	*	*		
	<i>Rosa</i> 'Piccolo' Tantau, 1984			*	
	<i>Rosa</i> 'Queen Elizabeth' Lammerts, 1954	*	*	*	*
	<i>Rosa</i> 'Rumba' Poulsen, 1959	*	*	*	
	<i>Rosa</i> 'Sangria' Meilland, 1966				*
	<i>Rosa</i> 'Satchmo' McGrady, 1970			*	*
	<i>Rosa</i> 'Schneewittchen' Kordes, 1958			*	
	<i>Rosa</i> 'Somerabend' Kordes, 1995			*	*
Tea hybrids	<i>Rosa</i> 'Alec's Red' Cocker, 1970	*	*		
	<i>Rosa</i> 'Barcarole' Laperriere, 1959			*	
	<i>Rosa</i> 'Big Purple' Stephens, 1985	*		*	
	<i>Rosa</i> 'Crimson Glory' Kordes, 1935	*			
	<i>Rosa</i> 'Duftzauber' Kordes, 1984			*	
	<i>Rosa</i> 'Flamingo' Kordes, 1979	*			
	<i>Rosa</i> 'Folklore' Kordes, 1977		*		
	<i>Rosa</i> 'Gloria Dei' Meilland, 1935	*	*		
	<i>Rosa</i> 'Holsteinperle' Kordes, 1987			*	
	<i>Rosa</i> 'Königin der Rosen' Kordes, 1964	*	*		
	<i>Rosa</i> 'Kronenbourg' McGredy, 1964		*	*	*
	<i>Rosa</i> 'Märchenkönigin' Kordes, 1985	*			
	<i>Rosa</i> 'Maria Callas' Meilland, 1965	*	*		
	<i>Rosa</i> 'Mascotte '77' Paolino, 1976		*	*	*
	<i>Rosa</i> 'Manou Meilland' Meilland, 1977	*		*	
	<i>Rosa</i> 'Mister Lincoln' Swim, 1964			*	
	<i>Rosa</i> 'Papa Meilland' Meilland, 1963			*	
	<i>Rosa</i> 'Parole' Kordes, 1991	*	*	*	
	<i>Rosa</i> 'Perfecta' Kordes, 1957		*	*	*
	<i>Rosa</i> 'Rose Gaujard' Gaujard, 1957			*	*
<i>Rosa</i> 'Sutter's Gold' Swim, 1950		*	*		
<i>Rosa</i> 'Tineke' Select, 1989	*	*			
<i>Rosa</i> 'Valencia' Kordes, 1966		*	*		
<b>TOTAL</b>		22	23	26	14

The most common rose cultivars, found in all four municipalities were white-red 'La Minuette' and pink 'Queen Elizabeth'. They were not mentioned in a paper by Janjić (1998), so it can be concluded that they were planted in recent years. This author, however, mentions *Rosa gallica* 'Tuscany Superb' in front of a building near the Faculty of Forestry in Novo Sarajevo municipality, *Rosa × mariae graebnerae* Ascherson & Graebner in several places in Novi Grad and Centar municipalities, *Rosa × micrugosa* Henkel in Novi Grad and Centar municipalities, and several cultivars, for which he did not list locations: 'Bischofsstadt Paderborn', 'Casino', 'Degel Hard', 'Edelweiss', 'Europawelle Saar', 'Eye Paint', 'Golden Times', 'Heinzelmannchen', 'Ingrid Bergman', 'Kabuki', 'Laura', 'Love Story', 'Anne Marie Trechslin', 'Nicole', 'Nordia', 'Prince de Monaco', 'Rustica', 'Satchmo', 'Sun King', 'Sophia Loren', 'Trumpeter' and 'Tchin Tchin'. Some of these findings date back in 1980s, and it is known that ornamental plants in public green areas were almost completely destroyed during the 1992-1995 war. The change in rose cultivar composition in public green spaces is very likely due to appearance of new, modern and cheaper plants in the market.

The cultivars mentioned in Janjić's work, which were also registered during this survey include 'Mr Lincoln' (found in Centar municipality) 'Duftzauber' (Centar municipality), 'Flamingo' (Novi Grad), 'Manou Meilland' (Novi Grad and Centar), 'Mascotte '77' (Novo Sarajevo, Centar and Stari Grad), 'Rumba' (Novi Grad, Novo Sarajevo and Centar) and 'Sutter's Gold' (Novo Sarajevo and Centar).

The ratio of rose types in public green spaces of surveyed municipalities is presented in Figure 1. The tea hybrids are present in high percentages (28.57% in Stari Grad, 50% in Novi Grad, 52.17% in Novo Sarajevo and 53.85% in Centar). Although they have showy flowers, tea hybrids demand more care than floribundas and species roses do, so they are not as suitable for public green spaces as some other types.

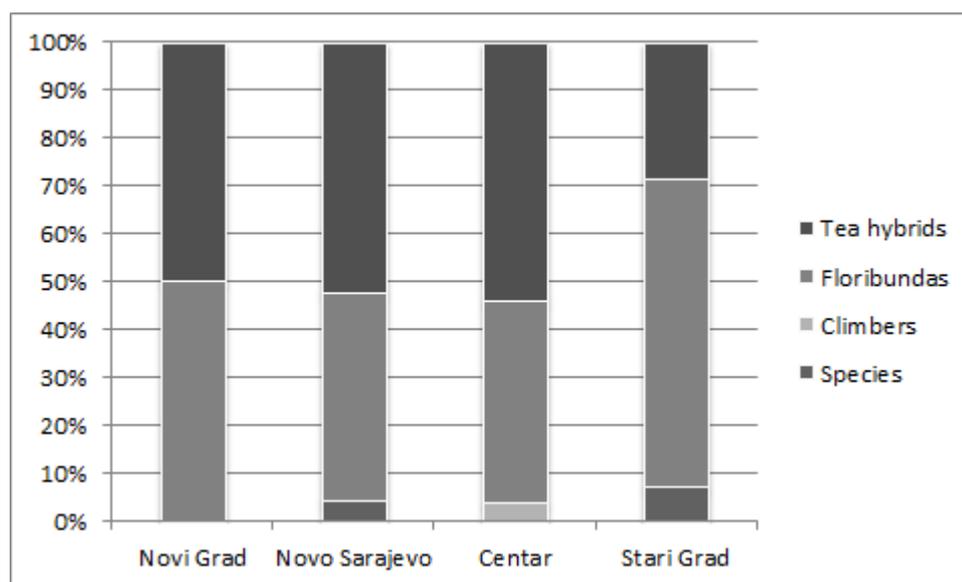


Fig. 1. The ratio of rose types in public green spaces of city of Sarajevo

In Novi Grad municipality roses were found in 3 public green spaces: green space in front of Novi Grad Municipality building, green space in front of Radio-television of Bosnia and Herzegovina and green space in Bulevar branilaca Dobrinje street. A total area under roses in Novi Grad municipality was 1612 m<sup>2</sup>. The most abundant rose cultivars were 'Queen Elizabeth' (50 shrubs) and 'Lilli Marlene' (37 shrubs).

In Novo Sarajevo municipality, roses were found in 7 public green spaces: green space in front of Faculty of Natural Sciences and Mathematics, two green spaces in front of Elektroprivreda building, green space in front of Faculty of Veterinary Medicine, green space near the Malta bridge, green space near the Grbavica stadium trolley station, and green space along the tram tracks near Otoka. A total area under roses in this municipality was 4680 m<sup>2</sup>. The most abundant rose cultivars in Novo Sarajevo municipality were 'La Minuette' (79 shrubs) 'Folklore' (77 shrubs) and 'Lilli Marlene' (62 shrubs).

In Centar municipality, roses were found in 12 public green spaces: Pionirska dolina zoo, green space in Patriotske lige street, green space in front of building of Government of Federation of Bosnia and Herzegovina, green space of Druga Gimnazija high school, green space in front of building of Centar Municipality, green space behind the Building of the Presidency of Bosnia and Herzegovina, Mali park, green space in front of Dr

Abulah Nakaš hospital, green space near Suada and Olga Bridge, green space in front of Holiday Inn hotel, green space along the tram tracks near Holiday Inn hotel and green space in Railway station. A total area under roses in this municipality was 33842 m<sup>2</sup>. The most abundant rose cultivars in Center municipality were 'Queen Elizabeth' (241 shrubs), 'Carte d'Or' (193 shrubs) and 'Somerabend' (78 shrubs).

In Stari Grad municipality, the roses were found in four public green spaces: green space in front of Embassy of Republic of Slovenia, At Mejdan park, green space in Čobanija street and Trg Oslobođenja – Alija Izetbegović square. A total area covered by roses in this municipality was 4299 m<sup>2</sup>. The most abundant rose cultivars in this municipality were 'Friesia' (76 shrubs), 'Lavaglut' (96 shrubs) and 'Lilli Marleen' (45 shrubs).

Generally, most rose shrubs in public green spaces of Sarajevo are in good condition, but planted sparsely, in irregularly shaped mixed groups, without paying attention on achieving good color and habit combinations.

This is particularly visible in At Mejdan park, where there are no there no compact, rich-blooming floribunda roses, but tall tea hybrids, with single flowers borne on top of long branches, which do not add to the overall appeal of the park. Most roses in green space of Druga Gimnazija high school and Mali park are planted under dense trees, where they do not grow as well as they could if they were in full sun. Some shrubs near roads and pedestrian zones were damaged, dry and with broken branches. It is recommended to increase the number of hardy rose types: floribundas and species roses, especially in places exposed to increased level of physiological stress (e.g. along roads), in order to reduce maintenance and improve overall aesthetics of public green spaces in Sarajevo.

## CONCLUSIONS

- During the survey in public green spaces in four municipalities of city of Sarajevo, a total of 47 roses, of which one species rose (2.13%), one climber (2.13%), 22 floribundas (46.81%) and 23 tea hybrids (48.94%), were recorded.
- The most common rose cultivars, found in all four municipalities were white-red 'La Minuette' and pink 'Queen Elizabeth'.
- The change in number and quality of rose cultivars recorded during this survey compared to the previous findings is probably related to appearance of large number of modern cultivars in the market.
- Tea hybrids are very numerous, but they are not as suitable for public green spaces as some other types.
- Generally, most rose shrubs in public green spaces of Sarajevo are in good condition, but planted sparsely, in irregularly shaped mixed groups
- It is recommended to increase the number of hardy rose types: floribundas and species roses, especially in places exposed to increased level of physiological stress (e.g. along roads), in order to reduce maintenance and improve overall aesthetics of public green spaces in Sarajevo.

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## EVALUATION OF BREAD WHEAT CULTIVARS FOR AGRONOMIC AND QUALITY TRAITS

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*Preliminary communications*

### Summary

The trial was conducted in randomized complete block design with 3 replications in Uludag University Faculty of Agriculture Agricultural Research and Application Center in year of 2013-2014. In this study to evaluate the performance in terms of agronomic and quality criteria in Bursa conditions, domestic and foreign origin 14 registered bread wheat are used that recommended for different regions of our country. The averages of used genotypes changed between 586.8-843.5 kg/da for grain yield, 66.3-122.6 cm for plant height, 6.7-11.2 cm for spike length, 14.7-19.1 for spikelet number per spike, 29.0-48.3 for grain number per spike, 1.2-2.1 g for grain weight per spike, 38.9-52.5 g for 1000 grain weight, 75.1-79.8 kg/hl for test weight, % 10.9-13.4 for protein content, 20.6-29.6 ml for sedimentation and % 24.5-34.7 for amount of gluten. The results of correlation study revealed that there were significant association between plant height and spike length, between spikelet number per spike and grain number per spike and grain weight per spike, between grain number per spike and grain weight per spike, between 1000 grain weight and grain yield and sedimentation and between protein content and amount of gluten. Cluster analysis was performed separately for agronomic and quality characteristics. Two main clusters formed in the dendrogram belonging to cluster analysis of agronomic traits and on the first of the main cluster 7 cultivars has been involved and 7 cultivars in the second. For quality features of the dendrogram two main clusters formed in the same way. While 2 cultivars in the second cluster 12 cultivars took place in the first cluster. When the trial results were evaluated in terms of agronomic traits in bread wheat varieties for grain yield, Pamukova and Tahirova varieties were observed to have a higher yield. For the quality characteristics, Sönmez-2001 are determined to have high values in terms of 1000 grain weight, the gluten content and protein content. Pamukova-97 and Tahirova-2000 has been observed that higher values than other varieties for sedimentation. Also the cultivar of Pehlivan for 1000 grain weight has been found to take place in the first place.

Key words: *Bread wheat, agronomic and quality traits.*

### INTRODUCTION

Wheat is the most important agricultural product used in human and animal nutrition that can adapt to the wide range of area in the world. Therefore, wheat breeding studies are ongoing intensively in our country and the all over the world, many commercial varieties are developed every year. But by the current wheat genetic diversity the potential yield power have approached the final frontier, a significant slowdown in the annual rate of increase in productivity has begun to be monitored (Aykut *et al.*, 2005; Kahrıman and Egesel, 2011).

Wheat quality is a relative concept, depends on the person and use purposes. Yield and quality of wheat significantly affected by genotype, environment and genotype x environment interactions (Peterson *et al.*, 1992). Wheat breeders should develop appropriate and acceptable varieties for each sector. For wheat breeding programs rapid and reliable tests are important for the detection of hereditary of yield and quality traits of wheat genotypes. Breeders use different quality tests as selection criteria such as thousand grain weight, test weight, color, hardness, ash content, flour yield, protein content, kneading time, kneading tolerance, gluten quality.

In this study to evaluate the performance in terms of agronomic and quality criteria in Bursa conditions, domestic and foreign origin 14 registered bread wheat are used that recommended for different regions (Southern Marmara, Thrace, Çukurova, Aegean, Southeastern Anatolia, Transitional Zone and Central Anatolia regions ) of our country.

### MATERIALS AND METHODS

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Fourteen bred wheat varieties (Adalaide, Alka, Avorio, Beşköprü, Golia-99, Gönen-98, Hanlı, Kate- A-1, Köksal- 2000, Marmara- 86, Pamukova-97, Pehlivan, Sönmez- 2001, Tahirova-2000) were grown on experimental field of Uludag University Faculty of Agriculture Agricultural Research and Application Center in year of 2013-2014. The trial was conducted in randomized complete block design with 3 replications.

Plant height (PH), spikelet length (SL), spikelet number per spike (SNS), grain number per spike (GNS), grain weight per spike (GWS), grain yield (GY), 1000 grain weight (TGW), test weight (TW), protein content (PC), sedimentation (S) and gluten content (GC) were investigated. Analysis of variance, correlation and cluster analysis were done by JUMP and MINITAB Statistical Computer Program.

## RESULTS AND DISCUSSION

According to the variance analysis, significant differences were determined in plant height and spike length in agronomic characteristics and in all of the quality properties except test weight (Table 1).

Tab. 1. Analysis of variance for traits examined in the trial.

Agronomic characteristics of bread wheat*												
Source of variation	DF	PH (cm)	SL (cm)	SNS	GNS	GWS (g)	GY (kg/da)	TGW (g)	TW (kg/ 100L)	PC (%)	S (ml)	GC (%)
Replication	2	9.91	1.56	2.38	13.74	0.02	17253	0.56	0.26	0.48	0.16	22.05
Genotype	13	591.14**	4.11**	4.75	85.64	0.25	16958	68.73**	4.88	1.02**	21.40**	25.70**
Error	26	21.357	0.50	1.78	31.56	0.13	8768	2.40	4.64	0.22	0.47	5.39
Total	41											
* Plant height (PH), spike length (SL), spikelet number per spike (SNS), grain number per spike (GNS), grain weight per spike (GWS), grain yield (GY), Thousand grain weight (TGW), test weight (TW), protein content (PC), sedimentation (S) and gluten content (GC).												

The average values of the traits are given in Table 2. The averages of used genotypes changed between 586.8-843.5 kg/da for grain yield, 66.3-122.6 cm for plant height, 6.7-11.2 cm for spike length, 14.7-19.1 for spikelet number per spike, 29.0-48.3 for grain number per spike, 1.2-2.1 g for grain weight per spike, 38.9-52.5 g for 1000 grain weight, 75.1-79.8 kg/hl for test weight, % 10.9-13.4 for protein content, 20.6-29.6 ml for sedimentation and % 24.5-34.7 for amount of gluten.

Tab. 2. The average values of the traits

Name of Variety	PH		SL		SNS	GNS	GWS	GY	1000 GW		TW	PC		S		GC	
Adalaide	93.0	fg	8.8	cde	16.4	42.6	1.8	774.5	47.3	cd	78.9	11.8	cd	25.6	de	28.5	cd
Alka	80.6	h	7.9	e	18.0	45.0	1.9	666.6	44.8	def	75.1	10.9	e	20.6	f	24.5	e
Avorio	91.0	fg	10.3	ab	17.6	30.0	1.2	688.0	42.0	gh	78.0	12.7	ab	26.6	cd	33.0	ab
Beşköprü	112.7	b	10.5	ab	19.1	44.8	1.9	701.9	46.7	cde	78.9	12.0	bc	27.6	bc	32.8	ab
Golia-99	66.3	ı	6.7	f	14.7	38.2	1.4	636.8	44.3	efg	76.0	11.6	cde	25.3	e	27.8	cde
Gönen-98	86.6	gh	8.9	cde	15.5	29.0	1.3	767.8	41.9	gh	77.2	11.8	c	25.3	e	28.9	c
Hanlı	103.1	cde	9.7	bcd	16.7	35.4	1.9	708.8	43.3	fgh	77.0	11.4	cde	24.6	e	26.9	cde
Kate- A-1	107.9	bc	9.8	bc	16.4	37.3	1.5	645.6	43.5	fg	78.4	11.5	cde	25.3	e	27.2	cde
Köksal-2000	104.8	cd	8.9	cde	17.7	39.0	1.8	586.8	46.6	cde	78.9	11.8	c	24.6	e	28.8	cd
Marmara-86	95.4	ef	9.4	bcd	16.2	35.4	2.1	734.9	48.1	cd	78.5	11.0	de	20.6	f	24.9	de
Pamukova-97	94.6	f	8.6	de	17.4	39.4	2.0	843.5	38.9	ı	79.2	11.7	cd	29.6	a	28.3	cde
Pehlivan	104.9	cd	9.8	bcd	18.3	38.5	2.0	633.1	52.5	a	77.8	12.0	bc	21.3	f	29.5	bc
Sönmez-2001	122.6	a	10.4	ab	17.4	39.7	1.9	614.7	51.9	b	78.1	13.1	a	24.6	e	34.7	a
Tahirova-2000	98.5	def	11.2	a	19.0	48.3	2.0	793.2	40.9	hı	79.8	12.1	bc	28.0	b	30.2	bc
<b>Mean</b>	<b>97.3</b>		<b>9.3</b>		<b>17.2</b>	<b>38.8</b>	<b>1.8</b>	<b>699.8</b>	<b>45.2</b>		<b>77.9</b>	<b>11.8</b>		<b>25.0</b>		<b>29.0</b>	

In general plant height has been found to vary between 80-100 cm in varieties cultivated in South Marmara region (Doğan, 2002). According to this our results showed similarity to the region's average values. Similar results were observed in the studies of Tayyar and Gül (2008) for plant height, Sakın *et al.* (2004), Ayçiçek and Yıldırım (2006), Kahrıman (2007) and Kaya and Şanlı (2009) for spike length, Ayçiçek and Yıldırım (2006), Tayyar (2008) for spike number per spike, Balcı and Turgut (2002), Kazan and Doğan (2005) and Kurt and Yağdı (2013 a) for grain weight per spike, Turan (2008) for grain weight per spike and Kahrıman *et al.* (2008) and Öztürk *et al.* (2009) for grain yield. The mean values for 1000 grain weight, test weight, protein content, sedimentation and gluten content were 45.2 g, 77.9 kg/100 L, %11.8, 25.0 ml and

%29.0, respectively. The varieties, in terms of quality criteria, examined separately, Sönmez-2001 cultivar was found to have high values in 1000 grain weight, protein content and gluten content while Pamukova-97 and Tahirova-2000 cultivars were determined to have higher values than the other varieties for sedimentation. Also the variety of Pehlivan for 1000 grain weight has been found to take in the first place. For the results of quality characters similar results were reported by in the studies of Kahrıman (2007), Kahrıman *et al.* (2008), Yazar and Karadoğan (2008) for 1000 grain weight, Yağdı (2004) and Kurt and Yağdı (2013 b) for test weight, Yağdı (2004), Doğan and Uğur (2005), Sayalsan *et al.* (2006) for protein content. The sedimentation values were found lower than the results of Egesel *et al.* (2009), Aydın *et al.* (2007) and Kahrıman (2007) and higher than the results Sayalsan *et al.* (2006), Aydoğan *et al.* (2008), Şahin *et al.* (2008). Kahrıman (2007) and Öztürk *et al.* (2009) were determined gluten content in respect of % 24.1- 35.1, % 25.3- 43.6 and % 33.2.

The results about correlation coefficients have been shown in Table 3.

Tab. 3. Correlation coefficients.

	PH	SL	SNS	GNS	GWS	GY	TGW	TW	PC	SV
SL	0.646**									
SNS	0.381	0.555**								
GNS	0.095	0.188	0.596**							
GWS	0.290	0.295	0.583**	0.577**						
GY	-0.094	0.041	-0.013	0.035	0.028					
TGW	0.393	0.107	0.108	0.031	0.248	-0.421*				
TW	0.283	0.439	0.171	0.230	0.138	0.297	-0.052			
PC	0.384	0.305	0.053	-0.136	-0.157	-0.165	0.172	0.256		
SV	0.089	0.186	0.121	0.106	-0.088	0.303	-0.577**	0.341	0.313	
GC	0.419	0.304	0.066	-0.102	-0.143	-0.198	0.196	0.153	0.900**	0.373

The results of correlation study revealed that there were significant association between plant height and spike length, between spikelet number per spike and grain number per spike and grain weight per spike, between grain number per spike and grain weight per spike, between 1000 grain weight and grain yield and sedimentation and between protein content and amount of gluten. In the high yielded area is more difficult to achieve the high protein content. This inverse relationship between grain yield and protein content has also been reported by several researchers (Marinciu and Sauleseu, 2008; Blanco *et al.*, 2012). Similar but insignificant relationship is also seen in our study. Yucel *et al.* (2009), Çifci (2012) and Gümüştaş (2014) were also reported similar results in their studies.

The diagrams of cluster analysis performed in this study are shown in Figure 1 and Figure 2. Two main clusters formed in the dendrogram belonging to cluster analysis of agronomic traits (K2 and K3) and on the first of the main cluster 7 cultivars has been involved and 7 cultivars in the second. For quality features of the dendrogram two main clusters formed in the same way (K2 and 3). While 2 cultivars in the second cluster 12 cultivars took place in the first cluster. Cluster analysis of agronomic and quality criteria were used in the study by Mangova and Petrova (2007), Kahrıman and Egesel (2011), Savii and Nedelea (2012) and Sabaghni *et al.* (2014).

## CONCLUSIONS

14 bread wheat cultivars performance is evaluated in Bursa conditions for some agronomic and quality traits. When the trial results were evaluated in terms of agronomic traits in bread wheat varieties for grain yield, Pamukova and Tahirova varieties were observed to have a higher yield. For the quality characteristics, Sönmez-2001 are determined to have high values in terms of 1000 grain weight, the gluten content and protein content. Pamukova-97 and Tahirova-2000 has been observed that higher values than other varieties for sedimentation. Also the cultivar of Pehlivan for 1000 grain weight has been found to take place in the first place.

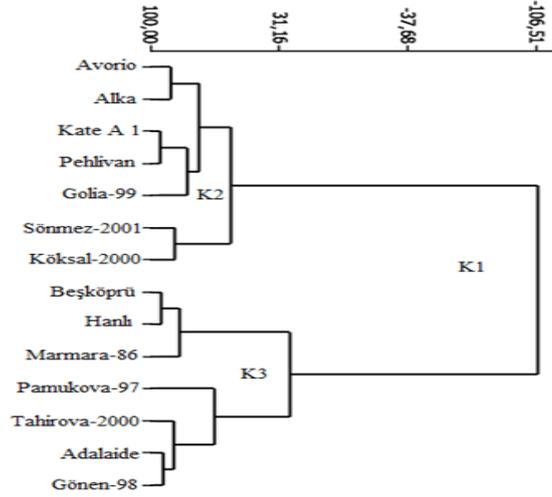


Fig. 1. The diagram of cluster analysis for agronomic traits

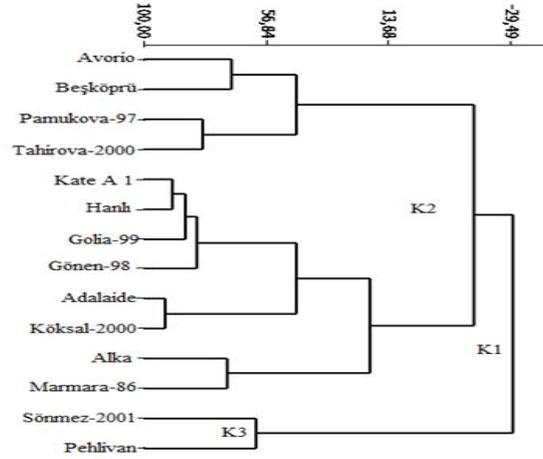


Fig. 2. The diagram of cluster analysis for quality traits

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## EFFECT OF ELEMENTAL SULPHUR AND ROCK PHOSPHATE APPLICATION ON PHOSPHORUS NUTRITION OF MAIZE

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*Scientific paper*

### Summary

In this study effect of elemental sulphur on availability of rock phosphate for maize plant was investigated. For this purpose, a greenhouse experiment was conducted during the year 2013. Three doses of elemental sulphur (S<sub>0</sub>: 0, S<sub>1</sub>: 100, S<sub>2</sub>: 300 mg S kg<sup>-1</sup>) and rock phosphate (P<sub>0</sub>: 0, P<sub>1</sub>: 36, P<sub>2</sub>: 72 mg kg<sup>-1</sup>) were used. Elemental sulphur applications increased amount of available sulphur concentration in soil. In addition, positive effect of rock phosphate applications on oxidation of elemental sulphur in the soil was determined statistically important (p<0.001). At the end of experiment the highest available sulphur concentration in soil was determined at S<sub>2</sub>P<sub>2</sub> application (60.57 mg SO<sub>4</sub>-S kg<sup>-1</sup>). Effect of elemental sulphur on availability of rock phosphate in the soil was found statistically important (p<0.001). Applications of elemental sulphur increased the amount of sulphur uptake but any increasing of phosphorus uptake by maize plant was not determined.

Key words: *Elemental sulphur, rock phosphate, sulphur oxidation*

### INTRODUCTION

For crop production phosphorus (P) is one of the essential nutrient. High crop production is strongly linked with the maintenance of sufficient amount of available P in soil (Elser *et al.*, 2007). Phosphorus is the main structural element of all major phospholipid component of cell membranes. Also, P is one of the basic structural member the most important macromolecules in the cell DNA and RNA. Furthermore phosphorus also located in the ATP molecule that is a kind of energy for all living organisms. P is an important component for coenzyme NAD and FAD which are important in photosynthesis and respiratory reaction (Kadıoğlu, 1998). Amount of available P in soil is very important for production. Two major forms of P exist in soil; organic and inorganic form. Soil inorganic P exists as different compounds of iron, calcium and aluminum and these compounds have different solubility rates in association with soil pH (Oelkers and Jones, 2008).

Major source of P is rock phosphate which is the basic material used in phosphate fertilizers. Like calcareous soils, phosphate rock contains mainly insoluble calcium phosphate compounds such as apatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH,F,Cl)). From rock phosphate, P release is an important matter. So, it is assumed that bacterially produced sulfuric acid as a result of biological S oxidation may solubilize P from insoluble calcium bounded compounds to soluble plant available P compounds.

Sulphur is an essential element for plant development and growth, since it is required for the synthesis of proteins, chlorophyll, oil and vitamins. Plants can take up sulphur from the soil as sulphate (SO<sub>4</sub><sup>2-</sup>) ions and from the atmosphere in a gaseous form as SO<sub>2</sub> through the stomata in leaves. Elemental sulphur can be used as an alternative S fertilizer in agriculture (Zhao *et al.*, 1999). When applied to soil, elemental sulphur is oxidized to sulphate (SO<sub>4</sub><sup>2-</sup>) by soil microorganisms. Physical factors, such as soil temperature and moisture, which affect microbial activity, play an important role in regulating S oxidation (Janzen and Bettany, 1987).

The aim of this study was to determine the effect of elemental sulphur as a fertilizer on solubility of rock phosphate.

### MATERIALS AND METHODS

Hybrid maize (*Zea Mays* L.) cultivar Fleuri AG 92149 was used as plant material. Experiment soil in this study was collected from 0-20cm depth of the field located in Uludag University Agricultural Application and Research Center. Some chemical and physical properties of experiment soil are determined; texture clay (Bouyoucos, 1951), CaCO<sub>3</sub> 1.96% (Richards, 1954), pH (1:2.5 water) 7.48 (Richards, 1954), EC 445 µS

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cm<sup>-1</sup> (Richards, 1954), organic matter 2.53% (Nelson and Sommers, 1982), total nitrogen (N) 0.14% (Bremner, 1965), extractable potassium (K), calcium (Ca), and sodium (Na) determined as 228, 96, 9262, mg kg<sup>-1</sup> (Pratt, 1965) and concentrations of micronutrients (Fe, Cu, Zn, Mn, B); 6.76, 1.48, 92.88, 3.52 mg kg<sup>-1</sup> respectively (Lindsay and Norwell, 1978). Available P was 15.15 mg kg<sup>-1</sup> (Olsen *et al.*, 1954) and extractable S was 12.75 mg kg<sup>-1</sup> (Fox *et al.*, 1964).

Experiment consist of three doses of elemental sulphur (S<sub>0</sub>: 0, S<sub>1</sub>: 100, S<sub>2</sub>: 300 mg S kg<sup>-1</sup>) and three doses of rock phosphate (P<sub>0</sub>: 0, P<sub>1</sub>: 36, P<sub>2</sub>: 72 mg kg<sup>-1</sup>) in a completely randomized block design in greenhouse conditions with 4kg air-dried soil per pot. Each application consists of three replications. Elemental sulphur and rock phosphate doses were applied and mixed thoroughly with soil before sowing.

The experiment was conducted for 4 weeks. Plant samples weighed and dried at 70°C until reaching a constant mass. Dried samples were weighed and wet digested by using HNO<sub>3</sub> + H<sub>2</sub>O<sub>2</sub> mixture. Phosphorus and sulphur concentrations of plant samples were determined by ICP-OES (Perkin Elmer Optima 2100) (Isaac and Johnson, 1998, Kovacs *et al.*, 1996; Wallinga *et al.*, 1989).

After plants harvested, soil samples were taken from pots, dried in shade and sieved with 2mm thin sieve. Available phosphorus was determined by spectrophotometer according to Olsen method (Olsen *et al.*, 1954) and also extractable sulphur concentration was determined by spectrophotometer by turbidimetric method (Fox *et al.*, 1964).

## RESULTS AND DISCUSSION

### Phosphorus and Sulphur Uptake of Maize Plant

The effect of interaction of elemental sulphur and phosphorus (P) applications on the amount of phosphorus uptake of maize plant determined significantly not important. Highest amount of P uptake is found in S<sub>1</sub>P<sub>1</sub> application (Table 1). Phosphorous uptake of plants increased with rock phosphate application but this incensement found statistically not important. Elemental sulphur applications irregularly changed the P uptake of plants.

Tab. 1. The difference of amount of P uptake by elemental sulphur applications in maize plant (mg pot<sup>-1</sup>)

Sulphur Doses	Phosphorus Doses			Mean
	P <sub>0</sub>	P <sub>1</sub>	P <sub>2</sub>	
S <sub>0</sub>	61.64	62.25	76.29	66.72
S <sub>1</sub>	77.76	78.78	73.73	76.75
S <sub>2</sub>	59.28	66.67	69.82	65.25
Mean	66.22	69.23	73.28	

Elemental sulphur applications increased the amount of sulphur (SO<sub>4</sub>-S) uptake of maize plants (p<0.001) (Table 2). The highest amount of SO<sub>4</sub>-S uptake is determined in S<sub>2</sub>P<sub>2</sub> application (20.65 mg kg<sup>-1</sup>). Interaction between rock phosphate and elemental sulphur applications was determined as statistically not important. With rock phosphate applications, SO<sub>4</sub>-S content of maize plant increased but this incensement is not found significant.

Tab. 2. The difference of amount of SO<sub>4</sub>-S uptake by elemental sulphur applications in maize plant (mg pot<sup>-1</sup>)

Phosphorus Doses	Sulphur Doses			Mean
	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	
P <sub>0</sub>	14.98	17.89	16.90	16.59
P <sub>1</sub>	13.38	17.57	18.81	16.59
P <sub>2</sub>	16.93	18.98	20.65	18.85
Mean	15.09 B	18.14 A	18.79 A	

Effect of the rock phosphate applications on P uptake of maize plants was found insignificant. Many researcher found that rock phosphate efficiency increase when soil reaction decrease (Nahas, 1996; Gyaneshwar *et al.*, 1998). In this study, reduce effect of elemental sulphur oxidation mechanism on soil reaction was not efficient (Fig. 1). Main reasons of the inefficiency are experiment soil texture and application amounts of elemental sulphur. For this reason, the increase of the availability of rock phosphate has been less than expected (Table 3).

### Soil Phosphorus and SO<sub>4</sub>-S Concentration

Available P content of soil was not change significantly with rock phosphate applications. It may be caused by soil pH level. In a previous study, researchers indicated that effect of rock phosphate associated with soil pH. The solubility of rock phosphate increase while soil pH decrease (Aydeniz ve Brohi, 1991). At the end of experiment pH status of soil are presented in Fig 1. Interaction between rock phosphate and elemental sulphur applications significantly changed soil pH ( $p < 0.001$ ).

Tab. 3. The difference of available P content of soil by elemental sulphur applications (mg kg<sup>-1</sup>)

Sulphur Doses	Phosphorus Doses			Mean
	P <sub>0</sub>	P <sub>1</sub>	P <sub>2</sub>	
S <sub>0</sub>	11.48	11.60	11.44	11.50 B
S <sub>1</sub>	13.41	13.82	13.47	13.57 A
S <sub>2</sub>	13.79	13.00	13.03	13.27 A
Mean	12.89	12.80	12.65	

The highest dose (S<sub>2</sub>) of elemental sulphur application significantly decreased the soil pH (S<sub>2</sub>P<sub>2</sub> 0,12 %) (Fig 1). However reduce effect of low dose (S<sub>1</sub>) of elemental sulphur applications on soil pH was not determined. Orman (2012) indicate that elemental sulphur applications significantly decreased the soil pH after 11 weeks experiment period. Orman and Kaplan (2011) reported that 3 weeks after application of 200 ppm elemental sulphur to calcareous sandy loam soil resulted in 0.18 unit decrease in soil pH, according to control soil. In their study Turan *et al.* (2013) applied three doses of elemental sulphur (0, 400, 800 mg S kg<sup>-1</sup>) to soil during eight weeks of experiment period. Researchers dedicate that lowest pH value of soil has founded at 800 mg S kg<sup>-1</sup> application. On the other hand, McCready and Krouse (1982) reported very low change in the soil surface pH and concluded that it was a result of the buffering capacity and the amount of CaCO<sub>3</sub> present.

Elemental sulphur applications significantly increased the soil SO<sub>4</sub>-S concentration ( $p < 0.001$ ) (Fig.2). The increasement show that elemental sulphur was oxidized in soil. The highest SO<sub>4</sub>-S concentration in soil was determined in S<sub>2</sub>P<sub>2</sub> application (60.57 mg kg<sup>-1</sup>). The highest dose of elemental sulphur increased the soil SO<sub>4</sub>-S concentration by about 380% compare to control application. Elemental sulphur applications were determined more effective on reduction of soil pH level in presence of rock phosphate.

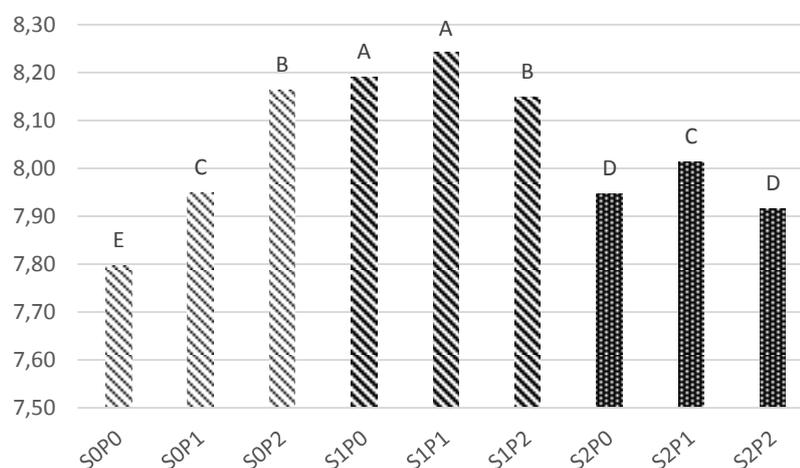


Fig 1. pH status of soils at the end of the experiment.

The relationship between elemental sulphur and rock phosphate could be explained by microbial activities. In a previous study, Bhatti and Yawar (2010) researched that effect of acidophilic iron- and sulfur-oxidizing bacteria (*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) on solubilization of phosphorus (P) from phosphate rock. Researchers stated that sulphur-mud (70.4% S<sup>0</sup>) used in experiment is an energy source of these bacteria.

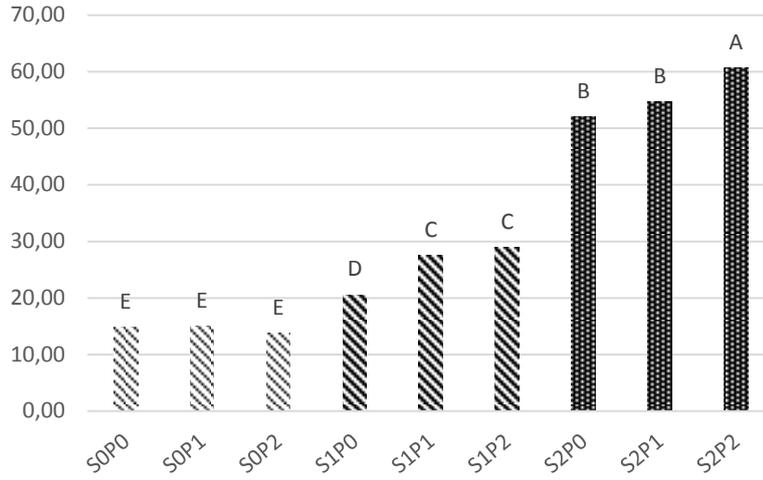


Fig.2. Effect of elemental sulphur applications on Soil SO<sub>4</sub>-S content (mg kg<sup>-1</sup>) (p<0.001).

### CONCLUSIONS

Phosphate rock dissolution and elemental sulphur oxidation is effected by the activity of soil microorganisms. Dissolution of rock phosphate is increased by reduction of soil pH which is dependent on rate of elemental sulphur oxidation. Data from present study points out that elemental sulphur decreased the soil pH and increased SO<sub>4</sub>-S concentration. However, this reduction in soil pH was not significantly sufficient to enhance the solubility of rock phosphate. Therefore, higher doses of elemental sulphur could be used as soil regulator to efficiently decrease the soil pH. Further research on elemental sulfur and rock phosphate is needed to understand the interaction between elemental sulphur, rock phosphate and soil microbial activity.

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## THE EFFECT OF DIFFERENT PLANT DENSITY ON YIELD, YIELD COMPONENTS AND SOME QUALITY PARAMETERS OF TWO HYBRID CORN (*Zea mays indentata* Sturt.) VARIETIES

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*Original scientific paper*

### Summary

Yield and quality of corn are greatly influenced by variety and plant density. This study was carried out to determine the effect of plant density on grain yield and some attributes of two corn varieties under irrigated conditions. Field experiments were conducted for two years on the Uludag University Research Farm, near Bursa. The experimental design was a randomized complete block design in a split-plot arrangement with three replications. Experiment factors were two corn hybrids (Shemal and ADA95-10) and four levels of plant densities (50.000, 65.000, 80.000 and 95.000 plants ha<sup>-1</sup>).

In this research there were not significant differences in number of ear per plant, ear length, grain yield and crude protein content between varieties but plant height, the first ear height, ear diameter, number of seed per ear and 1000 seed weight was significantly different between varieties. The grain yield of varieties ranged from 13707.9 kg ha<sup>-1</sup> to 13987.8 kg ha<sup>-1</sup>.

Results indicated that plant density had significant effect on yield and yield components of corn hybrids.

The plant height, the first ear height increased with increasing of plant densities. The ear length, ear diameter, seed number per ear, 1000 seed weight and crude protein content decreased by increasing plant densities. Seed yield were increased by increasing plant density. In this ecological conditions, 80.000-95.000 plants ha<sup>-1</sup> plant density had highest seed yield (15179.5, 14296.3 kg ha<sup>-1</sup> respectively) and 80.000, 95.000 plants ha<sup>-1</sup> plant density have the potential to give a significantly higher seed yield as compared with other planting density. But there is a need to executive research on plant density includes higher plant density and different corn hybrids for determined the optimum plant density for obtained highest seed yield.

Key words: *corn, Zea mays L., plant density, yield, yield components, quality*

### INTRODUCTION

Corn (*Zea mays* L.) belongs to family poaceae, is an important cereal crop of the world as well as of Turkey. It has high nutritional value as it contains about 72% starch, 10% proteins, 4.8% oil, 8.5% fibre, 3.0% sugar and 1.7% ash (Chaudhary, 1983). Corn is a vital food crop and gives a big volume of raw materials for farm animals and many agro-related industries in the world (Bello *et al.*, 2010; Randjelovic *et al.*, 2011). It ranks 3rd in cereal crops after wheat and rice (Ahmad *et al.*, 2010). To increase the corn production, there are needs for high yielding varieties, agronomical practices done on time etc. affecting directly the growth and productivity.

There are a number of biotic and abiotic factors those affect corn yield considerably; however, it is more affected by variations in plant density than other member of the grass family (Vega *et al.*, 2001). Corn differs in its responses to plant density (Luque *et al.*, 2006). Liu *et al.* (2004) also reported that corn yield differs significantly under varying plant density levels due to difference in genetic potential. Correspondingly corn also responds differently in quality parameters like crude starch, protein and oil contents in grains (Munamava *et al.*, 2006). Plant populations affect most growth parameters of corn even under optimal growth conditions and therefore it is considered a major factor determining the degree of competition between plants (Sangakkara *et al.*, 2004). The grain yield per plant is decreased (Luque *et al.*, 2006) in response to decreasing light and other environmental resources available to each plant (Ali *et al.*, 2003). Hybrids show different responses according to different plant densities which has a major impact on the yield of corn crop (Sarvari *et al.*, 2002). Keeping the importance of corn and the impact of plant density on its growth and yield, the present study was conducted to investigate the effect of different planting densities on growth and yield of hybrid corn (*Zea mays* L.) under the irrigated conditions of Bursa.

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## MATERIALS AND METHODS

Field experiments were conducted on clay loam soil at the Research and Experiment Station of Uludag University, near Bursa 40°11' North, 29°04' East 70 m above sea level. In both years, soil samples were taken prior to seeding and were analyzed. Soil test values indicated a pH of 7, none saline, low values in lime and organic matter and rich in potassium. This climatic zone is characterized as a Mediterranean type climate. Temperature, rainfall and relative humidity during the experimental period (May-September) are summarized (Table 1). The climatic data were provided by the Meteorological Station of Bursa. Although total precipitation in 2008 was only 33.6 mm upward the long-term mean, dry conditions existed in July and August. Precipitation in September was quite higher than the same months of long-term. Total precipitation in 2009 growing season was 42.1 mm below the long-term mean, and all of the months of this year were very dry. There were almost no differences between mean temperatures and relative humidities of experimental years and the long-term means.

Tab. 1. Precipitation, mean temperature and relative humidity in 2008, 2009 and long-term average in Bursa

Precipitation (mm)						
Year/Months	May	June	July	August	September	Total/mean
2008	22.1	28.8	0.2	0.1	132.2	183.4
2009	18.7	9.2	4.4	8.0	67.4	107.7
Long Term	43.4	33.6	18.9	13.8	40.1	149.8
Mean temperature (°C)						
2008	18.3	24.0	25.4	26.4	20.3	22.9
2009	16.1	24.1	25.9	24.5	19.8	22.1
Long Term	17.7	22.4	24.6	24.3	20.1	21.8
Relative humidity (%)						
2008	61.5	52.0	49.4	53.8	69.2	57.2
2009	59.9	50.9	54.7	55.9	69.0	58.1
Long Term	64.8	58.7	57.5	60.2	66.4	61.5

The experiment was performed using split-plot arrangement of treatment within randomized complete block design with three replications. Main plots involved plant density, split-plots contained varieties. Experiment factors were two corn hybrids (Shemal and ADA95-10) and four levels of plant densities (50.000, 65.000, 80.000 and 95.000 plants ha<sup>-1</sup>). The plant height, the first ear height, ear number per plant, ear length, ear diameter, seed number per ear, 1000 seed weight, grain yield and crude protein content were investigated.

The experimental site was prepared for planting by disk followed by cultivator tillage. Immediately after tillage, plots were seeded 5 to 6 cm deep using a hand with row spacing. Seedlings were carried out on 23 May 2008 and 21 May 2009. Twenty days after sowing seedling were thinned by hand after emergence to achieve the desired target plant densities. At sowing, 100 kg ha<sup>-1</sup> pure N, P and K (15-15-15 composite) was applied to each plot and this was followed by 200 kg ha<sup>-1</sup> N as urea (46% N) at the 6 leaf stage. Weed control and irrigation were performed as needed. Weeds were controlled either by hoes or manually. Plants were irrigated five or six times when necessary. First 3 applications were sprinkler; the others were furrow irrigation system.

Each plot were harvested by hand. Data were not collected for the outer rows in each plot to avoid any border effect. Ten plants were sampled randomly from each plot at harvest and the plant height, the first ear height, ear number per plant, ear length, ear diameter, seed number per ear, 1000 seed weight, grain yield and crude protein content were measured on ten samples. 1 g ground sample was used for the total nitrogen determination and were analyzed total nitrogen by Kjeldahl method. Then, Crude protein content was calculated. Before variance analysis, all data parameters of single year were averaged across years. All data obtained from measurements were subjected to analyses of variance by using JUMP program. The LSD was used to separate means of plant densities, hybrids and their interactions when the F-test was significant.

## RESULTS AND DISCUSSION

Data obtained from the research work averaged across years and subjected to variance analysis are given in Table 2a and Table 2b. Results of variance analysis indicated that the effects of plant densities and hybrids were significance on most of the parameters. Also, the effects of interactions on some parameters were observed significant.

Tab. 2a. Effect of different plant density on grain yield, yield components and quality parameters of two hybrid corn varieties (across of 2 years)<sup>1</sup>

Treatments	Components				
	Plant Height (cm)	First Ear Height (cm)	Ears/ Plant	Ear Diameter (cm)	Ear Length (cm)
<b>Hybrids (H)</b>					
Ada95-10	271.9 a	138.1 a	1.06	5.13 a	19.2
Shemal	266.2 b	117.8 b	1.00	4.85 b	19.9
<b>Plant Density (D) (plants ha<sup>-1</sup>)</b>					
50.000	260.8 c	122.4 b	1.09	5.14 a	21.1 a
65.000	265.8 bc	127.6 ab	1.04	5.08 a	19.7 b
80.000	268.8 b	130.0 a	1.02	4.96 ab	19.5 b
95.000	280.8 a	131.6 a	0.99	4.77 b	17.8 c
<b>H x D</b>					
Ada95-10 x 50.000	259.0 b	129.2	1.13	5.20	20.2
Ada95-10 x 65.000	267.0 b	138.5	1.07	5.19	19.5
Ada95-10 x 80.000	269.3 b	141.3	1.04	5.11	19.5
Ada95-10 x 95.000	292.3 a	143.2	1.02	5.01	17.5
Shemal x 50.000	262.7 b	115.7	1.04	5.08	22.1
Shemal x 65.000	264.7 b	116.7	1.00	4.96	19.8
Shemal x 80.000	268.3 b	118.7	1.00	4.82	19.5
Shemal x 95.000	269.3 b	120.0	0.97	4.53	18.1
<b>F test</b>					
Hybrids (H)	*	**	ns	**	ns
Plant Density (D)	**	*	ns	**	**
HxD	*	ns	ns	ns	ns

<sup>1</sup>Means of the same column followed by the same letter were not significantly different at the 0.05 level using LSD test.

\*, \*\*: F-test significant at  $p \leq 0.005$ , and  $p \leq 0.001$ , respectively. ns: not significant.

Plant height was significantly affected by corn hybrids and plant densities. Plant height increased with increasing plant densities. The maximum plant height (280.8 cm) was obtained with the highest plant density (95.000 plants ha<sup>-1</sup>). Similar results have been reported by Seyed Saharifi *et al.* (2009) and Konaşkan (2000). Means comparison for corn hybrids indicated the taller plants (271.9 cm) were measured from Ada95-10 hybrid. The previous studies indicated that there was genotypic difference in plant height by Gözübenli *et al.* (2001).

The first ear heights of corn hybrids were significantly different and the higher value (138.1 cm) was obtained from Ada 95-10 hybrid. On the other hand, the first ear heights of corn plants were affected by plant densities. The similar results were reported by the other researchers (Konaşkan, 2000; Cesurer and Ünlü, 2001; İdikut *et al.*, 2005). The first ear height increased with increasing of plant densities. Turgut (1997) reported results, which were in, confirm to our findings.

Plant density and corn varieties did not show any significant variation in respect of number of ear/plant (Table 2a). The number of ear per plant ranged from 0.97 to 1.13. The findings are in agreement with those reported by Ma *et al.* (2007) and Sanjeev and Bangarwa (1997) who reported that number of ear per plant had significantly affected by plant density.

Ear diameter decreased with increasing in plant density. The thickest ears (5.14 cm and 5.08 cm) were obtained low plant density (50.000 and 65.000 plants ha<sup>-1</sup>) and the thinnest ears (4.77 cm) were obtained at high plant density (95.000 plants ha<sup>-1</sup>) (Table 2a.). Konaşkan (2000) reported that plant densities affected ear diameter and thinner ears were obtained at high densities. Maximum ear diameter was recorded by Ada 95-10 hybrid (5.13 mm). Gözübenli *et al.* (2001) and Konaşkan (2000) indicated that ear diameter was affected by genotypes.

Ear length was decreased with increasing plant density. Low plant density (50.000 plants ha<sup>-1</sup>) had the longest ear (21.1 cm) and the shortest (17.8 cm) was in high plant density (95.000 plants ha<sup>-1</sup>) (Table 2a.). Similar results was also reported Zhang *et al.* (2006). The ear lengths were not affected by corn hybrids.

Tab. 2b. Effect of different plant density on grain yield, yield components and quality parameters of some hybrid corn varieties (across of 2 years)<sup>1</sup>

Treatments	Components			
	Seed/Ear	1000 seed weight g	Seed Yield kg.ha <sup>-1</sup>	Crude Protein Content %
<b>Hybrids (H)</b>				
Ada95-10	661.8 a	359.6 b	13987.8	9.6
Shemal	604.7 b	410.6 a	13707.9	9.2
<b>Plant Density (D) (plants ha<sup>-1</sup>)</b>				
50.000	695.6 a	411.5 a	12562.7 c	10.4 a
65.000	662.7 b	389.1 b	13352.8 bc	9.4 b
80.000	637.9 c	382.2 b	15179.5 a	9.2 b
95.000	536.6 d	357.6 c	14296.3 ab	8.7 b
<b>H x D</b>				
Ada95-10 x 50.000	745.8 a	381.0	12199.4	11.4 a
Ada95-10 x 65.000	684.2 b	361.0	13128.4	9.4 b
Ada95-10 x 80.000	664.0 bc	353.4	15740.7	9.1 bc
Ada95-10 x 95.000	553.1 f	342.8	14882.4	8.4 c
Shemal x 50.000	645.3 cd	442.0	12926.0	9.3 bc
Shemal x 65.000	641.3 d	417.3	13577.1	9.3 bc
Shemal x 80.000	611.9 e	410.9	14618.3	9.3 bc
Shemal x 95.000	520.2 g	372.3	13710.3	9.0 bc
<b>F testi</b>				
Hybrids (H)	**	**	ns	ns
Plant Density (D)	**	**	**	**
HxD	**	ns	ns	**

<sup>1</sup>Means of the same column followed by the same letter were not significantly different at the 0.05 level using LSD test.

\*,\*\*: F-test significant at  $p \leq 0.005$ , and  $p \leq 0.001$ , respectively. ns: not significant.

Data regarding the effect of corn hybrids and plant density on number of seed per ear are given in Table 2b. Across planting density the maximum number of seed per ear was recorded 50.000 plants ha<sup>-1</sup> (695.6) and minimum it was recorded at 95.000 plants ha<sup>-1</sup> (536.6). The number of seed per ear was increased with decreasing plant density. Our results are in agreement with those obtained by Seyed Sharifi *et al.* (2009) and Zhang *et al.* (2006) who reported that number of seeds per ear decreased with increasing plant density. The response of corn hybrids was significant to number of seed per ear. Means comparisons indicated that the maximum number of seed per ear (661.8) was recorded for the Ada95-10 hybrid. Number of seed per ear plays an important role to determining grain yield.

The 1000 seed weight of corn hybrids was significantly different and higher value (410.6 g) was determined in Shemal hybrid. It was significantly affected by different plant densities. 1000 seed weight decreased with increasing plant density and the highest seed weight (411.5 g) was determined at 50.000 plants ha<sup>-1</sup>. Low seed weight in high plant population density was probably due to availability of less photosynthates for seed development on account of high inter-specific competition which resulted in low rate of photosynthesis and high rate of respiration as a result of enhanced mutual shading (Zamir *et al.*, 2011).

The highest seed yield obtained from 80.000 and 95.000 plants ha<sup>-1</sup> plant density (15179.5, 14296.3 kg ha<sup>-1</sup> respectively) when plant density decreased, seed yield decreased too (Table 2b). The lowest seed yield obtained from 50.000 and 65.000 plants ha<sup>-1</sup> plant density (12562.7, 13352.8 kg ha<sup>-1</sup> respectively). Similar results in yield differences across planting density have been reported by Seyed Sharifi *et al.* (2009) and Zhang *et al.* (2006). Xue *et al.* (2002) reported that seed yield increased with increasing plant density from 54.000 to 94.000 plants ha<sup>-1</sup>. Yield increased with increasing plant density up to an optimum number for a corn genotype grown under a set of particular environmental and management conditions (Tollenaar, 1989).

Crude protein content of corn plant significantly changed depending on plant densities. The highest value was determined in 50.000 plants ha<sup>-1</sup> (10.4%). Other plant densities were the same statistics group (9.4%, 9.2% and 8.7%). Widdicombe and Thelen (2002) observed that crude protein contents of corn decreased with increased plant density. The crude protein content was not affected by corn hybrids.

## CONCLUSION

The aim of the research was to determine the effect of plant density on grain yield and some attributes of two corn varieties under irrigated conditions. Different plant density has a significant effect on grain yield of corn. Grain yield increased with increasing plant density up to 95,000 plants ha<sup>-1</sup>. In this ecological conditions, 80,000-95,000 plants ha<sup>-1</sup> plant density had highest grain yield (15179.5, 14296.3 kg ha<sup>-1</sup> respectively) and 80,000, 95,000 plants ha<sup>-1</sup> plant density have the potential to give a significantly higher grain yield as compared with other planting density. But there is a need to executive research on plant density includes higher plant density and different corn hybrids for determined the optimum plant density for obtained highest grain yield.

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## EFFECTS OF BORON RESOURCES AND INCREASING APPLICATION DOSES ON DRY MATTER, BORON AND POTASSIUM UPTAKE OF SUNFLOWER

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*Original scientific paper*

### Summary

Boron is one of the most essential micro elements for the plants growth and high grain yield. On the other hand, it is the only one element which has the levels of deficiency and toxicity very close to each other.

The current research was conducted in a greenhouse to determine the effects of boron resources and increasing application doses on dry matter, boron and potassium uptake of Sunflower (*Helianthus annuus* L.). For this purpose, seven boron doses (0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 mg B kg<sup>-1</sup>) were applied to soil as borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) and boric acid (B(OH)<sub>3</sub>).

According to the results, application of increasing amounts of boron doses elevated the boron uptake of sunflower and the highest value was found 6.16 mg pot<sup>-1</sup> at 16.0 mg B kg<sup>-1</sup>. Also there was an elevation on the dry matter and potassium uptake due to the increasing doses of boron, but the high application doses decreased this amounts. The highest amount of dry matter was taken from 2.0 mg B kg<sup>-1</sup> and the highest potassium uptake was taken from 8.0 mg B kg<sup>-1</sup>. Although the effects of application doses of boron were found statistically significant on dry matter, potassium and boron uptake, the effects of boron resources were only found significant on potassium uptake. According to the statistical analysis results, boric acid found much more effective than borax on potassium uptake.

**Key words:** *Boron uptake, potassium uptake, dry matter, sunflower.*

### INTRODUCTION

Boron is an essential nutrient element for plants (Gupta, 2007). Boron is a necessary component of the cell wall, it plays important roles in cell division, in synthesis of proteins, translocation of sugars and its available amounts in soil and irrigation water is an important determinant factor in agricultural production (Tanaka and Fujiwara, 2007, Gitanjali *et al.*, 2010). Boron requires special attention among the essential nutrient elements because of its deficiency and toxicity levels are too narrow. In soil solution B exists primarily as non-ionised boric acid B(OH)<sub>3</sub>, or ionic form B(OH)<sub>4</sub> which can be easily leached under high rainfall conditions (Shorrocks, 1997; Hu Brown, 1997; Yan *et al.*, 2006) leading to deficiencies in plants that grown there. On the contrary, high concentrations of boron may occur naturally in the soil or in groundwater, or be added to the soil from mining, fertilisers, or irrigation water. Under low rainfall conditions, B cannot be sufficiently leached and therefore may accumulate to levels that become toxic to plant growth (Reid, 2007). This is very often in arid and semiarid regions with high-boron groundwater, where the accumulation of B in top soil due to the evaporation of groundwater reaches toxic levels that reduce crop yields (Gupta *et al.*, 1985; Nable *et al.*, 1997, Tanaka and Fujiwara, 2007).

The amount of boron needed for normal crop production differs among various plants. Both deficiency and excess of boron will result in reduction of crop yield and quality (Yau and Ryan, 2008). Marschner (1995) describes boron (B); potassium (K) and calcium (Ca) as the key nutritional factors controlling fruit development and maturation. Plants vary widely in their nutrient requirements. Sunflower (*Helianthus annuus* L.) suffers from the lack of boron and tolerant for high boron concentrations according to Nable *et al.*, (1997). Dube *et al.* (2000) declared basal fading and distortion of young leaves with soaked areas and tissue necrosis as deficiency symptoms of boron on sunflower plant. The uptake of boron by plants can be affected by the presence of other nutrient elements in the soil. The most well-known was reported as calcium by Gupta (2007). Relation of potassium and boron was not as brief as calcium. There are various literatures of antagonistic and synergistic effects of boron on crops potassium concentrations (Gupta *et al.*, 1985; Prabha and Singaram, 1996; Alpaslan and Güneş, 2001; Davis *et al.*, 2003; Olson *et al.* 2004). In this study, it was aimed to

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determine the effects of boron resources and increasing application doses on dry matter, boron and potassium uptake of Sunflower (*Helianthus annuus* L.).

## MATERIALS AND METHODS

The experiment was conducted in completely randomized factorial design with three replications in greenhouse conditions. The soil used in this study was collected from the 0 to 20 cm depth of the field located in the Agricultural Research and Application Centre of Uludag University. The soil was classified as vertisol (Typic Haploxerert) according to U.S. soil taxonomy and as eutric vertisol according to the FAO/Unesco classification system (Özsoy and Aksoy, 2013). Some properties of the soil were shown in Table 1.

Tab. 1. Some properties of the soil used in the research.

Properties	Quantities	Properties	Quantities
Texture	Clay	Extractable Cations, mg kg <sup>-1</sup>	
Sand, %	31.71	Sodium (Na)	96
Silt, %	26.15	Potassium (K)	228
Clay, %	42.14	Calcium (Ca)	9262
pH	7.48	Magnesium (Mg)	987.6
EC, mS. cm <sup>-1</sup>	0.45	Extractable microelements, mgkg <sup>-1</sup>	
Lime, % CaCO <sub>3</sub>	1.96	Iron (Fe)	6.76
Organic matter, %	2.53	Copper (Cu)	1.48
Total nitrogen (N), %	0.14	Zinc (Zn)	3.52
Available sulphur (S), mgkg <sup>-1</sup>	12.75	Manganese (Mn)	92.88
Available phosphorus (P), mgkg <sup>-1</sup>	15.15	Boron (B)	0.56

The air-dried soil samples passed through a 4-mm sieve and put in to polyethylene covered plastic pots and adjusted to 3.5 kg with the soil. As basal fertilizer, 100 mg N kg<sup>-1</sup> as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), 80 mg P kg<sup>-1</sup> and 100 mg K kg<sup>-1</sup> as monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) were applied to the pots before planting. The applications consist of seven boron doses (0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 mg B kg<sup>-1</sup>) were applied to soil as borax and boric acid. Five hybrid sunflower (*Helianthus annuus* L.) grain of cultivar "Euralis es bella" were planted to the pots that were 20 cm in diameter and 18 cm deep. After the emergence the plants thinned and two sunflower plants were grown for 46 days which was long enough for the influence of the effects of the treatments. The aerial parts of the plants were harvested on 46<sup>th</sup> day. The leaf samples were immediately transferred to the laboratory in closed polyethylene bags. For the evaluation of the nutrient uptake of the plants, the plant materials were washed once in top water and then twice with deionised water. After washing, the plant materials were dried in a forced air oven at 70°C for 72 hours; weighted and ground with a laboratory mill. The ground plant materials were digested using a mixture of 3 ml of HNO<sub>3</sub> and 3 ml of H<sub>2</sub>O<sub>2</sub> in a microwave oven (Berghof MWS 2) (Wu *et al.*, 1997). Boron amounts were determined by ICP-OES (Perkin Elmer Optima 2100DV) (Isaac and Johnson, 1998). Potassium amounts were determined by flame emission (Eppendorf Elex 6361) (Horneck and Hanson, 1998). All of the analysis was conducted in triplicate. The mean values were compared using LSD (Least Significant Difference) multiple range test with the computer program *Tarist*.

## RESULTS AND DISCUSSION

The soil used in the experiment had clay texture and neutral pH. It was low in terms of lime, EC and boron. The soil was adequate in terms of nutrient elements. The effects of increasing doses of the boron resources on the dry matter yield, and on the uptake of boron and potassium were given in Table 2. Although the application doses of boron had statistically significant effects on dry matter and up taken amounts of boron and potassium, the resources of boron had no significant effect on dry matter and boron uptake of sunflower. But it had significant effect on potassium uptake.

According to the general appearance of the plants in the experiment, we can say the affirmative effects of boron resources on the development of the sunflower plants. The highest dry weight yield was taken from 1.0 and 2.0 mg B kg<sup>-1</sup> doses of both borax and boric acid respectively (22.69 and 22.11 g pot<sup>-1</sup>). But the increasing doses of both Borax and Boric acid affected the dry matter yields negatively and the highest doses gave the least results of dry matter yield (18.82 and 20.13 g pot<sup>-1</sup>). Research made with maize cultivars showed similar decrease on

the dry weight amounts (Güneş *et al.*, 2000). High amounts of boron may increase the thickness of the cell wall and this may cause difficulty on the uptake of the nutrient elements and leads a decrease on the dry weight yield.

Tab. 2. Effects of increasing doses of boron resources on dry matter, boron and potassium uptake of sunflower.

Boron Doses (mg B kg <sup>-1</sup> )	Dry Matter (g pot <sup>-1</sup> )			Boron Uptake (mg pot <sup>-1</sup> )			Potassium Uptake (mg pot <sup>-1</sup> )		
	Borax	Boric Acid	Mean	Borax	Boric Acid	Mean	Borax	Boric Acid	Mean
0	21.27	21.04	21.16ab	1.03	0.89	0.96d	625.54	717.82	671.68b
0.5	21.59	20.56	21.08ab	1.12	0.93	1.03d	648.74	693.84	671.29b
1.0	22.69	20.36	21.53a	1.15	1.11	1.13d	642.38	710.25	676.32b
2.0	22.37	22.11	22.24a	1.52	1.43	1.48d	648.66	726.81	687.73ab
4.0	21.57	20.84	21.20a	2.37	2.12	2.25c	702.64	713.82	708.23ab
8.0	20.50	21.56	21.03ab	3.45	3.23	3.34b	693.95	741.04	717.49a
16.0	18.82	20.13	19.48b	5.79	6.53	6.16a	646.44	696.28	671.36b
Mean	21.26	20.94		2.35	2.32		658.34B	714.27A	

Up taken boron amounts of the sunflower plants were elevated by the increasing doses of boron resources and the highest amounts were taken from the last doses (16.0 mg B kg<sup>-1</sup>) of both borax and boric acid respectively (5.79 and 6.53 mg pot<sup>-1</sup>). In our research results, the concentrations of boron ranged between 48.43 and 307.65 at borax, 42.30 and 306.01 at boric acid applications. The normal plant leaves contains about 40 mg to 100 mg of B kg<sup>-1</sup> dry weight. However, the leaves can contain 250 to 1000 mg B kg<sup>-1</sup> when B in soil approaches toxic levels. Boron concentrations greater than 300 mg kg<sup>-1</sup> indicates the presence of boron toxicity (Nable *et al.*, 1997). These results also confirm our findings. There was no statistically significant difference between boron resources (borax and boric acid) on up taken boron amounts.

The highest potassium uptake was found at 4.0 and 8.0 mg B kg<sup>-1</sup> doses of both borax and boric acid respectively (702.64 and 717.49 g pot<sup>-1</sup>). The increase on the boron uptake, negatively affected the potassium uptake of the sunflower plant. The amounts tended to decrease by the increasing doses of boron resources. Some literatures reported increases on the potassium concentrations with the boron applications but these increases were realised at small application doses (2 kg B ha<sup>-1</sup>) of boron (Sakal, 1988; Gezgin and Hamurcu, 2006). Singh *et al.* (1990) reported the decrease of nitrogen, phosphorus and potassium concentrations with the increasing application doses of boron on wheat. The ratio of potassium uptake and boron uptake also describes this decrease briefly in Figure 1. The toxicity symptoms of boron on the leaves look like as if they are lack of potassium. The values taken from the research also describes the deficiency symptoms of potassium at high boron up taken conditions. Parallel to our findings, Patel (1967) reported the increase of boron deficiency symptoms on tobacco when the ratio of K:B increased and also studies conducted in China showed that lower potassium levels enhanced boron accumulation in rape (*Brassica napus* L.) plant (Li *et al.*, 1989).

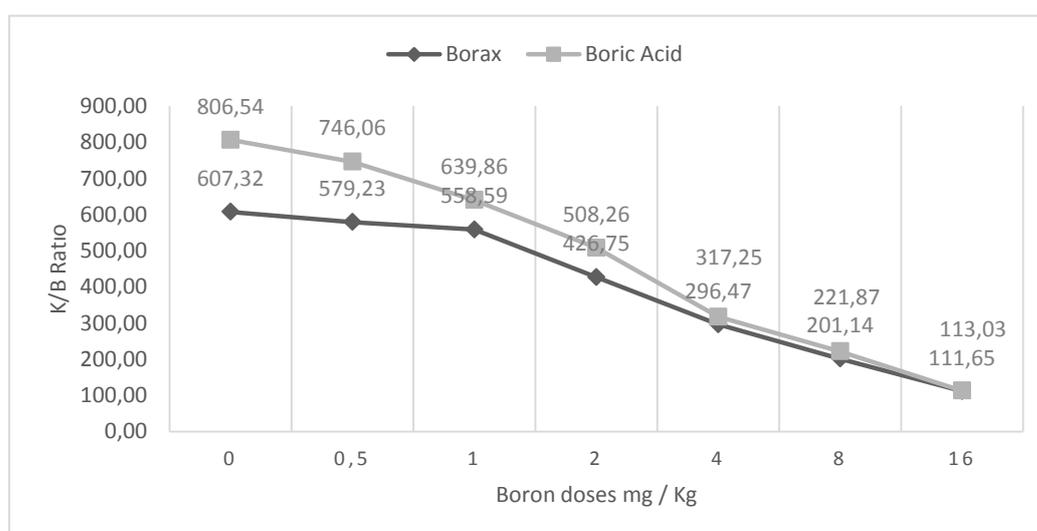


Fig. 1. Potassium uptake and boron uptake ratio

## CONCLUSIONS

Boron deficiency causes poor development and chlorosis symptoms in the sunflower plant. We can ameliorate the deficiency symptoms by boron fertilisers such as boric acid and borax. Both of these boron resources have similar effects. Increasing the amounts of boron stimulates the plant growth, the dry matter yield, the concentrations of the boron in the plant and their uptake. However, high doses have negative effect and decrease the plant growth and other parameters. The highest dose of boron resources not only lower dry matter yield in the plant but also decrease the uptake of potassium. We conclude that adequate boron may be required for the growth and quality of the sunflower plant. However, too high a concentration of boron will cause toxicity, degradation on the concentration of potassium and also on K/B ratio. These essential nutrients should be used in correct doses for increasing soil fertility and to boost up crop production so further researches should be done on the field.

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## EFFECTS OF DIFFERENT PLANT DENSITY AND NITROGEN RATES ON LEAF CHLOROPHYLL CONTENT, LEAF AREA INDEX AND FORAGE YIELD OF SILAGE MAIZE (*ZEA MAYS* L.)

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*Original scientific paper*

### Summary

This research was conducted to determine the effects of different plant density and nitrogen rates on leaf chlorophyll content, leaf area index and forage yield of silage maize grown during 2013/2014 growing season at Agriculture Faculty, Uludag University. The experiment was set up by split plot design in randomized blocks with three replications. A hybrid silage variety of maize, Sincero was used as plant material. Three different plant densities (119 050, 142 850 and 178 570 plants ha<sup>-1</sup>) and four different nitrogen rates (0, 250, 300 and 350 kg ha<sup>-1</sup>) were used in the experiment. The leaf chlorophyll contents (SPAD values) of plants were measured by the tool of SPAD-502. Leaf areas of plants were measured by a digital leaf area meter (LI-3000 Portable Area Meter Produced by LI-COR Lincoln, Nebraska, USA) and then calculated leaf area index. The results indicated that the forage yield increased as the plant densities increased and the highest yield was obtained at highest plant density (178.570 plants ha<sup>-1</sup>). The highest leaf area index was obtained at 142 850 and 178 570 plants ha<sup>-1</sup>. Leaf chlorophyll contents significantly decreased with increasing plant density and the highest value was found at 119 050 plants ha<sup>-1</sup>. The highest forage yield, leaf area index and chlorophyll contents were obtained at 300 kg N ha<sup>-1</sup>.

**Key words:** *Silage maize, nitrogen, SPAD, leaf area index, forage yield*

### INTRODUCTION

Maize (*Zea mays* L.) is the most important silage plants in the world because of its high yield, high energy forage produced with lower labor and machinery requirements than other forage crops (Roth *et al.*, 1995). Maize has good adaptation ability and a lot of cultivars have been used for production as a main crop and the second crop in Turkey. Plant density and nitrogen rate are very important parameters in forage maize production.

Forage maize responds differently to plant densities under different environmental and cultural factors which influence maize forage yield, leaf area index and chlorophyll content. Ayisi and Poswall (1997) suggested that high plant density increases total light interception by the crop canopy, which increased total dry matter and leaf area index. Yılmaz *et al.* (2007) reported that the highest forage yield (64.4 and 62.3 t ha<sup>-1</sup>) obtained at 114000 and 143000 plant density. Moosavi *et al.* (2012) indicated that with increasing plant density from 50000 to 140000 plants ha<sup>-1</sup>, leaf area index and total dry yield, increased 3.39 and 1.84 times, respectively.

Nitrogen fertilization is one of the most important agronomic practices and therefore there are numerous studies conducted with nitrogen fertilizer. Optimum rate of nitrogen fertilizer for forage maize cultivation depends on numerous variable factors such as environmental conditions, management systems and genotypes. Nitrogen fertilization of maize influences dry matter yield by influencing leaf area index, leaf area duration and photosynthetic efficiency (Muchow, 1988; Muchow and Davis, 1988). The increase or decrease in leaf area index has a direct effect on plant growth rate. This index is the main tool for enhancing photosynthesis capacity and assimilates production (Moosavi *et al.*, 2012). Tajul *et al.* (2013) reported that the highest leaf area index and SPAD values were found in the plants treated with 220 kg N ha<sup>-1</sup>. Karasu *et al.* (2009) reported maximum forage yield (95625 kg ha<sup>-1</sup>) at 300 kg N ha<sup>-1</sup>.

The objective of this study was to evaluate forage yield, leaf area index and leaf chlorophyll contents of second crop silage maize to plant densities and nitrogen rates.

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## MATERIALS AND METHODS

Field study was conducted during 2013/2014 growing season on clay loam soil at the Agricultural Research and Experiment Center of Uludag University, near Bursa (40° 11' N, 29° 04' E). Soil test values indicated a pH of 7, none saline, low values in lime and organic matter and rich in potassium. Total precipitation, average temperature and relative humidity were found as 234.2 mm, 22.06 and 72.31% in 2014; 139.3 mm, 21,08°C and 63.68% in long years (1975-2008), respectively.

The variety Sincero was used as plant material. Three plant densities (119 050, 142 850 and 178 570 plants ha<sup>-1</sup>) and four rates (0, 250, 300 and 350 kg N ha<sup>-1</sup>) were evaluated. The experimental design was a randomized complete block in a split plot arrangement with three replications. Main plots consisted of plant densities and sub plots consisted of nitrogen rates. Sub plot size was 5 by 3.5 m with 5 rows. Sub plots were planted at 0.70 m row spacing. Three-fold seeds for each plant density were sown at sub plots and hand-thinned to target plant densities. Half of the nitrogen rates with the starter amounts of P and K each at 100 kg ha<sup>-1</sup> were applied before planting. The rests of nitrogen rates were sidedressed when plants attained 40-50 cm heights. Weeds were controlled by a post-emergence application of 2,4-D at a rate of 2.0 l ha<sup>-1</sup> and mechanical hoeing whenever it was needed.

Ten plants from each subplot were selected for leaf chlorophyll content (SPAD value) and leaf area index at silking stage of maize. The leaf area was measured using a digital leaf area meter (LI-3000 Portable Area Meter Produced by LI-COR Lincoln, Nebraska, USA) and then calculated leaf area index. Leaf chlorophyll content may be used as an indirect indicator of crop N status. Chlorophyll meter values (SPAD) were taken using a portable SPAD meter (Model SPAD-502, Minolta crop, Ramsey, NJ). After removing border effects, two center rows of each sub plot were harvested and fresh-weighed insitu to determine forage yield when kernel was dough. The data were subjected to an analysis of variance using MINITAB (University of Texas, Austin) and MSTAT-C (Version 2.1 Michigan State University, 1991) software. Significant differences in treatments, primary effects and interactions were determined at a probability level of 0.05 and 0.01 by conducting an F-test. The LSD was used to group the means of plant density, nitrogen rate and their interactions for each component determined when the F-test was significant.

## RESULTS AND DISCUSSION

Results of variance analysis indicated that the effects of plant densities and nitrogen rates were of significance on forage yield, leaf area index and leaf chlorophyll content (SPAD value). Also, the effects of interaction on chlorophyll content (SPAD value) was observed significant (Table 1).

Tab. 1. Effect of plant densities and nitrogen rates on forage yield (kg ha<sup>-1</sup>), leaf area index and leaf chlorophyll content (SPAD value)

Plant Density (plants ha <sup>-1</sup> )	Forage Yield (kg ha <sup>-1</sup> )	Leaf Area Index	Chlorophyll Content (SPAD value)
119 050	57043 c	5.65 b	49.30 a
142 850	58033 b	6.31 a	46.77 ab
178 570	63821a	6.43 a	44.53 b
Nitrogen Rate (kg ha <sup>-1</sup> )			
0	34784 d	3.22 c	27.98 c
250	66028 c	6.40 b	51.77 b
300	69808 a	7.31 a	54.64 a
350	67910 b	7.60 a	53.08 ab
Plant Density (A)	*	*	**
Nitrogen Rate (B)	**	**	**
A x B	ns	ns	**

Means of the same column followed by the same letter were not significantly different at the 0.05 level using LSD test.

\*, \*\*: F-test significant at p ≤ 0.05, and p ≤ 0.01, respectively. ns: not significant

Forage yield was influenced by plant densities. Forage yield increased and reached maximum at 178 570 plants ha<sup>-1</sup> (Table 1). These results indicate a close relationship between forage yield and plant density. Numerous workers have determined different plant densities for maximum forage yield changing from 114 000 to 143 000 plants ha<sup>-1</sup> (Yılmaz *et al.*, 2007; Moosavi *et al.*, 2012). As planting density increased, so did the leaf area index and reached a peak value at 142 850 plants and ha<sup>-1</sup>, then then stayed stable at further plant density (Table

1). Tajul *et al.* (2013) reported that leaf area index decreased with increasing plant density, conforming to our results. Leaf chlorophyll contents significantly decreased with increasing plant density and the highest value was found at 119 050 plants ha<sup>-1</sup> (Table 1). Similar effects of plant density on leaf chlorophyll contents were reported by Tajul *et al.* (2013) in maize.

Nitrogen fertilization had significant effect on forage yield. Forage yield increased and reached maximum at 300 kg N ha<sup>-1</sup> and then declined as nitrogen rate increased further (Table 1). Similar results were reported by Karasu *et al.* (2009). The differences in leaf area index were markedly great and increased as nitrogen rate increased. Leaf area index was the lowest (3.22) at 0 kg N ha<sup>-1</sup> and the highest (7.31 and 7.60) at 300 and 350 kg N ha<sup>-1</sup> (Table 1). Similar effects of nitrogen fertilization were reported by Tajul *et al.* (2013) in maize. Leaf chlorophyll content increased and reached maximum value at 300 kg N ha<sup>-1</sup> then declined as nitrogen rate increased further. (Table 1). Tajul *et al.* (2013) reported that leaf chlorophyll content increased with increasing nitrogen rate, conforming to our results.

Maximum leaf chlorophyll content was obtained from 300 kg N ha<sup>-1</sup> with 119 050 plants ha<sup>-1</sup> (Table 2). Tajul *et al.* (2013) reported that planting density and nitrogen rate interaction effects on SPAD values were statistically significant.

Tab. 2. Plant density x nitrogen rate interactions for leaf chlorophyll content (SPAD value)

Nitrogen Rate (kg ha <sup>-1</sup> )	Plant Density (plants ha <sup>-1</sup> )		
	119 050	142 850	178 570
0	30.66 e	27.09 ef	26.18 f
250	54.12 a-c	51.04 cd	50.15 d
300	57.15 a	54.78 a-c	51.99 b-d
350	55.25 ab	54.16 a-c	49.81 d

Means of the same columns and lines followed by the same letter were not significantly different at the 0.05 level using LSD test.

## CONCLUSIONS

Second crop forage maize is a crop that is highly responsive to plant density and nitrogen fertilization. The results indicated that the forage yield increased as the plant densities increased and the highest yield was obtained at highest plant density (178.570 plants ha<sup>-1</sup>). The highest forage yield was obtained at 300 kg N ha<sup>-1</sup>.

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## THE EFFECTS OF DIFFERENT CURING METHODS ON SOME CHEMICAL PROPERTIES AND TOBACCO QUALITY OF IZMIR TYPE TOBACCO

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*Scientific paper*

### Summary

This research was carried out to investigate the effect of different curing methods (traditional curing and vento machine curing) on some chemical properties and quality of tobacco. In the trial, Izmir type tobacco was used and experimental design was Randomized Complete Block Design with three replications. In the study, total alkaloid (nicotine) (%), total reducing sugar (%), crude ash (%), total nitrogen (%) and tobacco quality were determined. Total alkaloid, total reducing sugar and total nitrogen were 0.667, 0.287 and 2.2% for traditional curing method, respectively whereas total alkaloid, total reducing sugar and total nitrogen were 0.287, 12.2 and 2.25% for vento machine methods, respectively. American grad tobacco rate in the traditional curing was higher than that of vento machine.

**Key words:** *Izmir type tobacco, curing, chemical properties, quality*

### INTRODUCTION

Tobacco is an industrial plant with its own characteristics such as seedling, cropping and processing, compared to other arable crops. Therefore, it is very important its quality criteria besides the high yield. To obtain a high-quality tobacco, each stage must be well known from seedling to harvest and from harvest to processing. The most important thing at this stage of the process is curing.

Oriental tobaccos are usually cured under the sun or in plastic covered tunnels. However, in recent years, net method has been used in curing system. A new method aimed at reducing post-harvest labor costs has been carried out by contracted farmers in the Black Sea Region, Eastern and Southern part of the Anatolia and Aegean Region.

The aim of this study was to investigate the effects of traditional and vento machine curing methods on some chemical properties and leaf quality of tobacco plant.

### MATERIAL AND METHOD

The research was carried out in Asagiilgindere village, Bergama, İzmir, Turkey in 2014. The soil was clay-loam texture. Organic matter and pH were 1.1% and 7.01, respectively.

Izmir type tobacco seed was used in the experiment. Tobacco seeds were sown (1.5 g/m<sup>2</sup>) into the soil consisting of sand, manure and mulch mixture (1:1:1) in February. Some cultural practices such as irrigation, weed control and ventilation were performed regularly. The seedlings were planted in the field by planting machine with 40x15 cm intervals on April 10<sup>th</sup>. Total experiment area was 8.0 ha<sup>-1</sup>. The experiment was designed in a randomised block complete design with three replication. After harvesting, tobacco leaves were separated in two equal parts for curing. Half of them were cured with traditional curing (sun-cured) and the others were cured with vento machine curing.

In the traditional curing method, tobacco leaves were stringed by hand. Strung tobacco was tied to a bamboo stick at both ends and also in the middle. These leaves were wilted in the shade one day before the curing operation. Then bamboo sticks were cured at the sun. Cured tobaccos were brought to the warehouse together with bamboo sticks, and these were stacked one over the other.

Harvested leaves were put in the machine supply unit for filling the string bag in the vento machine curing system. Tobacco leaves send from supply unit to vacuum unit and then transferred in the string bag. When the string bag reached the desired filling weight, the machine was stopped by machine operator and string bag was tied at both measurements. Tobacco leaves were wilted in the shade one or two days and hung them horizontal

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or vertical on the curing unit. Like the traditional curing methods, tobaccos were brought to the warehouse and stacked one over the other.

In this study some features were determined such as total N (Kaçar, 1972; Anon, 1986), total alkaloid (nicotine) (Anon, 1969), total reducing sugar (Lindsay, 1973), crude ash (Nelson, 1960), and tobacco expertise quality (Anon, 1971). The data for all traits were analyzed by the analysis of variance by Acıkgöz *et al.* (2004).

## RESULTS AND DISCUSSION

It was found that there were significant differences ( $p < 0.05$ ) between curing methods in terms of total alkaloid (nicotine) and total nitrogen rate (Table 1).

### Total alkaloid (nicotine) rate (%)

As shown in Table 1, total alkaloid rate was higher in traditional curing method (0.677%) than vento machine curing method (0.287%). High nicotine level in quality tobacco is not desirable. Peksüslü and Gencer (2001) reported that the nicotine content was very important for the quality and the rate of nicotine should be as low as between 0.51-0.85%. Nicotine content in Aegean tobaccos is between 0.25-1.79% in generally and our results were found similar to previous studies (Uz, 1997; Gencer, 2000; Korkmaz, 2006; Ekren, 2007).

Tab. 1. Effect of the traditional and vento machine curing methods on some chemical characters of tobacco (%)

Curing method	Total alkaloid (nicotine)	Total reducing sugar	Crude ash	Total nitrogen
Traditional	0.677	11.8	16.95	1.99
Vento machine	0.287	12.2	16.93	2.25
LSD <sub>0.05</sub>	0.262	ns	ns	0.263

\*  $p < 0.05$

ns: not significant

### Total reducing sugar rate (%)

As shown in Table 1, there were no significant differences in total reducing sugar content between vento machine curing method (12.2%) and traditional method (11.8%). Sugar content was found similar to the results obtained from many researchs (15.4-25.0%, Uslu *et al.*, 2005; 7.8-33.7%, Ekren, 2007; 8.2-15.1%, Kucukozden *et al.*, 2002). Although the values indicated by many researchers ranged within the large limits, it can be said that a good quality Izmir type tobacco should have sugar more than 15.0% (Sekin, 1979).

### Crude ash rate (%)

Crude ash rate was similar to each other and the values were 16.95% and 16.93% for traditional and vento machine curing method, respectively (Table 1). Ash content is one of the most important quality criteria in tobacco. Abdallah (1986) reported that there was a reciprocal relationship between leaf quality and ash content in oriental tobaccos.

Our results ranged between 19.93-16.95% and similar or higher results were found by many other researchers (Sekin *et al.*, 2002; Kucukozden *et al.*, 2002; Korkmaz, 2006).

### Total nitrogene rate (%)

There was significant difference between curing methods in total nitrogen rate (Table 1) nitrogen rates were 2.25% and 1.99% for traditional and vento machine curing methods, respectively. Nitrogen in tobacco is expressed as total N % and it is very important quality criteria. N is available in leaves as organic and inorganic compounds. Total nitrogen increases with N content of soils. Similar results were found by many other researchers (Gencer, 2001; Uslu *et al.*, 2005; Sekin *et al.*, 2002).

### Visual Tobacco Quality

Visual quality (expertise quality) is still the unique quality estimating method for tobacco leaves before the tobaccos are purchased. It is based on a system that classifies the leaves based on characteristics such as leaf position, texture, colour, aroma, leaf size, leaf damages and etc.

In this research, the visual quality was estimated by a tobacco expert who has been working in a tobacco company. American grad (AG) rate in traditional curing method (33.5%) was lower than in vento machine

(42.0%). Double Kappa (DKP) rate was found to be 25.2% and 24.6% in traditional method and vento machine method, respectively. When we compared traditional curing and vento machine for 3<sup>rd</sup> primings, it was found that American grad rate was higher than the other primings (1<sup>st</sup> and 2<sup>nd</sup>) (Table 2).

Tab. 2. Visual leaf quality in traditional and vento machine curing methods (%)

Leaf position	Traditional Method				Vento Machine Method			
	AG	BG	KP	DKP	AG	BG	KP	DKP
1	00.0	00.0	33.3	66.7	00.0	00.0	30.0	70.0
2	33.4	31.0	27.1	8.5	59.3	23.9	12.9	3.9
3	63.9	3.1	32.4	0.5	66.7	10.1	23.2	00.0
<b>Average</b>	<b>32.4</b>	<b>11.4</b>	<b>30.9</b>	<b>25.2</b>	<b>42.0</b>	<b>11.3</b>	<b>22.0</b>	<b>24.6</b>

Although visual quality estimation has been neglected or ignored in tobacco researchs in Turkey, it is a unique quality estimating way to evaluate the commercial tobacco crop in Aegean and other tobaccos, as well (Sekin, 1986).

## CONCLUSION

Considering the results, the all investigated chemical components were similar with the results of other researchers who worked with traditional and vento machine curing methods in Aegean tobaccos. When the visual quality traits was interpreted, American grad tobacco rate in vento machine was lower than in traditional method. However, Double kappa rate in vento machine was higher than the another method. According to the one year's result, it is said that the study must be carried out one more year and two years' results must be interpreted together.

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## SEEDLING RESPONSE OF GAMMA-RAY INDUCED ADVANCED BARLEY LINES TO THREE ISOLATES OF *Drechslera teres f. teres*

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*Scientific paper*

### Summary

Net form of net blotch caused by *Drechslera teres f. teres* is an important disease of barley (*Hordeum vulgare*) worldwide. Growing disease resistant plants is an important control measure. Barley mutants could be used as a source of genetic diversity. In this study, Turkish barley cultivar 'Tokak 157/37' which is commonly grown in Turkey was subjected to gamma irradiation using Cobalt-60 as the radiation source and mutants were obtained. Under greenhouse conditions, twenty-five advanced mutant barley lines were tested for their resistance status to 3 *Drechslera teres f. teres* single spore isolates obtained from Sivas, Eskişehir and Mersin provinces of Turkey. Virulence differences among isolates were observed. *Drechslera teres f. teres* isolate obtained from Sivas was the most virulent isolate whereas isolate obtained from Mersin was the least virulent. For evaluation of the lines a 1-10 scale was used. The reactions of the mutant lines to Sivas isolate was ranged between moderately resistant-moderately susceptible and susceptible with a mean scale value of 7.62. The reactions of the mutant lines to Eskişehir isolate was ranged between moderately resistant-moderately susceptible and moderately susceptible-susceptible with a mean scale value of 5.94. The reactions of the mutant lines to Mersin isolate was ranged between resistant and moderately resistant-moderately susceptible with a mean scale value of 2.80.

**Key words:** *Drechslera teres f. teres*, *Pyrenophora teres f. teres*, barley, Cobalt-60 irradiation, Turkey

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is the second most common cereal crop after wheat in Turkey. It is mainly used as animal feed and in malt production (Geçit *et al.*, 2009). Using disease resistant plants are among the most effective control methods. A rich genetical base in the barley plant is essential in obtaining disease resistant plants. Mutations can help creating variation in barley plants. Barley mutants could be used enriching the nutritional content and resistance to biotic and abiotic stress factors (Sağel *et al.*, 1994; Newman and Newman, 2008). A number of mutagenic agents could be used to induce mutations. One of the mutagenic agents is gamma rays. Cobalt-60 is used as the gamma irradiation source by many researchers (Sağel *et al.*, 1994; Tutluer *et al.*, 1995; Azamparsa *et al.*, 2015). A number of soybean, tobacco and chickpea varieties have been developed using this technique (Sağel *et al.*, 2013).

Net blotch is a common disease of barley in the World (Mathre, 1982). It is caused by the fungus *Drechslera teres* (teleomorph : *Pyrenophora teres*). The disease has two forms. *Drechslera teres f. maculata* causes spot form of the disease and *Drechslera teres f. teres* causes net form of the disease. Both forms are present in Turkey. Karakaya *et al.* (2014) and Aktaş (1997) reported spot form as more common, however, Damgacı (2014) reported net form as more common. In Turkey, Karakaya *et al.* (2014) found the disease in 79.9% and 72.4% of the barley fields inspected in 2012 and 2013, respectively.

In this study, 25 mutant barley lines obtained by gamma irradiation were tested for their seedling resistance status under greenhouse conditions to 3 *Drechslera teres f. teres* single spore isolates obtained from Sivas, Eskişehir and Mersin provinces of Turkey.

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## MATERIALS AND METHODS

Turkish barley cultivar ‘Tokak 157/37’ was subjected to gamma irradiation using Cobalt- 60 as the radiation source and mutants were obtained. Mutants with desirable agronomic characteristics were selected and carried to the further generations. In this study, 25 advanced mutant M<sub>8</sub> generation barley lines were used.

Isolation, inoculation, greenhouse conditions and evaluation procedures were similar to previous experiments (Usta *et al.*, 2014; Yazıcı *et al.*, 2015). Ten days after inoculation, plants were evaluated with a 1-10 scale developed for *D. teres* f. *teres* by Tekauz (1985). In this scale values were 1: R (Resistant), 2: R-MR (Resistant-Moderately Resistant), 3: MR (Moderately Resistant), 4: MR-MS: (Moderately Resistant-Moderately Susceptible), 5: MR-MS (Moderately Resistant-Moderately Susceptible), 6: MR-MS (Moderately Resistant-Moderately Susceptible), 7: MS (Moderately Susceptible), 8: MS-S (Moderately Susceptible-Susceptible), 9: S (Susceptible), 10: VS (Very Susceptible). Experiment was performed using randomized block design with three replications. Data were square root transformed before statistical analysis. Separate analysis of variance was performed for each isolate and means of mutant barley lines were separated by Least Significant Difference (LSD) test. For isolate comparisons, combined analysis of variance was performed and isolate means were separated with LSD test.

## RESULTS AND DISCUSSION

Mutant barley lines varied in their response to isolates. Statistically significant differences among the genotypes were observed ( $P<0.01$ ). Virulence differences among the isolates were also evident ( $P<0.01$ ) (Table 1). *Drechslera teres* f. *teres* isolate obtained from Sivas was the most virulent isolate whereas isolate obtained from Mersin was the least virulent (Table 1). The reactions of the mutant lines to Sivas isolate was ranged between moderately resistant-moderately susceptible and susceptible with a mean scale value of 7.62. Mutant barley lines # 9 and 13 showed a susceptible reaction to Sivas isolate. Lines # 1, 2, 3, 6, 7, 8, 10, 14, 17, 18, 22, 23 and 24 showed a moderately susceptible-susceptible reaction to Sivas isolate. Lines # 4, 5, 11, 12, 15, 16, 19, 21 and 25 showed a moderately susceptible reaction to Sivas isolate. Line # 20 showed a moderately resistant-moderately susceptible reaction to Sivas isolate. The reactions of the mutant lines to Eskişehir isolate was ranged between moderately resistant-moderately susceptible and moderately susceptible-susceptible with a mean scale value of 5.94. Lines # 22 and 23 showed a moderately susceptible-susceptible reaction to Eskişehir isolate. Lines # 7, 17, 20 and 24 showed a moderately susceptible reaction to Eskişehir isolate. Lines # 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 21 and 25 showed a moderately resistant-moderately susceptible reaction to Eskişehir isolate. The reactions of the mutant lines to Mersin isolate was ranged between resistant and moderately resistant-moderately susceptible with a mean scale value of 2.80. Lines # 13, 15, 22, 23 and 24 showed a moderately resistant-moderately susceptible reaction to Mersin isolate. Lines # 1, 2, 3, 10, 12, 14, 17, 18, 19, 20, 21 and 25 showed a moderately resistant reaction to Mersin isolate. Lines # 4, 5, 6, 8, 9, 11 and 16 showed a resistant-moderately resistant reaction to Mersin isolate. Line # 7 showed a resistant reaction to Mersin isolate.

There were statistically significant differences among the virulence of the isolates ( $P<0.01$ ). Pathogenic variation among the *Drechslera teres* f. *teres* isolates have been reported (Liu *et al.*, 2011; Tekauz 1990).

Variation among the mutant barley lines to isolates were observed. However, most of the lines showed reactions to Sivas isolate between moderately susceptible to susceptible range. Line number 20 showed a moderately resistant-moderately susceptible reaction to Sivas isolate. This isolate was the most virulent. Mutant barley lines should be tested with more isolates. Pathotypes of *D. teres* f. *teres* should be determined and resistance studies should be carried out with most virulent and/or most common pathotypes. Yazıcı *et al.* (2015) determined seedling reactions of 25 barley cultivars to 3 isolates of net form of net blotch under greenhouse conditions. The reactions of isolates to fungal isolates ranged between resistant-moderately resistant and moderately susceptible-susceptible. Cultivars ‘Bülbül 89’ and ‘İnce 04’ were found as the most susceptible cultivars followed by cvs ‘Çıldır 02’, ‘Özdemir 05’ and ‘Hamidiye 85’. Cultivar ‘Harman’ was found as the most resistant cultivar. Also in this study virulence differences among the isolates were observed.

Tab. 1. Seedling response of 25 mutant barley lines to 3 *Drechslera teres* f. *teres* isolates obtained from Sivas, Eskişehir and Mersin provinces of Turkey. Numbers are median of three replicatons. For evaluation a 1-10 scale developed by Tekauz (1985) was used. Numbers followed by different letters are statistically significant ( $P<0.01$ ).

Mutant barley lines	Sivas isolate	Eskişehir isolate	Mersin isolate
1	8,00 abc	6,00 cd	3,33 abc
2	8,00 abc	6,00 cd	2,66 bcde
3	7,66 bcd	5,66 cde	3,33 abc
4	7,00 def	4,33 g	2,00 de
5	7,00 def	4,66 fg	1,66 ef
6	7,66 bcd	4,66 fg	2,33 cde
7	7,66 bcd	7,00 ab	1,00 f
8	8,00 abc	6,00 cd	1,66 ef
9	8,66 a	6,00 cd	2,00 de
10	8,33 ab	5,33 def	2,66 bcde
11	7,00 def	4,33 g	2,33 cde
12	7,33 cde	6,00 cd	3,00 abcd
13	8,66 a	5,00 efg	4,00 a
14	8,00 abc	6,00 cd	3,33 abc
15	6,66 ef	5,33 def	3,66 ab
16	6,66 ef	6,00 cd	1,66 ef
17	8,00 abc	7,33 a	2,66 bcde
18	7,66 bcd	6,00 cd	3,33 abc
19	7,33 cde	6,00 cd	3,33 abc
20	6,33 f	7,00 ab	3,33 abc
21	7,33 cde	6,33 bc	2,66 bcde
22	8,00 abc	8,00 a	3,66 ab
23	8,00 abc	7,66 a	3,66 ab
24	8,33 ab	7,33 a	3,66 ab
25	7,33 cde	4,66 fg	3,33 abc
Mean	7,62 A	5,94 B	2,80 C

Azamparsa *et al.* (2015) determined seedling response of two barley cultivars and gamma ray-induced advanced barley lines to another important barley pathogen *Rhynchosporium commune*. Response of lines to isolates ranged between highly resistant and highly susceptible. Virulence differences among the isolates were observed.

Some researchers reported that virulence spectrum of *Drechslera teres* f. *teres* were wider than *Drechslera teres* f. *maculata* (Wu *et al.*, 2003). Variation among the barley genotypes were also observed. In some areas with the replacement of barley cultivars with new ones quick changes in the net blotch resistance was observed (Liu *et al.*, 2011). Steffenson and Webster (1992) emphasized the increase in the virulence of net blotch where sexual reproduction in fungus occurs. In our study, we observed clear differences among the virulence of the isolates. Pathotypes of *D. teres* f. *teres* should be determined and resistance studies should be carried out with most virulent and/or most common pathotypes.

## CONCLUSIONS

Clear variation among the *D. teres* f. *teres* isolates was present. The reactions of the mutant lines to isolates ranged between susceptible and resistant. Mutant barley line # 20 showed a moderately resistant-moderately susceptible reaction to the most virulent isolate. Studies should be carried out with more genotypes. Pathotypes of *D. teres* f. *teres* should be determined and resistance studies should be carried out with most virulent and/or most common pathotypes. Continuous monitoring of virulence of *D. teres* f. *teres* help developing appropriate strategies for barley resistance and breeding.

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## DETERMINATION OF THE SEEDLING REACTIONS OF ADVANCED BARLEY LINES TO SPOT BLOTCH DISEASE CAUSED BY *Cochliobolus sativus*

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Scientific paper

### Summary

Spot blotch caused by *Cochliobolus sativus* (anamorph: *Bipolaris sorokiniana*) is an important disease of barley (*Hordeum vulgare*). Finding genetic resistance among the barley germplasm is an important means of disease control. In this study, seedling reactions of 25 advanced barley lines to 5 single spore isolates of *Cochliobolus sativus* obtained from Yozgat (1 isolate), Hatay (1 isolate) and Kastamonu (3 isolates, K1, K2 and K3) provinces of Turkey were determined. Hatay isolate was obtained from wild barley (*Hordeum spontaneum*) while others were obtained from cultivated barley (*H. vulgare*). Infection responses of barley lines were assessed using a 1-9 scale. Four, five, eight, twelve and seventeen lines exhibited intermediate infection responses to Yozgat, Hatay, K1, K2 and K3 isolates, respectively. Remaining lines showed high infection responses. No line exhibited low infection response. Mean scale values of the Yozgat, Hatay, K1, K2 and K3 isolates were 6.28, 5.88, 5.72, 5.52 and 5.28, respectively. Barley lines #1 and #15 showed an intermediate infection response to all 5 isolates and line #24 showed an intermediate infection response to 4 isolates.

Key words: *Cochliobolus sativus*, *Bipolaris sorokiniana*, *Hordeum vulgare*, Spot blotch, Turkey

### INTRODUCTION

Spot blotch caused by *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoemaker) is an important disease of cereals including barley (*Hordeum vulgare* L.) in the world. The synonyms of the fungus are *Drechslera sorokiniana* (Sacc.) Subram. & B.L. Jain, *Helminthosporium sativum* Pammel, C. M. King, & Bakke and *Helminthosporium sorokinianum* Sacc. The disease agent also causes a root rot disease of cereals especially in drier climates (Mathre, 1982; Kumar *et al.*, 2002).

Barley is the most important cereal crop after wheat in Turkey. Barley production in Turkey is 7.900.000 tonnes (TÜİK, 2013). Barley is used generally as animal feed and in malt industry (Geçit *et al.*, 2009). Development of disease resistant genotypes is one of the most important control strategies. In this study, seedling reactions of 25 advanced barley lines to 5 single spore isolates of *Cochliobolus sativus* obtained from Yozgat (1 isolate), Hatay (1 isolate) and Kastamonu (3 isolates, K1, K2 and K3) provinces of Turkey were determined.

### MATERIALS AND METHODS

In this study, seedling reactions of 25 advanced barley lines to 5 single spore isolates of *Cochliobolus sativus* obtained from Yozgat (1 isolate), Hatay (1 isolate) and Kastamonu (3 isolates, K1, K2 and K3) provinces of Turkey were determined.

Hatay isolate was obtained from wild barley (*Hordeum spontaneum*) while others were obtained from cultivated barley (*H. vulgare*). Inocula were prepared from 10 day old fungal cultures grown on Potato Dextrose Agar. Conidia was scraped from plates using a paintbrush, filtered using a cheesecloth, adjusted to  $2 \times 10^4$  conidia/ml using a hemocytometer and sprayed to plants at the 2 leaf stage. For each 100 ml of inoculum 1 drop of Tween 20 was added (Aktaş and Tunalı, 1994; Arabi and Jawhar, 2003; Yazıcı *et al.*, 2015). Evaluations were made 7 days after inoculation using a 1-9 infection response (IR) scale developed by Fetch and Steffenson (1999). In this scale, nine IRs were classified into three categories: low (IRs 1 to 3), intermediate (IRs 4 and 5), and high (IRs 6 to 9). Experiment was performed using randomized block design with three replications. Data were square root transformed before statistical analysis. Separate analysis of variance was performed for each isolate and means of advanced barley lines were separated by Least Significant Difference (LSD) test. For

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isolate comparisons, combined analysis of variance was performed and isolate means were separated with LSD test.

## RESULTS AND DISCUSSION

Separate analysis of variance performed for each isolate revealed statistically significant ( $P < 0.01$ ) differences among the advanced barley lines. LSD test results performed at 0.01 level were presented in Table 1. According to the multiple comparison test results, four, five, eight, twelve and seventeen lines exhibited intermediate infection responses to Yozgat, Hatay, K1, K2 and K3 isolates, respectively.

Tab. 1. Seedling reactions of 25 advanced barley lines to 5 *Cochliobolus sativus* isolates under greenhouse conditions. For evaluation a 1-9 scale was used (Fetch and Steffenson, 1999). Numbers are median of 3 replications. Numbers followed by different letters are statistically significant ( $P < 0.01$ ).

Barley Lines	Isolates				
	Yozgat	Hatay	K1	K2	K3
1	5 a	5 a	5 a	5 a	5 b
2	6 b	6 b	6 b	6 b	5 b
3	5 a	5 a	5 a	6 b	6 c
4	6 b	6 b	6 b	5 a	6 c
5	6 b	6 b	6 b	6 b	5 b
6	6 b	6 b	6 b	5 a	5 b
7	6 b	6 b	6 b	5 a	5 b
8	6 b	6 b	6 b	6 b	6 c
9	8 d	7 c	5 a	6 b	6 c
10	7 c	6 b	5 a	6 b	6 c
11	7 c	7 c	6 b	6 b	6 c
12	6 b	6 b	5 a	5 a	6 c
13	6 b	6 b	5 a	5 a	5 b
14	6 b	6 b	5 a	6 b	5 b
15	5 a	5 a	5 a	5 a	5 b
16	6 b	6 b	7 c	5 a	5 b
17	7 c	6 b	6 b	6 b	5 b
18	7 c	6 b	6 b	6 b	6 c
19	7 c	5 a	6 b	5 a	5 b
20	7 c	6 b	6 b	5 a	5 b
21	7 c	6 b	6 b	5 a	5 b
22	6 b	6 b	6 b	6 b	5 b
23	7 c	6 b	6 b	6 b	5 b
24	5 a	5 a	6 b	5 a	4 a
25	7 c	6 b	6 b	6 b	5 b
Mean	6.28 A	5.88 B	5.72 BC	5.52 C	5.28 D

Remaining lines showed high infection responses ( $P < 0.01$ ). No line exhibited low infection response. Mean scale values of the Yozgat, Hatay, K1, K2 and K3 isolates were 6.28, 5.88, 5.72, 5.52 and 5.28, respectively. Barley lines #1 and #15 showed an intermediate infection response to all 5 isolates and line #24 showed an intermediate infection response to 4 isolates ( $P < 0.01$ ). Combined analysis of variance showed statistically significant ( $P < 0.01$ ) differences among the isolates. Multiple comparison test revealed that Yozgat isolate was the most virulent isolate and K3 isolate from Kastamonu was the least virulent isolate ( $P < 0.01$ ). Virulence differences among the isolates were also reported by different authors (Kumar *et al.*, 2002).

Although intermediate response was observed in reactions of genotypes to isolates no low reaction types were observed. More genotypes should be tested for their resistance status to *C. sativus*. Future studies should focus

on developing genotypes that exhibit low reaction types. For this purpose wild barleys could be used (Mathre, 1982).

Aktaş and Tunalı (1994) evaluated the resistance status of some barley cultivar and landraces to S96 race of *C. sativus*. Barley cultivars Tokak 157/35, Hamidiye 85, Anadolu 86, Obruk 86, Yerçil 147 and barley lines ABVD-118, ABVD-121, KABVD-2013, KVD-2104, KABVD-62-2149 showed a very susceptible reaction to the isolate used. Barley cultivars Ankara 86, Cumhuriyet 50, Zafer 160 and Yeşilköy 387 exhibited a susceptible reaction to this isolate. Barley cultivar Yıldırım and barley lines YEA-286-22, YEA-422-1, KABVD-2023, YEA-475-4 showed a moderately susceptible reaction to the isolate. Barley line KABVD-2017 showed a moderately resistant reaction to the isolate.

## CONCLUSIONS

Four, five, eight, twelve and seventeen lines exhibited intermediate infection responses to Yozgat, Hatay, K1, K2 and K3 isolates, respectively ( $P<0.01$ ). Barley lines #1 and #15 showed an intermediate infection response to all 5 isolates ( $P<0.01$ ). No line exhibited low infection response. Statistically significant ( $P<0.01$ ) differences among the isolates were observed. Future studies should focus on determining pathotypes of the fungus and obtaining genotypes that exhibit low infection responses. For this purpose, barley landraces and wild barleys could be used.

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## INTERACTIONS AMONGST *RHIZOBIUM* V5 ISOLATE, MYCORRHIZA INFECTION AND ROOT CHARACTERISTICS OF FORAGE PEA

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*Scientific paper*

### Summary

A greenhouse experiment was conducted in a randomized block design with four replications to evaluate the effect of forage pea on inoculation of *Rhizobium* sp. V5 isolate and mycorrhiza fungi (*Glomus mossae*) on root characteristics, nodule formation and mycorrhizal infection of forage pea. Maximum root biomass was obtained in the mycorrhizal fungi + *Rhizobium* sp. V5 isolate treatment. Total root length and mycorrhizal infection of roots were maximum for all the treatments. A positive association between mycorrhizal infection and *Rhizobium* isolate were observed.

Key words: *Glomus mossae*, *Rhizobium* sp. V5 isolate, forage pea, nitrogen fixation, root characteristics

### INTRODUCTION

Mycorrhiza is a mutual symbiosis between thallus of some fungi and the root of plants. Among the microorganisms living in the soil, arbuscular mycorrhiza fungi and *Rhizobium* bacteria are especially important (Toro *et al.*, 1998; Xavier and Germida, 2002). Legume biological nitrogen fixation forms a major proportion of total biological nitrogen fixation contributed by agricultural (Denison and Kiers, 2011). Legume require crop specific *Rhizobium* species and optimum conditions for nodule activity (Denison and Kiers, 2011). Forage pea (*Pisum sativum* L.) is one of the most important legume crops in forage. Therefore, the present study was conducted to evaluate the efficiency of the mycorrhizal fungus and *Rhizobium* isolate of forage pea (*Pisum sativum*).

### MATERIALS AND METHODS

A pot experiment was conducted under greenhouse conditions. The seeds were surface sterilized with 0.2% mercuric chloride solution for 2 min and inoculated with a *Rhizobium* isolate. The mycorrhizal inoculum was placed at a pot depth of 2-3 cm immediately prior to the sowing of the seeds to facilitate fungal colonization of plant roots. Mycorrhizal infection and length were estimated by the method of Giovannetti and Mosse (1980). Plant dry matter was measured, shoot dry weight was recorded after drying in an oven at 70°C (Alamgir-Kabi *et al.*, 2006). Phosphorous was determined by Jackson (1967).

### RESULTS AND DISCUSSION

In treatment, plants inoculated with mycorrhizal fungus (AMF) and *Rhizobium* isolate had significantly increased shoot fresh weight relative to the non-inoculated plants (Table 1).

Plants co-inoculated with *Rhizobium* isolate showed fresh weights similar to those of AMF root colonization than other treatments. These results are in agreement with previous findings by. A synergistic interaction between *Rhizobium* and mycorrhizal fungus has been reported for AM root colonization (Xaviers and Germida, 2002; Toro *et al.*, 1998). Soil microorganisms and their activities play important roles in the transformation of plant nutrients from unavailable to available forms and the improvement of soil fertility (Geneva *et al.*, 2010).

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Tab. 1. Effects of treatments on forage pea

Treatment	Root dry weight (g)	Plant height (cm)	Shoot wet weight (g)	Root height (cm)	% Mycorrhizal infection
Control (C)	1.05	64.	26	26.3	-
<i>Rhizobium</i> (R)	1.09	122.5	28.25	28.2	-
Gübre (G)	1.13	124	27	25.3	-
Mycorrhiza (M)	1.07	126.25	17.66	27.5	110*
M+R	1.10	135*	30.5*	30.33*	105
M+G	1.16*	107.5	26.75	27.7	107
R+G	1.14	115.25	29	29.1	-
M+R+G	1.13	131.5	28.5	27.3	112

\*p&lt;0.01

## CONCLUSIONS

Inoculation with *Rhizobium* isolate was increased nodule number. With respected to enhancement plant growth, root length, co-inoculation *Rhizobium* V5 isolate and mycorrhizal fungus resulted more effective than other combination tested. The presence of combined nitrogen and phosphour fertilizer limited the nodulation and mycorrhizal infection of pea.

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## THE CHARACTERIZATION OF MORPHOLOGIC AND AGRONOMIC TRAITS OF SOME WILD TYPE SUNFLOWER (*Helianthus L. spp.*) GENOTYPES

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*Original scientific paper*

### Summary

The study was carried out in 2013 and 2014 to determine the agronomic and morphologic characteristics of different wild type sunflower (*Helianthus L. spp.*) genotypes at Uludağ University Faculty of Agronomy's field and green house conditions. The 45 wild sunflower genotypes supplied from different sources (USDA-America; Germany, Canada) were used in the study. One month seedlings germinated with mechanical drawing application in a 1:1 portion soil and peat mixture violin were grown in field conditions.

Some of the agronomic (plant height, number of branches, number of leaves per plant, head diameter, stem thickness, length of branches) and morphologic (ray flower color, head angle, head shape, uniformity of flowering, uniformity of maturity, bract shape, pollen fertility, pubescence at general appearance, disk flower color, petiole position, branching, type of branching) characteristics were observed and measured two years.

The agronomic variation on the observed characters was found highly variable within wild sunflower genotypes. The plant height, the number of branches, the number of leaves, the head diameter, stem thickness, length of branches values changed respectively between 39 and 367 cm, 6 and 52, 3 and 39, 0.5 and 3.40 cm, 0.50 and 2.91 cm, 12 and 194 cm. There were no clear differences within observed morphologic characters.

### INTRODUCTION

Sunflower (*Helianthus annuus L.*) is the fourth most important oilseed crop in the world in terms of total yearly production, after soybean, rapeseed, and groundnut. It is also the first an important edible oil crop in Turkey, with harvesting areas of 609784 ha with production of 1523000 tons and an average grain yield of 24976 (Hg/Ha) for the 2013 period (FAOSTAT, 2015). *Helianthus*, a native to North, middle and South America contains 62 species with annual and perennial species (Anonymous, 2015).

In order to enhance a wide range of sunflower production, many agronomic and quality characteristics need to be improved in terms of their genetic structures. The classical breeding studies made from 1880 until today have been focused on major characters, such as high fat, pests and diseases resistance (Faure *et al.*, 2002; Seiler and Gulya, 2004; Vassilevska-Ivanova *et al.*, 2014). The narrow genetic base in sunflower causes a major problem at breeding and selection of desired sunflower lines. There is a need to inbred parental lines contained different genetic resources for selecting desired characters in terms of breeding. It is possible to select high yield, resistance to diseases, resistance to stress conditions, etc. important characters by using annual and perennial wild species of sunflower genotypes as starting material and make interspecific hybridization between them (Christov, 2012).

The loss of genetic variation in crops due to modernization of modern agricultural applications has been described as genetic erosion (Seiler and Marek, 2011). The base of genetic variation in cultivated sunflower is very narrow. This case restricts selection of suitable genotypes for breeding purposes. The wild genotypes are mandatory to use as starting material in the sunflower breeding programme for selection of suitable sunflower genotypes. While using SSR markers genetic diversity in cultivated sunflower was found as 0.47 by Mandel and his friends (2011), this diversity was 0.70 in limited numbers of wild species of *H. annuus L.*

Genotypes used in this study were obtained from America, Canada and Germany. Before wild sunflower species used by scientists related to plant breeding (breeders, disease specialist, etc.) in Turkey should be cultivated in our conditions. Despite the advances in molecular techniques, the level of differentiation detected by genetic markers may not be the same as the level of differentiation for quantitative traits (Presotto *et al.*, 2009). Determination of agronomic and morphologic characters (phenotypic characteristics) of wild genotypes is also extremely important for controlled crossing studies. The most appropriate combinations at hybridization will be

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determined in the genetic pool. The objectives of this study; the determination of the morphological and agronomic characteristics of the genotypes to be used in intraspecific and interspecific cross in Bursa /Görükle

## MATERIALS AND METHODS

This study was conducted at Uludağ University, Agricultural Faculty, Department of Field Crops' research and application fields and greenhouse conditions in 2013 and 2014. The 45 wild sunflower genotypes obtained from different sources (USDA-America, Germany, Canada) were used in the study. These genotypes are given below. *H. annuus* (USDA1, USDA2, USDA3, USDA4, USDA5, USDA6, USDA7, USDA8, USDA9, USDA10, USDA11, USDA12, USDA13, USDA14, USDA15, USDA16, USDA17, USDA18, USDA19, USDA20, USDA21, USDA22, USDA23, USDA24, USDA25, USDA26, USDA28 (in total 27), *H. petiolaris* (1), *H. petiolaris* subsp. *petiolaris* (3), *H. bolanderi* (1), *H. maximiliani* (4), *H. nuttalli* (1), *H. argophyllus* (5), *H. annuus* ssp. *lenticularis* (1), *H. petiolaris* subsp. *fallax* (1), *H. anomalus* (1). The soil in field where the trial is set up is the clay structure and poor in terms of nitrogen and in organic matter, rich in terms of potassium. Average temperature and total rainfall in the growing area was recorded as 20.96°C and 194.3 mm during the sunflower vegetation period (April-August) (Anonymous, 2014). The achenes were mechanically scarified by cutting off tip of achene to distal cotyledon end and transferred to 31 by 51 cm plastic multipot trays of 48 pots per tray containing sterile 1:1 peat : soil mixture (v:v) at 24 ± 2°C in 16h/8 h (light/dark) in the growth chamber. They were grown for 3 weeks in the growth cabinet and then seedlings were sown in to field with 2 rows of 2 m lengths with 0.4 m between rows and plants. After the sowing into field, seedlings were watered. Five kilograms of nitrogen per decare as ammonium nitrate was applied prior to sowing and a further 5 kg N/da was added when the plants were 30–40 cm in height. Sunflower growth stages were performed as described by Schneiter and Miller (1981). Paper bags were placed over the heads before ray flowers emergence (R4 stage of Schneiter and Miller, 1981). In order to obtain seed the wild genotypes were subjected to sib-mating. Bulk pollen from sib plants of the same genotype was applied by hand 2 to 3 times until the end of the flowering. Wild sunflowers grown in the field matured at different time periods. The 6 agronomic traits; plant height (cm), head diameter (cm), number of branches (number), number of leaves per plant (number), stem thickness (cm), length of branches (cm) and 11 morphological characters; ray flower color, head angle, head shape, uniformity of flowering, uniformity of maturity, bract shape, pollen fertility, pubescence at general appearance, disk flower color, petiole position, type of branching) were observed and measured in two years according to IBPGR (1985) and UPOV (2000). For observations and measurements, six plants within two rows were randomly selected from each genotype grown in the field. Mean data were presented with minimum, maximum mean, standard error, variance and standard deviations in the Table.

## RESULTS AND DISCUSSION

The maximum, minimum, mean, standard deviations, variance and coefficient of variation values for agronomic characters of sunflower genotypes obtained from different sources are given in Table 1.

According to the research results, there were large differences between wild sunflower genotypes in terms of measured agronomic characters (plant height, the main head diameter, the number of branches, the number of leaves the stem thickness the length of branches (Table 1).

Wild sunflower genotypes showed very large differences within themselves and among species grown Bursa-Görükle province in terms of agronomic characters. Similar work carried out by Presotto *et al.* (2009) in Argentina reported that wild sunflower genotypes within a population and between populations showed significant differences. Populations growing in different climate at natural conditions show differences in terms of agronomic such as growth period, plant height, leaf shape, plate morphology, etc. and morphologic characters. This is explained by phenotypic plasticity in environmental and ecological changes caused by different growing conditions (Richards *et al.*, 2006). The 35 characters of local sunflower populations (primitive cultivar) collected from various regions of Turkey were examined according to list and definition of IBPGR and UPOV by Tan and Tan (2011). The morphologic variation on the observed characters was found highly variable, while no variation on pollen fertility was observed in this study.

The mean values of 11 morphologic characters examined are given in Table 2. It is clear to see from the Table that there was no big variation within morphological characters examined in our study. All genotypes released the branching. The basal branching without central head was observed mostly. Taken overall status of pubescence at general appearance, the difference between genotypes was observed. The 2 of observed genotypes (*H. annuus* ssp. *lenticularis*, *H. petiolaris*) had extremely low pubescence, 4 of them (*H. annuus*, *H.*

*bolanderi*, *H. nuttalli*, *H. anomalus*) had low pubescence, 1 of them (*H. maximiliani*) had medium pubescence and 3 of them (*H. petiolaris* subsp. *petiolaris*, *H. argophyllus*, *H. petiolaris* subsp. *fallax*) had extremely high pubescence. When the ray flower color divided into 2 groups as yellow (1) and orange (2), 3 of them had orange color ray flower (*H. argophyllus*, *H. annuus* ssp. *lenticularis*, *H. petiolaris* subsp. *fallax*). The rest of them had yellow color ray flower. Considering colors of disk flowers, 3 genotypes had pale yellow color and dark yellow color, 4 genotypes had red color disk flowers. Considering the uniformity of flowering and uniformity of maturity at the same plant, only *H. petiolaris* subsp. *petiolaris* produced intermediate level uniform flowering, the rest of the genotypes produced highly variable flowering time and maturity. When the head shape of the character discussed the *H. bolanderi* genotype had the flat shaped head (1), whereas the *H. petiolaris* subsp. *petiolaris* genotype had convex shaped head (2). The rest of the genotypes had concave shaped head formation (3). According to the type of branching only *H. annuus* had fully branched with central head (3), the rest of the genotypes had fully branched without central head (Table 2).

## CONCLUSION

In conclusion there were differences within the genotypes in terms of morphologic and agronomic characters examined in Bursa /Görükle province while no clear differences within morphologic characters in wild type sunflower genotypes. The data obtained from this study will be used by the scientist as starting material who will work to create genetic variation and select the desired feature in hybrid sunflower breeding studies in the near future.

Tab. 2: The mean values of morphologic characters

Species	Branching (Scor)	Type of branching (Scor)	Pubescence (Scor)	Ray flower color (Scor)	Head flower colour (Scor)	Pollen fertility (Scor)	Flowering and Maturity uniformity (Scor)	Head angle (Scor)	Head shape (Scor)	Bract shape (Scor)	Petiole position (Scor)
<i>H. annuus</i>	1.0	3.1	1.1	1.0	1.9	1.0	1.1	2.2	2.9	1.0	3.6
<i>H. maximiliani</i>	1.0	4.0	2.2	1.0	1.9	1.0	1.4	2.0	3.0	1.0	3.6
<i>H. petiolaris</i> subsp. <i>petiolaris</i>	1.0	4.0	2.8	1.0	3.0	1.0	1.6	2.0	2.4	1.0	3.8
<i>H. argophyllus</i>	1.0	4.0	3.0	1.7	2.8	1.0	1.0	2.0	3.0	1.0	3.7
<i>H. annuus</i> ssp. <i>lenticularis</i>	1.0	4.0	0	2.0	2.0	1.0	1.0	2.0	3.0	1.0	3.3
<i>H. petiolaris</i>	1.0	4.0	0	1.0	3.0	1.0	1.0	2.0	3.0	1.0	3.3
<i>H. bolanderi</i>	1.0	4.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	3.0
<i>H. nuttalli</i>	1.0	4.0	1.0	1.0	1.0	1.0	1.0	2.0	3.0	1.0	4.0
<i>H. petiolaris</i> subsp. <i>fallax</i>	1.0	4.0	2.5	1.5	2.5	1.0	1.0	2.0	3.0	1.0	3.9
<i>H. anomalus</i>	1.0	4.0	1.0	1.0	1.0					1.0	4.0

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Tab. 1: The variation on the morphologic characters

Species		Plant height (cm)	Head diameter (cm)	Number of branches (number)	Number of leaves (number)	Stem thickness (cm)	Length of branches (cm)
<i>H. annuus</i>	Min.	59.0	0.50	6.0	3.0	0.50	13.0
	Max.	367.0	9.00	52.0	86.0	9.00	194.0
	Mean	211.0	2.16	24.2	12.3	2.16	82.7
	St. Dev.	5.1990	0.0675	0.7459	0.2645	0.0484	1.1705
	Variance	4270.6221	1.9766	87.9167	44.0156	0.7212	863.0850
	CV (%)	31.03	29.10	38.75	54.13	39.29	35.53
<i>H. maximiliani</i>	Min.	39.00	0.80	7.00	6.00	0.27	12.50
	Max.	181.00	3.00	39.00	36.00	2.29	88.00
	Mean	106.82	1.68	19.82	12.96	1.04	42.56
	St. Dev.	11.2936	0.0328	2.7160	0.7725	0.0980	2.3159
	Variance	2168.2794	0.1676	125.4044	39.9828	0.3267	359.3322
	CV (%)	43.59	24.69	56.49	48.81	54.98	44.54
<i>H. petiolaris</i> subsp. <i>petiolaris</i>	Min.	86.00	0.80	7.00	6.00	0.59	53.00
	Max.	144.00	3.40	22.00	17.00	1.90	109.00
	Mean	107.00	2.18	15.30	11.30	1.01	84.25
	St. Dev.	5.3728	0.0641	1.4686	0.5080	0.0777	2.0380
	Variance	288.6667	0.4113	21.5667	9.5480	0.1208	166.1410
	CV (%)	15.88	29.42	30.35	27.35	34.30	15.30
<i>H. argophyllus</i>	Min.	79.00	1.17	8.00	5.00	0.89	12.00
	Max.	301.00	6.30	41.00	39.00	2.91	113.00
	Mean	159.22	2.91	22.93	17.12	1.84	64.90
	St. Dev.	11.4150	0.0737	1.7870	0.6249	0.0784	2.3272
	Variance	3518.1795	1.2486	86.2251	41.7870	0.3322	584.8982
	CV (%)	37.25	38.41	40.50	37.76	31.76	37.26
<i>H. annuus</i> ssp. <i>lenticularis</i>	Min.	99.00	1.10	12.00	7.00	0.90	64.00
	Max.	124.00	3.40	12.00	16.00	1.11	115.00
	Mean	111.50	2.07	12.00	12.13	1.00	94.50
	St. Dev.	12.5000	0.1120	0.0000	1.1868	0.0494	5.2270
	Variance	312.5000	0.2508	0.0000	11.2679	0.0098	218.5714
	CV (%)	15.85	24.25	0.00	27.68	9.80	15.64
<i>H. petiolaris</i>	Min.	69.00	1.15	8.00	3.00	0.63	45.00
	Max.	96.00	2.50	9.00	14.00	1.11	93.00
	Mean	82.33	1.62	9.33	10.92	0.80	65.08
	St. Dev.	7.7960	0.0720	0.8819	0.8657	0.0858	4.7024
	Variance	182.3333	0.1556	2.3333	8.9924	0.0441	265.3561
	CV (%)	16.40	24.42	16.37	27.47	26.15	25.03

Tab. 1: The variation on the morphologic characters (Continued)

Species		Plant height (cm)	Head diameter (cm)	Number of branches (number)	Number of leaves (number)	Stem thickness (cm)	Length of branches (cm)
<i>H. bolanderi</i>	<b>Min.</b>	64.00	1.30	24.00	5.00	0.69	40.00
	<b>Max.</b>	64.00	2.10	24.00	10.00	0.73	56.00
	<b>Mean</b>	64.00	1.76	24.00	7.25	0.71	47.25
	<b>St. Dev.</b>		0.0718		1.1087	0.0200	3.5911
	<b>Variance</b>		0.0516		4.9167	0.0008	51.5833
	<b>CV (%)</b>		12.90		30.58	3.98	15.20
<i>H. nuttalli</i>	<b>Min.</b>	53.00	1.50	11.00	6.00	0.81	41.00
	<b>Max.</b>	68.00	2.40	13.00	11.00	0.90	133.00
	<b>Mean</b>	60.50	2.02	12.00	8.63	0.86	62.00
	<b>St. Dev.</b>	7.5000	0.0574	1.0000	0.6529	0.0194	10.4881
	<b>Variance</b>	112.5000	0.0659	2.0000	3.4107	0.0015	880.0000
	<b>CV (%)</b>	17.53	12.71	11.79	21.41	4.53	47.85
<i>H. petiolaris</i> subsp. <i>fallax</i>	<b>Min.</b>	99.00	1.70	13.00	10.00	0.65	57.00
	<b>Max.</b>	143.00	3.40	19.00	21.00	1.31	158.00
	<b>Mean</b>	113.75	2.26	15.50	15.88	0.95	105.25
	<b>St. Dev.</b>	10.0778	0.0598	1.3229	0.9304	0.0867	7.9527
	<b>Variance</b>	406.2500	0.1430	7.0000	13.8500	0.0601	1011.9333
	<b>CV (%)</b>	17.72	16.75	17.07	23.44	25.81	30.22
<i>H. anomalus</i>	<b>Min.</b>	54.00	1.60	17.00	5.00	0.55	24.00
	<b>Max.</b>	57.00	2.00	19.00	16.00	0.67	62.00
	<b>Mean</b>	55.50	1.80	18.00	10.25	0.60	40.25
	<b>St. Dev.</b>	1.5000	0.2000	1.0000	1.2783	0.0263	4.4791
	<b>Variance</b>	4.5000	0.0800	2.0000	13.0714	0.0028	160.5000
	<b>CV (%)</b>	3.82	15.71	7.86	35.27	8.79	31.48

## DETERMINATION OF FUNGAL AGENTS OF SOME VEGETABLES CULTIVATED GREENHOUSE AREAS OF UŞAK OF AGEAN REGION IN TURKEY

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*Preliminary communications*

### Summary

Turkey is an important greenhouse vegetable producing country with 6.274.000 t production on 615.000 decares (Tuik, 2014). Vegetable cultivation is increasing and is centered in the Mediterranean and Aegean Regions. In generally, the production is conducted either in open field or plastic tunnel systems. It is not common to apply pre-planting fumigation in greenhouse areas, usually crop rotation and sanitation are applied to avoid soil-borne pathogens. The diseases cause important economic losses in the region and they have become the major problems in production. Our survey's aim is to identify common soil-borne pathogens in greenhouse production areas. The surveys have done regularly during the seedling, flowering, fruiting stages. Plant samples were collected from provinces in Aegean Regions in Uşak, 2015. We surveyed 10% parts of planting areas is 627 da and in totally collected 200 symptomatic plant samples. The diseased plants were transferred to the laboratory they were cultured on potato dextrose agar including streptomycin media to detect the pathogens. *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Alternaria*, *Macrophomina* and *Botrytis* spp. were the commonly identified fungal and oomycetous organisms. In most cases, the samples were infected by more than one pathogen. The results are significant for developing certificated and productive seedling and seed production systems needed to be employed against soil borne pathogens in greenhouse.

Key words: *soil-borne pathogen, vegetable crops, greenhouse*

### INTRODUCTION

The need for food substance increases with the increasing world population, and in order to meet this food demand, agricultural production should be increased and the product amount obtained from unit area should be higher. Therefore, in order to obtain more products from the unit area, plants should be grown healthily and protected from diseases. For this purpose, it is important to determine fungal elements that cause diseases. As Turkey has suitable ecological conditions for vegetable-growing, it enables growing many horticulture species. It is also possible to grow many vegetable species in the greenhouse cultivation. Greenhouse cultivation includes production in greenhouses and low plastic tunnels. In Turkey, 6.274.000 tonnes of vegetable are produced within an area of 615.000 da within the scope of greenhouse cultivation (Anonymous, 2014). The city of Uşak meets 4% of the vegetable production of Turkey and 2% of the Aegean Region, and it comes to the forefront especially with root bulb and tuberous vegetables. Greenhouse cultivation includes 6.579 tonnes of vegetable production in a total area of 622 da (Anonymous, 2014). Greenhouse cultivation is common in the districts of Banaz, Eşme and Sivaslı. Almost all greenhouses are high tunnels. They are more commonly used for products such as tomato, cucumber, and lettuce. Within production values, tomato takes the first place with 3820 tonnes in an area of 216 da and cucumber takes the second place with 1497 tonnes in an area of 95 da. When leading districts in the city in terms of greenhouse cultivation are examined; tomato takes the first place in Banaz with 2822 tonnes in an area of 165 da whereas cucumber takes the first place in Eşme with 240 tonnes in an area of 16 da (Anonymous, 2014).

As the same products are repeatedly cultivated in greenhouses every year, some land-related disease factors lead to considerable losses. Thus, the non-performance of rotation of crops and the absence of effective and economical fighting methods in greenhouses increase more the severity of diseases caused by soilborne pathogens every year (Yücel, 1994). The most significant soilborne pathogens observed in these regions have been reported to be *Fusarium* spp., *Rhizoctonia* spp., *Verticillium* spp. (Yücel and Çınar, 1989; Yücel, 1995; Yücel *et al.*, 1998; Can *et al.*, 2004).

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In previous studies; *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Alternaria solani*, *Botrytis cinerea*, *Cladosporium fulvum*, *Leveillula taurica* and *Pyrenochaete lycopersici* were isolated from infected tomatoes; *S. sclerotiorum*, *Fusarium* spp., *Phytophthora capsici*, *B. cinerea*, *L. taurica*, and *A. Solani* were isolated from peppers; *S. sclerotiorum*, *B. cinerea*, *Fusarium* spp., *Verticillium* spp., *L. taurica*, and *A. Solani* were isolated from eggplants in vegetable greenhouses in the Mediterranean Region (Yücel, 1994).

In cities in the Central Anatolia Region where greenhouse vegetable cultivation is performed, *Fusarium oxysporum* and *Rhizoctonia solani*; *Cladosporium fulvum* (*Fulvia fulva*), *Alternaria solani*, *Botrytis cinerea*, *Phytophthora infestans* were found in tomatoes; *Phytophthora capsici* was found in peppers; *Pseudoperonospora cubensis*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *cucumerinum* were found in cucumbers; *Fusarium oxysporum* and *Rhizoctonia solani*, *Botrytis cinerea* were found in beans and *Leveillula taurica* was found in eggplants (Ozan and Aşkın, 2006).

In the city of Malatya; *Rhizoctonia solani*, *Fusarium solani*., *Fusarium oxysporum* *Pythophthora capsici*., *Alternaria solani*, *Macrophomina phaseolina*, *Pythium ultimum* var. *ultimum* were found in tomatoes; *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum*, *Pythophthora capsici*, *Pythium ultimum* var. *ultimum* were found in peppers; *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum*, *Pythophthora capsici*, *Pythium ultimum* var. *ultimum*, *Verticillium dahliae* Kleb. were found in eggplants, *Rhizoctonia solani*, *Fusarium solani*, *Fusarium oxysporum*, *Alternaria solani*, *Alternaria alternata*, *Macrophomina phaseolina* were found in beans as elements that cause diseases (Kırbağ and Turan, 2006).

In the city of Uşak, greenhouse vegetable growing has an important production potential. However, producers experience considerable product losses caused by some disease factors both in seedling period and during the production season. Therefore, these disease factors not only limit production but also lead to economic loss by increasing the production cost of producer. Therefore, these disease factors should be identified and suitable and effective fighting methods should be used against these factors. However, there are no studies on the identification of these fungal disease factors that impose a problem in greenhouse vegetable cultivation in our city. This is a pre-study intended for identification of fungal disease factors observed in some vegetables in the city of Uşak.

## MATERIALS AND METHODS

### MATERIALS

The material of the study consisted of fungal disease factors obtained from pepper (*Capsicum annum* L.), tomato (*Lycopersicum esculentum* Miller), cucumber (*Cucumis sativus* L.), bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa*) and eggplant (*Solanum melongena* L.) vegetables (and their isolations) which are cultivated in greenhouses in districts of Merkez, Banaz, Eşme, Karahallı, Sivaslı, and Ulubey in the city of Uşak.

### METHODS

In this study, visits were paid to seedling, blooming, and fruit periods in the areas of greenhouse vegetable cultivation (pepper, tomato, bean, cucumber, cabbage, lettuce, and eggplant) in the city centre of Uşak and its districts (Banaz, Eşme, Karahallı, Sivaslı, and Ulubey).

Received samples were labelled, placed into plastic bags and ice boxes and brought to the laboratory, where their isolation studies were performed within 24-48 hours. Strawberry seedlings brought to the laboratory were washed under tap water, small parts were taken from their diseased and partly healthy parts from their roots and root collars, and surface disinfection was performed on these parts. While 2% sodium hypochlorite (NaOCl) was used for the surface disinfection of tissue parts taken from stems, 1% sodium hypochlorite (NaOCl) was used for the tissue parts taken from the roots. Afterwards, the samples were rinsed with sterile distilled water and dried on sterile drying paper. Pieces were analysed under potato dextrose agar (PDA-Merck). Purification was performed on colonies that looked different in terms of development in PDA. Then, the diagnoses of the fungi obtained as a result of the macroscopic and microscopic analyses were made according to the relevant literature (Agrios; 2005; Nelson *et al.*, 1983; Leslie and Summerell, 2006; Sneh *et al.*, 1998; Ndiaye *et al.*, 2007; Holliday 1989; Smith *et al.* 1988).

## RESULTS AND DISCUSSION

Isolates of *Colletotrichum* spp., *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Macrophomina phaseolina* and *Botrytis* spp. factors were obtained from diseased vegetable samples (pepper, tomato, bean, cucumber, cabbage, lettuce, and eggplant) as a result of the isolation studies.

In isolations conducted on roots and root collars; as damping agents, it was found as follows; *Fusarium* spp., and *Rhizoctonia* spp. in pepper, tomato and lettuce seedlings, only *Fusarium* spp. in bean, and only *Rhizoctonia* spp. in cabbage; similarly *Alternaria* spp., *Macrophomina phaseolina* in the roots and root collars of pepper and tomato seedlings. In the cities of Adana and Mersin in the Eastern Mediterranean Region where tomato is cultivated in greenhouses, *Fusarium oxysporum* was found to cause tomato root-crown rot disease and tomato fusarium wilt (Çolak and Biçici, 2011).

The factors *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Macrophomina phaseolina* were isolated in peppers and tomatoes, also *Botrytis* spp. was isolated in tomatoes, *Colletotrichum* spp. was isolated in peppers and cucumbers, *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp. were isolated in lettuce, *Fusarium* spp., *Rhizoctonia* spp. were isolated in eggplants, *Fusarium* spp. was isolated in beans and cucumber; and *Rhizoctonia* spp. was isolated in cabbage. In Central Anatolia Region, *Fusarium oxysporum* and *Rhizoctonia solani*; *Cladosporium fulvum*, (*Fulvia fulva*), *Alternaria solani*, *Botrytis cinerea*, *Phytophthora infestans* were identified in tomatoes, *Phytophthora capsici* was identified in peppers, *Pseudoperonospora cubensis*, *Botrytis cinerea*, *Fusarium oxysporum* f.sp. *cucumerinum* were identified in cucumbers; *Fusarium oxysporum* and *Rhizoctonia solani*, *Botrytis cinerea* were identified in beans; and *Leveillula taurica* was identified in eggplants (Ozan and Aşkın, 2006).

In the cities of Antalya, Mersin and Samsun; *Fusarium oxysporum* f. sp. *melongenae* (Fomg), and *Verticillium dahliae* Kleb. were found to cause wilt disease in eggplant greenhouses (Altınok *et al.*, 2012).

## CONCLUSION

*Colletotrichum* spp, *Fusarium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Macrophomina phaseolina* and *Botrytis* spp. fungal disease factors were identified in some vegetables cultivated in greenhouses in the districts of Merkez, Banaz, Eşme, Karahallı, Sivaslı, and Ulubey in the city of Uşak.

When obtained results were assessed, it is necessary to perform detailed surveys in greenhouse vegetable cultivation areas in the city of Uşak, identify the fungal disease factors on samples taken from isolations of diseased plants and determine if they are pathogens, precautions intended for control methods to against such pathogen disease factors should be determined and producers should be informed on the matter.

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## THE EFFECTS OF DIFFERENT PLANTING METHODS ON TOBACCO QUALITY AND YIELD ON IZMIR TYPE TOBACCO

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*Scientific paper*

### Summary

The research was carried out to investigate the effect of different planting methods (traditional and double cross planting) on some chemical characters, yield and tobacco visual quality in 2014. In the trial, Sarıbaglar and Birlik-124 tobacco varieties were used and experimental design was Randomized Complete Block Design with three replications. In the study, total alkaloid (nicotine) (%), total reducing sugar (%), raw ash (%), total nitrogen (%), yield (kg ha<sup>-1</sup>) and tobacco visual quality were determined. In Suleymanli village, total alkaloid 0.5%, total reducing sugar 11.5%, raw ash 16% and yield 200 kg ha<sup>-1</sup> were found in the traditional planting methods whereas total alkaloid 0.3%, total reducing sugar 15.0%, raw ash 20.5% and yield 313 kg ha<sup>-1</sup> were determined in the double cross planting methods. In Kapakli village, total alkaloid 0.9%, total reducing sugar 11.5%, raw ash 17% and yield 156 kg ha<sup>-1</sup> were found in the traditional planting methods while total alkaloid 0.7%, total reducing sugar 13.1%, raw ash 16.4% and yield 165 kg ha<sup>-1</sup> were determined in the double cross planting methods. American grad tobacco rate in double cross planting method in Suleymanli was higher than in Kapakli.

Key words: *Tobacco, planting, chemical characters, quality and yield*

### INTRODUCTION

Tobacco which still has a great economic importance in the world and Turkey provides extensive employment opportunities from growing to evaluation stage to a certain part of our people and is produced as a family enterprise for centuries in our various regions.

Turkey has a production of 90.000 tons tobacco compared with the production of 7.3 million tons in the world. Besides is number one with the 73.284 kg of high quality oriental tobacco production in Turkey in the world. According to 2013 statistics, 50.877 kg tobacco which is more than that of 50% of total production of Turkey, has been obtained from Aegean Region of Anatolia. Furthermore the region has sufficient agricultural land to meet requirement of the World's total oriental tobacco. However there is a significant decrease in tobacco production the Aegean region as Turkey's total tobacco production (Anonym a).

There are many factors causing the decline in production. Such as reduction in the number of families producing tobacco, farmers usually be over the age of us 45 years and older consists of farmers, price policy, anti-smoking policy and the challenges of the agricultural practices. As well as, to grow tobacco in the same field every year and to use low purity seed are among the main factors in the decrease of the yield.

Production of tobacco in Turkey, from 1969 until 2002, was organized by the Law No. 1177. Since January 9, 2002, contract production method has been taken over with the Law No 4733 shortly known as 'Tobacco Act'.

Tobacco has an important role in Turkey's economy and social life of producers. Tobacco production and industrial facilities provide livelihood to nearly 3 million people. A large part of tobacco production is exported and it provides approximately 400-500 million dollars to Turkey's economy in every year. In addition to this, Aegean Region has an important role in terms of foreign sales and more than 79.80% of exported tobacco is provided from the Aegean Region (Anonym a).

For this pilot study, Akhisar location which is important tobacco production area in Aegean Region was chosen. Trials were carried out Süleymanli and Kapakli villages of Akhisar. Double cross planting method was applied to Birlik-124 and Sarıbaglar tobacco varieties and effects of the methods on yield and quality were examined.

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## MATERIAL AND METHOD

The research was carried out in Suleymanli and Kapakli villages, Akhisar, Manisa, Turkey in 2014. The soil was sandy loam and clayey loam texture. Some physical and chemical characteristics of the soil are shown in the Table 1.

Tab. 1. Some physical and chemical characteristics of the soil at the experiment plot (0-40 cm depth)

Villages	Texture	pH	Total nitrogen (%)	Organic matter (%)
Suleymanli	Sandy-clayey loam	7.66	0.067	1.238
Kapakli	Sandy-loam	7.44	0.062	1.032

Akhisar climate is mild Mediterranean climate type. The highest monthly average temperatures were recorded 29.0°C in August, by the Directorate of the Turkish State Meteorological Services in Akhisar. There was no rain during the growing period in summer. Birlik-124 and Saribaglar tobacco cultivars were used in Süleymanli and Kapakli villages, respectively. Traditional and double cross planting methods were used in both villages. Plant spacing of 40x10 cm and 36 x 10 cm were used in traditional and double cross planting methods, respectively. Tobacco seeds were sown (1.5 g/m<sup>2</sup>) into the soil consisting of sand, manure and mulch mixture (1:1:1) in February. Some cultural practices such as irrigation, weed control and ventilation were performed regularly. When the tobacco seedlings reached 15-20 cm plant height they were transferred to the experimental plots at the end of the May in traditional and double cross planting methods in Suleymanli whereas at the end of the April in traditional method and beginning of the June in double cross planting in Kapakli.

The experiment was designed in a randomized complete block design with three replications. After the planting the seedling was irrigated. There was no irrigation but hoeing was done twice in vegetation period in both villages. The plants were harvested when the tobacco leaves had maturing. Harvesting was done 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> priming.

In this study some features were determined such as total N (Kacar, 1972; Anon, 1986), total alkaloid (nicotine) (Anon, 1969), total reducing sugar (Lindsay, 1973), crude ash (Nelson, 1960), and yield and tobacco visual quality (Anon, 1971). The data for all traits were analyzed by the analysis of variance by Acikgöz *et al.* (2004).

## RESEARCH RESULTS AND DISCUSSION

### Total Alkaloid (Nicotine) Rate (%)

The total alkaloid (nicotine) is one of the most important quality criteria in tobacco and high nicotine amount is not desired for Aegean tobaccos. As shown in Table 2, nicotine content was affected by planting methods at  $p < 0.01$  significance level. The highest nicotine was obtained in traditional method in Kapakli. Double cross planting method had lower total alkaloid than traditional method in both villages. Nicotine level is the most important for smoking quality. Uz (1997) claimed that nicotine rate increased from lower parts of the plant to upper parts. In this study, nicotine content changed between 0.3-0.9%. These results were consistent with the results of other researchers (Yazan, 1989; Gencer, 2000; Korkmaz, 2006; Ekren, 2007).

Tab. 2 . Effect of the traditional and double cross planting methods on some chemical characters of tobacco (%)

Chemical components	Süleymanli			Kapakli		
	Traditional	Double planting	cross	Traditional	Double planting	cross
Total alkaloid (nikotine)	0.5 <sup>a</sup>	0.3 <sup>b</sup>		0.9 <sup>a</sup>	0.7 <sup>b</sup>	
LSD	0.038**			0.065**		
Total reducing sugar	11.5 <sup>b</sup>	15.0 <sup>a</sup>		11.5 <sup>b</sup>	13.1 <sup>a</sup>	
LSD	1.481**			0.421**		
Crude ash	16.0 <sup>b</sup>	20.5 <sup>a</sup>		17.0	16.4	
LSD	1.696**			ns		
Total nitrogen	2.4 <sup>a</sup>	2.1 <sup>b</sup>		2.8 <sup>a</sup>	2.4 <sup>b</sup>	
LSD	0.290*			0.272*		

\*\*  $p < 0.01$

\*  $p < 0.05$

ns: not significant

### Total Reducing Sugar Rate (%)

Different planting methods had significant effect ( $p < 0.01$ ) on total reducing sugar and it ranged from 11.5 to 15.0 % in the trial (Table 2). The maximum dose was found to be 15.0% for double cross planting method in Süleymanlı village. Total reducing sugar in double cross planting was higher amount than traditional method. There was an inverse relationship between the proportion of nicotine and reducing sugar for oriental tobacco plant and increasing the amount of reducing sugar is known to have a positive impact on tobacco quality (Sekin, 1979; Egilmez, 1988).

### Crude Ash Rate (%)

As shown in Table 2, the difference of crude ash values in Kapaklı were not found to be statistically significance whereas the crude ash rates showed differences at  $p < 0.01$  significance level in Süleymanlı. The values ranged from 16 to 20.5%. The highest crude ash content (20.5%) was found in double cross planting in Süleymanlı. While high crude ash was found in Kapaklı for traditional method the highest crude ash rate was obtained from double cross planting method in Süleymanlı.

Our results were found similar to previous studies (Uz, 1987; Tuncay *et al.*, 1995; Gencer, 2001; Salman *et al.*, 2005; Uslu *et al.*, 2005). Ekren (2007) also found that ash content decreased towards the upper leaves from lower leaves.

### Total Nitrogen Rate (%)

The results of the total nitrogen were shown in Table 2. It was significantly affected by planting methods at  $p < 0.05$  significance level. The highest total N content (2.8%) was obtained in traditional method in Kapaklı whereas the lowest total N was found in double cross planting method in Süleymanlı. Total nitrogen contents in Kapaklı were higher than in Süleymanlı. In Aegean tobaccos, total N was found to be between 1.49-2.88% (Yazan, 1989; Irget, 1995; Sekin *et al.*, 2002; Ekren, 2007).

### Yield ( $\text{kg ha}^{-1}$ ) Tobacco Visual Quality

As shown in Figure 1, yield in Süleymanlı village was affected by planting method at  $p < 0.01$  significant level whereas there were not significant differences between planting methods in Kapaklı. Yield ranged between 156-313  $\text{kg ha}^{-1}$ . The highest yield was obtained (313  $\text{kg ha}^{-1}$ ) in Süleymanlı. Yield and tobacco expertise quality was affected by environmental conditions, application methods and soil type. Many researchers have reported that yields ranged from 156 to 313  $\text{kg ha}^{-1}$  in Aegean tobaccos (Otan *et al.*, 1989; Er, 1994; Uz, 1997; Ekren, 2007).

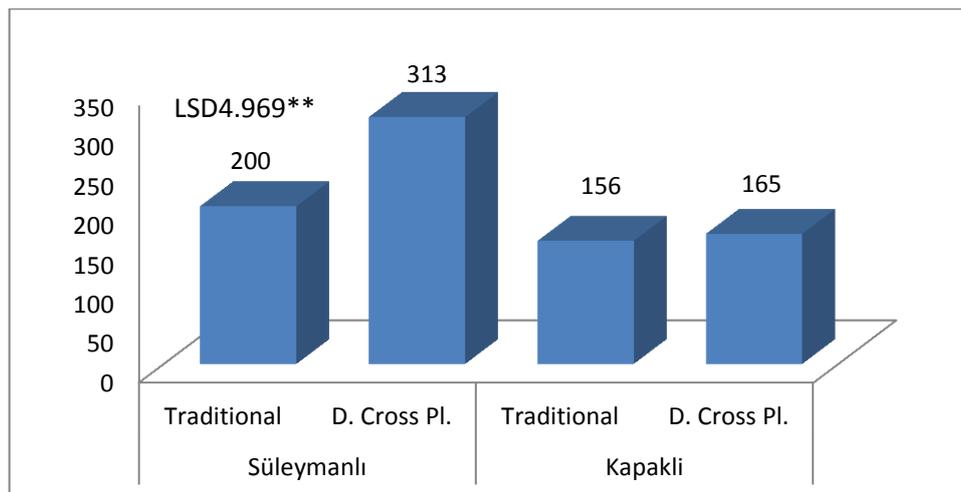


Fig. 1. Effect of the Traditional and Double Cross Planting Methods on Yield ( $\text{kg ha}^{-1}$ )

American grad (AG) was found the dominant in both methods and villages for leaf quality component. According to the villages, when we compared to both methods, American Grad was higher in traditional method than the double cross planting. Kappa rate was determined as the lowest quality criteria in the research (Figure 2).

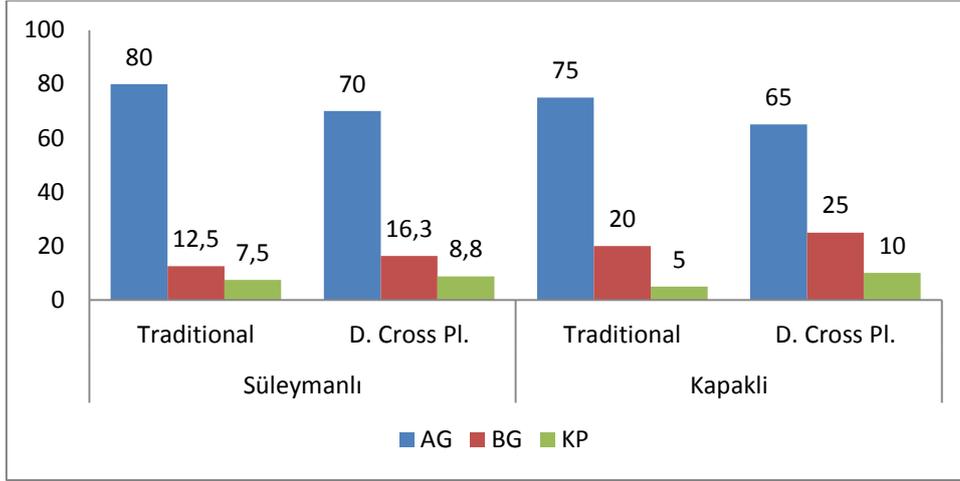


Fig. 2. Effect of the Traditional and Double Cross Planting Methods on Leaf Quality

### CONCLUSION

Considering the results, the all investigated chemical components were similar with the results of other researchers who worked with traditional and double cross planting methods in Aegean tobaccos. When the visual quality traits were interpreted, American grad quality was increased whereas BG and kappa tobacco rate was decreased in double cross planting. According to the one year's result, it is said that the study must be carried out one more year and two years' results must be interpreted together.

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## PESTALOTIOPSIS SPECIES ISOLATED FROM TEA AND KIWIFRUIT PLANTS IN TURKEY

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Scientific paper

### Summary

Tea (*Camellia sinensis*) and kiwifruit (*Actinidia deliciosa*) plants are important crops in Black Sea region of Turkey. *Pestalotiopsis* species cause diseases in a number of plants including tea and kiwifruit. A survey was conducted in Black Sea region of Turkey in order to determine *Pestalotiopsis* species causing diseases of tea and kiwifruit in 2014. Thirty-six and 10 single spore isolates were obtained from diseased tea and kiwifruit leaves, respectively. Morphological characters of these isolates were examined. It appears that at least two different *Pestalotiopsis* species from tea including *Pestalotiopsis theae* and at least two different *Pestalotiopsis* species from kiwifruit are present. Both concolor and versicolor isolates were observed.

Key words: *Camellia sinensis*, *Actinidia deliciosa*, *Pestalotiopsis*, Turkey

### INTRODUCTION

Tea is a popular drink both in the World and Turkey. In Turkey, tea plants (*Camellia sinensis* (L.) O. Kuntze) are grown in Black Sea region. Turkey is the sixth tea growing country in the world. All of this tea is produced in Black Sea region of Turkey and especially in Rize province (Anonymous, 2009a).

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C. S. Liang et A. R. Ferguson) is recently introduced to the Black Sea region of Turkey and kiwifruit growing areas are increasing. In Turkey, kiwifruit growing studies started in 1988 (Yalçın *et al.*, 1998). In Black Sea region of Turkey, kiwifruit plants are becoming an important alternative crop to hazelnut and tea which are widely grown in the region.

There are diseases affecting tea and kiwifruit plants (Anonymous, 2009b; Chen and Chen, 1982; Maharachchikumbura *et al.*, 2013; Doğu and Karakaya, 2008). *Pestalotiopsis* species are a large group of plant pathogens causing diseases in a number of plants including tea and kiwifruit (Guba, 1961; Park *et al.*, 1997; Maharachchikumbura *et al.*, 2014; Maharachchikumbura *et al.*, 2013).

In this study, *Pestalotiopsis* species causing diseases on tea and kiwifruit plants in Black Sea region of Turkey were investigated.

### MATERIALS AND METHODS

A survey was conducted in Black Sea region of Turkey in order to determine *Pestalotiopsis* species causing diseases of tea and kiwifruit in 02-10 September, 2014. Fourteen tea plantations in Artvin, Rize, and Trabzon and 14 kiwifruit plantations in Artvin, Rize, Trabzon and Ordu provinces of Turkey were inspected for the presence of diseases and diseased leaves were taken. Leaves were taken to laboratory, organized and photographs were taken. Small pieces containing both diseased and healthy sections of leaves were surface sterilized using 1% NaOCl and rinsed with steril distilled water. They were placed to Petri dishes containing Potato Dextrose Agar (PDA) and incubated under room conditions until they sporulate. *Pestalotiopsis* species were selected and their colony morphologies and some cultural characteristics were determined. Conidia, three median cells, apical appendages and their numbers and one basal appendage were measured. For each morphological and cultural character 20 measurements were made.

### RESULTS AND DISCUSSION

*Pestalotiopsis* species caused leaf spots of varying sizes in tea and kiwifruit leaves. In tea leaves, most of the time fruiting structures of the fungi were clearly visible.

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Thirty-six and 10 single spore isolates were obtained from diseased tea and kiwifruit leaves, respectively (Tables 1 and 2). *Pestalotiopsis* species isolated from tea grew well in PDA and readily formed conidiomata of varying sizes. In some isolates pigment production is also observed. Most of the tea isolates resembled each other and they were identified as *Pestalotiopsis theae* (Mordue and Holliday, 1971; Maharachchikumbura *et al.*, 2013). However, there were some isolates that showed differences. In Table 1, morphological and cultural characteristics of 15 tea isolates are presented. All conidia of tea isolates were concolor, on the other hand both concolor and versicolor conidia were present among the kiwifruit isolates (Tables 1 and 2). Forked apical appendages in some tea isolates were present. Mean conidial length of tea isolates ranged between 35.37-24.67  $\mu\text{m}$ . Appendages of most of the tea isolates were knobbed.

In Turkey, *Pestalotia theae* (= *Pestalotiopsis theae*), causing a disease of tea plants has been reported (Gürcan, 1975; Çakır and Ceylan, 1988). In a study performed by Samsun Plant Protection Institute, in addition to *Pestalotia theae*, *Pestalotiopsis natrassi* has been reported causing a disease of tea plants (Çakır and Ceylan, 1988). Karakaya *et al.* (2011a) reported that *Pestalotiopsis theae* was common in Rize region and they reported that at least 2 *Pestalotiopsis* species cause tea diseases in Rize, Turkey. Our study confirms this report. *Pestalotiopsis theae* (= *Pestalotia theae*), *Pestalotiopsis longiseta* (= *Pestalotia longiseta*), *Pestalotiopsis adusta*, *Pestalozzia* (= *Pestalotiopsis*) *palmarum*, *Pestalotiopsis furcata*, and other unidentified *Pestalotia*=*Pestalotiopsis* species cause diseases on tea plants (Anonymous, 2009b; Chen and Chen, 1982; Hamaya, 1981; Maharachchikumbura *et al.*, 2013). *P. hangzhousensis*, *P. microspora* and *P. neglecta* also isolated from tea plants (Wei *et al.*, 2005; Wei *et al.*, 2007).

Kiwifruit leaf spots and blights caused by *Pestalotiopsis* species were common in Black Sea region. Both concolor and versicolor isolates were present among the kiwifruit isolates. Mean conidial length of kiwifruit isolates ranged between 26.18-20.22  $\mu\text{m}$ . Kiwifruit isolates also formed abundant black conidiomata of varying sizes.

Both kiwifruit and tea isolates formed abundant spores in PDA. Some kiwifruit and tea isolates exhibited different growth patterns in PDA.

Karakaya *et al.* (2011b) found that diseases caused by *Pestalotiopsis* species in Rize region of Turkey were common. They suggested that there were more than one species of *Pestalotiopsis* causing diseases on kiwifruit plants. Our results confirm their findings.

Karakaya (2001) reported a *Pestalotiopsis* sp. causing disease of kiwifruit plants in Artvin-Arhavi region of Turkey. This area is also located in the Black Sea region. *Pestalotiopsis neglecta*, *P. longiseta* and *P. menezesiana* reported causing diseases of kiwifruit plants in Japan and Korea (Park *et al.*, 1997; Ushiyama *et al.*, 1996).

Keith *et al.* (2006) isolated *Pestalotiopsis* species from guava and alternative hosts. Although majority of the isolates appeared similar morphological and cultural differences were noted. Researchers concluded that species cannot grouped using only conidia and colony morphology.

There are problems in the *Pestalotiopsis* taxonomy (Guba, 1961; Sutton, 1980; Nag Raj, 1993). To overcome these problems molecular tools can help (Jeewon *et al.*, 2002; Keith *et al.*, 2006; Maharachchikumbura *et al.*, 2014).

Tab. 1. Some of the *Pestalotiopsis* species isolated from tea in Black Sea region of Turkey and some characteristics of the isolates

Isolate	Conidia (µm)		Median Cell (µm)	Apical Appendages (µm)	Basal Appendages (µm)	Some characteristics of the isolates
	Width	Length				
14Ç19	(6)7.15(8.5)	(25)29.56(35)	(17.25)18.5(22.5)	(17.5)31.3(35)	(5)6.18(9.75)	Concolor, 2-4 apical app., knobbed
14Ç17	(5)6.8(7.5)	(25)29.25(42.5)	(17.5)19.18(26.25)	(6.25)27.72(38.75)	(1.25)6.57(10)	Concolor, mostly 3 apical app., knobbed
14Ç54	(6.25)7.92(10)	(22.5)30.66(37.5)	(16.25)20.93(23.75)	(12.5)32.23(37.5)	(2.5)5.63(7.5)	Concolor, 2-4 apical app., some forked, knobbed
14Ç82	(5)7.75(10)	(25)29.42(35)	(15)19.2(21.25)	(11.25)29.08(40)	(4.75)6.28(8)	Concolor, mostly 3 apical app., forked app. present, knobbed
14Ç91	(5)7.18(8.75)	(25)28.08(32.5)	(17)18.73(21.25)	(22.5)32.34(37.5)	(5)6.53(7.5)	Concolor, mostly 3 apical app., knobbed
14Ç43	(5)7.25(7.5)	(22.5)26.93(35)	(16.25)18.85(22.5)	(22.5)28.58(32.5)	(5)5.85(8.75)	Concolor, 2-4 apical app., knobbed
14Ç58	(6.25)7.42(8.75)	(27.5)31.7(40)	(17.5)21.08(25)	(22.5)34.75(45)	(5)6.35(7.5)	Concolor, 2-4 apical app., knobbed
14Ç49	(5)7.67(8.75)	(25)29.31(35)	(15)19.31(25)	(25)32.39(42.5)	(2.5)6.72(8.75)	Concolor, 2-4 apical app., knobbed
14Ç56	(5)7.2(7.5)	(22.5)27.43(32.5)	(15)17.92(22.5)	(21.25)30.52(37.5)	(2.5)6.78(10)	Concolor, 2-4 apical app., knobbed
14Ç92	(5)7.25(8)	(25)30.18(37.5)	(17)19.36(22.5)	(15)28.75(41.25)	(5)6.82(10)	Concolor, 2-4 apical app., some forked, knobbed
14Ç61	(6.25)7.23(9.5)	(25)30.01(35)	(16.25)19.78(24.5)	(21.25)28.72(41.25)	(2.5)6.22(7.5)	Concolor, 2-4 apical app., knobbed
14Ç74	(5.25)6.86(7.5)	(26.25)28.75(35)	(16.25)19.51(22.5)	(18.75)29.05(40)	(1.25)6.75(10)	Concolor, 2-4 apical app.
14Ç45	(5.75)7.03(7.5)	(25)30.87(37.5)	(14)20.48(23.75)	(10)31.58(42.5)	(0.25)3.75(7.5)	Concolor, 2-4 apical app., knobbed
14Ç28	(5)6.09(8.75)	(27.5)35.37(37.5)	(20)22.03(25)	(17.5)27.16(35)	(3.75)6.53(8.75)	Concolor, 2-4 apical app.
14Ç53	(7)7.87(10)	(27.5)29.25(37.5)	(17.5)20.06(22.5)	(25)34.87(45)	(2.5)6(10)	Concolor, 2-4 apical app., knobbed

Tab. 2. *Pestalotiopsis* species isolated from kiwifruit in Black Sea region of Turkey and some characteristics of the isolates

Isolate	Conidia		Median Cell (µm)	Apical Appendages (µm)	Basal Appendage (µm)	Some characteristics of the isolates
	Width (µm)	Length (µm)				
14K66	(5)6.57(8)	(12.5)20.22(25)	(12.75)13.67(25)	(7.75)12.75(28.5)	(2.5)4.88(8.75)	Concolor, 2-4 apical app.
14K59	(5)6.06(8.75)	(20)23.98(27.5)	(12.5)14.87(17.5)	(5)11.63(17.5)	(4.25)4.92(6.25)	Concolor, 2-4 apical app.
14K5	(5)5.68(7.5)	(15)22.35(25)	(11.25)13.48(16.25)	(5)13.44(18.75)	(2.5)5(7.5)	Versicolor, 1-4 apical app. (mostly 3)
14K41	(5)5(6.25)	(20)22.87(25)	(12.5)14.32(15)	(7.5)30.52(17.5)	(2.5)4.16(6.25)	Concolor, 2-4 apical app.
14K122	(5)6.36(7.5)	(21.25)23.31(27.5)	(13.75)12.19(17.5)	(5)9.61(12.5)	(1.25)3.82(7.5)	Versicolor, 2-4 apical app.
14K126-1	(7)8.03(10)	(22.5)26.18(27.5)	(13.75)17(20)	(15)28.25(42.5)	(2.5)5.37(7.5)	Versicolor, 2-4 apical app.
14K126-2	(7.5)8.25(10)	(22.5)25.25(30)	(13.75)16.68(20)	(18.75)26.52(37.5)	(2.5)5.43(7.5)	Versicolor, mostly 3 apical app.
14K126-5	(5)7.25(7.5)	(21.25)25.68(30)	(13.75)17.06(17.5)	(17.5)26.95(37.5)	(2.5)5.06(10)	Versicolor, 2-4 apical app.
14K126-3	(5)6.91(7.5)	(17.5)22.43(26.25)	(12.5)14.12(17.5)	(7.5)16.87(27.5)	(2.5)4.88(10)	Versicolor, 1-4 apical app.
14K126-6	(6.25)7.38(8.75)	(22.5)24.63(27.5)	(15)16.06(18.75)	(17.5)25.42(35)	(3.75)5.19(7.5)	Versicolor, 2-3 apical app.

## CONCLUSIONS

It appears that at least two different *Pestalotiopsis* species from tea including *Pestalotiopsis theae* and at least two different *Pestalotiopsis* species from kiwifruit are present in Black Sea region of Turkey. Molecular tools can help identifying these isolates to the species level. Diseases caused by *Pestalotiopsis* species are important. The biology of the disease causing agents should be studied and disease resistant genotypes should be developed.

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## A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT IRRIGATION WATER LEVELS ON THE GRAIN YIELD AND RELATED CHARACTERISTICS OF QUINOA (*Chenopodium quinoa* Willd.)

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*Original scientific paper*

### Summary

A pot study was carried out to determine the effect of different irrigation water levels on the grain yield and some other yield components of quinoa crop (cv. Q-52) under glasshouse conditions in 2014. Five irrigation treatments were applied in the experiment, the first treatment was 100% of the field capacity (FC) as a control, and, the others were received 80%, 60%, 40% and 20% of the FC, respectively as deficit irrigation treatments. Results indicated that deficit irrigation significantly reduced the grain yield and other growth parameters of quinoa compared to the control, but the highest plant height, harvest index and grain yield were determined in the 80% of FC treatment. There was no any significant difference between 100% and 60% of FC in terms of grain yield, however, the highest dry root weight was found in the 60% of FC treatment. The results demonstrate that quinoa has good adaptation to drought conditions with high phenological flexibility; however, these strategies result in lower maximum grain yield. Water stress negatively affected the crude protein content of the quinoa grain.

**Keywords:** *Quinoa, deficit irrigation, harvest index, grain yield, crude protein content.*

### INTRODUCTION

Drought stress is one of the growing concerns in agriculture management around the world. Adopting tolerant plants to drought conditions could be an appropriate approach to this problem. An interesting crop for production under drought conditions is quinoa (*Chenopodium quinoa* Willd.). Quinoa is a traditional Andean pseudo-cereal increasingly attracting attention because of its adaptability to produce in these unfavorable soil and climatic conditions. It has been cultivated in the Peruvian and Bolivian Andes, where it is grown at altitudes of more than 3000 m above sea level for more than 7000 years (Geerts *et al.*, 2006). Quinoa has a very high nutritional value, and, apart from the high protein content, the grains are also rich in vitamins and minerals. Worldwide results also confirm quinoa's potential for growing as an alternative crop in the regions where drought, high temperature, salt stress conditions or poor soil quality are the major constraints on efficient agricultural productivity (Jacobsen *et al.*, 2003; Razzaghi *et al.*, 2012; Yazar *et al.*, 2015). However, soil moisture plays an important role in determining the time and rate of quinoa seed germination and seedling growth (Gonzalez *et al.*, 2009). Due to its significant nutrient values and ability to grown in different agro-ecological conditions (Stikić *et al.*, 2015), the organization of the United Nations for Food and Agriculture has declared the year 2013 as "The Year of Quinoa". Quinoa is a native field crop to Latin America which has the potential to grow with fewer inputs, water and tolerate a variety of biotic and abiotic stresses as compared to common cereals (Basra *et al.*, 2014) and the crop is well adapted to grow under conditions of extreme aridity and low temperatures occurring in Andean highlands of Bolivia, Peru, Chile and Argentina (Aguilar and Jacobsen, 2003; González *et al.*, 2011), however, is a new introduction to Turkey. Therefore, getting to know the possibility of production of this crop is important not only in Turkey, but also in all over the world. The objective of this preliminary study was to evaluate the responses to drought stresses of the quinoa "cv. Q-52" determining growth parameters, grain yield and crude protein content.

### MATERIALS AND METHODS

A pot study was conducted at the greenhouse of Field Crops Dept., Faculty of Agriculture Ege University during 2014 season from March to July. The physical properties of soil used were (sand: 80.2%, silt:18.0%, clay:1.8%) and chemical properties were (pH:5.83, salt:0.03%, OM:2.27%, total N:0.092%, available CaCO<sub>3</sub>, P, K were, 1300 ppm, 2.54 ppm and 40 ppm, respectively). A randomized complete block design experiment with three replications

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for each treatment was established. Seeds of quinoa (cv. Q-52) crop were sown on 26<sup>th</sup> March 2014 at 1- to 2- cm depth in plastic pots filled with 4 kg silty soil. When the first two leaves emerged, a hand-thinning was carried out to give a seedling rate of one plant per pot. Five irrigation treatments were applied in the experiment. The first treatment was 100% of the field capacity (FC, %) as a control. The other treatments were received 80%, 60%, 40% and 20% of the FC, respectively as deficit irrigation treatments.

The deficit irrigation treatments were applied after two weeks from cultivation in the pots and continued till the end of the experiment. During the experiment, all of the pots were weighed daily, and the amount of water lost was replaced to maintain the soil water content by tap water (Farahani and Chaichi, 2012). The plants were harvested when the quinoa crops had physiological maturity of seeds (~13% moisture). Every plant in each pot was used for the measurements set out below: plant height (cm); the plant was measured from the soil surface to the top level of the plant before harvest. The harvest index (%) was calculated as the grain yield per plant over the total dry, above ground biomass per plant at harvest (g/g). 100 quinoa grains were weighted and converted for calculation of 1000 grain weights. At the end of the experiment, the soil was gently washed from roots, and the roots were oven dried at 70°C until they reached a constant mass to measure the dry weight. Grain samples grounded to fine powder and were taken for determination of the crude protein (CP) content (Kjeldahl N% x 6.25). All data were statistically analyzed using analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1990). Probabilities equal to or less than 0.01 were considered significant. If ANOVA indicated differences between treatment means, a LSD test was performed to separate them.

## RESULTS AND DISCUSSION

The results of the pot experiment are presented in Table 1. The highest plant height (78.6 cm) was obtained from 80% of FC application, whereas the lowest was 42.9 cm for 20% of FC application. The plant height of the quinoa plant was significantly affected by irrigation treatments, and significantly decreased with decreasing irrigation levels, but there were no significant differences among 100%, 80% and 60% of FC treatments. The highest average harvest index (48.8%) was obtained from 80% of FC treatment, whereas the lowest (25.6%) was in 20% of FC. Harvest index of quinoa was significantly decreased with decreasing irrigation treatments, but there was no any significant difference between 80% and 100% of FC. Different irrigation water levels did not show any significant effect on 1000 grain weight of quinoa in the study. The values of 1000 grain weight ranged from 2.22 to 2.29 depending on the irrigation levels. There were significant differences between irrigation treatments concerning the grain yield of quinoa. The highest grain yield (12.4 g plant<sup>-1</sup>) was found at 80% of FC level, whereas the lowest yield (3.6 g plant<sup>-1</sup>) was 20% of FC level. The grain yield of quinoa decreased with decreasing irrigation water levels, but there was no any significant difference among 100%, 80% or %60 of FC treatments with regard to grain yield per plant. Dry root weight of quinoa per plant significantly decreased with decreasing FC level and reached its minimum values by applying 20% of FC treatment (8.5 g plant<sup>-1</sup>). Deficit irrigation practices affected crude protein content of quinoa grain significantly. CP content in grain was progressively increased with the increasing levels of irrigation up to 100% of FC. The highest CP content of the grain recorded by 100% of FC treatment which was 16.0%, but statistically similar to 80% of FC (15.9%) in the study.

Tab. 1. Effect of different irrigation water levels on the grain yield and some yield characteristics of quinoa

Irrigation levels	Plant height (cm)	Harvest index (%)	1000 grain weight (g)	Grain yield (g plant <sup>-1</sup> )	Dry root weight (g plant <sup>-1</sup> )	Crude protein content (%)
FC-100%	75.5 a	48.2 a	2.22	11.5 a	16.3 b	16.0 a
FC-80%	78.6 a	48.8 a	2.23	12.4 a	17.8 b	15.9 a
FC-60%	74.4 a	44.6 b	2.26	10.8 a	21.5 a	14.5 b
FC-40%	57.4 b	32.6 c	2.29	6.3 b	12.3 c	11.6 c
FC-20%	42.9 c	25.6 d	2.28	3.6 c	8.5 d	10.5 c
Mean	65.7	40.0	2.25	8.9	15.3	13.7
LSD (.01)	6.3	3.5	ns	1.8	2.2	1.3
CV(%)	3.7	3.4	2.9	7.8	5.5	3.9

In our pot study, water deficit such as 40% or 20% decreased plant height, harvest index, grain and dry root weights compared to control which applied of 100% of FC. These morphological changes in growth can be considered as a morphological adaptation of the plant to water and environmental stresses to reduce transpiration and to induce a lower consumption of water (Banon *et al.*, 2003; Farahani and Chaichi, 2012). Many studies

showed that grain yield of quinoa have been affected negatively by water deficit (Fischer *et al.*, 2013; Basra *et al.*, 2014). Applying water stress during the vegetative growth stage and supplying full irrigation in the rest of growing period allowed the root system to develop and non-stress conditions during the rest of growing period allowed the plant to be able to cover its needs for water and nutrient supply and optimize its photosynthesis and carbon translocation, therefore increase its productivity (Hirich *et al.*, 2014).

This result was revealed by Hirich *et al.* (2014) who reported that root dry matter values increased with water shortage, furthermore Stikić *et al.* (2015) obtained that soil drying at vegetative stage promotes a larger and deeper root system. Several researches showed therefore that deficit irrigation application during vegetative growth stage induced root system growth and development for quinoa (Geerts *et al.*, 2006). Full irrigation supply during the rest of crop cycle accelerated plant growth and development and improved yield and productivity. Applying half required water supply for crop has improved crop productivity by inducing its root system development, full irrigation during flowering and grain filling gave chance to plant to uptake more water and nutrients through its developed root system, as result crop produced more shoots and flowers intercepting more radiations by its large leaf area and producing higher yield (Hirich *et al.*, 2012; Razzaghi *et al.*, 2012).

Yazar *et al.* (2015) informed that grain yields of quinoa (cv. Titicaca) were slightly reduced by irrigation water salinity up to 30 dS m<sup>-1</sup> compared with fresh water irrigation. Salinity and drought stress together interfered considerably with crop grain and biomass yields. However, salinity stress alone did not interfere with grain and biomass yield significantly; therefore, quinoa may also be defined as a crop tolerant to salinity. Yield parameters such as aboveground biomass, grain yield and harvest index suggested a good adaptation of quinoa cv. Titicaca to Mediterranean environments.

One of the first signs of water shortage is the decrease in turgor which causes a decrease in both growth and cell development, especially in the stem and leaves (Bosque Sanchez *et al.*, 2003). The growth of cells is the most important process that is affected by water stress and the decrease in the growth of cells leads to decrease the plant height. Growth reduction as a result of water deficit has been widely reported by many researchers (Rostamza *et al.*, 2011; Hassan *et al.*, 2013). Moreover, reducing both stomatal conductance and biomass from aerial parts could be involved in the ability of plant to resist drought conditions (Martyniak, 2008; Díaz-López *et al.*, 2012). In the case of deficit irrigation, plants have mechanisms for preventing turgor loss such as stomata closure and osmotic adjustment accompanied by decreases in elasticity (Álvarez *et al.*, 2009). Decreasing the harvest index under water deficit could be a result of a reduction in the chlorophyll content, and consequently, photosynthesis efficiency, as reported by some researchers (Bosque Sanchez *et al.*, 2003; Geerts *et al.*, 2008; Farahani and Chaichi, 2012).

In our study, deficit irrigation had a negative effect on protein content of quinoa. As a result of vegetative growth reduction, the absorption of nutrient elements could be decreased (Pascale *et al.*, 2001). These results support the growth reduction obtained in our pot study at deficit irrigation because that effect may be resulted from a deficiency of nutrients, as our results shown, and that high irrigation level could compensate for nutrient deficiency (Silber *et al.*, 2003; Stikić *et al.*, 2015). Inorganic material solutes involved in the plant osmotic adjustment may lead to a decrease in leaf osmotic potential to maintain turgor and this is also an important adaptive mechanism in plants subjected to deficit irrigation (Bosque Sanchez *et al.*, 2003; Geerts *et al.*, 2008; Farahani and Chaichi, 2012).

## CONCLUSIONS

Considering overall results of our pot experiment, it could be concluded that deficit irrigation or water stress significantly decreases plant growth and yield. The highest plant height (78.6 cm), harvest index (48.8%) and grain yield (12.5 g plant<sup>-1</sup>) were determined in the 80% of FC treatment. However, the highest dry root weight (21.5 g plant<sup>-1</sup>) was found in the 60% of FC treatment. The grain yield of quinoa decreased with decreasing irrigation water levels, but there was no any significant difference among 100%, 80% or %60 of FC treatments. The results demonstrate that quinoa has good adaptation to drought conditions with high phenological flexibility; however, these strategies result in lower maximum grain yield. Water stress negatively affected the crude protein content of the quinoa grain. Future experiments on quinoa crop should be conducted on field conditions with various agronomical treatments to be sure that results are relatively consistent over time.

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## RESEARCH RESULTS ON QUINOA (*Chenopodium quinoa* Willd.) CROP FOR ARABLE LANDS UNDER MEDITERRANEAN CLIMATIC CONDITIONS IN AEGEAN REGION

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*Original scientific paper*

### Summary

Quinoa member of *Chenopodiaceae* family is a seed crop that has been cultivated for thousands of years in the Andean region for its nutritious grain and leaves. The crop, a pseudo-cereal, contains gluten-free high-quality protein, so it can play an important role in the diet of people suffering from celiac disease and it is adaptable to the conditions of marginal lands. Since intensive works on quinoa crop introductions, adaptations and cultivation techniques for specific regions are also necessary; three individual field experiments were conducted in Izmir, Mediterranean part of Turkey during 2012 and 2014. Six different sowing dates (1 March, 15 March, 1 April, 15 April, 1 May, 15 May) and four different row spacings (17.5 cm, 35 cm, 52.5 cm, 70 cm) and also seven different nitrogen levels (0, 50, 75, 100, 125, 150 and 175 kg N ha<sup>-1</sup>) were tested on the quinoa crop in different years. Results indicated that sowing dates, plant populations and N levels affected the growth and productivity of quinoa due to differences in temperature, precipitation and relative humidity over the year and there were significant differences among the all treatments in terms of grain yield. The highest grain yields were obtained when quinoa sown in the first half period of April at 35 cm row spacing under 150 kg ha<sup>-1</sup> N application.

Keywords: *Quinoa*, sowing date, row spacing, nitrogen dose, grain yield.

### INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) member of *Chenopodiaceae* family is a seed crop that has been cultivated for thousands of years in the Andean region for its nutritious grain and leaves. It is an annual broad-leaved plant, also adaptable to the conditions of marginal lands, allotetraploid (2n=4x=36), 1–2 m tall with deep penetrating roots and can be cultivated from sea level to over 4000 m above sea level (Jacobsen *et al.*, 1999). The crop, a pseudo-cereal, contains gluten-free high-quality protein, so it can play an important role in the diet of people suffering from celiac disease (Jacobsen, 2003). The protein of quinoa seed is rich in essential amino acids, particularly methionine, threonine and lysine, which are the limiting amino acids in most cereal grains (Bhargava *et al.*, 2007). The Organization of the United Nations for Food and Agriculture (FAO) has declared the year 2013 as the year of quinoa. Quinoa is a native field crop to Latin America which has the potential to grow with fewer inputs, water and tolerate a variety of biotic and abiotic stresses as compared to common cereals (Basra *et al.*, 2014), and is a new introduction to Turkey. Therefore, getting to know the possibility of production of this crop is important not only in Turkey, but also in all over the world. Nevertheless, information on the productivity and quality of quinoa crop under intensive farming management in Mediterranean environment is not well documented (Lavini *et al.*, 2014). In the development of quinoa as an arable break crop, sowing date, sowing rate, row spacing and fertilizer application are identified as agronomic variables having a high priority for investigation. The aim of this article was to evaluate the results of different experiments conducted in the western part of Turkey with quinoa related to sowing dates, row spacing and nitrogen levels on the grain yield and some other yield components of the crop in the area.

### MATERIALS AND METHODS

Three individual field experiments on quinoa crop were conducted in Izmir/Turkey region between 2012 and 2014. All studies were carried out on silty-clay loam soil with 7.8 pH in Bornova experimental field of Ege University (38°27.236 N, 27°13.576 E) located at about 20 m above sea level with typical Mediterranean climate characteristics.

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**Experiment 1: Effect of different sowing dates on the grain yield and some yield components of quinoa.**

The field studies were carried out during 2012 and 2013 summer growing season at Bornova. The experiment was carried out with a randomized complete block design with three replicates; 6 different sowing dates (1 March, 15 March, 1 April, 15 April, 1 May, 15 May) were tested on quinoa crop. Seeds (Q-52 cultivar) were sown by hand in rows 70 cm apart at a depth of 1-2 cm on related dates at a rate of 20 kg ha<sup>-1</sup> (Jacobsen, 2003). Each plot was consisted of 4 rows with 5 m length. 50 kg ha<sup>-1</sup> N, 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O was applied before sowing, and an extra 30 kg ha<sup>-1</sup> N was applied at 35 days after emergence as NH<sub>4</sub>NO<sub>3</sub> (Jacobsen *et al.*, 1999). Drip irrigation system was installed on the field during the both growing seasons. No insecticide was used to control insects. Hand hoeing was done twice after emergence for controlling weeds. Plants shoots were harvested at maturity stage (~13% moisture) (Jacobsen, 2014) during June, July and August in both years, collecting mid 2 rows of plots in order to avoid border effects. 30 plants from the central rows in each replication were randomly tagged and data were recorded on these plants for the following traits (Bhargava *et al.*, 2007); number of plant at harvest (m<sup>-2</sup>), main inflorescence length (cm), plant height (cm), harvest index (%), 1000 grain weight (g), grain yield (kg ha<sup>-1</sup>).

**Experiment 2: Effect of different row spacings on the grain yield and some yield characteristics of quinoa.**

The field studies were carried out during 2013 and 2014 summer growing season at Bornova. The experiment was carried out with a randomized complete block design with three replicates; 4 different row spacings (17.5 cm, 35 cm, 52.5 cm, 70 cm) were tested on quinoa crop. Seeds (Q-52 cultivar) were sown by hand in related row spacing at a depth of 1-2 cm on 8<sup>th</sup> of April 2013 and 7<sup>th</sup> of April 2014 at a rate of 20 kg ha<sup>-1</sup> (Jacobsen, 2003). 12, 6, 4 and 3 rows with 5 m length were sown for 17.5 cm, 35 cm, 52.5 cm and 70 cm row spacing, respectively. 50 kg ha<sup>-1</sup> N, 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O was applied before sowing, and an extra 30 kg ha<sup>-1</sup> N was applied at 35 days after emergence as NH<sub>4</sub>NO<sub>3</sub> (Jacobsen *et al.*, 1999). Overhead sprinkler system was installed on the field during the both growing seasons. No insecticide was used to control insects. Hand hoeing was done once after emergence and fluzifop-p-butyl (500 g ha<sup>-1</sup> of a.i.) was applied at 3-5 leaf stage of crops to control narrow leafed weeds in both years. Plants shoots were harvested at maturity stage (~13% moisture) at 22<sup>nd</sup> of July 2013 and 21<sup>st</sup> July 2014, collecting mid rows of plots in order to avoid border effects. Above mentioned characteristics (in Experiment 1) were determined in the study.

**Experiment 3: Effect of different nitrogen levels on the grain yield and some yield components of quinoa.**

The field studies were carried out during 2013 and 2014 summer growing season at Bornova. Q-52 cultivar of quinoa was used as crop material. The experiment was carried out with a randomized complete block design with three replicates; 7 different nitrogen levels (0, 50, 75, 100, 125, 150 and 175 kg N ha<sup>-1</sup>) were tested on the quinoa crop. Seeds were sown by hand in rows 35 cm apart at a depth of 2-3 cm on 8<sup>th</sup> of April 2013 and 7<sup>th</sup> of April 2014 at a rate of 20 kg ha<sup>-1</sup> (Jacobsen, 2003). Each plot was consisted of 6 rows with 5 m length. Half a dose of N fertiliser (urea) was applied before sowing, and the rest of N was applied at 7-10 leaf stage as NH<sub>4</sub>NO<sub>3</sub>. All plots even control were fertilised using 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> before sowing (Jacobsen *et al.*, 1999). Following establishment, plants were hand-thinned to 9-10 cm apart on rows so that the final populations were 28.6 plants m<sup>-2</sup>. Overhead sprinkler system was installed on the field during the both growing seasons. No insecticide was used to control insects. Hand hoeing was done once after emergence and fluzifop-p-butyl (500 g ha<sup>-1</sup> of a.i.) was applied at 3-5 leaf stage of crops to control narrow leafed weeds in both years. Plants shoots were harvested at maturity stage (~13% moisture) during July in both years, collecting mid 4 rows of plots in order to avoid border effects (net 5.6 m<sup>-2</sup>). Above mentioned characteristics (in Experiment 1) plus crude protein content (N% x 6.25) were determined in the study.

All data were statistically analyzed using analysis of variance (ANOVA) with the Statistical Analysis System (SAS, 1998). Probabilities equal to or less than 0.05 were considered significant. If ANOVA indicated differences between treatment means, a LSD test was performed to separate them.

**RESULTS AND DISCUSSION**

**Experiment 1:** There were significant differences between sowing dates in terms of number of plant at harvest, plant height, main inflorescence length, harvest index (HI), 1000 grain weight and grain yield in Table 1. According to two years average results, the highest number of plant at harvest in unit area was obtained in 1<sup>st</sup> or 15<sup>th</sup> of April, while 1<sup>st</sup> of March was the lowest. Plant height of quinoa increased by delaying sowing date till 15<sup>th</sup> April but later on decreased again. Our results indicate that low temperature in March (germination problems) or

weed competition in late sowings affected negatively plant height and number of harvestable crop in unit area (Shams, 2012; Jacobsen, 2014; Hirich *et al.*, 2014). Harvest index was also affected by sowing dates and years, and the highest HI was obtained when quinoa was sown in April and the lowest value was recorded when quinoa was sown in 1st March. In the study, progressing sowing date from March to May caused a limited but significant decrease on 1000 grain weight in both growing season. Grain yield of quinoa had the same tendency as HI; the highest yield was harvested when quinoa was sown in 1<sup>st</sup> or 15<sup>th</sup> of April while the lowest was obtained when quinoa was sown in 1st March or 15th May.

Tab. 1. Effect of different sowing dates on the yield and some yield components of quinoa.

Sowing dates (SD)	2012	2013	2 yrs av.	2012	2013	2 yrs av.	2012	2013	2 yrs av.
	Number of plant at harvest (m <sup>-2</sup> )			Plant height (cm)			Main inflorescence length (cm)		
1 March	15.0	18.3	16.7	63.8	68.5	66.2	35.6	31.4	33.5
15 March	18.3	23.0	20.7	77.1	80.9	79.0	40.9	38.0	39.5
1 April	27.7	28.0	27.8	97.8	111.7	104.7	46.2	52.9	49.6
15 April	27.0	30.7	28.8	101.1	107.5	104.3	46.6	53.3	49.9
1 May	26.3	28.0	27.2	96.5	99.4	98.0	44.3	43.4	43.8
15 May	22.0	27.0	24.5	85.9	81.5	83.7	33.2	28.6	30.9
Mean	22.7	25.8	24.3	87.0	91.6	89.3	41.1	41.3	41.2
LSD(.05)	Year:1.1 SD:1.7 int: ns			Year:2.7 SD:4.7 int:6.7			Year: ns SD:1.6 int:2.3		
	Harvest index (%)			1000 grain weight (g)			Grain yield (kg ha <sup>-1</sup> )		
1 March	39.5	44.2	41.9	3.52	3.47	3.49	1389	1623	1506
15 March	45.5	47.3	46.4	3.49	3.41	3.45	1703	2045	1874
1 April	47.6	51.6	49.6	3.44	3.34	3.39	2094	2263	2179
15 April	49.6	54.1	51.9	3.42	3.28	3.35	1944	2388	2166
1 May	46.5	49.4	47.9	3.35	3.25	3.30	1778	1941	1860
15 May	42.2	43.4	42.8	3.27	3.20	3.24	1688	1735	1711
Mean	45.1	48.3	46.7	3.42	3.32	3.37	1766	1999	1883
LSD(.05)	Year:0.6 SD:1.1 int:1.6			Y:0.03 SD:0.05 int:ns			Year:36 SD:62 int:87		

int: interaction, ns: not significant

**Experiment 2:** There were significant differences between row spacings with regard to number of plant at harvest, plant height, main inflorescence length, harvest index, 1000 grain weight and grain yield in Table 2. According to two years average results, number of harvestable crop in unit area decreased when the row spacing increased from 17.5 cm to 70 cm.

Tab. 2. Effect of different row spacings on the yield and some yield components of quinoa.

Row spacing (RS)	2013	2014	2 yrs av.	2013	2014	2 yrs av.	2013	2014	2 yrs av.
	Number of plant at harvest (m <sup>-2</sup> )			Plant height (cm)			Main inflorescence length (cm)		
17.5 cm	63.0	62.0	62.5	73.8	76.5	75.2	39.3	37.2	38.3
35.0 cm	36.7	37.3	37.0	87.1	90.9	89.0	42.9	43.3	43.1
52.5 cm	27.3	28.0	27.7	92.8	102.5	97.6	46.2	47.9	47.1
70.0 cm	22.3	23.3	22.8	97.1	110.7	103.9	52.6	53.9	53.3
Mean	37.3	37.7	37.5	87.7	95.1	91.4	45.3	45.6	45.4
LSD(.05)	Year: ns RS:2.6 int: ns			Year:1.2 RS:3.1 int:4.38			Year: ns RS:1.8 int: ns		
	Harvest index (%)			1000 grain weight (g)			Grain yield (kg ha <sup>-1</sup> )		
17.5 cm	52.1	48.5	50.3	3.07	3.29	3.19	2673	2876	2775
35.0 cm	51.2	46.9	49.1	3.17	3.34	3.25	2745	3208	2976
52.5 cm	49.3	43.6	46.5	3.28	3.39	3.34	2513	2777	2645
70.0 cm	48.2	43.2	45.7	3.34	3.52	3.43	2407	2544	2475
Mean	50.2	45.6	47.9	3.23	3.39	3.30	2585	2851	2718
LSD(.05)	Year:1.7 RS:2.4 int: ns			Year:0.03 RS:0.04 int: ns			Year:45 RS:64 int:91		

Plant height and main inflorescence length increased by decreasing plant density till 52.5 cm row spacing but later decreased. In the study, increasing row spacing from 17.5 cm to 70 cm caused a significant decrease on harvest index. 1000 grain weight of quinoa seed was increased with the decreasing rate of plant density. The highest average grain yield of quinoa was obtained from 35 cm row spacing being 2976 kg ha<sup>-1</sup>, whereas the lowest average grain yield obtained from 70 cm row spacing being 2475 kg ha<sup>-1</sup>. Some reports shows that plant population in unit area in quinoa depends on various factors like genotype grow habit, sowing date, climatic

conditions and soil fertility (Risi and Galwey, 1991; Spehar and da Silva Rocha, 2009; Lavini *et al.*, 2014). Our findings are in agreement with those researchers.

**Experiment 3:** There were significant differences between N levels in terms of plant height, grain yield per plant, crude protein content of seed, harvest index, 1000 grain weight and grain yield in Table 3. In the study, the plant height of quinoa increased noticeably by increasing N fertilizer rate up to 175 kg N ha<sup>-1</sup> in both seasons. Many researchers informed that the plant height of quinoa increases with the increasing N level are mainly due to the role of nitrogen in stimulating metabolic activity which contribute to the increase in metabolites amount and consequently lead to internodes elongation and increase plant height with the increasing N rate (Jacobsen, 2014; Erley *et al.*, 2005; Shams, 2012).

Tab. 3. Effect of different nitrogen levels on the yield and some yield components of quinoa.

N levels (ha <sup>-1</sup> )	2013			2014			2 yrs av.		
	2013	2014	2 yrs av.	2013	2014	2 yrs av.	2013	2014	2 yrs av.
	Plant height (cm)			Grain yield (g plant <sup>-1</sup> )			CP content of seed (%)		
0 kg	43.8	53.1	48.5	2.4	3.3	2.9	8.2	7.6	7.9
50 kg	48.4	58.9	53.6	4.1	5.0	4.5	9.8	8.3	9.1
75 kg	57.1	72.2	64.6	6.1	6.1	6.1	12.1	10.0	11.1
100 kg	66.2	75.1	70.7	8.7	8.4	8.6	14.5	11.7	13.1
125 kg	77.1	80.4	78.7	9.2	10.0	9.6	15.4	14.2	14.8
150 kg	82.8	93.0	87.9	9.2	11.2	10.2	16.1	15.5	15.8
175 kg	87.1	101.1	94.1	7.9	9.1	8.5	17.1	16.0	16.5
Mean	66.1	76.2	71.2	6.8	7.6	7.2	13.3	11.9	12.6
LSD(.05)	Y:1.8	N:3.4	YxN:4.8	Y:0.2	N:0.3	YxN:0.5	Y:0.5	N:0.9	YxN:ns
	Harvest index (%)			1000 grain weight (g)			Grain yield (kg ha <sup>-1</sup> )		
0 kg	12.3	14.4	13.3	3.38	3.33	3.36	867	988	927
50 kg	15.8	19.6	17.7	3.34	3.26	3.30	1190	1490	1340
75 kg	34.4	36.8	35.6	3.28	3.18	3.23	1855	1936	1896
100 kg	43.2	44.6	43.9	3.28	3.14	3.21	2378	2513	2446
125 kg	43.6	46.2	44.9	3.21	3.11	3.16	2675	2939	2807
150 kg	44.6	48.5	46.6	3.13	3.06	3.10	2599	3308	2953
175 kg	38.8	41.4	40.1	3.11	3.04	3.08	2361	2677	2519
Mean	33.3	35.9	34.6	3.25	3.16	3.20	1989	2264	2127
LSD(.05)	Y:0.7	N:1.3	YxN:ns	Y:0.03	N:0.05	YxN:ns	Y:32	N:59	YxN:84

Increases were significant among the N fertilizer rates till 125 kg N ha<sup>-1</sup>, but there was not any significant difference between 125 and 150 kg N ha<sup>-1</sup> in 2013 season, while the increases were significant among the N rates till 150 kg N ha<sup>-1</sup> in 2014. In the study, the results of two years average monitored that increased N levels from 0 to 150 kg N ha<sup>-1</sup> increased grain yield per plant but later on decreased. The harvest index of quinoa increased by increasing N treatments till 150 kg N ha<sup>-1</sup> level but later on decreased. The two years average indicated that N fertilization practices affected crude protein (CP) content of quinoa grain significantly for both years. CP content in grain was progressively increased with the increasing levels of N up to 175 kg N ha<sup>-1</sup>. The highest CP content in the grain recorded by 175 kg N ha<sup>-1</sup> treatment which was 16.5% higher than that in the control treatment, but statistically similar to 150 kg N ha<sup>-1</sup> treatment (15.8%).

In this study, increasing N levels caused a limited but significant decrease on 1000 grain weight in both growing season. Basra *et al.* (2014) stated that thousand grain weight of quinoa (2.1 g) was not affected by N fertilization from 0 to 120 kg ha<sup>-1</sup>. In our study, the grain yield of quinoa increased with the increasing N level from 0 to 125 kg N ha<sup>-1</sup> in the first year, however, the 150 kg N ha<sup>-1</sup> treatment caused the highest grain yield compared with the other N treatments in the second year, whereas rate of increase diminished the grain yield at the highest (175 kg N ha<sup>-1</sup>) rates in both years. These results could be explained by the increasing vegetative growth and decreasing inflorescence due to the N application. Some researchers (Erley *et al.*, 2005; Bhargava *et al.*, 2006) explained that plant height, maturation period and yield of quinoa and amaranth increased under optimum soil conditions, but at high levels of N fertilizer, grain yield was decreased caused by plant lodging. On the contrary, lodging was not observed in our experiment in both years. According to the two years results, the grain yield was enhanced to 357% at 150 kg N ha<sup>-1</sup> compared to control (0 kg N ha<sup>-1</sup>) in the study.

## CONCLUSIONS

It should be emphasized that quinoa, a new introduction to the Mediterranean coastal part of Turkey is a promising pseudo-cereal crop material with an acceptable level of adaptability and grain yield peculiarities, considering the potential harsh effect of global warming in near future. In the first experiment, sowing dates affected the growth and productivity of quinoa due to differences in temperature, precipitation and relative humidity over the year and there were significant differences among the sowing dates in terms of plant height, harvest index and grain yield. The highest grain yields were obtained when quinoa sown in the first half period of April. In the second experiment, results indicated that row spacing significantly affected the plant height, main inflorescence length, grain yield and other related traits. The highest grain yield was obtained from the plots with 35 cm row spacing. And the third experiment, the results of our two-year study testing the effect of seven different N levels on quinoa crop showed that it was possible to produce an average of 2.95 t ha<sup>-1</sup> quinoa grain yield with an average of 16% crude protein content under 150 kg N ha<sup>-1</sup> treatment in the regions with Mediterranean-type climates. Future experiments on quinoa crop should be conducted at different locations with various agronomical treatments and additional fertilizer rates to be sure that results are relatively consistent over time.

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## MICROBIAL AND ENZYME ACTIVITY AS INFLUENCED BY FERTILIZATION IN RASPBERRY PRODUCTION

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*Original scientific paper*

### Summary

Microorganisms play a very useful role in improving the soil fertility and become a subject of researches during the several past decades. However, because of unfavorable effects of chemical fertilization on soil fertility, the alternative methods for fertilization were developed. The aim of this paper was to estimate the influence of mineral and organic fertilizers on microbial and enzyme activity in raspberry production. Samples were collected from soil under raspberry in village Donja Večerska (Vitez municipality, Bosnia and Herzegovina) in March 2015. Four treatments were examined: sheep manure, sheep manure+mineral fertilizer, mineral fertilizer, and control (unfertilized soil). Chemical properties, microbial and enzyme activity of soil were determined by standard methodology. The results showed the low to moderate humus content, neutral PH reaction, low available phosphorus and high potassium content. Microbial number was higher in surface compared with subsurface layer. Except of fungi, highest microbial activity was noticed in treatment with sheep manure. The lowest microbial activity, except of *Azotobacter* sp. and actinomycetes number, was recorded in variant with mineral fertilizer. Enzyme activity differs between examined treatments. This investigation confirms that application of sheep manure, has stimulative effects on microbial activity under the conditions of the experiment and can be used in improvement of soil fertility.

Key words: *fertilization, microbial activity, raspberry*

### INTRODUCTION

Soil is the ecosystem of a various organisms which include both flora and fauna (Nakhro and Dkhar, 2010). Except of them, microorganisms may play a crucial role in ecosystems; they can alter characteristics of the environment, participate in transformation of nutrients, which lead to the greater plant development (Sylvia *et al.*, 2005). Microbial populations is a more reactive than other living organisms in soil ecosystems (Panikov, 1999). Changes in structure of microbial populations is one of the parameters of environmental disturbance caused by organic or conventional plant production (Poudel *et al.*, 2002).

Among the different agrotechnique practices which can influence the soil fertility is the use of chemical fertilization. However, this practice caused several environmental problems (Adesemoye and Kloepper, 2009). The alternative method is organic fertilization, primarily including the use of organic manures (Mohammadi *et al.*, 2011). Its application increased total N and organic matter content (Mann and Ashraf, 2000). Recent reports have addressed the use of sheep manure in improvement of qualitative properties of vegetables (Djilani and Senoussi, 2013; Massri and Labban, 2014). Some researchers also found that integral use of organic and inorganic fertilizers is suitable for improvement of crop productivity (John *et al.*, 2004). These informations are very useful for plant production in Central Bosnia Canton, where raspberry is one of the most important agricultural product of Bosnia and Herzegovina. Thus, the aim of this paper to examine the impact of different fertilizers on vertical distribution of microorganisms in soil under raspberry.

### MATERIALS AND METHODS

Two types of fertilizers (organic and mineral) in three treatments were used in this research: i) SM-sheep manure (25t/ha); ii) SMF- sheep manure (25t/ha) + mineral fertilizer NPK (INA, Kutina, Croatia) 7:20:30 (700kg/ha); and iii) MF-mineral fertilizer NPK (INA, Kutina, Croatia) 7:20:30 (700kg/ha). Control treatment

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(C) was unfertilized soil. Sampling of surface (0-20 cm) and subsurface (20-40 cm) layer was conducted in March 2015, in village Donja Večerska (N 44°09'17.65" E, 17°45'13.96", altitude 500m) in Vitez municipality (Central Bosnia Canton, Bosnia and Herzegovina). After preparing of composite samples from 10 separate samples, chemical and microbiological analyses were performed. Soil pH value was determined by ISO 10390 method (1994), humus content by dichromate method (Mineev *et al.*, 2001), available P and K using the Al method (Egner *et al.*, 1960) and carbonates content by Scheibler calcimeter.

Total number of bacteria was determined using the 0.1x-TSA, ammonification bacteria using the nutrient agar, oligonitrophiles and *Azotobacter* sp. using the Fyrodov's agar, fungi by rose bengal streptomycin agar (Peper *et al.*, 1995) and actinomycetes by starch ammonia agar. Number of microorganisms (CFU/g absolute dry soil) was determined after drying of composite samples at 105°C for 2 h. All experiments were performed in triplicate. Dehydrogenase activity (DHA) was determined by Casida *et al.* (1964), while phosphatase activity (PHA) using the Tabatabai (1994) method.

## RESULTS AND DISCUSSION

Our results showed differences in chemical characteristics and abundance of microorganisms. These properties depend on fertilizer treatments and depth of sampling.

In most of samples, soil pH value decreased with increased soil depth (tab. 1), with neutral or slight acidic values. Moderate humus content in samples was detected. In most of samples available phosphorus content was low, while available potassium content was high. Carbonates content decreased with decreased pH value.

Tab. 1. Chemical characteristics of soil under raspberry

treatments	depth (cm)	pH in H <sub>2</sub> O	pH in KCl	humus (%)	P <sub>2</sub> O <sub>5</sub> (mg/100g)	K <sub>2</sub> O (mg/100g)	CO <sub>3</sub> <sup>2-</sup> (%)
SM	0-20	7.57	6.92	3.52	21.58	142.3	0.8
	20-40	7.33	6.59	2.63	10.61	108.1	0.5
SMF	0-20	7.04	6.30	3.63	31.63	172.2	0.3
	20-40	7.10	6.26	2.11	5.92	69.2	0.2
MF	0-20	6.79	6.14	3.53	2.19	25.1	0.0
	20-40	6.70	6.05	1.73	1.07	12.0	0.0
C	0-20	6.55	5.81	2.52	1.68	13.0	0.0
	20-40	7.99	7.44	2.00	1.89	4.5	1.2

Heterotrophic soil microbial populations mediate important processes that control C and N cycling; they also represent a link between plants and ecosystem (Zak *et al.*, 2003). These microorganisms use plant exudates or other plant materials for food (Liu *et al.*, 2008). Microbial and biochemical indicators can react to changes during soil management (Gil-Sotres *et al.*, 2005), especially after use of organic fertilizers (Dinesh *et al.*, 2010).

In all treatments, number of microorganisms decreased with increased depths of sampling (tab. 2). Similar conclusion was previously observed (Fierer *et al.*, 2003). Total number of bacteria was highest in surface layer of SM and SMF treatments. In MF treatment, lowest values of total bacterial number were observed. Previous researches showed that organic manures increase soil microbial activity about 16 to 20% compared to mineral fertilizers (Dinesh *et al.*, 2010). Organic manures increase productivity of soil (Lazcano *et al.*, 2012), while use of inorganic fertilizers resulted in poor microbial activity and soil fertility (Kang *et al.*, 2005).

The number of ammonification bacteria (total and sporogenous) was higher in the organic production in comparison with the conventional system. Similar observation was confirmed previously (Lehocka *et al.*, 2008). Compared to other treatments, lowest number of ammonification bacteria was detected in MF treatment (tab. 2).

Abundance of oligonitrophiles was lower compared to other bacterial groups. Moreover, its abundance was higher in manure treatments than in MF and C treatment. Organic residues of soil, primarily containing proteins, are more suitable substrate for ammonification bacteria compared to oligonitrophiles (Miletić *et al.*, 2012).

Biological nitrogen fixation has an important role in the accumulation of nitrogen in soil organic matter, plant and microbial biomass (Kahindi *et al.*, 1997). These results showed that number of *Azotobacter* sp. was from 0.40 to 16.52 CFUx10<sup>2</sup>/g of soil (tab. 2). Also, its number was higher in organic manure treatments compared to MF and C treatments. *Azotobacter* sp. is sensitive to acid pH values of soil. Gonzales-Lopez *et al.* (1991) suggested that optimal growth of *Azotobacter* sp. was estimated at pH of 7.

Tab. 2. Number of bacteria in soil under raspberry

treatments	depth (cm)	Total number of bacteria	Ammonification bacteria		oligoni-trophiles	<i>Azotobacter</i> sp.
			total	sporo-genous		
		CFU x 10 <sup>5</sup> /g absolute dry soil				
SM	0-20	38.7	17.4	4.0	6.4	16.52
	20-40	19.0	8.8	1.8	2.9	7.50
SMF	0-20	35.2	13.3	3.3	3.6	9.87
	20-40	17.3	6.4	1.3	1.8	4.28
MF	0-20	22.0	7.3	1.1	2.1	5.66
	20-40	10.6	4.0	0.5	1.0	2.21
C	0-20	24.8	8.6	1.6	2.2	1.50
	20-40	13.1	4.2	0.8	1.1	0.40

Actinomycetes are microorganisms widely distributed in various environments and involved in degradation of organic matters and production of compounds of commercial interest (Saugar *et al.*, 2002). The highest number of actinomycetes was recorded in SM and SMF treatments (tab. 3). Niewiadomska *et al.* (2010) suggest that application of mineral fertilizers had no effects on number of actinomycetes. Also, Dong *et al.* (2014) suggest that highest actinomycetes PLFA biomarker content was noticed in soil fertilized with organic matter.

Tab. 3. Number of actinomycetes and fungi in soil under raspberry

treatments	depth (cm)	actinomy-cetes	fungi
		CFU x 10 <sup>4</sup> /g absolute dry soil	
SM	0-20	6.6	2.9
	20-40	2.8	1.4
SMF	0-20	2.6	3.1
	20-40	1.3	1.4
MF	0-20	2.3	3.3
	20-40	1.1	1.5
C	0-20	1.5	4.9
	20-40	0.7	2.2

Fungi are an important group of soil microorganisms (Saravanakumar and Kaviyarasan, 2010), and its number depends on various factors (Marschner *et al.*, 2003). Number of fungi was higher in surface compared to subsurface layer (tab. 3). As showed previously, fungal biomass tends to decrease with increasing depth (Ekelund *et al.*, 2001). In organic manure treatments, lower number of fungi was obtained compared to MF and C treatments. In contrast, Lee *et al.* (2013) suggest that application of manures has more stimulative effects for growth of fungi compared with mineral fertilizers.

Tab. 4. PHA and DHA in soil under raspberry

treatments	depth (cm)	PHA (µg PNP/g/h)		DHA (x10 <sup>-5</sup> µg TPF/g/h)
		acid	alkaline	
SM	0-20	3.55	3.56	1.25
	20-40	2.87	0.45	1.79
SMF	0-20	3.25	3.79	1.31
	20-40	3.80	2.95	1.90
MF	0-20	3.55	2.75	0.86
	20-40	3.41	2.72	1.75
C	0-20	3.39	3.72	1.99
	20-40	3.39	3.44	1.28

Soil phosphatases play an important role in the dephosphorylation of organic P compounds (Šarapatka, 2003). Our results showed similar values of acid phosphatase in all treatments (tab. 4). In most of samples PHA decreased with increasing depths, which is also confirmed in previous research (Samuel *et al.*, 2010). Dehydrogenase activity (DHA) gives crucial information about biological properties of soil (Wolinska-Stepniewska, 2012). Except of C treatment, DHA was higher in subsurface layer compared to surface layer (tab. 4). In surface layer of C treatment highest DHA was recorded. In subsurface layer, in treatments with organic manure, highest DHA was observed. Although DHA is often correlated with total number of bacteria (Skujins, 1976), some authors suggest that DHA is not a parameter of microbial activity, because environmental factors have more significant impact on microbial number compared to its activity (Singh and Rengel, 2007).

## CONCLUSIONS

As a conclusion for this research, organic sheep manure treatments had the highest values of microbial activity. We also conclude that manuring with organic sheep manure provides the most beneficial effects for quality of soil among the different fertilization treatments. Further research will be focused on plant growth promoting effects of microbial populations in soil under raspberry.

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## EXPERIENCE WITH ROOT PRUNING OF CV. 'WILLIAMS' PEAR (*Pyrus communis* L.)

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*Original scientific paper*

### Summary

The relationship between growth and yield can be maintained using different technologies. Since chemical growth retardants were largely banned in Europe physical methods of reducing tree vigour have become more widely used – including root pruning. Root pruning is now one of the most popular methods to control pear vigour. By root pruning we can reduce growth, have influence on yield and reduce the occurrence of pests, particularly pear sucker (*Cacopsylla pyri*, *Cacopsylla pyrisuga* and *Cacopsylla pyricola*). We made root pruning at 'Williams' pear cultivar on 30<sup>th</sup> March 2014. Angle of a knife, with which we pruned roots, was 32.5 degrees, distance from knife to a tree was 40 cm and depth of knife was 40 cm. The experiment included three treatments: root pruning on two sides of the tree, root pruning on one side of the tree and the control, where the roots were not pruned. Root pruning on the both sides of the tree had significant influence on lower growth of one-year shoots. Root pruning on the both sides of the tree also had significant influence on larger fruit dimensions (height, width), fruit weight, soluble solids content and titratable acids. Number of fruit and yield per tree in 2014 did not decrease because of root pruning. Root pruning on one side of the tree resulted in lower fruit firmness.

Key words: *pear*, *Pyrus communis*, *root pruning*, *yield*, *growth*

### INTRODUCTION

The growth control of pear trees is of great importance in pear orchard management for sustainable production. The balance between vegetative and reproductive growth is associated with improved production efficiency and fruit quality. Excessive vegetative growth often causes an increased competition among and within individual pear trees for resources, and thus may reduce the yield and quality of fruit (Wang *et al.*, 2014). There are many horticultural ways to maintain a permanent balance between growth and fruiting: rootstocks (that control directly vegetative growth), dormant pruning, summer pruning, root pruning, branches orientation, scoring, girdling and bark inversion, plant growth regulators, deficit irrigation, fertilisation, but, to date, none have proven to be universally successful (Sharma *et al.*, 2009). Among these approaches, rootstocks have provided pear growers with trees of reduced stature suited to a wide range of planting densities. The lack of suitable dwarfing rootstocks compatible with pear have further added to the problem (Sharma *et al.*, 2009). Traditional manual shoot pruning is time consuming and expensive, therefore alternative methods for controlling canopy growth have been used in pear orchards. Significant control of fruit tree growth could be obtained by improved management of fertilizers in orchards, including timing of fertilizer application. Use of plant growth regulators continues to offer the most efficient and cost effective technique for regulating shoot growth and controlling tree size (Sharma *et al.*, 2009). In earlier years, chemical growth retardants have been widely used to control excessive shoot growth of pear trees. However, this has resulted in increased chemical residues on the fruit and negative impact on the environment (Asín *et al.*, 2007). Therefore, the use of chemical growth inhibitors has nowadays been prohibited in European pear production. Instead of chemical inhibitors, root pruning has proven effective as an alternative to control vegetative growth of fruit trees (Geisler and Ferree, 1984; Schupp and Ferree, 1990; Ferree *et al.*, 1992; Khan *et al.*, 1998; Vercammen *et al.*, 2005; McArtney and Belton, 2011). Root pruning reduces growth, average length of shoots, average length of annual growth, pruning time, preharvest drop, and fruit size while increasing productivity, fruit firmness and soluble solids concentration and improving fruit color. Root pruning also had a strong influence upon the shoot growth, number of bearing branches, yield of tree, cumulative yield, trunk cross sectional area, the ratio of the yield to a trunk cross-section (Ferree, 1992; Ferree and Rhodus, 1993; Mitre *et al.*, 2012).

Root pruning not only decreased water uptake but also nutrient uptake by the pear trees, and both could have caused reduced vegetative and generative growth of the trees (Wang *et al.*, 2014). An important factor is the

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timing of root pruning and root pruning in the dormant season or at full bloom was more effective in reducing shoot elongation than at June drop (Schupp and Ferree, 1987).

The aim of this research was to determine the effects on growth and fruiting of root pruning used as horticultural technique to reduce vegetative growth of pear tree.

## MATERIALS AND METHODS

The study was conducted on 23-years-old 'Williams' pear trees on quince MA rootstock (*Cydonia oblonga* Mill.), growing on sandy loam soil and trained as slender spindles, during the 2014 growing season at a commercial orchard in Bistrica ob Sotli (Slovenia). The trees were spaced at 3.8 x 1.4 m. Thirty trees (10 trees per treatment) were selected according to similar crop load.

The experiment encompassed the following treatments: (1) Root pruning on both sides, where the trees were root-pruned on both sides of the tree, (2) Root pruning on one side, where the trees were root-pruned on one side of the tree and (3) Non-root pruning, where the trees were not root-pruned. Root pruning was done on 30<sup>th</sup> March 2014 using a tractor mounted BAB Root cutter (BAB Bamps, Brustem-St.Truiden, Belgium) that cuts the roots to a depth of approximate 40 cm in a distance of 40 cm from the trunk of pear trees. The angle of root pruning knife was set on 32.5°. Fruit were harvested at commercial maturity on 18<sup>th</sup> August 2014. The orchard was managed according to standard commercial practice for integrated fruit production (i.e., pruning, spraying, irrigation, etc.) (Ministry of Agriculture and Environmental, 2014).

Physical characteristics such as trunk circumference, flower buds number, length of one-year shoots, fruit weight, fruit firmness and yield per tree were determined, and chemical analyses (soluble solids, titratable acidity) were performed at harvest on 20 randomly selected fruit. Trunk diameters were measured 20 cm above the graft union. The fruit were weighed using a precision scale with an accuracy of 0.01 g. Soluble solids contents were determined in the juice of each sample using a refractometer (model WM-7, Atago, Tokyo, Japan) at 20°C and titratable acidity with an automatic titrator (719 S Titrimo; Metrohm, Herisau, Switzerland) (mg malic acid/100 g of the juice). Firmness was measured with a digital penetrometer (model 53205, T.R. Turoni SRL, Forli, Italy) with an 8-mm-diameter plunger (measured on four sides of each fruit). The skin was removed before firmness was measured. Statistical significance was determined by one-way analysis of variance (ANOVA) using the Statgraphics Plus 4.0 program (Manugistics, Rockville, MD, USA). Differences between treatments at observed parameters were tested with a LSD test at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Trunk circumference, flower buds number, yield/tree and length of one-year shoots in different root pruning treatments at cv. 'Williams' were shown in Fig. 1. Root pruning was done on 30<sup>th</sup> March, so root pruning didn't affect the trunk circumference and lower buds number, which were measured just before root pruning on one or both sides. It is also evident that that the root pruning did not negatively affect the yield/tree. Root pruning on both sides has significantly reduced the length of one-year shoots what is in accordance with Ferree (1992) and Asin *et al.* (2007). Also root pruning on one side reduced the length of one-year shoots, but difference between control and root pruning on one side was not statistically significant.

The fruits from the trees where the roots were pruned from both sides had greater fruit weight compared to control fruits (Fig. 2). Ferree and Knee (1997) reported that by root pruning fruit size was reduced at apple cultivar 'Golden Delicious'. Fruit firmness was significantly lower from trees where roots were pruned on one side of the tree compared to control fruits. Root pruning on both sides of the tree had significantly greater soluble solids content, while there were not significant differences between root pruning on both sides of the tree and control in titratable acidity. Ferree (1992) noted that root-pruned trees produced firmer fruit with an increased soluble solids concentration.

When we compared soluble solids content and titratable acidity at root pruning on one and both side of the tree we could conclude that soluble solids content and titratable acidity were significantly higher at root pruning on both sides of the tree.

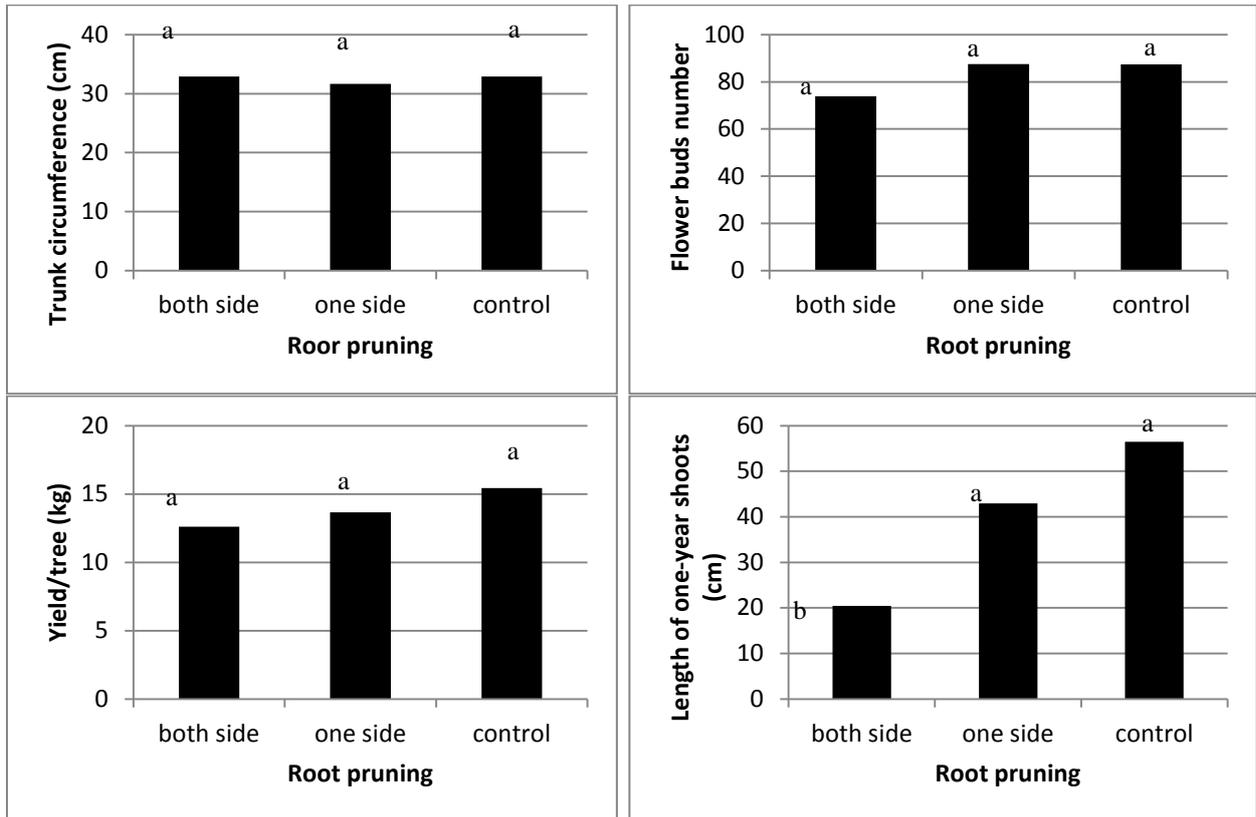


Fig. 1. Trunk circumference, flower buds number, yield/tree and length of one-year shoots in different root pruning treatments at cv. 'Williams'. Different letters indicate statistically significant differences in root pruning treatment at  $p \leq 0.05$ .

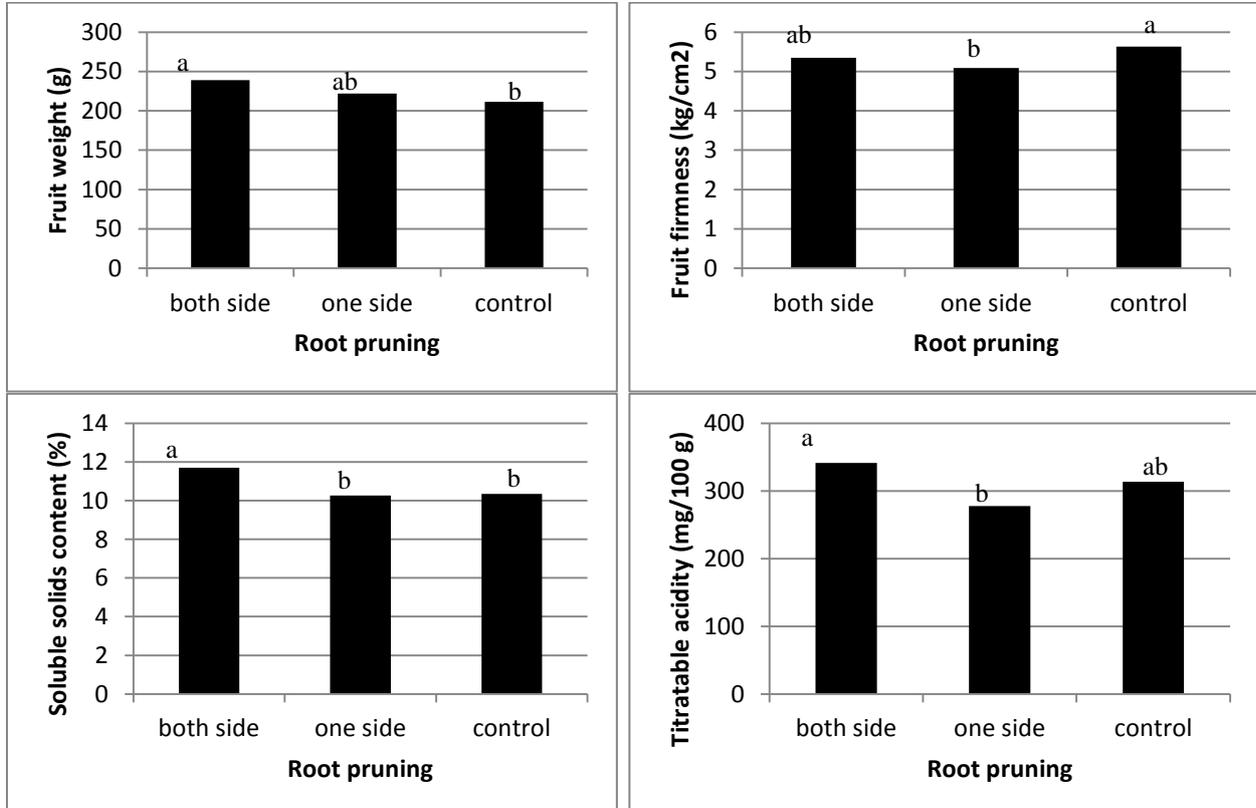


Fig. 2. Average fruit weight (g), fruit firmness (kg/cm<sup>2</sup>), soluble solids content (%) and titratable acidity (mg malic acid/100 g of the juice) in different root pruning treatments at cv. 'Williams'. Different letters indicate statistically significant differences in root pruning treatment at  $p \leq 0.05$ .

## CONCLUSIONS

According to previous information and the results of the experiment, we can conclude that root pruning could be successful horticultural way to maintain a permanent balance between yield and growth. By root pruning we can reduce growth and have no negative influence on yield. Root pruning on the both sides of the tree had significant influence on lower growth of one-year shoots, larger fruit dimensions (height, width), fruit weight, soluble solids content and titratable acids. Number of fruit and yield per tree did not decrease because of root pruning. But when we prune the roots we must be careful to prune the roots only at vigorous trees, otherwise we could completely stop the growth of trees. We don't want to completely stop the growth, because of lower number of annual shoots which consequently reduces the yield. An important factor is also the timing of root pruning.

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## INFLUENCE OF SELECTION PRESSURE ON THE FREQUENCY OF TRIPLOID GENOTYPES AMONG DIFFERENT TRADITIONAL APPLE GERMPLASMS

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*Original scientific paper*

### Summary

Triploid apple cultivars are often favored by human selection due to their increased fruit size, even though they possess higher requirements in terms of pollination compared to their diploid counterparts. However, similar to the sensory characteristics, preferences for the size and shape of apple fruit are not universal, but culturally dependent. In order to investigate the influence of selection pressure on the frequency of triploid genotypes among different traditional apple germplasms, 141 apple accessions maintained in *ex situ* collections in Norway and 115 traditional and international apple accessions from Bosnia and Herzegovina (B&H) were analyzed using 8 microsatellite markers. Genotypes possessing more than two alleles per locus were deemed to be triploid. The results of SSR analyses from this and comparable studies indicate that the triploid apple cultivars are much more frequent among the traditional apple germplasm from Southern Europe than they are among the traditional apple germplasm from Northern Europe. The possible causes for these results are also discussed.

Key words: *triploids, SSR, apple, Northern Europe*

### INTRODUCTION

Apple (*Malus × domestica* Borkh.) genome consists out of 17 chromosomes and while most of the cultivars are diploid ( $2n=34$ ), the occurrence of triploid genotypes ( $2n=3x=51$ ), which possess 51 chromosomes is not uncommon. Triploids are a result of fusion between an unreduced gamete (gamete with the somatic chromosome number) and a normal gamete (gamete with a haploid chromosome number) (Einset, 1952). Some well-known apple cultivars belong to this group: 'Boskoop', 'Kaiser Wilhelm', 'Gravensteiner', 'Jonagold' etc. Triploids are unsuitable pollinators because of the issues that arise in their meiotic division. Namely, since these cultivars have an uneven number of chromosomes, they also have a reduced ability to produce viable pollen. Also, unlike diploid cultivars the triploid ones possess three *S* alleles, thus increasing the possibility of cross incompatibility during pollination. However, human selection favors triploids due to the overall increase in fruit size (Brown, 1992).

Differentiating triploids from diploid apple genotypes based on morphological traits (fruit size, pollen viability etc.) is often time-consuming and unreliable. Flow cytometry, a method that measures the genome size is the most commonly applied approach for identifying apple triploids. However, this method requires fresh plant material and oftentimes has to be severely optimized. In diversity studies which rely on the use of codominant DNA markers, such as microsatellites or SSR (simple sequence repeats), detection of more than two different alleles per locus (a third allele) serves as an indicator of a samples triploid state. By combined use of SSR markers and flow cytometry, microsatellite markers have in recent studies proved to be a reliable technique for identifying triploids (Ramos Cabrer *et al.*, 2007; Garkava-Gustavsson *et al.*, 2008).

The frequency of triploid cultivars in a high intensity apple worldwide production is relatively low. However their occurrence in traditional germplasm of this specie can be quite high, albeit highly variable from country to country. Two molecular studies conducted on traditional apple germplasm in Spain reported a frequency of triploid accession just below 30% (Pereira-Lorenzo *et al.*, 2007; Ramos Cabrer *et al.*, 2007), while a study on traditional apple germplasm in Sweden reported the frequencies of these genotypes around 10% (Garkava-Gustavsson *et al.*, 2008). These results indicate a dichotomy in the frequency of triploid genotypes among

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traditional apple germplasms from Northern and Southern Europe. In order to investigate this further, the occurrence of triploids among 141 apple accessions maintained in *ex situ* collections in Norway and 115 traditional and international apple accessions from Bosnia and Herzegovina (B&H) was analyzed using microsatellite markers.

## MATERIAL AND METHODS

A total of 141 accessions, maintained in *ex situ* collections in Norway, were sampled for this study. Among the sampled accession most are considered traditional Norwegian cultivars, while a part represented foreign cultivars with a long tradition of cultivation in Norway. All accessions were genotyped using a set of eight SSR markers, previously used by Gasi *et al.* (2010). The molecular data obtained from the analyses of the Norwegian accession was later pooled together with the SSR data obtained on 100 traditional apple accessions from Bosnia and Herzegovina and 15 modern international reference cultivars ('Pink Lady', 'Topaz', 'Fuji Nagafu', 'Fuji Yataka', 'Golden Reinders', 'Gala Galaxy', 'Pinova', 'Pilot', 'Jonagold Wilmut', 'Jonagold', 'Piros', 'Braeburn', 'Melrose', 'Elstar' and 'Granny Smith').

### SSR analyses

Tissue samples for DNA analyses were collected from a single tree for each accession. Genomic DNA was isolated from 70 to 80 mg of leaf powder using CTAB method (Cullings, 1992; Doyle and Doyle, 1987). Eight primer pairs (Table 1), used for SSR amplifications have been previously published by Gianfranceschi *et al.* (1998) and Liebhard *et al.* (2002). PCR amplification of SSR sequences was performed in a Veriti™ Thermal Cycler (Applied Biosystems, Foster City, California, USA) using fluorescent labeled primers, which enabled the detection of PCR products using ABI 310 automated sequencer (Applied Biosystems). All PCR amplifications were performed as described in Gianfranceschi *et al.* (1998). PCR product (1 µl) was added to a master mix containing 9 µl of deionized formamide and 0.5 µl GeneScan-350 Rox size-standard (Applied Biosystems). Samples were heated at 95°C for 5 min and immediately cooled down on ice. SSR profiles were scored using GeneMapper Software ID v3.2 (Applied Biosystems).

Tab. 1. SSR loci and the DNA sequence of primer pairs used in the analyses.

SSR locus	forward primer	reverse primer
CH04E02	GGCGATGACTACCAGGAAAA	ATGTAGCCAAGCCAGCGTAT
CH05E04	AAGGAGAAGACCGTGTGAAATC	CATGGATAAGGCATAGTCAGGA
CH01H02	AGAGCTTCGAGCTTCGTTTG	ATCTTTTGGTGCTCCCACAC
CH01H10	TGCAAAGATAGGTAGATATATGCCA	AGGAGGGATTGTTTGTGCAC
CH05E03	CGAATATTTTCACTCTGACTGGG	CAAGTTGTTGTACTGCTCCGAC
CH01H01	GAAAGACTTGACAGTGGGAGC	GGAGTGGGTTTGAGAAGGTT
CH02D08	TCCAAAATGGCGTACCTCTC	GCAGACACTCACTCACTATCTCTC
CH02C02b	TGCATGCATGGAAACGAC	TGGAAAAAGTCACACTGCTCC

### Biostatistical analyses

The allele frequency and the frequency of triploids were calculated with the aid of population genetics software SPAGeDI 1.2 (Hardy and Vekemans, 2002) (available at [www.ulb.ac.be/sciences/ecoevol/](http://www.ulb.ac.be/sciences/ecoevol/)).

## RESULTS AND DISCUSSION

Since none of the used primer pairs displayed the ability to amplify more than one locus, the presence of a third allele on a single locus was interpreted as indicative of a triploid state (Fig. 1). Among the Norwegian apple accession, overall 17 genotypes displayed three alleles on at least one of the eight analyzed loci (Table 2). Therefore, the frequency of triploid accession was calculated at 12%. On the other hand, the frequency of triploids among traditional apple accessions from Bosnia and Herzegovina was on average much higher (40%). Chi square test, taking into account the overall number of analyzed apple accessions from Norway and Bosnia and Herzegovina, revealed that the examined B&H apple germplasm holds significantly more triploids ( $P < 0.001$ ).

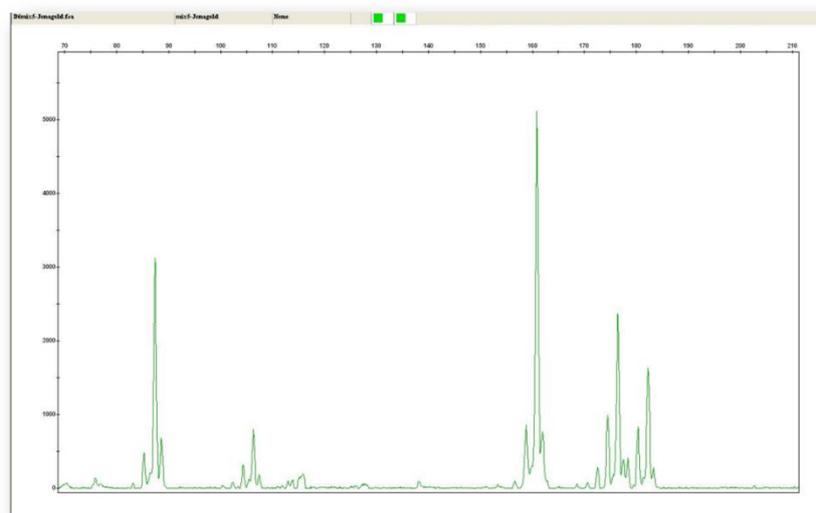


Fig. 1. A single apple accession showing two and three alleles respectively on two of the eight analyzed SSR loci.

The obtained results indicate that the frequency of triploids is much higher among apple germplasm from countries located in southern parts of Europe in comparison to the apple accessions from countries in far north of Europe. This is in concordance with the results published on Spanish and Swedish apple germplasm (Pereira-Lorenzo *et al.*, 2007; Ramos Cabrer *et al.*, 2007; Garkava-Gustavsson *et al.*, 2008). The differences in the frequency of triploids, noted among apple germplasm from two countries located in the South of Europe (B&H - 40% and Span - 30%), on the other are smaller and probably due to cultural differences.

Tab. 2. The Norwegian accessions with registered triploid state

Accession name	<i>Ex situ</i> collection
Ribston	Lier
Bramleys Seedling	Ås
Close	Ås
Kjolbergeple	Ås
Tommos	Ås
Dr Nansen	Njøs
Gravenstein Fusa	Njøs
Keiserkrone	Njøs
Leiknes	Njøs
Leinestrاند	Njøs
Leriseple	Njøs
Mostereple	Njøs
Nanna	Njøs
Rival	Njøs
Rosenstrips Sogn	Njøs
Maglemer	Hjeltnes college
Storesteinseple	Hjeltnes college

The results obtained in this study indicate a stark difference in selection pressure that has influenced the frequency of triploids among the examined germplasms. The main reasons for this are probably linked to the environmental conditions and anthropological factors that did not favor the cultivation of triploids in Northern Europe. Considering the two main characteristics that triploid apple cultivars have in common (pollination issues and large fruit) it is possible to deduce the underlying factors which resulted in this state. The problematic pollination present among triploids is far more pronounced in climatic conditions that are not favorable to insect

mediated pollination (cold climates with a shorter vegetation and plentiful precipitation). Consequently there is a clear production disadvantage of cultivating triploid genotypes in Northern Europe, when compared to Southern Europe. The increased fruit size present among triploid cultivars is an attractive trait when the fruit is used for fresh consumption. However, if the fruits are used as a raw material for processing, for instance in cider production which is very common in Scandinavia, large fruit size becomes irrelevant compared to the overall productivity. Therefore, it is reasonable to conclude that differences in the end use of the apple fruits might as well influenced the selection pressure which affected the frequency of triploid genotypes among different traditional apple germplasms.

## CONCLUSIONS

The results of the SSR analyses from this and comparable studies indicate that the triploid apple cultivars are much more frequent among the traditional apple germplasm from Southern Europe than they are among the traditional apple germplasm from Northern Europe. This is probably due to the effect of climatic conditions on the pollination rates and differences in the traditional end use of the fruit.

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## DETERMINATION OF THE DISEASES OCCURRING ON NATURALLY GROWING WILD BARLEY (*Hordeum spontaneum*) FIELD POPULATIONS

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Scientific paper

### Summary

Wild barley (*Hordeum spontaneum*) is a progenitor of cultivated barley and it grows naturally in Turkey. *Hordeum spontaneum* populations are good source of disease resistance. Wild barleys can be used in barley breeding programs in order to obtain disease resistant genotypes. A total of 40 naturally growing *Hordeum spontaneum* field populations in Şanlıurfa, Mardin, Şırnak, Siirt, Diyarbakır, Gaziantep, Kilis and Hatay provinces of Turkey were examined for the presence of diseases and their severities in 2015. Nine *Hordeum spontaneum* populations were disease free. The following diseases were found: Scald caused by *Rhynchosporium commune*, powdery mildew caused by *Blumeria graminis* f. sp. *hordei*, both forms of net blotch caused by *Drechslera teres* f. *teres* and *D. teres* f. *maculata*, semi loose smut caused by *Ustilago nigra*, loose smut caused by *Ustilago nuda*, brown rust (leaf rust) caused by *Puccinia hordei* and barley stripe caused by *Drechslera graminea*. Scald was the most commonly encountered disease followed by powdery mildew and net blotch. The incidence (number of plants infected of those examined) and severity values of diseases varied. It appears that a wide range of variation exist in terms of disease resistance status of naturally growing *Hordeum spontaneum* populations.

Key words: *Hordeum spontaneum*, *Rhynchosporium commune*, *Blumeria graminis* f. sp. *hordei*, *Drechslera teres*, *Drechslera graminea*, *Puccinia hordei*, *Ustilago nuda*, *Ustilago nigra*

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is the second cereal crop after wheat for Turkey in terms of the area sown and production. Barley production in Turkey is 7.900.000 tonnes (TÜİK, 2013). Cultivated barley (*Hordeum vulgare*, syn. *Hordeum vulgare* ssp. *vulgare*) is one of the earliest domesticated crops. It is assumed that non brittle barley was first domesticated by selecting from rachis forms of wild barley (*Hordeum spontaneum* Koch, syn. *Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) Thell.) in Fertile Crescent (Harlan, 1992; Zohary *et al.*, 2012; Badr *et al.*, 2000; Azhaguvel and Komatsuda, 2007). Wild barley is the progenitor of modern day barley cultivars possessing a rich source of genetic variation for various biotic and abiotic stresses (Nadha and Singh, 2014; Fetch *et al.*, 2003; Tuberosa and Salvi, 2006). Wild barleys and cultured barleys have different characteristics and they can hybridize each other. This creates a large variation in the genetic base of barley. Wild barleys and barley landraces are important new variation sources for tolerance to different stress sources. Since they possess large genetic variation, wild barleys and barley landraces are considered as a gold mine against various biotic and abiotic stress factors (Ellis *et al.*, 2000; Ceccarelli and Grandi, 2000; Kü, 1996; Fetch *et al.*, 2003; Yitbarek *et al.*, 1998). In this study disease resistance status of naturally growing *Hordeum spontaneum* populations were examined at 40 locations of Turkey.

### MATERIALS AND METHODS

A survey was carried out between 8-12 April 2015, in order to determine diseases occurring on naturally growing *H. spontaneum* populations in Şanlıurfa, Mardin, Şırnak, Siirt, Diyarbakır, Gaziantep, Kilis and Hatay provinces of Turkey. A total of 40 naturally growing *H. spontaneum* populations were examined in these provinces. At least 100 plants were visually inspected for the presence of diseases at each location. Most of the diseases were identified based on characteristic symptoms and morphology of spores (Mathre, 1997;

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Zillinsky, 1983). Diseased leaves with scald, net blotch and barley stripe symptoms were placed to blotter and forming conidia were observed under the microscope. For scald isolation Bean Agar was used. Net blotch and barley stripe pathogens were isolated using Potato Dextrose Agar. Spores of *Blumeria graminis* f. sp. *hordei* and *Puccinia hordei* were examined under the microscope. For *Ustilago* species, germinated spores in Water Agar were examined under microscope and production of either basidiospores or hyphae were observed (Mathre, 1997; Niks *et al.*, 1989). For determining disease severity, a 1-9 scale developed by Saari and Prescott was used (Saari and Prescott, 1975).

## RESULTS AND DISCUSSION

No disease was found in 3 locations at Şanlıurfa, 2 locations at Siirt, 2 locations at Mardin, 1 location at Diyarbakır and 1 location at Hatay provinces (Table 1).

Scald caused by *Rhynchosporium commune* was the most commonly encountered disease among *H. spontaneum* populations. Zaffarona *et al.* (2011) named the *Rhynchosporium* isolates infecting cultivated barley and other *Hordeum* spp. and *Bromus diandrus* as *R. commune*. *R. secalis* is retained for *Rhynchosporium* isolates infecting rye and triticale. Scald was found in 20 locations. Scald was found in 9 locations in Şanlıurfa, 2 locations in Mardin, 3 locations in Siirt, 4 locations in Diyarbakır, 1 location in Kilis, and 1 location in Hatay. (Table 1). In these locations disease incidence percentages (number of plants infected of those examined) ranged between 1 and 50 and diseases severity values ranged between 3 and 5 (Table 1).

Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* was found in 16 locations. Powdery mildew was found in 5 locations in Şanlıurfa, 2 locations in Mardin, 2 locations in Şırnak, 1 location in Siirt, 3 locations in Diyarbakır, 1 location in Gaziantep, 1 location in Kilis and 1 location in Hatay. In these locations disease incidence percentages ranged between 1 and 50 and diseases severity values ranged between 3 and 5.

Net blotch caused by *Drechslera teres* f. *teres* (net form of net blotch- *Dtt*) and *Drechslera teres* f. *maculata* (spot form of net blotch- *Dtm*) was found in 13 locations. Both forms of the disease was found. Net blotch was found in 6 locations in Şanlıurfa, 1 location in Şırnak, 2 locations in Siirt, 1 location in Gaziantep, 2 locations in Kilis and 1 location in Hatay. In these locations, for *Dtm*, disease incidence percentages ranged between 1 and 40 and diseases severity values ranged between 3 and 5. For *Dtt*, disease incidence percentages ranged between 1 and 35 and diseases severity values ranged between 3 and 5.

Brown rust (leaf rust) caused by *Puccinia hordei*, barley stripe caused by *Drechslera graminea* and smut diseases caused by *Ustilago* spp. were found in 2 locations. Brown rust incidence was 1% in Mardin and 50% in Şanlıurfa. Disease severity values of brown rust were 3 and 5 at these locations. Barley stripe disease was found in 2 locations in Siirt. Semi loose smut caused by *Ustilago nigra* was found in Şırnak and loose smut caused by *Ustilago nuda* was found in Siirt. Barley stripe disease and smut incidences were low being 1% at all locations.

In some locations only one disease was found, however, in some locations more than one disease was present even in the same plant. Large variation in disease resistance among the *H. spontaneum* populations was observed. Variation was present among the plants of *H. spontaneum* even in one location. Generally disease incidence and severity values were low, however, in some locations high disease incidence of some diseases were observed. In nine locations no disease was observed in *H. spontaneum* populations. This shows the heterogenous nature of disease resistance in *H. spontaneum* populations examined. Disease resistance status of *H. spontaneum* genotypes should also be tested under controlled conditions and promising genotypes could be tested under field conditions.

Fetch *et al.* (2003) assessed the diversity of *H. spontaneum* accessions from Israel and Jordan for their reaction to six fungal pathogens of importance to cultivated barley in the United States and Canada. They found a high level of diversity both across collection sites and within a collection site for disease reaction in the 116 accessions of *H. spontaneum* evaluated at the seedling stage. In our survey, we also observed diversity for disease reactions across collection sites and within a collection site. The frequency of resistance in accessions from Jordan and Israel was high for Septoria speckled leaf blotch (77 and 98%, respectively), leaf rust (70 and 90%), net blotch (72 and 68%), and powdery mildew (58 and 70%); intermediate for spot blotch (53 and 46%); and low for stem rust (2 and 26%). They found two *H. spontaneum* accessions resistant to all six pathogens. They concluded that *H. spontaneum* is an extraordinarily rich source for cultivated barley improvement.

Tab. 1. Diseases occurring on naturally growing wild barley (*Hordeum spontaneum*) field populations (*R. comm.*: *Rhynchosporium commune*, *Dtm*: *Drechslera teres* f. *maculata*, *Dtt*: *Drechslera teres* f. *teres*, *Bgh*: *Blumeria graminis* f. sp. *hordei*, Inc.: Incidence, Sev.: Severity)

No	Province	District	Location No	Disease situation	<i>R. comm.</i>	<i>R. comm.</i>	<i>Dtm</i>	<i>Dtm</i>	<i>Dtt</i>	<i>Dtt</i>	<i>P. hordei</i>	<i>P. hordei</i>	<i>Bgh</i>	<i>Bgh</i>	<i>U. nigra</i>	<i>U. nuda</i>	<i>D. graminea</i>
					Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Inc.	
1	ŞANLIURFA	CEYLANPINAR	15-9	absent													
2	ŞANLIURFA	CEYLANPINAR	15-12	absent													
3	ŞANLIURFA	VİRANŞEHİR	15-14	present	40	5					50	5					
4	ŞANLIURFA	CENTRAL	15-15	present	40	5							1	3			
5	ŞANLIURFA	CENTRAL	15-16	absent													
6	ŞANLIURFA	CENTRAL	15-17	present	5	5	1	3					2	3			
7	ŞANLIURFA	CENTRAL	15-19	present			1	3					2	3			
8	ŞANLIURFA	HARRAN	15-20	present	3	3	1	3									
9	ŞANLIURFA	CENTRAL	15-21	present	5	3	1	3									
10	ŞANLIURFA	CENTRAL	15-22	present	3	5											
11	ŞANLIURFA	CEYLANPINAR	15-23	present			20	3	20	3							
12	ŞANLIURFA	CEYLANPINAR	15-24	present	40	5							30	5			
13	ŞANLIURFA	VİRANŞEHİR	15-25	present			1	3									
14	ŞANLIURFA	CEYLANPINAR	15-26	present	30	5							20	5			
15	ŞANLIURFA	VİRANŞEHİR	15-27	present	1	3											
16	MARDİN	DERİK	15-28	present	15	5					1	3					
17	MARDİN	KIZILTEPE	15-29	absent													
18	MARDİN	KIZILTEPE	15-30	present									1	3			
19	MARDİN	CENTRAL	15-31	absent													
20	MARDİN	CENTRAL	15-32	present	1	3							5	3			

Tab. 1. Diseases occurring on naturally growing wild barley (*Hordeum spontaneum*) field populations (*R. comm.*: *Rhynchosporium commune*, *Dtm*: *Drechslera teres* f. *maculata*, *Dtt*: *Drechslera teres* f. *teres*, *Bgh*: *Blumeria graminis* f. sp. *hordei*, Inc.: Incidence, Sev.: Severity) (continued)

No	Province	District	Location No	Disease situation	<i>R. comm.</i>	<i>R. comm.</i>	<i>Dtm</i>	<i>Dtm</i>	<i>Dtt</i>	<i>Dtt</i>	<i>P. hordei</i>	<i>P. hordei</i>	<i>Bgh</i>	<i>Bgh</i>	<i>U. nigra</i>	<i>U. nuda</i>	<i>D. graminea</i>
					Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.	Inc.	Inc.	Inc.
21	ŞIRNAK	İDİL	15-36	present					1	3			1	5	1		
22	ŞIRNAK	CİZRE	15-37	present									1	3			
23	SİİRT	TİLLO	15-38	present	5	3							1	3		1	1
24	SİİRT	TİLLO	15-39	present	2	3			1	3							1
25	SİİRT	CENTRAL	15-40	absent													
26	SİİRT	CENTRAL	15-41	present	1	3											
27	SİİRT	CENTRAL	15-42	absent													
28	SİİRT	CENTRAL	15-43	present					3	5							
29	DİYARBAKIR	BİSMİL	15-49	present	3	3											
30	DİYARBAKIR	CENTRAL	15-52	present									3	3			
31	DİYARBAKIR	CENTRAL	15-53	present	10	5							1	3			
32	DİYARBAKIR	CENTRAL	15-56	absent													
33	DİYARBAKIR	CENTRAL	15-57	present	2	3											
34	DİYARBAKIR	CENTRAL	15-58	present	50	5							1	3			
35	GAZİANTEP	ŞAHİNBEY	15-59	present			3	5					50	3			
36	KİLİS	CENTRAL	15-62	present	3	5	40	5	35	5							
37	KİLİS	CENTRAL	15-63	present			5	3	1	3			2	3			
38	HATAY	KIRIKHAN	15-71	absent													
39	HATAY	KIRIKHAN	15-74	present	20	5											
40	HATAY	KIRIKHAN	15-75	present			2	5					10	3			

## CONCLUSIONS

It appears that a large variation exist in terms of disease resistance among the *Hordeum spontaneum* populations. Heterogenous nature of disease resistance status of these populations should be explored. Resistant genotypes could be used in breeding disease resistant barleys. Scald, powdery mildew and net blotch appear to be common diseases. Breeding studies should focus on these diseases to develop resistant barley varieties.

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## A PRELIMINARY STUDY ON THE EFFECT OF DIFFERENT SEED COAT TREATMENTS ON THE GERMINATION OF CILIATE MEDICK (*MEDICAGO CILIARIS* L.)

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*Original scientific paper*

### Summary

This study was conducted in the Seed Technology Laboratory of Ege University, Faculty of Agriculture, Department of Field Crops, Pasture and Forage Crops Section Turkey. Aim of the study was to remove the seed coat hardness of Ciliate Medick for enhancing germination. Pre-sowing seed treatments (scarification by sandpaper, acid treatment with %95-98 H<sub>2</sub>SO<sub>4</sub> for 5-10-15 minutes and soaking for 24-48 hour) were used. All observations and measurements were conducted following ISTA regulations and notifications.

According to our results, it was observed that germination of tested seeds highly increased within the acid treatment for 10-15 minute duration, while scarification treatment also gave satisfactory germination rate.

Keywords: *Medicago ciliaris* L., germination, pre-sowing seed treatments

### INTRODUCTION

Annual medics are specific the Mediterranean region as native legumes. They are also wide spread species of alfalfa around the world. In the environmental conditions of Mediterranean region these plants are able to grow successfully. Lesin and Lesins (1979) identified totally 34 species of alfalfa. These species are growing in different parts of the world and effectively are able to grown in both western and eastern Australia. It had been said that these species were carried by the first immigrants to Australia on early 1800. In southern Australia on 1930s alfalfa species were used to establish semi-arid grasslands through the cereals cultivating by rotation. Currently in Australia, nearly 30 million hectares are covered with these species in rotation with cereals. In those regions with harsh and low precipitation growth conditions annual medic species produce high rate of seeds. However, the seed coat hardness is a feature for being several times re-germinated, therefore these seeds can be observed as perennial species as well. In United States alfalfa usage for covering land in many regions became popular (Crawford *et al.*, 1989; Walsh *et al.*, 2001).

Annual medic species apparently are appropriate plants to grow in those mild regions of Mediterranean with winter precipitations and dry summer. In this regions with sufficient precipitations and mild environment those species are able to germinate and grow properly. However, recent researches in the past 10 years show a successful growth of these species over the summer time. Annual medic species are adopted with high capability of adaptation as similar as the perennial alfalfa species. For example in the regions with only 275-375 mm annual precipitations those species are able to grown successfully (Uzun and Aydın, 2004; Avcıoğlu *et al.*, 2009).

The first stage of plant development and yield production is to sow the seeds and let them germinate in the suitable conditions (Avcıoğlu and Soya, 2005). Seed coat has many functions such as regulation of imbibitions and hence plays a significant role during germination. Species that have thick seed coat do not germinate even if the ideal conditions for germination exist. This is called physical dormancy due to hard seed coat or hardseededness (De Souza and Filho, 2001; Galussi *et al.*, 2013).

The hardness of ciliate medick seeds (*Medicago ciliaris* L.) can negatively affect the germination. In this study, we aimed to determine three pre-sowing seed treatments (control as no treatment, scarification by sandpaper, acid treatment with %95-98 H<sub>2</sub>SO<sub>4</sub> for 5-10-15 minutes and soaking for 24-48 hour) for enhancing the germination of *Medicago ciliaris*.

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## MATERIALS AND METHODS

Ciliate medick seeds were provided by Ege University, Faculty of Agriculture, Department of Field Crops, Pasture and Forage Crops Section Seed Bank Reserves, Bornova/Izmir/Turkey.

Seven different treatments were tested for germination of ciliate medick seeds: control treatment, scarification, acid scarification (10 and 15 minutes) and soaking in water (24 hour and 48 hour).

Scarification is a method to make seeds weaker and softer which could be appear to penetrate water easily. Seeds were carefully manually rubbed between sandpaper (Glass paper No. 1) for 2 min (Wang *et al.* 2011). Sulphuric Acid (95-98%) was applied on hard seeds for 5, 10 and 15 minutes, washed by tap water and prepared for germination (Sehirali, 1986). For treatment where seeds were soaked in water distilled water was used for 24 and 48 hours

100 seeds were placed in each petri dish in four replication (Can *et al.*, 2009).The seeds were laid in petri dishes containing moisture-retaining paper wetted with sterilized-distilled water for keeping moist during germination period and then stored in incubator for germination under 20°C.

All observations and measurements were conducted following ISTA regulations and notifications (Anonym, 2006). The seeds with emerged radicula and cotyledon were counted 4 and 14 days after treatments (Can *et al.*, 2009). The results obtained from the study were statistically analyzed using ANOVA and differences between treatment means were compared using the LSD test at 1% probability.

## RESULTS AND DISCUSSION

Significant differences were detected among all treatments for first (4) and final (14) count days compared to control (Figure 1). The lowest germination rates was detected on seeds soaked with water (16.00-18.33%), and the highest germination rate were obtained from acid treatments of 10 and 15 minutes (74.67,74.67-76.67,78.67%) by first (4) and final (14) days respectively, although there was no significant difference between acid treatments for 10 or 15 minutes.

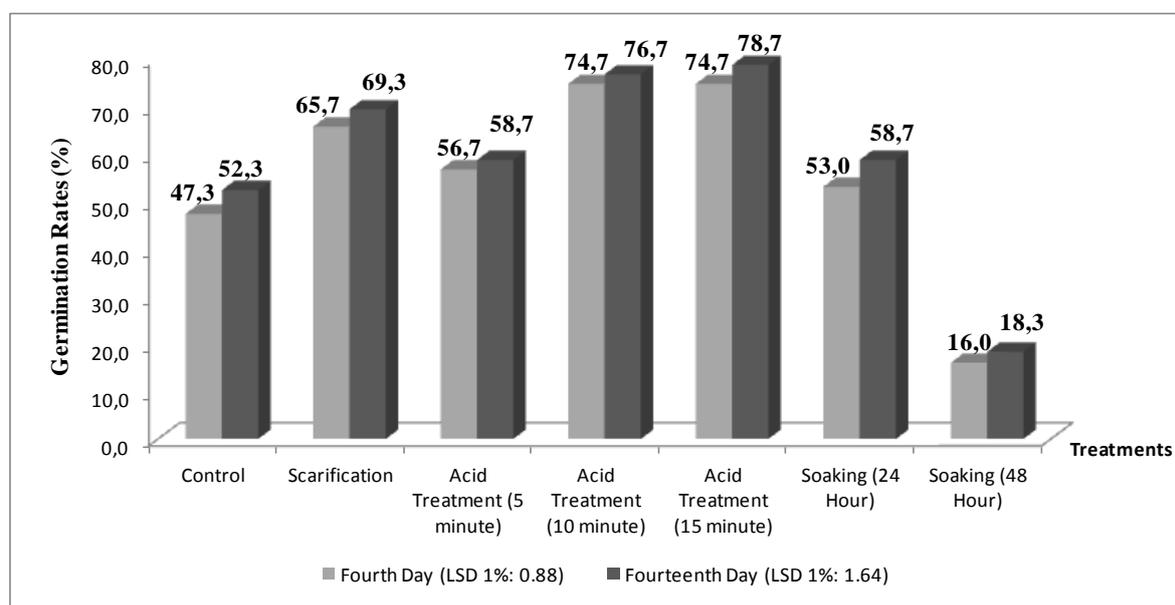


Fig. 1. Germination rates of *Medicago ciliaris* seeds (%).

The first and final counts showed that germination rates have reached the highest value when seeds were acid treated for 10 or 15 minutes (insert value %). The most widely used chemical substance for successfully breaking down hard legume seeds is sulphuric acid in concentrations from to 50-98% (Ramdeo, 1971; Phipps, 1973; Argel and Patton, 1999; Pandita *et al.*, 1999; El-Siddig *et al.*, 2001) although immersion time varied among authors. When seeds were soaked in concentrated 98% sulphuric acid for 30 minutes for enhancing germination of *Colutea armena*. excellent the best were obtained (82.8%) although Olmez *et al.* (2007) tested other treatments such as floating in hot water (100°C) followed by continual cooling for 24 hours in the same water, soaking in tap water (20±1°C) for 24 hours and cold stratification for 7, 14 and 21 days.

Sensitivity of five alfalfa cultivars to sulphuric acid was also shown by Tomer and Maguire (1989) where 85% germination was obtained when seeds were treated for 30 minutes. Similarly with results from our study Martin and De La Cuadra (2004) reached up to 89% germination of *M. polymorpha* seeds acid treated for 15 minutes and Balouchi and Sanavy (2006) reached 96% germination of *M. polymorpha* and *M. rigidula* species when seeds were soaked in acid for 10 minutes.

Results from our study indicated that soaking water treatment for 24-48 hours appeared to have negative impact on *Medicago ciliaris* germination especially when seeds were soaked for 48 hours (value in %). This triggered by penetrating too much water into the seeds and performing a circumstance to loss vitamins and amino acid from the seed by enhancing seeds membrane integration. In consequence, this might be a main issue in terms of nutrition deficiency in seed and reduce the germination. Germination is a complex physiological process controlled by a large number of genes, which are affected by several environmental factors, such as light, temperature and the duration of seed storage and coat (Koornneef *et al.*, 2002; Galussi *et al.*, 2013).

In our study, seeds treated with sand paper gave the higher germination rate (value in %) compare to control but lower than acid treatments. Our findings are in accordance with the results obtained by Wang *et al.* (2011) who also enhanced germination of *Vigna* species using scarification method. Uzun and Aydın (2004) declared very similar statements for scarification treatment. Mechanical disruption was found to be very effective way to eliminate seed dormancy in legumes although positive effect was changeable among species. In addition, Can *et al.* (2009) pointed out that effectiveness of scarification with sandpaper was also reported on some annual medics (*M. rigidula*, *M. rotata*, *M. orbicularis* and *M. scutellata*). Many researchers indicated that the most used methods for enhancing germination include chemical scarification with sulphuric acid and mechanical scarification with sand paper (Tomer and Singh, 1993; Fu *et al.*, 1996; Pandita *et al.*, 1999).

## CONCLUSIONS

According to our results, statistically best results in ciliate medic germination was obtained when seeds were treated with sulphuric acid in duration 10-15 minutes. Mechanical scarification gave also satisfactory germination rates. Soaking ciliate medic seeds in water for 24 and 48 hours did not enhanced germination.

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## EVALUATION RESISTANCE OF *X.PHASEOLI* AND *P.PHASEOLICOLA* IN *P.VULGARIS* LINES

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*Original scientific paper*

### Summary

In recent years, common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* and halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* have serious yield losses in Turkey. In this work, we investigated resistance to *X.phaseoli* and *P.phaseolicola* in bean lines. Plants of each bean line were also inoculated in the greenhouse with suspensions of the pathogens. Two bean lines were found to be resistance to *P.phaseolicola* and bean lines were found to be sensitivity to *X.phaseoli* using hypersensitivity and pathogenicity tests.

Keywords: *Pseudomonas phaseolicola*, *Xanthomonas phaseoli*, bean, line

### INTRODUCTION

Common bean play an important role in crop production systems in Turkey (Dursun *et al.*, 2002) and bean is a major source of protein for human consumption, its helps to maintain soil fertility through biological nitrogen fixation. Bacterial diseases are a major constraint in limiting Turkey common bean production. Halo and common blights are two bacterial diseases causing serious decrease in yield and quality of bean production in Turkey (Benlioğlu *et al.*, 1994).

Common bacterial blight caused by *Xanthomonas phaseoli* (Xcp) (Islam *et al.*, 2002) and halo blight caused by *Pseudomonas phaseolicola* (Psp) are most destructive bean diseases (Rodrigues *et al.*, 1999). The purpose of the current investigation was to evaluate of new bean lines against pathogenic bacteria.

### MATERIAL AND METHOD

14 new bean lines were used in this study. *Xcp* and *Psp* isolates were grown at Nutrient broth (NB) for 24 h at 27°C, 150 rpm. The concentrations of cells in bacterial suspensions were estimated turbidimetrically and were adjusted to 10<sup>8</sup> cfu/ml. Three seeds were sown in each 20 cm diameter pots containing sterile soil. The seeds of bean lines (*Phaseolus vulgaris* L.) were obtained from the Blacksea Agricultural Research Institute Institute, Samsun, Turkey.

Bean plants (*Phaseolus vulgaris* L.) that were about 2 weeks old and had only primary leaves were spray inoculated with suspensions of the bacteria. One week interval, spray treatment was applied twice. Each treatment was replicated with three pots of plants (2 plants per pot). After inoculation, the bean plants were either covered with plastic bags to maintain a high relative humidity conditions, when appropriate left uncovered and placed in a growth chamber. Plants were incubated in the greenhouse for symptoms development. Pathogenecity was evaluated according to Schaad (1994). Duncan's multiple range (DMR) test was applied for the difference between lines.

### RESULTS AND DISCUSSION

Dry beans (*Phaseolus vulgaris* L.) play an important role in crop production systems in Turkey (TUIK, 2013a). Bacterial diseases are a major constraint in limiting Turkey dry bean production (Benlioğlu *et al.*, 1994). *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola*, *Xanthomonas syringae* pv. *phaseolicola*, *Xanthomonas axonopodis* pv. *phaseolis* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* are seed-borne pathogens of dry beans (*Phaseolus vulgaris* L.) etiological agents of bacterial brown spot, halo blight and common blight diseases respectively (Benlioğlu *et al.*, 1994).

Incidence and severity of these diseases vary annually, being influenced by biological and climatic factors as well as management practices (Legard *et al.*, 1987; Schwartz *et al.*, 2005). Common blight is a world wide

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problem in bean production and may be highly destructive during extended periods of warm, humid weather, resulting in yield and seed quality losses (Schwartz *et al.*, 2005). Halo blight is distributed worldwide and is favoured by cool, wet weather early in the season. In Turkey, serious crop losses have been observed (Benlioğlu *et al.*, 1994).

Resistance reactions of bean seeds to *Xcp* and *Psp* were detected under greenhouse conditions. Occurrence of bacterial diseases in bean lines were tested, seven (TB116, TB199, F5Ç290, F5Ç153, F5Ç224, Matterhorn, Zülbiye) lines were highly susceptible, three (TB137, TB155 and FÇ250) were susceptible, two (F5Ç264 and Weighing) lines were moderate susceptible, one line (TB156) was resistant and one line (TB117) was highly resistant to ( $p<0.01$ ) *P. phaseolicola*. 13 bean lines (TB137, TB156, TB199, F5Ç290, F5Ç153, F5Ç224, F5Ç264, F5Ç250, Matterhorn, Weighing, Zülbiye) were sensitive, one (TB155) line was highly susceptible ( $p\leq 0.05$ ) to *X. phaseoli*.

Tab. 1. Reactions shown against *P. phaseolicola* ve *X. phaseoli* of bean lines

Bean lines	<i>P. phaseolicola</i>	<i>X. phaseoli</i>
1.TB.116	5a	4b
2.TB.117	1f	4b
3.TB.137	4c	3d
4.TB.155	4b	5a
5.TB.156	2e	3d
6.TB.199	5a	3d
7.F5Ç.290	5a	3d
8.F5Ç.153	5a	3d
9.F5Ç.224	5a	4b
10.F5Ç.264	3d	3d
11.FÇ.250	4b	3d
12.Matterhorn	5a	4b
13.Weighing	3d	4bc
14.Zülbiye	5a	4c

In previous studies, different bean seeds were tested to *X. phaseoli* and *P. phaseolicola*, differential interactions between bean lines were obtained (Bozkurt and Soylu, 2001; Mabagala, 1997). In our study, TB156 and TB117 were resistant to *P. phaseolicola* (Table 1). Rodriguez *et al.* (1999) reported that the effect on bean yields is common bacterial blight caused by *X. phaseoli* and halo blight caused by *P. phaseolicola*.

## CONCLUSIONS

In this study, tested bean lines are new and new bean lines were evaluated for resistance *Xcp* and *Psp* under greenhouse conditions. Thus, the result of this study indicated that, TB156 and TB117 bean lines were highly resistant to *P. phaseolicola*. Improvement of local lines resistant is important for long term control of diseases. These lines will be important resistance source in bean breeding studies in Turkey.

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## WEATHER CONDITIONS AND YIELDS OF MAIZE IN FEDERATION OF BOSNIA AND HERZEGOVINA WITH EMPHASIS ON TUZLA CANTON

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*Review paper*

### Summary

In the 15-year period 2000-2014 maize was grown in Federation of Bosnia and Herzegovina (FB&H) on the area of average 61405 ha (maize for grain 48208 ha and green maize 13197 ha). Average yields of maize for grain was 3.99 t ha<sup>-1</sup> with considerable variation among the years from 2.18 to 4.92 t ha<sup>-1</sup>. In three “the poor” years (2000, 2003 and 2012) yield was less than 3.0 t ha<sup>-1</sup> (average 2.66 t ha<sup>-1</sup>), while in three “the good” years (2006, 2008 and 2009) it was above 4.5 t ha<sup>-1</sup> (average 4.74 t ha<sup>-1</sup>). Precipitation quantity in the April – September period of “the poor” years in Tuzla was 320 mm (3-year average) or 39% of the long-term mean (LTM) 1961-1990, while in “the good” years it was 492 mm. Mean air-temperature at the same period of the favorable years was 17.5°C or for 1.0°C lower than in the unfavorable years. The most unfavorable for maize growing in FB&H and the wide region were the growing seasons 2000 and 2012. Precipitation in April – September period (Tuzla) of 2000 was only 282 mm or 54% of LTM. Also, in three summer months (June-August) precipitation was only 98 mm or third part of LTM, while air-temperature was 22.0°C or for 4.0°C higher than LTM. Under these unfavorable conditions, maize yield in FB&H was only 2.18 t ha<sup>-1</sup>. The growing season 2012 was similar to 2000 and especially dry was in July and August (8 mm precipitation and mean air-temperature 23.6°C or for 4.2°C above LTM). Maize yields in Tuzla Canton in 2005 - 2012 period were in range from 2.6 t ha<sup>-1</sup> in 2012 to 4.4 t ha<sup>-1</sup> in 2008 and 2009. However, by statistical analysis were found significant negative correlation only between yield and monthly temperature as follows: August -0.68\*\* , April-August -0.52\* (in FB&H), June -0.62\*, July -0.72\*\* (in TC). Alleviation of unfavorable effects of “poor” years for maize yields is possible by irrigation and by adequate soil management (ploughing and addition of majority NPK fertilizers in autumn instead of spring, weed control, growing more drought tolerant hybrids etc.).

**Key words:** *Maize, weather conditions, grain yield, Federation of B&H, Tuzla Canton*

### INTRODUCTION

Maize is important field crop on the arable lands of Federation of Bosnia and Herzegovina (FB&H) and Tuzla Canton (TC). In general, yields of maize in this area are low with included very high variation of annual yields. In the 2000-2014 period (SY, 2005-2014), average maize harvested area (maize for grain) in FB&H was 48 208 ha and low deviation was found among the years. Average grain yield of maize in this period was 3.99 t ha<sup>-1</sup> with variation among the years from 2.18 to 4.92 t ha<sup>-1</sup>. Weather conditions during the individual growing season are main reason of considerable variation of annual yields in the mentioned period. In general, the low yields of maize are in close connection with the lower precipitation and the higher air-temperature in July and August (Shaw, 1988; Markulj *et al.*, 2010; Kovacevic *et al.*, 2013). Aim of this study was survey variation of grain yields of maize in FB&H from 2000-2014 period and in TC from 2005-2014 period (the earlier data are not available) with emphasis on the role of weather conditions.

### MATERIAL AND METHODS

#### *Collection of the data and statistical analysis*

The publications of the Institute for Statistics of FB&H in Sarajevo (The Statistical Yearbooks from 2001 to 2014; SB, 2015) were used as source for maize harvested area and yield data, while Federal Hydrometeorological Institute was source of meteorological data (Tuzla Weather Bureau). With aim of scientific evaluation of weather and yield data, simple correlation analysis was performed using Minitab<sup>®</sup> Statistical Software (2007). Monthly data of precipitation and mean air-temperature were correlated with yields.

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*Description of the analyzed area*

FB&H is one of two entities of Bosnia and Herzegovina (B&H) and it is covering 26 110 km<sup>2</sup>. The most part of this area is covered by forests while for intensive field crop production is suitable mainly the northern lowland area of Sava valley. Climate of FB&H is temperate continental with the warm summers and the cold winters with exception of the southern part which is characterized by the Mediterranean climate.

Tuzla Canton (TC) is one of ten cantons of FB&H. TC covering territory of 2 649 km<sup>2</sup> or 10.14% of FB&H and 5.30% B&H territory. Agricultural area participating with 49% of total TC territory. TC is situated in the northeastern part of B&H between geographical coordinates 44 and 45°N, 18 and 19°E. With the geographical point of view, TC is in the southern part marked mountain area with the mountains Konjuh, Javornik and Ozren, in the central part are wide lowlands of Spreca and Tinja rivers, while in the northern part are situated the mild rolling area. In the northwest-southeast direction are situated Skipovac, Trebava and Majejica mountains. Elevation of TC is in range from 150 m on the north to 1328 m (Konjuh) on the south. Climate of TC is temperate continental with evident marked differences among winter, spring, summer and autumn periods and averages (1961-1990: Tuzla) annual precipitation 895 mm, air-temperature 10.0°C (1961-1990). The coldest month is January (-0.8°C), while the warmest is July (19.3°C) (www.vladatk.kim.ba).

**RESULTS AND DISCUSSION**

FB&H has on disposal of 405 thousands hectares (average 2000-2014) of cultivable land in category of arable land and gardens (Table 1) and about 20% of this area is situated in TC. Even about 48% of arable land and gardens are unfarmed in level of FB&H, while in TC this contribution is about 29%. Maize harvested area in FB&H is 61405 ha and share of maize for grain is close to 75%, while rest is the green or forage maize using for livestock feeding as green maize or silage. About 50% of total maize harvested area in FB&H is situated in TC. Maize contributions in total and farmed arable land area of FB&H are 15% and 29% respectively, while in level of TC are 37% and 52%, respectively (Table 1).

Tab. 1. Arable land and gardens and areas covered by maize in FB&H and TC (SY, 2005-2014; SB, 2015; Softic, 2012, 2013)

Arable land and gardens (ArLG) in FB&H and Tuzla Canton: Total and unfarmed land (UnF) and areas of maize for grain and silage (green maize)										
Year	Federation of B&H (FB&H)					Tuzla Canton (TC)				
	ArLG (000 ha)		Maize harvested area (ha)			ArLG (ha)		Maize harvested area (ha)		
	Total	UnF	Grain	Green	Total	Total	UnF	Grain	Green	Total
2000	425	208	47919	4364	52283	data not available				
2001	412	193	46539	3481	50020					
2001	410	190	49453	6016	55469					
2003	416	200	50906	6991	57897					
2004	416	202	48556	9402	57958					
2005	411	199	48511	12673	61184	82364	22094	22028	data not available	
2006	409	196	48355	14321	62676	80086	19524	21916		
2007	400	195	47154	15341	62495	82978	23599	20881		
2008	400	188	50023	16385	66408	79128	21974	21530		
2009	391	186	48685	16717	65402	79814	22769	22003	8351	30354
2010	398	196	46714	16408	63122	80862	23119	20629	8953	29582
2011	390	181	48620	18408	67028	84846	25697	21844	9592	31436
2012	396	188	48558	19135	67693	84 347	24 954	22 102	9760	31862
2013	402	194	49317	18812	68129	87 244	29 403	21 715	8773	30488
2014	396	188	43811	19497	63308	86 134	24770	19497	10528	30025
	FB&H : Averages 2000-2014					TC: Averages 2005 (2009)-2014				
	405	194	48208	13197	61405	82780	23790	22015	9326	30625

In the period 2000-2014 maize for grain were grown in FB&H on average 48 208 ha with low deviation among the years. At the same period, average yield of maize grain was 3.99 t ha<sup>-1</sup> with considerable variation of the annual yields from 2.18 to 4.92 t ha<sup>-1</sup>. Yield of maize grain in TC had similar trend as in FB&H, with variation among the years for 2005-2014 period from 2.6 to 4.4 t ha<sup>-1</sup>. These differences are mainly result of weather

conditions. In three “the poor” years (2000, 2003 and 2012) annual yields of maize were below  $3.0 \text{ t ha}^{-1}$  (average  $2.66 \text{ t ha}^{-1}$ ), while in three “the good” years (2006, 2008 and 2009) above  $4.5 \text{ t ha}^{-1}$  (average  $4.74 \text{ t ha}^{-1}$ ). Average precipitation in April-September period in “the poor” years were 320 mm or 39% below the long-term mean 1961-1990 (LTM), while in “the good” years 492 mm or only 5% lower than LTM. At the same period, mean air-temperatures were  $18.5^\circ\text{C}$  and  $17.5^\circ\text{C}$ , for “the poor” and “the good years”, respectively (Table 2).

In accordance with the global climatic changes, air-temperature during maize growth of the first 15 years of 21<sup>st</sup> century in Tuzla were  $17.4^\circ\text{C}$  (Table 2) or for  $1.3^\circ\text{C}$  above LTM 1961-1990, with amplitude from  $16.3^\circ\text{C}$  (2001 and 2004) to  $18.9^\circ\text{C}$  (2012). At the same period, precipitation quantity was in average 569 mm (LTM 521 mm) with variation among the years from 282 mm (2000) to 1020 mm (2014).

The extremely unfavorable for maize growth in the analyzed 15-year period in FB&H and the countries of the wide region were 2000 and 2012. The 2000 growing season was characterized by the lowest amount of precipitation (282 mm or 45% of LTM) but mutually similar distribution of precipitation among the months and average air-temperature  $18.3^\circ\text{C}$ . Annual grain yield of maize in FB&H was only  $2.18 \text{ t ha}^{-1}$ . However, the 2012 growing season was characterized by considerable more variation of precipitation and temperatures among the months compared to 2000. In the April-September period of recent 2012 precipitation in Tuzla was 320 mm or 54% of LTM. At the same period, average air-temperature was  $18.9^\circ\text{C}$  or for  $2.8^\circ\text{C}$  higher. Also, in three summer months (June-August) precipitation was 64 mm or about 20% of LTM. The warmest was July with average air-temperature  $23.9^\circ\text{C}$  or for even  $4.0^\circ\text{C}$  above LTM. August of 2012 was without precipitation with average temperature  $23.3^\circ\text{C}$  or  $4.4^\circ\text{C}$  higher than LTM. Under these conditions of 2012 grain yield of maize in FB&H was  $2.90 \text{ t ha}^{-1}$ . Also, low yields were found in the countries of the region (Kovacevic *et al.*, 2013).

The favorable years for maize growth were 2006 and 2008. Main characteristics of precipitation regime in these years were average (2006) and above average (2008) rain quantities (April-September) and both years with adequate precipitation at the critical stage of maize growth (July and August). Also, the 2009 growing season was favorable for maize in spite of the lower precipitation, but majority of precipitation were fall in three summer (June-August: 269 mm or 73% of total amount for April-September period). As result of favorable weather conditions, annual yield of maize grain in 2006, 2008 and 2009 were  $4.67 \text{ t ha}^{-1}$ ,  $4.63$  and  $4.92 \text{ t ha}^{-1}$  in FB&H and  $4.30 \text{ t ha}^{-1}$ ,  $4.40 \text{ t ha}^{-1}$  and  $4.40 \text{ t ha}^{-1}$  in TC, respectively. However, by statistical analysis (Table 2) were found significant negative correlation only between yield and monthly temperature as follows: August -  $0.68^{**}$ , April-August  $-0.52^*$  (in FB&H), June  $-0.62^*$ , July  $-0.72^{**}$  (in TC).

Alleviation of unfavorable effects of “the poor years” on maize yields is possible directly by irrigation in the critical stages of maize growth (Josipovic *et al.*, 2012) and by indirect preventive soil management practices, for example by ploughing in autumn/winter instead in spring period. By increase of fertilizers consumption and their adequate distribution on arable land also is possible to contribute stabilization of yields among years. According FAO data (2015), in the 5-year period 2006-2010 average consumption of nitrogen and phosphorus nutrients in B&H were only 16.1 kg N and 1.3 kg  $\text{P}_2\text{O}_5$  per hectare of arable land and permanent crop area.

Despite this usual amounts of fertilizers in Germany were 137.6 kg N and 21.1 kg  $\text{P}_2\text{O}_5$ , France 109.4 kg N and 22.3 kg  $\text{P}_2\text{O}_5$ . Distribution of fertilizers also is inadequate by omission of autumn term of their application. By presowing soil tillage treatments or under drought conditions is impossible to incorporate fertilizers in the deeper soil layers. As in B&H prevail soils with very low and low content of available P (Markovic and Supic, 2003; Markovic *et al.*, 2006) application of the higher P rates were useful for maize yield increases (Komljenovic *et al.*, 2010; Jovic *et al.*, 2013).

Jovic *et al.* (2012) were increased yield of maize for 14% by liming soil of Posavina Canton with the hydratized lime in amount of  $14 \text{ t ha}^{-1}$ . Komljenovic *et al.* (2013, 2015) applied hydratized lime in amount of  $10 \text{ t ha}^{-1}$  in combination with four rates of P fertilization (0, 500, 1000 and  $1500 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ) on soil of Lijeve Polje. Yield of maize was increased by liming for 32% (3-year average) with variation in the effectiveness in liming from 18% to 47% among years. Effects of P fertilization were the lower and, depending on the year, among 5% and 18%. The highest effect of P fertilization was under drought stress in 2011.

Komljenovic *et al.* (2010) applied four rates of P fertilizers (up to  $1750 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ) in form of triple superphosphate on the hydromorphic soil in the northern Bosnia. Maize was grown four years in monoculture. P fertilization resulted mainly in considerable yield increase of 17%. In the other experiment performed in Knespolje area maize responded during three years considerable to P fertilization by yield increases from 17% to 32% (Komljenovic *et al.*, 2006, 2008).

Tab. 2. The harvested area and yields of maize in Federation of B&amp;H and Tuzla Canton and weather data in Tuzla (SY, 2001-2014; Hodzic, 2015)

The harvested area (ha) and grain yields of maize (t ha <sup>-1</sup> ) in Federation of B&H (FB&H) and Tuzla Canton (TC) and weather data in Tuzla											
Year		Maize for grain		Tuzla: precipitation (mm) and mean air-temperature (°C)							
		FB&H	TC	The month							
				April	May	June	July	Aug.	Sept.		
2000	ha t ha <sup>-1</sup>	47 919 2.18		mm °C	59 13.7	52 16.7	47 19.7	31 20.9	20 22.9	73 15.7	282 18.3
2001	ha t ha <sup>-1</sup>	46 539 3.61		mm °C	93 9.9	67 16.3	338 16.7	95 20.3	45 20.7	304 13.8	942 16.3
2002	ha t ha <sup>-1</sup>	49 453 4.06		mm °C	122 10.2	175 16.5	80 19.8	68 21.1	80 19.6	100 14.4	625 16.9
2003	ha t ha <sup>-1</sup>	50 906 2.90		mm °C	34 10.2	72 18.0	47 22.3	118 21.2	33 22.8	55 15.0	359 18.3
2004	ha t ha <sup>-1</sup>	48 556 4.38		mm °C	163 11.3	104 13.4	98 18.2	135 20.4	70 19.6	63 14.9	633 16.3
2005	ha t ha <sup>-1</sup>	48 511 4.50	22 028 4.0	mm °C	82 10.6	73 15.4	194 17.6	227 20.1	187 18.4	63 16.0	826 16.4
2006	ha t/ha	48 355 4.67	21 916 4.3	mm °C	97 11.6	74 14.9	134 18.2	61 21.4	196 18.6	33 16.6	595 16.9
2007	ha t ha <sup>-1</sup>	47 154 3.74	20 881 3.5	mm °C	18 12.2	89 16.7	58 21.4	36 22.7	73 21.4	106 13.5	380 18.0
2008	ha t ha <sup>-1</sup>	50 023 4.63	21 530 4.4	mm °C	54 11.8	100 16.4	113 20.1	128 20.8	28 20.8	90 14.5	513 17.4
2009	ha t/ha	48 685 4.92	22 003 4.4	mm °C	32 13.0	56 17.3	160 18.5	22 21.3	87 21.0	11 17.4	368 18.1
2010	ha t ha <sup>-1</sup>	46 714 4.40	20 629 4.2	mm °C	102 11.2	177 15.1	257 19.0	110 21.1	85 20.7	126 14.9	857 17.0
2011	ha t ha <sup>-1</sup>	48 620 4.20	21 844 4.1	mm °C	29 12.1	86 14.8	74 19.2	147 21.2	13 22.0	14 19.4	363 18.1
2012	ha t ha <sup>-1</sup>	48 558 2.90	22 102 2.6	mm °C	92 11.5	137 14.8	56 21.4	8 23.9	0 23.3	27 18.3	320 18.9
2013	ha t ha <sup>-1</sup>	49 317 4.60	21 715 4.1	mm °C	31 12.8	168 15.7	74 18.6	55 20.9	36 21.6	79 15.0	443 17.4
2014	ha t ha <sup>-1</sup>	43 811 4.10	19 497 3.5	mm °C	187 11.5	339 14.5	64 18.5	112 20.4	183 19.6	135 15.7	1020 16.7
Mean	ha t ha <sup>-1</sup>	48 208 3.99	22 015 3.91	mm °C	80 11.6	118 15.8	120 19.3	90 21.2	76 20.9	85 15.7	569 17.4
Average 1961 – 1990 (Saric et al., 1996)				mm °C	76 10.4	92 14.8	111 17.7	94 19.3	84 18.9	64 15.4	521 16.1
				Correlation coefficients (r)							
				April	May	June	July	Aug.	Sept.	Total	
Precipitation : yield		Fed.		0.10	0.15	0.32	0.32	0.48	-0.11	0.35	
		TC		-0.29	-0.34	0.53	0.34	0.21	-0.05	0.12	
Temperature : yield		Fed.		-0.03	-0.30	-0.52*	-0.34	-0.68**	0.22	-0.52*	
		TC		0.23	0.36	-0.62*	-0.72**	-0.48	-0.17	-0.47	

## CONCLUSION

In the last 15-years period (2000-2014) maize in FB&H was grown on average 61405 ha (maize for grain 48208 ha and green maize 13197 ha) or about 15% of arable land. Average yields of maize for grain was 3.99 t ha<sup>-1</sup> with considerable variation among the years from 2.18 to 4.92 t ha<sup>-1</sup>, mainly affected by weather conditions. About 50% of total maize harvested area in FB&H is situated in Tuzla Canton. In general, drought and higher

air-temperatures, particularly in summer months are in close connection with the lower yields of maize. The most unfavorable for maize growing in FB&H and the wide region were the growing seasons 2000 and 2012. Under unfavorable conditions caused by drought and high temperatures, maize yield in FB&H was only 2.18 and 2.90 t ha<sup>-1</sup>, for 2000 and 2012, respectively. However, by statistical analysis were found significant negative correlation only between yield and monthly temperature as follows: August -0.68\*\*, April-August -0.52\* (in FB&H), June -0.62\*, July -0.72\*\* (in TC). Alleviation of unfavorable effects of “poor” years for maize yields is possible by irrigation and by adequate soil management (ploughing and fertilization with NPK fertilizers in autumn instead of spring, weed control, growing more drought tolerant hybrids etc.).

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## THE EFFECT OF VARIETY AND DIFFERENT CULTIVATION SYSTEMS ON THE CONTENT OF LYCOPENE AND VITAMIN C IN TOMATO (*Lycopersicon esculentum* Mill.)

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*Original scientific paper*

### Summary

Tomatoes and tomato products have a special place in the food industry due to its nutritional value. It is a significant source of vitamins B and C, copper and iron, and mineral substances potassium, calcium, magnesium, sodium and lycopene. Lycopene is an active substance of carotenoids family which plays an important role in preventing the development of tumors of the prostate, digestive system, breast, lung, and heart diseases and blood vessels.

During 2013 and 2014 tests were carried out on the effect of different cultivation systems (organic, integrated and conventional farming systems) and different varieties on the content of lycopene and vitamin C in tomatoes. The trials were set up in the greenhouses, on a private property in Kakanj. The aim of the research was to analyze the content of lycopene and vitamin C in tomato varieties Volovsko srce and Relly F<sub>1</sub> at different cultivation systems. The trials were set according to the standard method of the experiment by block system with three replications.

The results show that the highest content of lycopene and also vitamin C was achieved with conventional methods of growing cultivar Volovsko srce.

Keywords: *organic, integral and convencional farming, lycopene, tomato*

### INTRODUCTION

Lately, there are more and more medical research on the positive effect of tomato on human health. The most medicinal substances contains red, naturally on the sun ripe tomato fruit. What makes tomatoes especially important and quality is lycopene, the active substance from the family of carotenoids. Lycopene is the pigment responsible for the red color of tomato fruits. It plays a major role as micronutrients, and as a protector against a broad spectrum of cancer cells.

Tomatoes and tomato products have a special place in the food industry due to its nutritional value. Tomato is a significant source of vitamin C, also vitamin B,  $\alpha$ -tocopherol,  $\beta$ -carotene, mineral substances copper, iron, potassium, calcium, magnesium, sodium, then the phenolic compounds, carboxylic acids, and maleic, fumaric, ascorbic and oxalic (Gastelum-Barrios *et al.*, 2011).

Content of lycopene and vitamin C in tomato fruits depends of the system of farming - organic, integrated and conventional, of sorts, stages of maturity, and agro-ecological conditions of place where is farming and maturation of the fruits (Bramley, 2000).

During 2013 and 2014 tests were carried out on the effect of different cultivation systems (organic, integrated and conventional farming systems) and different varieties on the content of lycopene and vitamin C in tomatoes.

### MATERIALS AND METHODS

In order to address these issues in a protected area is seted two factorial field experiment. The study was conducted on vegetable *Lycopersicon esculentum* Mill., during the two growing seasons (two-year researchs).

During 2013 and 2014 the trials were set up in the greenhouses, on a private property of organic producer, in Kakanj municipality. This area is characterized by a continental climate, summers are hott and winters are cold. The altitude is 405 meters.

In greenhouses are cultivated varieties Volovsko srce and hybrid Relly F1 in three ways of cultivation (organic, integrated and conventional). Production of tomato seedlings was seedlings, and the trials were set according to

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the standard method of the experiment by block system with three replications. Planting was in the double row of strips, the distance between the strips was 80 cm, between rows in strips 60 cm, and between plants in a strips 50 cm. The mulch material is used black polyethylene film and precrop was spinach. The experiment had a total of 90 plants.

In carrying out of organic cultivation method in this research, both years of tests it was fertilized with manure in the spring (on 10 m<sup>2</sup> - 40 kg of manure). Each plant received 500 g lumbrihumus in the ground. Irrigation was the system "drop by drop", every 2-6 days with a dose of 1000 liters (depending on weather conditions). Because it was an organic method of cultivation, the plants were treated with herbal cocktails (2 times).

It was a cocktail made with nettle and comfrey. 3 kg of comfrey leaves are immersed in 45 liters of water, covered and let it rest for 28 days, to get acid. After 28 days fertilizer is used dilutet with water 1:10 l. For nettle fertilizer it is necessary 1 kg of nettle and 10 litres of water, immerise and let it for 15 days. After that use on the same way as comfrey fertilizer.

In integrated way of cultivation it is also fertilized with manure in the spring, and each seedling has received 500 g lumbrihumus in the zone of the root system. Plants are fertilized liquid fertilizer Folifertil 3 times, before flowering, before jamming and before ripening fruit.

In conventional way of cultivation it is fertilized on the same way as in the previous two methods of cultivation. The complex NPK fertilizer were entered directly into the ground, combined 15:15:15. The crop is fed 3 times with Folifetilom and protected with chemical fungicide Dithane (30 g in 10 liters of water) once, against aphids is used Mospilan (2.5 g in 10 liters of water).

Every day was performed tearing of laterals, cleaning of weeds, removing the lower leaves, decapitation, trimming (removing the top of the plants when they are grown to the construction of the greenhouses). Trees are formed on one branch.

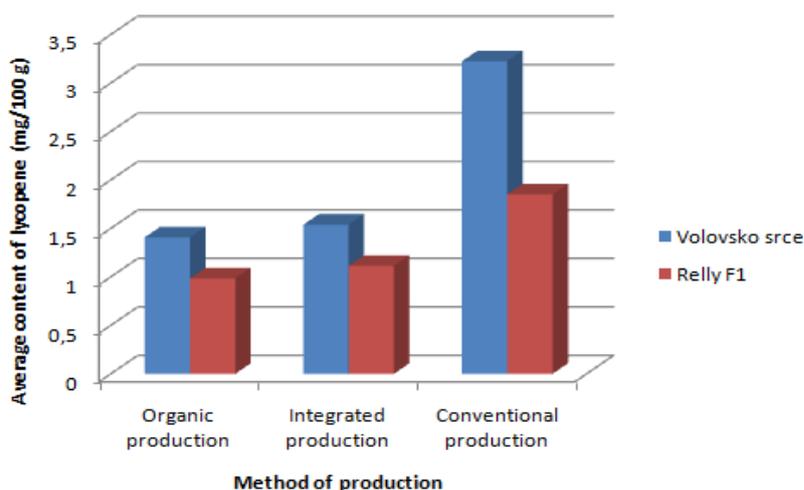
To determine the lycopene it was used spectrophotometric method (Ravelo-Perez *et al.*, 2007) and to determinate vitamin C we used metod with o-phenylenediamine (OFDA metod).

Average values of the parameters in the study after two years of research were statistically processed with computer, using software SPSS and Excel. In data processing was used analysis of variance, and to test the difference between the middles was used Tukey's test.

## RESULTS AND DISCUSSION

### - Lycopene content

The highest content of lycopene is redorded in conventional farming at sort Volovsko srce (3.225 mg/100 g) and hybrid Relly F<sub>1</sub> (1.852 mg/100 g of tomato fruit). Lower average lycopene content was recorded in the integrated cultivation method, where that content in cultivar Volovsko srce was 1.539 mg/100 g, and the hybrid Relly F<sub>1</sub> 1.114 mg/100 g. The lowest content of lycopene was in organic way of cultivation, at sort Volovsko srce 1.408 mg/100 g, and at hybrid Relly F<sub>1</sub> (0.985 mg/100 g) (Graph 1).



Graph 1. The content of lycopene (mg/100 g of moisture) depending on varieties and cultivation methods

From the Analysis of variance datas on differences in the content of lycopene statistically significant effect had method of production but variety and interaction of these factors had no statistical impact on the content of lycopene.

The average content of lycopene in conventional way of farming, after two years of research was the highest (2.543 mg/100 g), and it was significantly higher than content in integrated way of farming (1.367 mg/100 g), and the most significantly higher than content in organic way of production (1.227 mg/100 g).

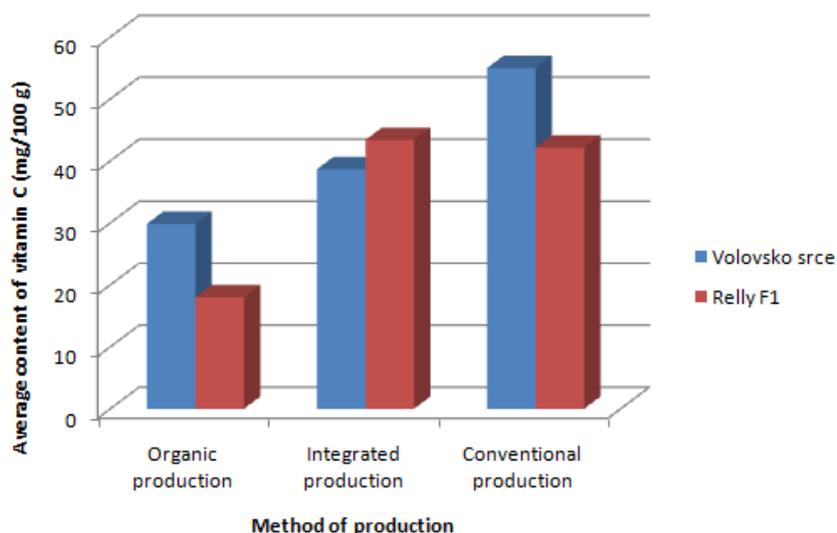
The lycopene content in tomato fruits produced on conventional method of production was 10.02 mg/100 g, while at organic tomato fruit that content was lower (6.85 mg/100 g) (Lumpkin, 2005). This shows that our research coincide with other authors.

The content of lycopene is different in some varieties and hybrids, so you can say it's varietal characteristics. Cultivar Cherry recorded significantly higher content of lycopene (77.4 mg/kg fresh weight) in relation to the hybrid Delfine F<sub>1</sub> (59.2 mg/kg of fresh weight) (Sass-Kiss *et al.*, 2005).

### - Vitamin C content

The average content of vitamin C depending of the variety and cultivation methods is ranged from 18.02 to 54.97 mg/100 g of tomato fruit.

The highest content of Vitamin C is recorded in conventional farming at sort Volovsko srce (54.97 mg/100 g) and hybrid Relly F<sub>1</sub> (42.165 mg/100 g of tomato fruit). Lower average Vitamin C content was recorded in the integrated cultivation method, where that content in cultivar Volovsko srce was 38.33 mg/100 g, and the hybrid Relly F<sub>1</sub> 43.39 mg / 100 g. The lowest content of Vitamin C was in organic way of cultivation, at sort Volovsko srce 29.84 mg/100 g, and at hybrid Relly F<sub>1</sub> (24.36 mg/100 g) (Graph 2).



Graph 2. The content of Vitamin C (mg/100 g of moisture) depending on varieties and cultivation methods

From the Analysis of variance datas on differences in the content of Vitamin C statistically significant effect had method of production but variety and interaction of these factors had no statistical impact on the content of lycopene.

The average content of vitamin C in the conventional way of farming, after two years of research, was the largest (48.57 mg/100 g), which is significantly higher content in comparison with integrated method of production (27.10 mg/100 g), and the most significantly higher than the content in organic cultivation methods (1.227 mg/100 g).

The higher amount of vitamin C, lycopene and antioxidants was observed in organically produced tomatoes (Maria Raquel Alcantara Miranda *et al.*, 2013).

## CONCLUSIONS

Every day, consumers become aware of the nutritional value of food, and also they are interested to eat health food. Datas from this research demonstrates the importance of tomato and his components on human health, and

they will help consumers to make right decisions of consuming tomato, and tomato products. These data demonstrates the importance of variety on lycopene and vitamin C content, but also method of the production is very important factor which affects on tested parameters.

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## FIRST EXPERIENCES ON THE OPEN SOURCE TECHNOLOGY PLATFORM VIPS IN BOSNIA AND HERZEGOVINA

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*Original scientific paper*

### Summary

VIPS (norw. *Varsling innen planteskadegjørere* – pest notification) is an Open Source technology platform developed for international collaboration on prognosis and decision support, where results from forecasting models can be easily distributed to users anywhere.

In Bosnia and Herzegovina this technology was introduced as a part of a project financed by the Norwegian ministry of Foreign affairs with focus on ICT (Information and Communication Technologies) development as a tool for improving Integrated Pest Management in this country. Within the project a network of 8 meteorological stations was established in the main fruit growing area of Tuzla and Gradačac. The system includes forecasting models relevant for diseases and pests on apple orchards. After a two-year work on the implementation of the project and the validation process conducted in apple orchards in the region of project implementation, the VIPS system shows the first remarkable results.

This paper aims to present the first results of field monitoring and conducted validation process of forecast models for apple scab (*Venturia inaequalis* (Cooke) Winter) and codling moth (*Cydia pomonella* L.), but also to elaborate the ecological and economical benefits of this system for improving Integrated Pest Management in Bosnia and Herzegovina.

*Key words: apple orchard, forecast models, IPM, meteorological stations, VIPS*

### INTRODUCTION

During the last decade there has been an increased use of pesticides in order to avoid great yield reductions due to plant diseases and pests. With the frequent use of fungicides, insecticides and herbicides in agriculture production, their negative effects to the environment have come to the fore. The negative side effects of different kinds of pollution have become too obvious, so that a different approach for pest management was necessary. The economic importance of pests and diseases of agricultural crops, the availability of new, highly effective pesticides, and the negative effects of insecticides, fungicides and herbicides have focused attention on forecasting pest outbreaks (Sigvald, 2012). Forecasting models that predict the likelihood of pest or disease outbreak can assist crop growers in determining when or if pesticides are needed and represent an important part in integrated pest management (IPM).

In order for Bosnia and Herzegovina (B-H) to have access to EU markets for their agricultural products it will be necessary to implement IPM in B-H farming. IPM represents a broad-based approach that integrates practices for pest control by focusing on a reduced/adjusted usage of chemicals, but also on alternative methods of regulation alone, or with need-based pesticide use. Therefore the main aim of this approach is to suppress the pest population below the economic injury level (EIL) and not in the total eradication of the same by the use of chemical treatments. The princip of „need-based pesticide use“ refers to the EIL and is determined by the potential risk of great yield reduction due to plant diseases and pests. To facilitate the timely decision on pesticide application it is necessary to identify the pest, the potential risk and economic justification. The same is possible by using computer-based tools in IPM. One role of ICT (Information and Communication Technologies) in IPM is to provide information for decision support in plant protection.

VIPS (norw. *Varsling innen planteskadegjørere* – pest notification ) is a Norwegian web based forecasting and information service developed for integrated management of pests and diseases in cereals, vegetables and fruit crops. The system presents risk alerts, leaving the final decisions on choice of pesticide and dose to the grower in collaboration with the local extension service. At present, 24 models are running in VIPS, covering all pest and disease models used in Norway (Trandem and Netland, 2013). In 2014, a totally reconstructed and

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internationally flexible version of VIPS was tested in Sweden and Bosnia and Herzegovina. The system allows local adaptations, including multi language support, incorporation of models and other services (Skog *et al.*, 2015). During the last two years a network of 8 meteorological stations was established in the main fruit growing area of Tuzla and Gradačac. The system includes forecasting models relevant for diseases and pests in apple orchards (apple scab and codling moth). Models that are used are a combination of commercial models, ones developed within VIPS and software offered from producers of meteorological stations. In 2015, the testing continued in apple orchards in Bosnia and Herzegovina, focusing on predictions for apple scab (*Venturia inaequalis*, AS) and codling moth (*Cydia pomonella* L., CM). This paper aims to present the first results of field monitoring and conducted validation process of forecast models for apple scab and codling moth, but also to elaborate the ecological and economical benefits of this system for improving Integrated Pest Management in Bosnia and Herzegovina.

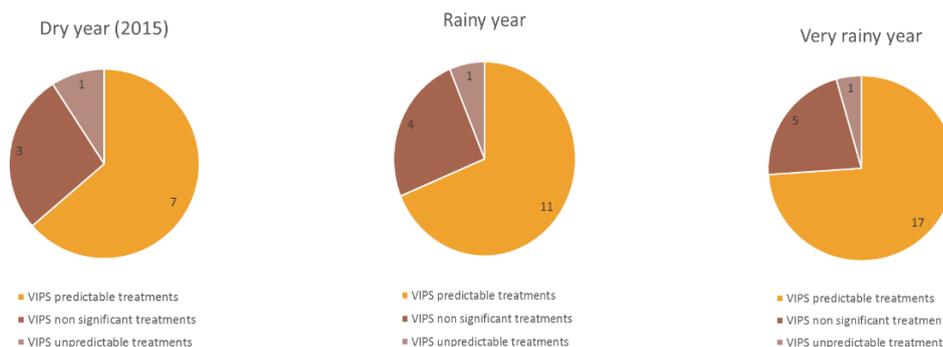
## MATERIAL AND METHODS

As part of this work were carried out two main activities; field monitorings and a survey among b-h farmers in the area of project implementation, Tuzla and Gradačac.

Monitoring activities for the target plant disease and pest were all conducted during the growing season 2015. Model validation entailed monitoring of the targeted pest and disease and therefore are necessary to check the reliability of the model. For the purpose of validation of codling moth prognosis model, traps were placed in seven orchards in the area of Gradačac. For this purpose commercial pheromone RAG traps were used (Csalomon, Hungary). The traps were placed in the same apple orchards as the meteorological stations.

The validation for apple scab was focused on risk assessment for secondary infections, where the estimation of disease severity was done according to the McKinney formula (Campbell and Neher, 1994). For this purpose leaf and fruit samples were collected at the end of the growing season.

To be able to estimate the use and benefit of the VIPS forecasting model it was necessary to conduct a survey among the apple growers in the area of Tuzla and Gradačac. This survey comprises information about the spray schedule for apple orchards, precisely fungicide treatments for apple scab and the use of the VIPS forecasting models. A comparison was made in one orchard which completely incorporates the use of forecasting disease outbreaks for spray timing and one apple orchard in the same area that is based on conventional plant protection methods. With these informations a case scenario was created. This case scenario is based on the data collected from the apple growers in the area of project implementation and regular pesticides prizes for 2015. It represents an estimation of the benefits for using ICT based prognosis models in integrated pest management. The estimations are only based on fungicide treatments against apple scab.



Graph 1: Case-scenario for the use of pesticides with and without the use of teh VIPS prognosis model

Tab. 1: Results of conducted survey on the use of pesticides in apple orchards in the area of Tuzla and Gradačac

Description	Dry year (2015)	Rainy year	Very rainy year
<b>Treatment cost (BAM*/ha)</b>	<b>363,64</b>	<b>536,82</b>	<b>789,62</b>
No of treatments	7,00	11,00	17,00
VIPS efficiency (%)	70,00	70,00	70,00
VIPS corrected treatments	4,90	7,70	11,90
Treatment cost (BAM/ha) with operational VIPS	109,09	161,05	236,89
<b>Cost difference (BAM/ha)</b>	<b>254,55</b>	<b>375,77</b>	<b>552,73</b>
VIPS area			
Apple orchards (ha)	131,00	131,00	131,00
Total cost without VIPS (BAM)	47.636,84	70.323,42	103.440,22
Total cost with operational VIPS (BAM)	14.291,05	21.097,03	31.032,07
Cost difference (BAM)	33.345,79	49.226,39	72.408,15

\*BAM (Bosnian Convertible Mark) 1 BAM = 0,5113 EUR

## RESULTS AND DISCUSSION

Results of the monitoring activities indicate a high precision of VIPS forecasting models for apple scab and codling moth, and therefore its suitability for use in agro-ecological conditions of B-H. The computer - based potential risks assessments for CM were highly accurate. According to the VIPS prognosis system, which incorporates a growing degree day model for CM, most imagos (adult insects) were caught in pheromone traps on the estimated days with high risk potential. The monitoring results have shown the development of 2 generations of this economically important apple pest, but also a development possibility of a third generations depending on temperature conditions.

On the other side, the results of risk assesment of secondary infection with apple scab have shown a disease severity of 46% in the tested apple orchard. High accuracy of VIPS prognosis models for apple scab was determined for primary infections and first few weeks of possible secondary infections. Due to the characteristics of the incorporated prediction model for apple scab, the VIPS systems focuses solely on ascospore release from perithecia. The later conducted survey among apple growers has shown that most of the treatments in orchards are aimed for the effective management of apple scab. According to the collected data, 16 treatments were conducted this year for apple scab management in the area of Gradačac. In orchards which used the VIPS prognosis model the number of treatments was 11. Taking into account that this year was relatively dry, the number of treatments in conventional orchards would be twice as high in a moderate rainy year. To represent the benefits of the VIPS model two case-scenarios were created on base of the data for 2015. In table 1 are shown the number of treatments with and without the use of VIPS for a dry, rainy and very rainy year. The estimations are made on the basis of one hectar and the total of 131 hectar under apple orchards in Gradačac. Estimations with operational costs of VIPS are based on apple production cost with the use of fungicides for 131 hectar. With the use of the VIPS prognosis model it is possible to achieve a cost difference of 254,55 BAM (Bosnian Convertible Mark) only for the reduced use of fungicides againts apple scab. Additionally, this has a large benefit for the environment since that the ICT based prognosis model can reduce the number of treatments on 11 for a dry year (2015), particularly 16 treatment for a rainy year. Ecological benefits are reflected in reduced/adjusted use of pesticides, biodiversity protection, lower impact on environment, protection of beneficial insects and similar.

## CONCLUSION

First experiences have shown a significant economic impact of ICT based forecast systems on the production cost for a apple orchard, which are up to 33.345,79 BAM/ha with the operational VIPS system. The prognosis system reflects in economical benefits, ecological benefits and the improvement of IPM in Bosnia and Herzegovina. Farmers and farmers associations have a large interest in the system, which leads to the development of new forecast models according to the farmers needs. Monitoring results for apple scab and codling moth have shown a high accuracy of the prognosis model.

## ACKNOWLEDGMENT

The project „Applying ICT to Bosnia-Hercegovina's agricultural sector to improve integrated pest management (IPM)“ was financed the Norwegian ministry of Foreign affairs, respectively under the HERD program. We want to thank Bioforsk for the succesfull cooperation on this project.

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## GA<sub>3</sub> ENHANCED SEED GERMINATION OF *SOLANUM TORVUM*

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*Original scientific paper*

### Summary

*Solanum torvum* has been used as rootstock for grafted transplant production of tomato and aubergine. Seeds of the species germinate slowly and some cases at low percentages. This study aimed for improving seed germination of *Solanum torvum* by using different combination of GA<sub>3</sub> (Gibberellic acid) concentrations (250-1000 ppm) and alternating temperatures (15-30°C) for various periods. The highest germination as 81% was obtained from 15°C / 7 days, 30°C / 7 days over 28 days. Germinated seeds (2 mm radicle) were taken from Petri dishes and stored at 5°C and 10°C for 5, 10, 15 and 20 days on wet Whatman papers. Seed germination test was conducted after each storage combination. Germinated seeds had high germination after 5 and 10 days at 5°C 86 and 80% respectively. Longer storage of germinated seeds reduced germination percentages to 62% at 5°C, and 60% at 10°C by 20 days. Seedling emergence percentages were also high (98%) after storage at 5°C for 10 days as those germinated and not stored. Results indicated that GA<sub>3</sub> is promoting effect on germination and germinated and stored seeds can be used as alternative seed enhancing treatment in *Solanum torvum*.

Key words: *Seed enhancement, dormancy, seed storage, emergence*

### INTRODUCTION

Grafting technologies have been used in vegetable transplants in order to increase yield, disease tolerance, adaptability to adverse soil conditions and tolerance to low temperatures (Lee *et al.*, 2010). *Solanum torvum* is an African originated species which is used since the 1920s, as a rootstock for tomato and aubergine cultivars to increase nematode tolerance and cool season adaptability of scion after grafting. In grafting technology, fast and uniform germination (emergence) is especially important to get maximum efficiency in grafted transplant production. Obtaining uniform thickness of rootstock and scion enhances the proliferation of cambium tissues. When germination is spread over time, different sized seedlings occurred and the thickness of hypocotyls varied. This hinders the fast and efficient grafted transplant production.

Seed germination in *Solanum torvum* is slow and erratic (Hayati *et al.*, 2005). Different priming methods are used to improve seed germination of various crop species (Khan, 1992). In *Solanum torvum*, KNO<sub>3</sub>, heat treatments and soaking were suggested as efficient priming methods (Hayati *et al.*, 2005). Moreover, GA<sub>3</sub> and alternating temperatures are also known as germination promoters (Baskin and Baskin, 2004). This study was designed to enhance germination of *Solanum torvum* seeds through GA<sub>3</sub> and alternating temperature combination treatments.

### MATERIALS AND METHODS

*Solanum torvum* seeds (cv. Terra) were obtained from Terra Seed Company in Antalya / Turkey in 2013. Seeds were kept at 5°C between experiments, in hermetic packets. Three GA<sub>3</sub> concentrations and different alternating temperatures were used as in Table 1. Four replicates of 50 seeds were put in petri dishes (9 cm diameter) and kept in appropriate temperatures for periods over 28 days. Seeds with radicle of 2 mm length were considered as germinated. As part of the treatment, seeds were soaked at 20°C for 12 hours and then washed for 30 minutes under running tap water. After washing, the seeds were dried by holding them at 50°C for one hour and then at 0.1 KNO<sub>3</sub> at 20°C for 5 days. Control seeds were germinated at 25°C for over 28 days in distilled water.

In the second stage of the experiment, the initial germination percentage was higher and the same trend was observed when the first stage of the germination test was reconducted. Eight hundred (800) germinated seeds with a 2 mm radicle length were isolated onto moist paper in the plastic box (10 x 10 x 5 cm). The seeds were then separated into 8 different boxes with 100 germinated (2 mm long radicle) seeds. Four boxes with 400 germinated seeds were stored at 5°C in the dark (in the plastic boxes) and the other four boxes were stored at

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10°C under the same conditions. After 5, 10, 15 and 20 days of storage, one box from each of the storage periods were taken and germinated between filter papers (two on the bottom, one on the top and seeds were between the two layers) at 25°C. Normal and abnormal seedling percentages were calculated after 21 days. Control seeds which had 2 mm long radicle but not stored were also germinated under the same conditions.

In the third stage of the experiment, the best storage temperature and duration (5°C, 10 days), that seeds germinated 2 mm long were separated from germination medium and sown into a mixture of peat moss and perlite medium after storage to determine their potential of emerging under field conditions. Seeds that were not stored but had 2 mm long radicle were also sown in the same medium. Seedling emergence percentage (%), fresh weight (mg/plant), seedling emergence rate (day), seedling dry weight (mg/plant) were determined.

Statistical comparison of treatment after seed germination was done by using ANOVA in the first stage of the experiment. Seed germination and seedling emergence percentages of germinated and stored seeds and those that were not stored (control) were compared by using SPSS package program.

## RESULTS

Among the 26 different treatments, the highest seed germination percentage was obtained from seeds kept for 7 days at 15°C, and subsequently 7 days at 30°C in 1000 ppm GA<sub>3</sub> with 81% after 28 days.

The second best treatment was in the same GA<sub>3</sub> concentration, with 15°C and 25°C, at 7 days intervals with 56% (Table 2). Some other treatments had germinations between 4-7%. Control seeds in distilled water and over 20 treatments had no germination.

In the second phase of the experiment where emerged seeds with a 2 mm long radicle were stored at 5°C and 10°C over 20 days. The highest percentage of normal seedlings (82%) was obtained from seeds stored at 5°C for 5 days. The percentage of normal seedlings from germinated seeds reduced from 82% to 44% as the storage duration extends to 20 days at 5°C, and from 67% to 42% at 10°C (Table 3). In general, total and normal germination percentages were lower at 10°C storage than at 5°C in all storage durations. The total germination percentages of seeds which were not subjected to storage but had 2 cm radicle length was 82% out of which 75% was evaluated as normal seedling; which is not significantly different from those stored at 5°C, for 5 and 10 days ( $P < 0.05$ ) (Table 3).

In the third phase of the experiment, the quality of the seeds that were germinated but not stored was compared with those that were stored at 5°C / 10 days. Emergence rate, fresh weight, dry weight, root length, root fresh and dry weight, number of main leaf, total and normal seedling emergence percentages were higher in those seeds which were germinated but not stored compared to those that were germinated and stored at 5°C / 10 days. However, the difference was significant at 5% level in 5 characters, but not in the others (Table 4). Neither normal emergence nor total seedling emergence were significantly different between germinated but non-stored and germinated and stored at 5°C for 10 days (Table 4).

## DISCUSSION

The result of the present work indicated that GA<sub>3</sub> has promotive effect on *Solanum torvum* seed germination and radicle germinated seeds can be stored at 5°C, up to 10 days without losing germination and emergence capability. GA<sub>3</sub> and alternating temperatures are common dormancy breaking treatments (Coons *et al.*, 1989; Ellis and Baretta, 1994; Geneve, 2003; Baskin and Baskin, 2004). GA<sub>3</sub> enhances, cell enlargement and cell division in embryo (Jones and Varner, 1967) and is used for breaking dormancy in various seeds (Groot and Karrsen, 1987; Finch-Savage and Leubetner Metzger, 2006). Our result agreed with previous results that GA<sub>3</sub> helps to enhance radicle protrusion through seed coat and facilitates seedling emergence. *Solanum torvum* has small seeds (1000 seeds = 2 grams) compared to the other solanum species like tomato (1000 seeds = 4 grams). Embryonic development may not be well completed on the mother plant at maturation. So GA<sub>3</sub> may enhance germination of less developed embryo. GA<sub>3</sub> concentration of 1000 ppm was found to be more effective in enhancing seed germination than those of 500, and 250 ppm GA<sub>3</sub>. According to the ISTA (2008) rules, 1000 ppm GA<sub>3</sub> is recommended for breaking seed dormancy in seed testing practices. Seven days intervals of alternating temperatures of 15°C and 30°C at 1000 ppm GA<sub>3</sub> gave the highest seed germination. At the same GA<sub>3</sub> concentration with 4 and 3 day, or 1 day intervals over 28 days did not effectively promote germination. Similarly 30°C / 20°C alternating temperature with 7 days, 4 and 3 days or daily intervals did also fail to promote germination (Table 2). The response of alternating temperatures may be species dependent.

Storage of germinated seeds was done successfully in brassicas (Finch-Savage, 1981). Seeds of cauliflower and cabbage were dried slowly to a certain level of seed moisture percentages (16%, 6%). Then, seeds are stored at just above freezing temperature (1-2°C). We did not dry seeds in our work. We wanted to test the storability of germinated seeds under humid environment (100% RH). Results showed that germinated seeds can be stored at 5°C at relatively high temperature for up to 10 days as it recorded a higher percentage of normal seedlings as those of germinated but non-stored ones. Storage of germinated seeds is important for fluid drilling methods. This work is not aimed at fluid drilling but it was conducted to test the potential effects of GA<sub>3</sub> in enhancing germinability and seedling emergence and quality. As seen in the results, we achieved our goal. Storage of germinated seeds had, were similar to previous works of the similar aim (Finch-Savage, 1981).

## CONCLUSIONS

Enhancing emergence in grafting technologies helps to produce fast and efficient grafted transplants. Rootstock seeds are slow germinated, so priming methodologies can help to enhance germination. Various other seed treatments different from GA<sub>3</sub> and alternating temperatures did fail to promote germination in *Solanum torvum* (Table 1). However, KNO<sub>3</sub> treatments are found to be effective in ensuring high germination. However, their work was carried out under tropical environmental conditions (Thailand). Level of maturity and temperature of environment can be other effective factors that react to priming treatments. The periods necessary to attain certain levels of maturity in *Solanum torvum* seeds, can be different between the southern part of Turkey which has a subtropical climate, and Thailand which is a tropical region. Moreover, *Solanum torvum* is a cultivar which can respond to priming treatments.

## ACKNOWLEDGEMENT

We thank TUBITAK (The Scientific and Technological Research Council of Turkey, Project No: 20130167) for the financial support.

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Tab. 1. Seed treatments used in the study

Alternating Temp. / Days				GA <sub>3</sub>		
Temp.	Days	Temp.	Days	250 ppm	500 ppm	1000 ppm
15°C	7 days	30°C	7 days			+
15°C	4 days	30°C	3 days			+
15°C	1 day	30°C	1 day			+
15°C	7 days	25°C	7 days			+
15°C	4 days	25°C	3 days			+
15°C	1 day	25°C	1 day			+
15°C	7 days	30°C	7 days		+	
15°C	4 days	30°C	3 days		+	
15°C	1 day	30°C	1 day		+	
15°C	7 days	25°C	7 days		+	
15°C	4 days	25°C	3 days		+	
15°C	1 day	25°C	1 day		+	
30°C	7 days	15°C	7 days	+		
30°C	4 days	15°C	3 days	+		
30°C	1 day	15°C	1 day	+		
15°C	7 days	25°C	7 days	+		
15°C	4 days	25°C	3 days	+		
15°C	1 day	25°C	1 day	+		
30°C	7 days	20°C	7 days			+
30°C	4 days	20°C	3 days			+
30°C	1 days	20°C	1 day			+
50°C	1 day					
5°C	1 day					
0.1% KNO <sub>3</sub>	20 °C	5 days				
Control	25 °C	distilled water				

Tab. 2. The treatments that positive response were obtained in germination percentage of *Solanum torvum* seeds

Treatments (Alternating temperature / GA <sub>3</sub> concentrations)	Germination Percentage
<b>1000 ppm</b>	
15°C / 7 days – 30°C / 7 days	81a
15°C / 1 days – 30°C / 1 days	7c
15°C / 7 days – 25°C / 7 days	56b
30°C / 7 days – 20°C / 7 days	2c
30°C / 4 days – 20°C / 3 days	4c
<b>250 ppm</b>	
30°C / 7 days – 15°C / 7 days	4c
30°C / 4 days – 15°C / 3 days	7c

\*The means with different letters in the same column are significantly different (P&lt;0.05)

Tab. 3. The effect of storage temperature and duration of germinated seeds on subsequent germination percentages of *Solanum torvum* seeds

Storage conditions		Germination percentage % (25°C, 21 days)	
Temperature	Day	Normal	Total
5°C	5	82a	86a
	10	76b	80ab
	15	55de	64d
	20	44f	62d
10°C	5	67bc	77bc
	10	62cd	72c
	15	45ef	70c
	20	42f	60d
Germinated but not stored seeds		75ab	82a

\* The means with different letters in the same column are significantly different (P&lt;0.05)

Tab. 4. The effect of storage (5°C, 10 days) of germinated seeds of *Solanum torvum* seeds on seedling quality after sowing perlite and turf mixture

Seedling parameters	Germinated but not stored seeds		Germinated and stored at 5°C/10 days	
Emergence rate (day)	14.50a		10.52b	
Fresh weight (mg/plant)	19.50a		12.7b	
Dry weight (mg/plant)	2.00		1.72	
Main leaf number (plant)	2.20a		2.01b	
Emergence percentage (%)	Normal	Total	Normal	Total
	82	97	84	98

\* The means with different letters in the same column are significantly different (P<0.05)

## USING COMPUTER BASED DETERMINATION TECHNIQUES FOR AGRICULTURAL PURPOSES

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*Professional paper*

### Summary

Setting and using quality standards in agriculture is very important part of agricultural production. Using human resources in agricultural production has become expensive and may cause errors. Mechanization is necessary in order to producing and marketing large amounts of products.

Even though there are a lot of systems and machines in every stage of production process which are widely used in conventional agriculture, there are still needs for mechanized quality based classification systems.

Computer based determination techniques became popular in last decade. Image and signal processing techniques with machine learning algorithms offers objective, solid, and high accuracy solutions to producers for quality based classification without human related errors. This study aimed to discuss those machine learning and data processing techniques in order to use them to classify agricultural products.

Keywords: *image processing, signal processing, machine learning*

### INTRODUCTION

Today, to produce large amounts of goods, industry needs faster, accurate and low operating cost solutions. Computer based determination methods (CBDM) are reasonable to determine quality factors rapid and cheaper. Although there are many CBDM, some of the methods has been widely used by developers when compared the others. The methods described in this paper include two main subjects, image processing and signal processing. Image and signal processing can help measuring quality characteristics. Capability of those methods are enough to develop determination algorithms to satisfy industry needs.

#### Image Processing

The material that processed in image processing is a digital image of an object. To process data, processing stages should be applied on digital image. Those stages are elements of image processing method. To better understanding of quality characteristics of an object and to transform it to analyzable data, computer algorithms should be used. Common algorithms and effects are listed below.

#### Binary image

Binary images are images that has two possible pixel data, “0” and “1”, usually “0” represents black and “1” represents white. Therefore, binary images displayed as black and white. An example of binary image is shown in Fig.1.



Fig. 1. A digital color image, and a binary image respectively.

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Binary images has less data than the original ones. Because of this feature of binary images, they are more suitable to calculate them in uncomplicated algorithms. Using binary images simplify algorithms and that makes run algorithms faster.

To create a binary image, thresholding method is used while converting original image to binary. Thresholding is determining a threshold value to obtain a binary image. Threshold value determines which range of pixel data will be 1 and others will be 0 after binary conversion. An example of thresholding has shown in Fig. 2.



Fig. 2. Different threshold levels of same image

In Fig. 2 there are 5 different levels of thresholds. Compared to others in d level “TÜRK LİRASI” text is more readable than the others. Although there are many of thresholding algorithms, Otsu’s method has common usage in image processing field (Sezgin and Sankur, 2004).

For aid understanding, the following is a Java algorithm, The input is an array of bytes, srcData that stores the greyscale image.

**File Excerpt: OtsuThresholder.java**

```
// Calculate histogram
int ptr = 0;
while (ptr < srcData.length) {
    int h = 0xFF & srcData[ptr];
    histData[h] ++;
    ptr ++;
}

// Total number of pixels
int total = srcData.length;

float sum = 0;
for (int t=0 ; t<256 ; t++) sum += t * histData[t];

float sumB = 0;
int wB = 0;
int wF = 0;

float varMax = 0;
threshold = 0;

for (int t=0 ; t<256 ; t++) {
    wB += histData[t]; // Weight Background
    if (wB == 0) continue;

    wF = total - wB; // Weight Foreground
    if (wF == 0) break;

    sumB += (float) (t * histData[t]);

    float mB = sumB / wB; // Mean Background
    float mF = (sum - sumB) / wF; // Mean Foreground

    // Calculate Between Class Variance
    float varBetween = (float)wB * (float)wF * (mB - mF) * (mB - mF);
```

```

// Check if new maximum found
if (varBetween > varMax) {
    varMax = varBetween;
    threshold = t;
}
}

```

Fig. 3. Java implementation of Otsu's tresholding method (Greensted, 2010)

### Color Channels

Although many digital images, such as photos usually have three color channels which are Red Green and Blue, there is also CMYK (Cyan, Magenta, Yellow, Black) and HSI (Hue Saturation Intensity) for different purposes. Following figure shows Red, Green and Blue channels of an digital RGB image.



Fig. 4. Color channels of a digital image

In processing an image, different channels are used for different purposes. As you can see in figure, Blue channel has the highest contrast between coins and background. Therefore Blue channel can be used for apart coins from background. Following figure shows specific threshold applied Blue channel. Color histograms can also be used to determine contrast of a given image.

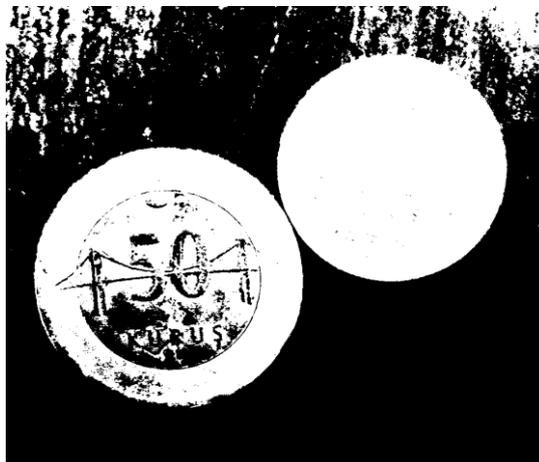


Fig. 5. Threshold applied to Blue color channel

Following figure describes an example of using binary image, thresholding and color channels methods for agricultural quality determination. In figure; Original image of a wounded pear (a), Blue color channel of original image (b), focus mask created according to blue channel (c), focused area of original image (d), Red channel of focused area (e), binary image created from specific threshold level applied to Red channel (f), black areas bigger than a specific sizes are marked (g), determined marks on original image (h).

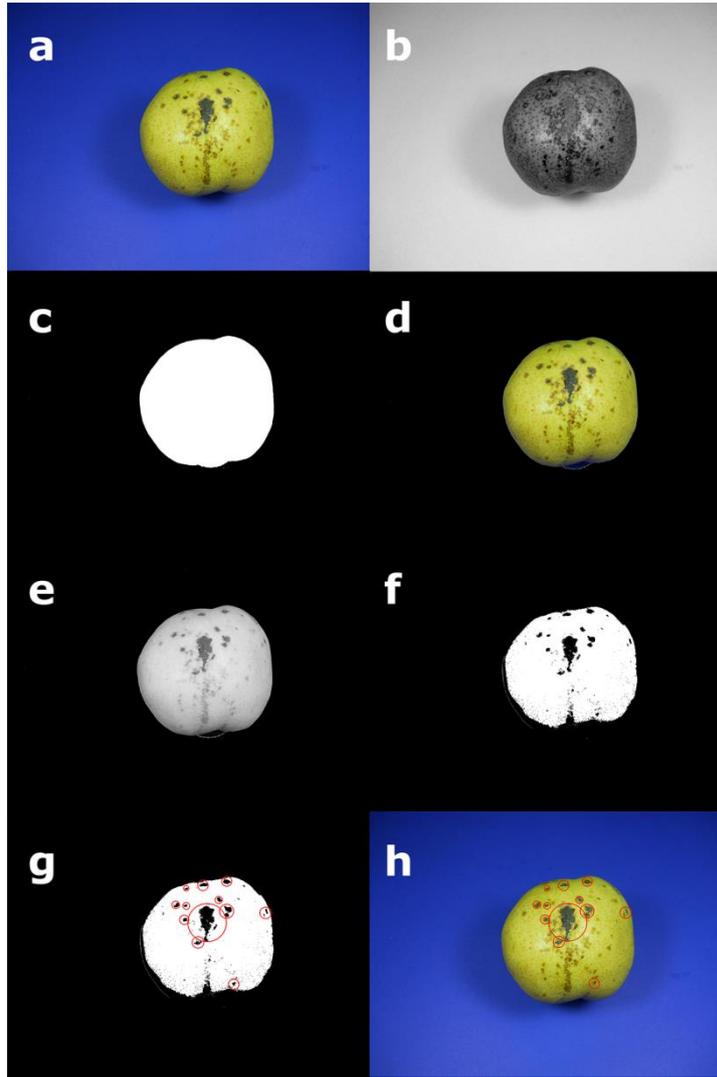


Fig. 6. Explanation of wound determination algorithm

### Signal Processing and Machine Learning Methods

Similar to other data types, digital image data could be process as signals. For example, Fourier analysis method could be applied to a digital image to understand its signal behaviors. Beside all of the methods described in this paper, today, Machine Learning methods are also used by developers. Machine learning uses computer algorithms to learn and predict based on given data (Rouse, 2011). Machine learning builds a statistical model from given data, and then calculates a response for unseen data (Anonym, 2015). Today Machine learning could be used by agricultural industry to produce fast and high quality products by separating lower and high quality products fast and reliable.

### CONCLUSION

Methods described in this paper could be applied to solve problems encountered in agricultural production. CBDM could be used any stage of production such as seeding, cultivating, harvesting or quality standardization. Even though costs of the licensed software might be expensive in set up, long term operating is affordable, compared to human-operated systems. CBDM gives the opportunity of doing agriculture related time consuming works faster and canalize the labor to other operations that mechanization not able to handle or reduce employment costs.

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## ANALYSIS OF WEED IN MAIZE AND SUNFLOWER CROPS IN BAČKA

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*Original scientific paper*

### Summary

In 2010 and 2011, taxonomic, ecological and phytogeographic analysis of weed flora in maize and sunflower crops was studied at two localities in Bačka. Studies revealed the presence of 19 species of weed plants that included in 13 families, 11 orders, 5 sub-classes and 2 classes from division Magnoliophyta. By monitoring of the life forms, the prevalence of annual weeds – therophytes (78.95%) was determined, while perennial geophytes and hemicryptophytes were equally represented by 10.53%. Study of the present maize and sunflower floral elements showed that dominant are plants belonging to the group of floristic elements of wide distribution – a cosmopolitan with 42.10% of the total number of determined weed species. Subeurasian, Eurasian, and adventive floral elements were presented equally with 10.53% each, and European, sub-European, Eurasian – Submediterranean, sub Pontic-Mediterranean-Submediterranean, East Pontic-sub-Mediterranean and Subcircumpolar with 5.26% each.

Keywords: *weed flora, crops*

### INTRODUCTION

Weeds represent a threat to all crops, but they have special significance in row crops, where a large inter-row distance enables undisturbed development of weeds, especially in the first phases of a crop development. It was established that in comparison to pests and diseases, weeds cause the highest losses in maize yield (Rajcan and Swanton, 2001) and that in the period 2001-2003 at the global level losses in maize yield were over 10% (Oerke, 2006), while Daugovish *et al.* (2003) determined that weeds can reduce sunflower yield for over 50%. In our country, weed flora of maize and sunflower agrophytocoenoses is floristically diverse and rich, but they all do not have equal significance in weed infestation. In building of a characteristic complex of maize and sunflower weed community are included: *Ambrosia artemisiifolia*, *Amaranthus retroflexus*, *Chenopodium album*, *Convolvulus arvensis*, *Hibiscus trionum*, *Setaria glauca*, *Setaria viridis*, *Solanum nigrum* and *Sorghum halepense*. In maize crops with great abundance and coverage are distinguished also: *Abutilon theophrasti*, *Cirsium arvense*, *Cynodon dactylon*, *Digitaria sanguinalis*, *Rubus caesius*, *Panicum crus-galli*, *Polygonum aviculare*, *Polygonum lapathifolium* and *Polygonum persicaria* and in sunflower: *Bilderdykia convolvulus*, *Chenopodium hybridum*, *Datura stramonium*, *Polygonum lapathifolium*, *Polygonum persicaria*, *Sinapis arvensis*, *Sonchus oleraceus*, *Stachys annua* and *Xanthium strumarium*.

At certain localities, a species of wide distribution such as: *Amaranthus albus*, *A. blitoides*, *Anagallis arvensis*, *Aristolochia clematidis*, *Bilderdykia convolvulus*, *Capsella bursa-pastoris*, *Chenopodium hybridum*, *Cychorium intybus*, *Datura stramonium*, *Eragrostis megastachya*, *Erigeron canadensis*, *Galinsoga parviflora*, *Polygonum lapathifolium*, *Portulaca oleracea*, *Reseda lutea*, *Roripa silvestris*, *Sinapis arvensis*, *Sonchus arvensis*, *Sonchus oleraceus*, *Stachys annua*, *Verbena officinalis* and *Xanthium strumarium* may dominate, as well as the others (Konstantinović, 1999). The basic consequence of weed species the presence of in row crops is weed reduction and increase in product price (Stefanović and Šinžar, 1993), and therefore, for their control knowledge on weed community of these crops is necessary.

### MATERIALS AND METHODS

During 2011 and 2012, taxonomic analysis of weed flora of maize and sunflower crops was studied at two localities in Bačka, in two terms, by the beginning of April and by the end of May, the beginning of June. The collected plant material was preserved and determined according to Šinžar i Janjić (1995), Knežević (1988), Kojić (1986), Jávorka and Csapody (1975), floristic elements were given according to Gajić (1980), taxonomic and nomenclature, ecological index and life forms according to Landolt (1977).

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## RESULTS AND DISCUSSION

Taxonomic analysis of weed flora in maize and sunflower crops revealed the presence of 19 weed plants included 13 families, 10 orders, 5 subclasses and 2 classes from division Magnoliophyta (Table 1).

The class Magnoliopsida (dicots) included 12 families 19 plant species, and the class Liliopsida (monocots) included only the family *Poaceae* with 2 weed species.

Tab. 1. Taxonomic affiliation of the analysed weed flora in maize and sunflower crops

Division	Class	Subclass	Order	Familia	Species
Magnoliophyta	Magnoliopsida	Caryophyllidae	Caryophyllales	<i>Amaranthaceae</i>	<i>Amaranthus retroflexus</i> L.
				<i>Chenopodiaceae</i>	<i>Chenopodium album</i> L.
				<i>Portulacaceae</i>	<i>Portulaca oleracea</i> Linn.
			Polygonales	<i>Polygonaceae</i>	<i>Polygonum aviculare</i> L.
					<i>Polygonum lapathifolium</i> L.
					<i>Polygonum persicaria</i> L.
		<i>Bilderdykia convolvulus</i> (L.) Dum.			
		Dilleniidae	Capparales	<i>Brassicaceae</i>	<i>Sinapis arvensis</i> L.
			Malvales	<i>Malvaceae</i>	<i>Abutilon theophrasti</i> Medic.
					<i>Hibiscus trionum</i> L.
		Euphorbiales	<i>Euphorbiaceae</i>	<i>Euphorbia cyparissias</i> L.	
		Asteridae	Asterales	<i>Asteraceae</i>	<i>Ambrosia artemisifolia</i> L.
	Lamiidae	Rubiales	<i>Rubiaceae</i>	<i>Galium verum</i> L.	
		Solanales	<i>Solanaceae</i>	<i>Datura stramonium</i> L.	
				<i>Solanum nigrum</i> L.	
			<i>Convolvulaceae</i>	<i>Convolvulus arvensis</i> L.	
	Lamiales	<i>Lamiaceae</i>	<i>Stachys annua</i> L.		
Liliopsida	Commelinididae	Poales	<i>Poaceae</i>	<i>Echinochloa crus-galli</i> L.	
<i>Sorghum halepense</i> (L.) Pers.					
Σ	2	5	10	13	19

Tab. 2. Ecological index, life form and floral elements of weed flora in maize and sunflower crops according to Landolt (1977).

Species	Ecological index										Life form	Floral elements
	F	R	N	H	D	S	L	T	K			
<i>Abutilon theophrasti</i> Medic.	2	3	4	3	4	-	4	5	3	Th	Evr.-subm.	
<i>Amaranthus retroflexus</i> L.	2	3	4	3	3	-	4	4	3	Th	Adv.	
<i>Ambrosia artemisifolia</i> L.	2	3	4	2	2	+	4	5	3	Th	Adv.	
<i>Bilderdykia convolvulus</i> (L.) Dum	2	3	3	3	4	-	4	4	3	Th	Subevr.	
<i>Chenopodium album</i> L.	2	3	4	3	4	-	4	3	3	Th	Kosm.	
<i>Convolvulus arvensis</i> L.	2	4	3	3	4	-	4	4	3	G	Kosm.	
<i>Datura stramonium</i> L.	3	3	4	4	4	+	4	5	2	Th	Kosm.	
<i>Euphorbia cyparissias</i> L.	2	3	2	3	4	-	4	3	3	H	E.Az.	
<i>Echinochloa crus-galli</i> L.	3	3	5	3	4	-	3	4	3	Th	Kosm.	
<i>Galium verum</i> L.	2	4	2	3	5	-	4	4	4	H	E.Az.	
<i>Hibiscus trionum</i> L.	3	3	3	3	4	-	4	5	4	Th	Pont.-is.-subm.	
<i>Polygonum aviculare</i> L.	3w	3	4	3	5	-	4	3	3	Th	Kosm.	
<i>Polygonum lapathifolium</i> L.	3w	3	4	3	3	-	5	3	3	Th	Subcirk.	
<i>Polygonum persicaria</i> L.	3	3	4	3	3	-	4	3	3	Th	Evr.	
<i>Portulaca oleracea</i> Linn.	3w	3	4	3	4	-	4	4	3	Th	Kosm.	
<i>Sinapis arvensis</i> L.	3	4	4	3	4	-	4	4	3	Th	Subevr.	
<i>Solanum nigrum</i> L.	3	4	4	3	4	-	4	4	3	Th	Kosm.	
<i>Sorghum halepense</i> (L.) Pers.	1	2	3	3	3	-	4	5	3	G	Kosm.	
<i>Stachys annua</i> L.	2	4	2	3	4	-	4	4	4	Th	Subpont.-subm.	

Considering the distribution of floral elements present in maize and sunflower weed flora, the dominant presence of plants from the group floristic elements of wide distribution was noticed – they were cosmopolitan, with 8 plant species, which makes 42.10% of the total number of the studied weed species (Tab. 2). Subeurasian, Eurasian, and adventive floral elements were presented equally with 10.53% each, and European, sub-European, Eurasian – Submediterranean, sub Pontic-Mediterranean-Submediterranean, East Pontic-sub-Mediterranean and Sub circumpolar with 5.26% each. Similar data were obtained by Milošević *et al.* (2008) during analysis of row crops flora, in which the dominance of plants from the group of floristic elements of wide distribution was also established (Eurasian, cosmopolitan and adventive).

On weed occurrence in the field, in addition to environmental factors, the greatest impact has soil humidity. Maize and sunflower crops have moderate needs for water, and can be classified intomesophytes. It is therefore not surprising that in these crops emerge weeds with the identical needs, such as: *Amaranthus retroflexus*, *Abutilon theophrasti*, *Datura stramonium*, *Hibiscus trionum*, *Sinapis arvensis* and *Solanum nigrum*, that also belong to the group of mesophytes. Among all factors, lack of water, i.e. competition between maize and weeds for water causes the greatest yield reduction (Norris, 1996).

In relation to life forms, weed community of the studied row crops include mainly therophytes, i.e. annual weeds (almost 78.95%), mostly due to deep pre-sowing tillage and inter-row cultivation and tillage which reduces possibility of emergence of perennial weeds – geophytes (10.53%) and hemicryptophytes (10.53%) that in such a case occur in equal measure. The ecological analysis of the found weed species in maize and sunflower crops indicates the presence of the greatest number of weeds that grow successfully in moderately dry to moderately humid soil. There are 9 weed species with the ecological index  $F_2$  (47.37%), as well as with the index  $F_3$ . Of all studied weed species, only *Sorghum halepense* belongs to the group of xerophytes with the ecological index for habitat water regime  $F_1$  (5.26%). The average value of the ecological index for humidity is 2.42, and thus it may be concluded that the studied soil was moderately dry.

In regard to the ecological index for soil chemical reaction, 13 weed species have value  $R_3$  (68.42%), i.e. neutral to slightly acidic habitat are suitable to them. Only 4 weed species have the value of the ecological index  $R_4$  with 26.32%, and only *Sorghum halepense* endures sour habitat and the value of the ecological index for pH  $R_2$  is suitable for it. The average value of the ecological index for soil chemical reaction is 3.21 which suggest that the studied soil was neutral to slightly acid.

The majority of established weeds suit soil relatively or moderately rich in nitrogen compounds (11 weed species have the value  $N_4$  with 57.89%, and 4 ones have  $N_3$  value representing 21.05%). Soils that are poor in content of biogenic mineral matters are convenient for growth of 3 weed species ( $N_2$  is represented with 15.79%), and the greatest need for this ecological factor has *Echinochloa crus-galli* ( $N_5$ ). The average value of the ecological index for the content of biogenic mineral matters (especially nitrogen compounds) is 3.53, which means that the habitat is relatively rich in the content of biogenic mineral matters.

The analysis of the ecological indices for the content of organo-mineral compounds, such as humus indicated the domination of weed species with the value of ecological index  $H_3$  (17 weed species; 89.47%). The exception is *Ambrosia artemisiifolia* to which are suitable habitats with low content of organo-mineral compounds ( $H_2$  5.26%) and *Datura stramonium* which is indicator of habitat relatively rich in content of organo-mineral compounds ( $H_4$  5.26%). The average value of this ecological index is 3 which leads to the conclusion that the studied habitat is characterized by the medium content of organo-mineral compounds.

Unsalinated soil is suitable to all weeds except to the species *Ambrosia artemisiifolia* and *Datura stramonium* which are indicators of the salinated environment.

In crop and weed community, competition for light is of great importance. Due to its high growth, in competition for light, except in early growth phases, maize is in advantage in relation to weeds of low growth. However, there are weeds such as *Abutilon theophrasti*, *Chenopodium album*, *Amaranthus spp.* and others that grow to the height of over a meter, and disturb maize in its light supply (Lindquist *et al.*, 1998). Species such as *Sorghum halepense*, *Echinochloa crus-galli* and other disturb light supply in its lower half, or up to two third of the stem height.

Weed species with the ecological index for light  $L_4$  are dominant in the studied weed flora (17 species; 89.47%). These species successfully grow exposed to direct light but are tolerant to weaker or stronger over shadow. One weed species was found with the ecological index  $L_3$  and one with the ecological index  $L_5$ . The average value of this ecological index is 4, which means that dominate plants that belong to the transition group between half sciophytes and heliophytes and represent light indicators. This was expected since these are typical weeds of row crops.

The competition intensity will determine a content of the factor that is in deficit. In conditions with optimal

water supply of soil and on areas on which sufficient quantities of nitrogen are applied, the competition for light between maize and the species *Abutilon theophrasti* is a factor that will determine yield height (Lindquist and Mortensen, 1999). Weed species *Abutilon theophrasti* has a higher efficiency of light absorption and its translation into the dry matter which gives it the advantage in the competition for light.

The analysis of ecological indices for temperature shows domination of plants with the value  $T_4$  (9 species; 47.37%). Weeds with the index  $T_3$  are represented with 5 species and 26.31%, as well as weeds with  $T_5$ . There is an absence of weed species with values  $T_2$  and  $T_1$ . The average value of the ecological index for temperature is 4, which means that thermophilic plants are dominant. Ecological indices for continental climate show that the majority of weeds have the value  $K_3$  (15 species; 78.95%).  $K_4$  has 3 species (15.79%) and  $K_2$  has 1 species. The average value of  $K$  is 3.10 which indicate that the studied habitat is in moderate continental climate.

One of the important modes of mutual impact between plants and crops is the occurrence of allelopathy, which is manifested as the negative effect of weeds to the crop or vice-versa. Weed species *Ambrosia artemisiifolia* L. is an inevitable weed in maize and sunflower crops, as well as in other row crops. The studies showed that the above-ground part of ragweed contains high percentage of phenolic compounds, the matter that in higher concentration inhibit maize seed germination, elongation of leaves, as well as the growth of root of this culture (Đurđević *et al.*, 2004). Johnson grass (*Sorghum halepense*) in maize crops has the allelopathic effect which is the cause of yield reduction in this crop. Weed species *Abutilon theophrasti* and *Datura stramonium* are strong competitors in sunflower crops. Apart from the competitive activity, in sunflower and other crops these species showed also allelopathic inhibiting effects which explain their invasiveness.

## CONCLUSIONS

Taxonomic analysis of weed flora in maize and sunflower crops revealed the presence of 19 weed plants included 13 families, 10 orders, 5 subclasses and 2 classes from division Magnoliophyta. The class Magnoliopsida (dicots) included 12 families with 19 plant species, and the class Liliopsida only the family *Poaceae* with 2 weed species. Based upon the ecological analysis of weed flora under maize and sunflower crops, it can be concluded that the studied soil is moderately dry, neutral to weak acid pH value, unsaturated, relatively rich in content of biogenic mineral matters and with the medium content of organo-mineral compounds. It is under the influence of moderate continental climate. Here are dominant weeds with the increased needs for light and temperature. The noticed domination of annual weeds is the consequence of regular and frequent soil tillage. The domination of the cosmopolitan weed species that have a wide areal distribution is also expressed. Subeurasian, Eurasian, and adventive floral elements were presented equally (10.53%), and European, sub-European, Eurasian – Submediterranean, sub Pontic-Mediterranean-Submediterranean, East Pontic-sub-Mediterranean and Sub circumpolar with 5.26% each.

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## THE PHENOLOGY OF FLOWERING AND RIPENING OF NEWLY INTRODUCED CHERRY CULTIVARS IN ECOLOGICAL CONDITIONS OF ČAPLJINA

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*Original scientific paper*

### Summary

Flowering phenology depends on the variety and weather conditions before and during the flowering.

The aim of this research was to determine the progress, duration and abundance of flowering of four newly introduced cherry varieties ('Van', 'Lala star', 'Sunburst' and 'Black star') in the area of Čapljina, Nerezi location. The research was carried out during the period of 2010-2011. The flowering period of the variety 'Early Burlat' was used for comparison to the flowering periods of introduced varieties 'Van', 'Black star', 'Lala star' and 'Sunburst', since it is a standard flowering period. Different durations of flowering period of the observed cherry varieties in 2010 and 2011 (from 7 to 13 days), have not been statistically significantly different.

Flowering order of cherry varieties studied in this research was uniformed per years, with the following flowering order 'Van' – 'Black star' – 'Lala star' — 'Early Burlat' – 'Sunburst'.

Based upon observation of flowering period, it has been concluded that the variety 'Van' belongs to the group of early flowering varieties, then 'Black star' and 'Lala star' varieties that have medium flowering period, and 'Sunburst' variety with medium late flowering, showing satisfying adaptation to ecological conditions of Čapljina. There were significant differences in the time of harvest beginning of cherry varieties in 2010 and 2011, which is result of agro ecological conditions.

Studied varieties by the ripening time, belong to the groups of medium early and medium late varieties.

*Key words: cherry, ecological conditions, phenology of flowering*

### INTRODUCTION

Knowing the flowering timing of cherry varieties is one of the aspects necessary for finding of quality variety compositions in certain ecological conditions. Fruit flowering is depending on hereditary biological characteristics of the variety and meteorological factors (Radičević, 2013).

Although the beginning of flowering of cherry is influenced by air temperature, order of flowering beginning of varieties grown in same ecological conditions is influenced by hereditary characteristics (Stančević, 1967). Same author say that variety influence is emphasised in years with earlier beginning of flowering, and flowering last longer in those years.

Duration of flowering is shorter if air temperatures are higher, precipitation lower and weather mild windy.

Ripening period of cherry varieties depends, besides genetic basis on weather conditions as well, and it is determined in relation to 'Burlat' variety which ripens in the second cherry week, it is taken as the standard and marked with „0“. Varieties which are ripening before are marked as –, and those which are ripening after variety 'Burlat', are marked with +.

Larger period of fruit ripening, actually availability of varieties from the earliest to the latest is one of the main aims in the production. Earlier varieties reach higher market price, though the sensitivity on fruit cracking is bigger, smaller fruits and lower flesh randman which is making them suitable for the local markets. Late varieties have better pomological characteristics such as size, firmness, colour and taste, but lower sensitivity on fruit cracking (Sansavini and Lugli, 2008).

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## MATERIALS AND METHODS

Two years research (2010-2011) was conducted in the cherry orchard at the locality Nerezi owned by the company Dominant d.o.o. from Čapljina. Locality Nerezi is positioned southwest from Čapljina, altitude 1,5 m to 9 m. Orchard was planted in 2005 and it is in the phase of full fruit bearing, planting distance is 4.0 x 3.6 m. Rootstock is Sante Lucie 64 (SL 64), training system is pyramid, semi-intensive.

Research comprehended four introduced cherry varieties: 'Black star', 'Lala star', 'Sunburst' and 'Van'. Besides these varieties, variety 'Early Burlat' is also presented with three trees with the same age and grafted on the same rootstock, therefore it was used as standard for flowering time and fruit ripening.

Flowering phenology was monitored in two years period, in the accordance with method of Stančević (1967), visual observations and recording the following characterises:

- beginning – date when 20% of flower was opened;
- main (full) flowering – date when 90-100% of flower was opened;
- end of flowering – date when 90% of petals are dropped.

Examined varieties are grouped in one of four groups in the accordance with the time of flowering (Kobel, 1954).

In order to determine fruit ripening of examined varieties in two years of research, the moment of full ripening was recorded – date when the fruits reached the best quality for consumption. Varieties were grouped in one of six weeks in accordance with ripening time and in comparison to ripening of 'Early Burlat' variety.

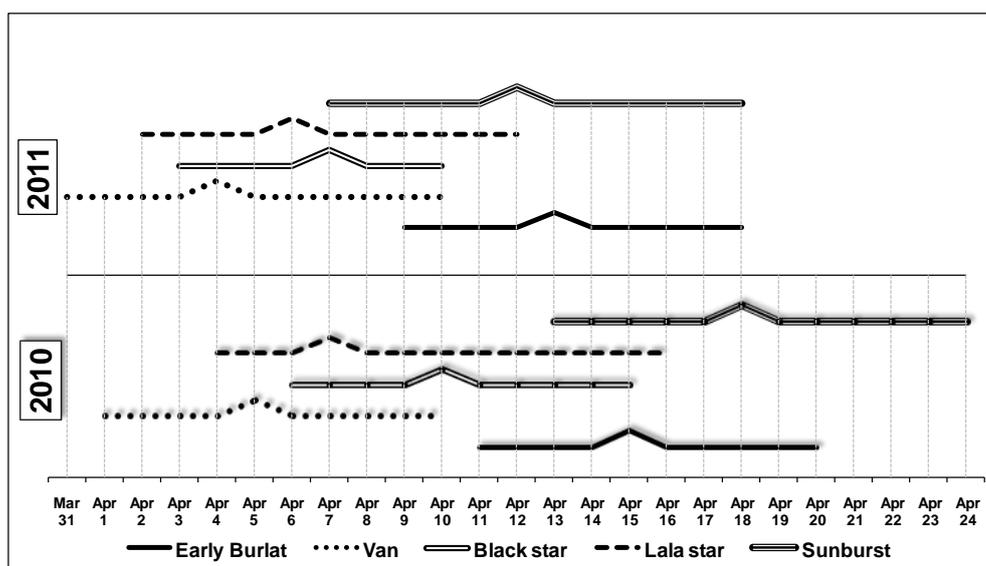
## RESULTS AND DISCUSSION

### Flowering period

Knowing the flowering period of cherry varieties is one of the aspects necessary for determination of the best quality variety compositions in certain agro-ecological conditions that provide the best possibilities for realization of full bearing.

This paper comprehend differences in the periods of beginning of flowering of examined varieties through years, and especially earlier flowering spotted in 2011, which was result of quite high April temperatures.

Although beginning of flowering of cherry is under influence of air temperature, the order of flowering of the varieties in the same ecological conditions is influenced with hereditary characteristics (Stančević, 1967). Flowering period of varieties 'Van', 'Black star', 'Lala star' and 'Sunburst' given for comparison function with flowering period of variety 'Early Burlat' as standard.



Graph 1. Flowering periods of cherry varieties 'Early Burlat', 'Van', 'Black star', 'Lala star' and 'Sunburst' in 2010 and 2011 (▲ – date of full flowering)

Through the data presented in the Graph 1, it may be concluded that cherries of all five examined varieties in 2011 started flowering period earlier then in 2010. Variety classification per flowering periods, in comparison to

‘Early Burlat’ variety, though it was done on the basis of flowering time in two years period, was in the accordance with the results of Konstantinos *et al.* (2011).

In 2010 variety ‘Van’ (01.04.) was the first one to start flowering, and the latest flowering was observed for variety ‘Sunburst’ (13. 04.). ‘Van’ variety started flowering as the first in 2011 as well (31. 03.), but ‘Early Burlat’ (09. 04.) was the latest flowering variety. Averagely ‘Van’ variety was the earliest in both observed years (31. 03. – 01. 04.). The rest of the flowering order was: variety ‘Lala star’ (02. 04. – 03. 04.), variety ‘Black star’ (03. 04. – 06. 04.) and at the end variety ‘Early Burlat’ (09. 04. – 11. 04.) and variety ‘Sunburst’ (07. 04. – 13. 04.).

In both observed years, periods of full flowering was observed for ‘Van’ variety (05. 04. 2010 and 04. 04. 2011). Full flowering in 2010 was latest for the variety ‘Sunburst’ (18. 04.), and in 2011 the latest one was the variety of ‘Early Burlat’ (13. 04.). There was no matching of flowering periods of varieties ‘Van’ and ‘Early Burlat’ in 2010, while flowering periods of all examined varieties in 2011 were matching in at least one day (09. 04. – 10. 04.).

Hodun and Hodun (2002) observing flowering characteristics of 80 cherry varieties and established that for the most of varieties flowering lasted 8 to 10 days.

Duration of flowering period in 2010 was 9 days for the varieties ‘Early Burlat’, ‘Van’ and ‘Black star’, 11 days for the variety ‘Sunburst’ and 13 days for the variety ‘Lala star’. in 2011, the shortest flowering period was observed for the variety ‘Black star’ (7 days), and the rest of the order by duration of flowering was variety ‘Early Burlat’ (9 days), varieties ‘Van’ and ‘Lala star’ (10 days) then variety ‘Sunburst’ (11 days).

Table 1. represents variance analyses of durations of flowering periods of five examined varieties of cherry at the observed locality in 2010 and 2011. It was two-factor analyses (2 years x 5 varieties) with one replicate per experimental treatment.

Tab. 1. Variance analyses for duration of flowering period (days) of examined cherry varieties in 2010 and 2011

Source of variability	Deviance	Df	Variance	F <sub>exp</sub>	F <sub>crit</sub> 0.05
Variety	14.40	4	3.60	6.00	6.39
Year	0.10	1	0.10	0.17	7.71
Error	2.40	4	0.60		
Total	16.90	9			

Results of variance analyses are presented with Table 1, and they indicate that there was no statistically significant influence of observed year or variety on different durations of flowering period. Flowering order of cherry varieties studied in this research was uniformed per years, with the following flowering order ‘Van’ – ‘Black star’– ‘Lala star’– ‘Early Burlat’– ‘Sunburst’.

Important problem in investigation of flowering phenology and its influence on correct formation of flowering composition is determination of time match of certain varieties in full flowering phenophase, as a condition of possible pollination.

Since, the varieties examined in this research ‘Black star’, ‘Lala star’ and ‘Sunburst’ are self fertile varieties, matching of their flowering phenophase could not be limiting factor for pollination compatibility. It is also necessary to emphasise self fertility of these varieties because of creating of possibilities for their cultivation in mono variety orchards.

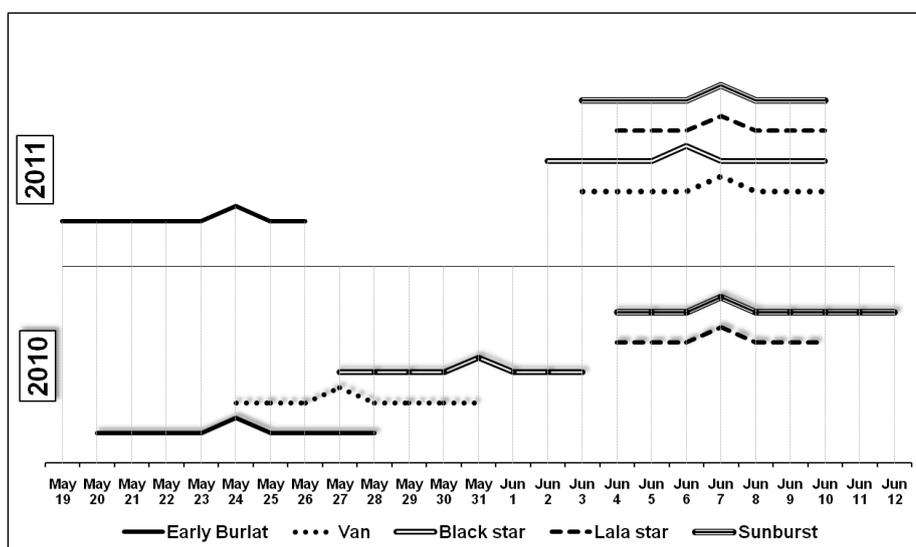
The exception is the variety „Van“, as well as the standard variety Early Burlat which are cross-fertile, therefore it is necessary to take care about selection of the pollinator varieties. This research shows that this was a well selected variety composition with matched flowering phenophases between examined varieties.

Based upon examination of flowering period we may conclude the following: ‘Van’ variety belong to the early flowering group, varieties ‘Black star’ and ‘Lala star’ medium flowering and variety ‘Sunburst’ belongs to the group of medium late flowering.

#### Ripening period

The beginning of ripening of all fruit types, cherry as well is mostly influenced by the weather conditions (temperature and precipitation) in the decade prior to ripening as well as during the ripening itself. According to Stančević (1968), the order of ripening of varieties cultivated in the same ecological conditions is influenced with genetic characteristics. In this research, the order of ripening of certain varieties is equable; actually there were no inversions in the order by observed years.

Data on ripening periods of examined cherry varieties are presented with Graph 2.



Graph 2. Ripening periods of observed cherry varieties in 2010 and 2011 (▲ – date of full harvest)

As it can be seen in the Graph 2, there were significant differences in harvest beginning of examined varieties of cherry in 2010 and 2011. In 2010, it was successive harvest beginning of the varieties. ‘Early Burlat’ (harvest beginning 20. 05.), ‘Van’ (harvest beginning 24. 05.) and ‘Black star’ (harvest beginning 27. 05.), while ‘Lala star’ and ‘Sunburst’ varieties had quiet late harvest beginning period (04. 06.).

Variety ‘Early Burlat’ in 2011, had harvest beginning date on 19. 05., while the other four varieties had later and more equable harvest beginning period (02. 06. to 04. 06.). It is interesting that in both years, dates of full harvest were matching for varieties ‘Early Burlat’ (24. 05.), ‘Lala star’ (07. 06.) and ‘Sunburst’ (07. 06.). Similar results for ‘Burlat’ variety were observed by Aliman *et al.* (2013) in the research from 2006 for the area of Mostar. Ripening period of ‘Van’ variety responds to the results of Tomasović *et al.* (2011).

Examined varieties by their ripening periods belong to groups of medium early and medium late varieties. Duration of harvest period for observed cherry varieties in 2010 was 6 days for variety ‘Lala star’, 7 days for varieties ‘Van’ and ‘Black star’ and 8 days for varieties ‘Early Burlat’ and ‘Sunburst’. Variety ‘Lala star’ in 2011 had the shortest ripening period (6 days), followed by varieties Early Burlat, ‘Lala star’ and ‘Sunburst’ (7 days) and variety ‘Black star’ (8 days).

Results of variance analyses for duration of harvest period of all five observed cherry varieties are presented in Table 2.

Tab. 2. Variance analyses for of duration of harvest period (days) observed cherry varieties in 2010 and 2011 with LSD test

ANOVA						LSD test (LSD <sub>0.05</sub> = 0.68)	2010	2011
Source	Deviance	Df	Variance	Fexp	Fcrit <sub>0.05</sub>	‘Early Burlat’	8a	7b
Variety	4.60	4	1.15	7.67*	6.39	‘Van’	7b	7b
Year	0.40	1	0.40	2.67	7.71	‘Black star’	8a	8a
Error	0.60	4	0.15			‘Lala star’	6c	6c
Total	5.60	9				‘Sunburst’	8a	7b

Results of variance analyses presented in the Table 2. indicate that variety had statically significant influence on different durations of harvest period, while this indicator was not statistically influenced by the year of monitoring. In the accordance with the variance results, it was necessary to test significance of differences between different harvest periods of examined cherry varieties.

In the accordance with those results, in those ecological conditions, harvest period had duration of 8 days (varieties ‘Early Burlat’ and ‘Sunburst’ in 2010 and variety ‘Black star’ in 2011) and it was significantly longer from the harvest period that the other varieties had in both years. Besides to that, duration of harvest period of variety ‘Lala star’ in both years (6 days) was significantly lower from the duration of harvest period of all other varieties which had in both years 7 and 8 days.

## CONCLUSIONS

Based upon two years of research of phenological characteristics of introduced cherry varieties 'Early Burlat', 'Black star', 'Lala star', 'Van' and 'Sunburst', the following conclusions can be made:

- Flowering period of 'Van' variety was medium early, varieties 'Black star', 'Lala star' and 'Sunburst' medium late flowering.
- Varieties had equable flowering, by the order 'Early Burlat' → 'Van' → 'Black star' → 'Lala star' → 'Sunburst' in both research years.
- Time match during flowering phenophase of the varieties 'Black star', 'Lala star' and 'Sunburst' was not limiting factor for pollination, since they are self fertile varieties.
- The exception is the variety „Van“, as well as the standard variety Early Burlat which are cross-fertile, therefore it is necessary to take care about selection of the pollinator varieties. This research shows that this was a well selected variety composition with matched flowering phenophases between examined varieties.
- According to the period of fruit ripening observed cherry varieties belong to the varieties of late early ('Van') and medium late ripening ('Black star', 'Lala star' and 'Sunburst').
- Duration of harvest period for examined cherry varieties was 6 – 8 days for all varieties, though variety 'Lala star' in both years (6 days) was statically significantly lower to the harvest period of all the other varieties had in both years (7 and 8 days).
- Examined cherry varieties were presented as adaptable on existing agro-ecological conditions of Čapljina and they can be recommended to farmers for commercial production.

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## THE IMPACT OF FERTILIZERS ON MICROBIAL DIVERSITY OF SOIL UNDER RASPBERRY

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*Original scientific paper*

### Summary

Microbial processes are crucial in nutrient cycling and transformation of organic matter in soil. Improvement of soil fertility by organic fertilizers utilization has always been a key principle of modern agriculture. Thus, the aim of this paper was to determinate the impact of different fertilizers on microbial diversity of soil under raspberry. A field trial was carried out in March of 2015 in Šantić village in Central Bosnia Canton (Bosnia and Herzegovina) in four fertilization variants: pigeon manure, sheep manure, pigeon manure+mineral fertilizer, and mineral fertilizer. Also, one variant of experiment was soil flooded in 2014. Control variant was unfertilized soil. Chemical characteristics, microbial and enzyme activity of soil was performed using the standard methodology. Samples have neutral pH, moderate to high humus content, low content of available phosphorus and moderate to high available potassium content. The number of microorganisms was higher in surface, especially in variant fertilized with pigeon manure, compared with subsurface layer and other variants in the soil. The lowest total number of bacteria and ammonification bacteria number was noticed in flooded soil. In variants with mineral fertilizer, low microbial and enzyme activity was detected. According to microbial number and enzyme activity, this research confirms the validity of organic fertilizers application in contemporary raspberry production.

Key words: *microbial diversity, raspberry, soil supplements*

### INTRODUCTION

Raspberry is one of the most profitable fruit, especially in some Balkan countries. Successful production of raspberry is often linked with several suitable conditions, such as climate factors in locality, presence of refrigerators, good transport of fresh fruits (Kljajić, 2012) etc. Because agricultural plants require nutrients for optimal growth, one of the methods for improvement of soil fertility is use of chemical fertilizers (Miransari, 2011). Although appreciable increase of organic carbon and available nutrients content in soil with increase in chemical fertilizers level was obtained (Sarkar, 1998), its overuse lead to the unfavorable environmental consequences (Yang *et al.*, 2009). Thus, alternative methods of soil fertilization were developed. One of these methods is introduction of organic amendments, which have a number of benefits (Mohammadi *et al.*, 2011). Organic manures increased organic matter of soil and total nitrogen content (Mann and Ashraf, 2000). Application of organic manure stimulate the development of specific microbial populations which plays important role in transformation of organic matter in soil (Lazcano *et al.*, 2012), followed with changes in microbial profil (Zhong *et al.*, 2010). Also, shifts in microbial populations structure were detected in anaerobic conditions caused from flooding (Mentzer *et al.*, 2006). Nevertheless, most plants and their microorganisms play a significant role in soil processes (Taylor *et al.*, 2009). In sustainable plant production, interactions between microorganisms and plants play a important role in mobilization and transformation of nutrients and its uptake by plants (Hayat *et al.*, 2010). Thus, the aim of this paper was to determined microbial populations structure of soil under raspberry treated with different fertilizers.

### MATERIALS AND METHODS

A field trial was carried out in Santic village (17°29'26.27" E, 44°16'44.62" N, altitude 757m) in Travnik municipality (Central Bosnia Canton, Bosnia and Herzegovina) on fluvisol in four variants: i) PM-pigeon manure (10t/ha); ii) SM-sheep manure (15t/ha); iii) PMF-pigeon manure+mineral fertilizer (10t/ha+400 kg/ha

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NPK 7:20:30 INA Kutina, Croatia); and iv) MF-mineral fertilizer (400 kg/ha NPK 7:20:30 INA Kutina, Croatia). Fifth variant was soil flooded in 2014 (FS). Control variant was unfertilized soil (C). Sampling of soil in surface (0-20 cm) and subsurface (20-40 cm) layer was performed in March of 2015. After preparing of composite soil sample from 10 separate samples, soil pH was determined using the digital pHmeter (ISO 10390, 1994), humus content by dichromate method (Mineev *et al.*, 2001), available K and P by Al method (Egner *et al.*, 1960), and carbonates by Scheibler calcimeter.

Total number of bacteria was determined using the 0.1x tryptic soy agar, ammonification bacteria on meat peptone agar, *Azotobacter* sp. and oligonitrophiles on Fyodor Agar, fungi on rose bengal streptomycin Agar (Peper *et al.*, 1995) and actinomycetes on starch-ammonia Agar. The total microbial number (CFU/g absolute dry soil) was calculated after drying of composite samples at 105°C for 2 h. Dehydrogenase (DH) activity was determined using the Casida *et al.* (1964) method, while phosphatase (PH) activity by method of Tabatabai (1994). All experiments were performed in triplicate.

## RESULTS AND DISCUSSION

The results showed that chemical and microbiological properties depend on the variants and depth of sampling. Except of PM and PMF variants, pH values (in H<sub>2</sub>O) were higher in subsurface compared with surface layer (tab. 1). Various humus content in samples was detected (from 1.19 to 5.14%). In most of samples, content of available phosphorus was low, while available potassium amount was high. Carbonates content was lower in more acidic samples.

Tab. 1. Chemical properties of samples

variants	depth (cm)	pH H <sub>2</sub> O	pH KCl	Humus (%)	P <sub>2</sub> O <sub>5</sub> (mg/100g)	K <sub>2</sub> O (mg/100g)	CO <sub>3</sub> <sup>2-</sup> (%)
PM	0-20	7.88	7.35	5.14	17.24	76.2	1.1
	20-40	7.75	7.25	3.48	2.04	16.8	1.1
SM	0-20	7.60	7.37	3.60	2.35	30.2	0.9
	20-40	7.75	7.27	1.58	2.30	40.0	0.7
PMF	0-20	7.77	7.37	3.79	8.93	25.1	0.9
	20-40	7.71	7.28	3.35	3.37	12.6	0.5
MF	0-20	6.82	6.38	5.04	10.77	42.1	0.3
	20-40	7.19	6.31	2.83	1.84	49.2	0.1
FS	0-20	6.90	5.80	1.19	1.02	9.2	0.0
	20-40	7.98	7.41	3.00	6.02	12.2	0.8
C	0-20	7.61	7.27	2.99	1.12	35.1	1.2
	20-40	8.10	7.58	1.84	1.84	17.9	1.1

Microorganisms play a very significant role in soil ecosystems (Nakhro and Dkhar, 2010). They can improve the characteristics of the environment and stimulate growth of agricultural plants (Sylvia *et al.*, 2005). As can be seen from presented data, number of all examined groups of bacteria was higher in surface compared with subsurface layer (tab. 2). Similar results have been reported previously by other authors (Bhattarai *et al.*, 2015). Presented data can be linked with favorable conditions in surface layer of most samples (higher humus content, available P and K content). Lowest total number of bacteria was recorded in FS variant (11,5x10<sup>5</sup> CFU/g absolutely dry soil), and highest in PM variant (45,1x10<sup>5</sup> CFU/g absolutely dry soil). Application of organic manures caused increase of total number of bacteria, while increase of mineral fertilizer amount caused decrease of microbial number. Similar result was obtained previously (Dabek-Szreniawska *et al.*, 2006).

Ammonification bacteria are non-specific microbial population and its number in terrestrial ecosystems is from 10<sup>5</sup> to 10<sup>7</sup>/g of soil (Alexander, 1977). Similar trend was reported in our research (from 3.2 to 22.7x10<sup>5</sup> CFU/g absolutely dry soil). Some authors suggested that organic practices have stimulative effects on abundance of ammonification bacteria (Lehocka *et al.*, 2008), which is in accordance with our results.

Amount of oligonitrophiles was lower compared with total number of bacteria and ammonification bacteria, which can be linked with composition of soil organic residues, primarily containing proteins. These proteins are more favourable substrate for ammonification bacteria compared with oligonitrophiles (Miletić *et al.*, 2012).

On the other side, our results showed that estimated number of *Azotobacter* sp. was from 1.13 to 19.23 CFUx10<sup>2</sup>/g. *Azotobacter* sp. is very sensitive to acidic pH values and this conclusion was confirmed in this research. Mazinani *et al.* (2012) suggested that highest number of *Azotobacter* sp. was detected in soils with pH from 7.0 to 7.4, which is similar with our findings (tab. 2).

Fungi play an important role in transformation of organic matter in soil ecosystems into suitable forms, nutrient cycling and disease suppression (Hoorman, 2011). Actinomycetes are also responsible for decomposition of organic compounds (Seong *et al.*, 2001).

Tab. 2. Number of bacteria in soil under raspberry

variants	depth (cm)	Total number of bacteria	Ammonification bacteria		oligoni-trophiles	<i>Azotobacter</i> sp.
			total	sporo-genous		
		CFU x 10 <sup>5</sup> /g absolutely dry soil				CFU x 10 <sup>2</sup> /g absolutely dry soil
PM	0-20	45.1	22.7	4.1	7.8	19.23
	20-40	27.0	11.3	2.3	3.5	10.08
SM	0-20	40.5	18.7	3.8	6.1	17.13
	20-40	15.7	8.9	1.9	3.0	8.12
PMF	0-20	37.0	14.3	2.2	3.9	10.66
	20-40	20.7	6.7	1.3	1.7	4.10
MF	0-20	24.1	7.8	1.1	2.1	3.66
	20-40	11.9	3.2	0.6	1.1	1.13
FS	0-20	11.5	6.8	1.6	3.2	7.36
	20-40	6.3	4.0	0.8	1.3	4.51
C	0-20	27.7	9.2	1.4	2.6	11.67
	20-40	12.8	4.3	0.7	1.2	4.61

In our research, number of fungi and actinomycetes was higher in surface compared with subsurface layer (tab. 3), which is in accordance with other authors (Rouag *et al.*, 2013). Highest number of fungi in MF variant was observed, while lowest number in PMF variant was detected. Fungal number in control variant was lowest compared with other variants, which was also concluded in previous research (Cwalina-Ambroziak and Bowszys, 2009). Actinomycetes show their optimum growth in alkaline environments (Basilio *et al.*, 2003), which is confirmed in this research (tab. 3). In FS and organic manure variants high number of actinomycetes was recorded, while in MF and C variants number of actinomycetes was lowest. Niewiadomska *et al.* (2010) suggest that mineral fertilization had no effects on number of actinomycetes.

Tab. 3. Number of fungi and actinomycetes in soil under raspberry

variants	depth (cm)	fungi	actinomycetes
		CFU x 10 <sup>4</sup> /g absolutely dry soil	
PM	0-20	3.2	7.5
	20-40	1.6	3.2
SM	0-20	2.9	6.1
	20-40	1.3	2.5
PMF	0-20	2.8	5.5
	20-40	1.1	2.3
MF	0-20	3.4	3.2
	20-40	1.4	1.5
FS	0-20	2.4	6.5
	20-40	1.4	3.2
C	0-20	2.3	3.8
	20-40	1.2	1.6

Dehydrogenase activity is commonly used indicator of soil biological activity (Burns, 1978). In surface layer, highest DH activity was recorded in PMF variant, which is in accordance with previous research (Šimon i Czako, 2014). In same layer, lowest DH activity was noticed in MF variant (tab. 4). On the other hand, higher values of DH activity in FS variant compared to non-flooded soils were recorded, which is discussed previously (Benckiser *et al.*, 1984).

Phosphatase are capable of catalysing hydrolysis of phosphoric compounds and play critical roles in the soil system (Dick *et al.*, 2000). Our results showed similar acid PM activity in surface layer in all variants. However, highest acid PM activity was recorded in variants with organic manures, as well as in control. Lemanowicz *et al.* (2014) also suggest that highest acid PM activity was noticed in treatments with organic manures compared to mineral fertilizers. In FS and PMF variants highest alkaline PM activity was observed, while in MF variant lowest activity of this enzyme was detected, which is in accordance with previous research (Okur *et al.*, 2009).

Tab. 4. Dehydrogenase (DH) and phosphomonoesterase (PH) activity in soil under raspberry

variants	depth (cm)	DH ( $\times 10^{-5}$ $\mu\text{g TPF/g/h}$ )	PH ( $\mu\text{g PNP/g/h}$ )	
			acid	alkaline
PM	0-20	1.75	4.09	3.55
	20-40	0.56	3.70	3.35
SM	0-20	1.48	3.92	3.57
	20-40	3.66	2.04	2.90
PMF	0-20	2.61	3.88	3.75
	20-40	2.17	3.91	4.02
MF	0-20	0.92	3.64	3.46
	20-40	1.58	3.45	3.65
FS	0-20	2.09	3.80	3.95
	20-40	1.80	3.90	0.57
C	0-20	1.92	4.10	3.62
	20-40	2.11	2.91	0.48

## CONCLUSIONS

These results showed that organic manures increased microbial activity of topsoil stronger than other types of used fertilizers. The lowest microbial activity was noticed in variants with mineral fertilizers, as well in flooded soil. We suggest that organic manures provides the more useful effects for soil quality and can be used in other locations in Bosnia and Herzegovina in order to increase the production of raspberry.

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## RESPONSE OF WINTER WHEAT TO LIMING WITH FERTDOLOMITE

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*Original scientific paper*

### Summary

The stationary field experiment with increasing rates (0, 5, 10, 20, 30, and 40 t ha<sup>-1</sup>) of liming material Fertdolomite containing 24.0% CaO, 16.0% MgO, 3.0% N, 2.5% P<sub>2</sub>O<sub>5</sub> and 3.0% K<sub>2</sub>O was set up in 2008 on the acid soil with in the central Croatia. The experiment was conducted in a randomized block design in four replicates. In the following years (2009 – 2014) on the experimental plots the usual agricultural practices was applied and the annual field crops were rotated. Aim of this study was testing the response of winter wheat (cultivar *Srpanjka*) on liming in the 2013/2014 growing season. Grain yield, some agronomic traits and wheat grain quality parameters were evaluated. Precipitation amount on the experimental area during winter wheat growing period (Oct.-June) was slightly lower than long term average, but monthly distribution of precipitation were unfavourable because of drought in December-March period and oversupply in April-May. Mean air-temperature were by 1.9°C higher than usual. Average grain yield of wheat in the experiment was 5.33 t ha<sup>-1</sup>, with range from 4.71 on the treatment without liming to 6.10 t ha<sup>-1</sup> on the 40 t ha<sup>-1</sup>. Application of 20 t ha<sup>-1</sup> increased yield by 15%, and additionally by 15% on the highest liming rate. The results showed that yield increase may be attributed to higher ear density on limed plots. Grain proteins content and sedimentation value raised at higher Fertdolomite rates. Response of wheat to liming was affected by the weather characteristics during growing season.

Key words: *liming, winter wheat, grain yield, weather characteristics*

### INTRODUCTION

Soil acidity is a widespread problem in the world and one of the very important limiting factors of reduced soil fertility and crops yield. In Croatia, acid soils cover an area of about 30% compared to the total agricultural land (Mesić *et al.*, 2009). Liming is a remedial procedure necessary in acidic soils to improve conditions for plants growth. Most common, problems associated with acid soils could be solved by liming. The most obvious benefits occur when lime applications increase soil pH, exchangeable cations, organic matter and improve soil biological activity (Andrade *et al.*, 2002; Soon and Arshad, 2005; Abd El-Azeem *et al.*, 2013; Brmež *et al.*, 2014). Liming considerably improve acid soil properties, especially regarding soil phosphorus accessibility as plant available phosphorus can raised several time with increased pH after liming (Rastija *et al.*, 2007; Rastija *et al.*, 2010a). Applying lime could have long-term effect on soil properties and crops yield (Tang *et al.* 2003; Bakina *et al.*, 2011; Antunović *et al.*, 2014). Combination of agrotechnical practice and liming could also have the influence on plants growth. Soon and Arshad (2005) conducted that combining liming with no-till resulted in a 4-year mean increase of 27% in grain yield of barley, rapeseed and field pea. However, effects of liming on plant growth and yield are commonly in the interaction with growing season and weather conditions (Rastija *et al.*, 2010b). Soil N transformations and fluxes can be also affected by soil pH and for example, increasing the pH of acid soils by liming resulted in increased N mineralization (Soon and Arshad, 2005). Even though application of liming materials is considering as a soil amendment, not a type of fertilization, Fertdolomite contains a certain share of macronutrients as N, P and K, which have positive influence on plant growth.

The aim of this study was testing the response of winter wheat in the 2013/2014 growing season to Fertdolomite application. The other relevant information regarding management of the experiment were elaborated in previous studies (Kovacevic *et al.*, 2015a, 2015b).

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## MATERIAL AND METHODS

The stationary field experiment with increasing rates of liming material Fertdolomite (trade name) containing 24.0% CaO, 16.0% MgO, 3.0% N, 2.5% P<sub>2</sub>O<sub>5</sub> and 3.0% K<sub>2</sub>O were set up in 2008 on the acid soil in the central Croatia (45°30' N, 17°11' E). Fertdolomite was applied in the following amounts: 0, 5, 10, 20, 30, and 40 t ha<sup>-1</sup>. The experiment was conducted in a randomized block design in four replicates. The size of basic plot was 40 m<sup>2</sup>. In the following years (2009 – 2014) on the experimental plots the usual agricultural practices was applied and the annual field crops were rotated. Soil sampling was performed in the autumn of 2013, before soil fertilization and preparing for winter wheat sowing. Soil pH (in 1M KCl) was determined according to ISO (1994) and plant available phosphorus by AL-method (Egner *et al.*, 1960).

Area of 1.0 m<sup>2</sup> was harvested from each plot for determinations of wheat grain yield and ears number. Protein, starch, wet gluten and sedimentation value were determined by Near Infrared transmission spectroscopic method on Foss Tecator Infratec 1241 Grain Analyzer. The data were statistically analysed by ANOVA and treatment means were compared using t-test and at 0.05 probability level.

Tab. 1. Monthly precipitation (mm) and mean air temperatures (°C) in the winter wheat growing season 2013/2014 on the experimental area compared to the long term mean 1961-1990 (LTM)

Month/ Year	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Total
	Monthly precipitation (mm)									
2013/2014	22	122	1	43	8	37	123	191	54	601
LTM	64	82	66	55	49	58	77	86	99	635
	Monthly mean air-temperatures									Mean
2013/2014	13.0	6.7	2.9	4.7	5.4	9.1	12.3	14.9	19.6	9.8
LTM	10.9	5.8	1.4	-0.4	2.1	6.2	11.0	15.7	18.9	7.9

For the interpretation of weather conditions during growing season, meteorological data from station near experimental area were used (SHS, 2014). Precipitation amount during winter wheat growing period (October-June) was slightly lower than long term average (601 mm vs. 635 mm), but monthly distribution of precipitation were unfavourable because of drought in the first part of season and oversupply in the spring. In December-March period there were 91 mm or only 40% compared to long-term mean (228 mm), but precipitation in April-May were 314 mm or by 70% higher, while temperature was by 3.2°C higher (Table 1). Mean air-temperature during whole season were by 1.9°C higher than usual.

## RESULTS AND DISCUSSION

Soil analysis showed that liming considerably improved soil chemical properties five years after Fertdolomite application. The soil pH increased from very acid (4.98) to the adequate values of 6.16 at 20 t ha<sup>-1</sup> and 6.48 at 40 t ha<sup>-1</sup>. As a result, phosphorus availability was enhanced from very low level (6.54 mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup>) at the treatment without liming to a high value of 31 mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> at the highest liming rate.

Liming significantly affected wheat ears number and consequently grain yield. The highest yield was attained on the highest liming rate, but moderate amounts was also effective (Table 2). Average wheat grain yield in the experiment was 5.33 t ha<sup>-1</sup>, with differences among treatments from 4.71 (no liming) to 6.10 t ha<sup>-1</sup> (40 t ha<sup>-1</sup>). For significant yield increase of 15% it was necessary 20 t ha<sup>-1</sup> of Fertdolomite, while the highest rate increased yield additionally by 15% or by 30% compared to the control. Yield increase is mainly result of significant ear density increases from 564 to 732 ears per square m (Table 2). Many authors reported about high relationship between wheat yield and soil pH improving after liming.

Response of wheat to liming is considerably influenced by the weather characteristics during growing season, mainly by precipitation amounts and air temperatures. In general, according to our experiences, moderately lower precipitation, particularly in autumn/winter period and mild winter are favourable for winter wheat growing (Marijanovic *et al.*, 2010; Pepo and Kovacevic, 2011). In the 2013/2014 growing season unusually high amount of precipitation was recorded in April and especially in May (Table 1), during stem elongation and ear formation, what could reflect on yield components formation. Moreover, in such wet conditions increased incidence of some diseases was recorded.

In the 2011/2012 growing season also wheat was grown in this field experiment and by the 20 t ha<sup>-1</sup> and higher Fertdolomite rates wheat grain yields were slightly decreased compared to the control. This may be associated

with too high ears densities and water deficit in tillering and stem elongation phase of wheat (Kovacevic *et al.*, 2015b).

Liming effect on quality parameters of wheat grain were either without impact, as in case with test weight and wet gluten content, or with somewhat lower effect compared to those on the yield. By the application of 20 and more tones of Fertilomite per hectare, protein content in grain was significantly increased above 12%. Also, sedimentation value was increased by the higher rates of Fertilomite. There is generally an inverse relationship between grain yield and protein contents, but in our study were increased both, yield and protein contents (Table 2).

Tab. 2. Residual impact of liming with Fertilomite on winter wheat grain yield, ears density and some grain quality parameters

Fertilomite (t ha <sup>-1</sup> )	Grain yield (t ha <sup>-1</sup> )	Ears density (ears/m <sup>2</sup> )	Test weight (kg)	Thousand grain weight (g)	Proteins (%)	Starch (%)	Wet gluten (%)	Sediment. value (ml)
0	4.71	564	73.0	32.2	11.3	70.5	24.9	31.3
5	4.91	532	73.3	31.7	11.4	71.2	23.8	30.4
10	5.17	605	73.2	31.3	11.8	70.3	25.4	32.4
20	5.40	602	73.7	31.5	12.2	70.1	26.3	33.7
30	5.67	631	73.9	31.4	12.1	70.1	25.2	32.2
40	6.10	732	73.1	30.4	12.2	69.5	26.0	33.3
Mean	5.33	612	73.4	31.4	11.8	70.3	25.3	32.2
LSD <sub>0,05</sub>	0.51	65	ns	1.6	0.7	ns	ns	2.3

In the previous six years of the experiment (Kovacevic *et al.*, 2015a, 2015b), maize was grown in four years (2008, 2010, 2011, 2013) and in remaining two years spring barley (2009) and winter wheat (2011/2012). The growing season characteristics, mainly precipitation and temperature regimes, considerably affected maize yield (Kovacevic *et al.*, 2013) and efficiency of Fertilomite. For example, maize yield in 2010 and 2011 were increased up to 12% and 9%, respectively, while in 2013 yield were increased up to 17%. However, in the first year of testing, application of the highest Fertilomite decreased grain yield 9% due to overliming.

Liming alone or in combination with phosphorus fertilization is mainly usual management practice contributing to increases of field crop yields on acid soils in Croatia as well as in neighbouring Bosnia and Herzegovina (Markovic *et al.*, 2008; Komljenovic *et al.*, 2010, 2013; Kovacevic and Rastija, 2010; Andric *et al.*, 2012; Kovacevic and Loncaric, 2013). Tang *et al.* (2003) reported that re-liming after 15 years of once limed soil have positive effect on barley biomass and grain yield.

## CONCLUSION

Residual effects of liming on wheat grain yield were ascertained. Average grain yield of wheat was 5.33 t ha<sup>-1</sup>, with differences among treatments from 4.71 to 6.10 t ha<sup>-1</sup>. Higher yield could be attributed to ear density increasing. The highest yield was achieved on the highest liming rate, but results showed that moderate amounts of liming materials were also effective five year after application. Liming also improve some bread-making quality parameters, as grain proteins content and sedimentation value raised at higher Fertilomite rates. However, Fertilomite application didn't affect test weight and content of starch and wet gluten. The results also showed that liming effects are under great influences of environmental conditions.

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## DISTRIBUTION OF JAPANESE KNOTWEED (*Reynoutria japonica* Houtt.) IN THE CITY OF SARAJEVO

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*Original scientific paper*

### Summary

Japanese knotweed (*Reynoutria japonica* Houtt.) is a perennial herbaceous plant of the family Polygonaceae with annual, glabrous stems that rapidly arise from strong rhizomes to form a dense thicket. It was introduced in Europe in 1825 as an ornamental, but has rapidly spread and today it is one of the most troublesome weeds in many European countries. Japanese knotweed forms dense stands that shade and crowd out native vegetation, representing a serious threat to the biodiversity, and is regarded as one of the most invasive plant species in Europe. The literature sources on this species in area of the city of Sarajevo are very scarce, so the aim of this paper is to record the sites where Japanese knotweed is present, in order to be able to monitor its populations in the future.

Key words: *Reynoutria japonica*, invasive species, distribution, Sarajevo

### INTRODUCTION

Japanese knotweed (*Reynoutria japonica* Houtt., Polygonaceae) is a perennial herbaceous dioecious plant native to Japan, Korea, northwest China, Taiwan and Vietnam (Ainsworth & Weiss, 2002). It was introduced in England in 1825 as an ornamental plant for hedges, but has rapidly spread in other parts of Europe, where it was grown in parks and gardens (Bailey & Conolly, 2000). By the end of 19<sup>th</sup> century it became naturalized in most European countries (Alberternst & Böhmer, 2011). Today, Japanese knotweed is one of the most troublesome weeds (Townsend, 1997; Novak & Kravarščan, 2011) and is listed by the World Conservation Union as one of the world's worst invasive species. This plant grows extremely fast and forms dense stands, which shade and crowd out native vegetation representing a serious threat to the biodiversity (Tokarska-Guzik *et al.*, 2005; Novak & Kravarščan, 2011). It also has very strong, thick rhizomes, which are very deep, can grow over 1 m during one season and damage urban infrastructure by displacing foundations, walls, pavements and drainage works (Johnson, 2006). Japanese knotweed is mostly found in large groups along roads, railway tracks, in disturbed areas and especially along watercourses (Lodeta & Novak, 2010; Alberternst & Böhmer, 2011). This plant can easily tolerate a wide variety of environmental conditions, including wide range of soil types, pH, salinity, drought and shade.

Although it was believed that all European populations have developed from a single female plant cloned for horticultural production, several authors (Hruškova & Hofbauer, 1999; Forman & Kesseli, 2003; Bram & McNair, 2004) have proved that Japanese knotweed produces viable seeds. However, vegetative reproduction by pieces of rhizome or stem is far more common in non-native range (Alberternst & Böhmer, 2011).

Japanese knotweed mostly spreads by garden waste, or moving soil contaminated by rhizomes from one location to another, especially during construction or similar works. The eradication methods include frequent mowing in order to weaken the plant, burning and herbicide application, although two latter methods may not be recommended in urban areas or near watercourses.

There are no detailed data on distribution of Japanese knotweed in the city of Sarajevo, so it was impossible to follow the dynamics of its spread and development over past years.

The aim of this paper is to map populations of *Reynoutria japonica* Houtt. in urban area of city of Sarajevo in order to ensure its monitoring and control in the future.

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## MATERIALS AND METHODS

The research was conducted in urban green spaces of city of Sarajevo during vegetation period (May to September) of 2014 and 2015. It was attempted to cover as much area as possible, so the survey covered green areas along roads, tram and railway tracks, construction sites and other disturbed sites, banks of Miljacka river, public green spaces (parks, avenues, schoolyards, green spaces around buildings). Gardens of private houses in peripheral areas of the city were not completely surveyed due to tall walls and fences around them and not being able to contact the owners. All *Reynoutria japonica* stands were photographed, mapped and assigned rough size classes (0-10 m<sup>2</sup>, 10-50 m<sup>2</sup> and >50 m<sup>2</sup>).

## RESULTS AND DISCUSSION

During this research, a total of 190 *Reynoutria japonica* stands were recorded in urban area of city of Sarajevo (Table 1). Most of them were found in western part of the city, in municipality of Novi Grad, followed by municipality of Novo Sarajevo, then Centar, and the smallest number of established stands was found in municipality of Stari Grad, in the eastern part of the city.

Tab. 1. The overview of number and size of *Reynoutria japonica* stands in Sarajevo in 2014 and 2015

Municipality	Approximate stand size			TOTAL
	0-10 m <sup>2</sup>	10-50 m <sup>2</sup>	>50 m <sup>2</sup>	
Novi Grad	73	43	8	124
Novo Sarajevo	30	12	3	45
Centar	12	2	0	14
Stari Grad	7	0	0	7
<b>TOTAL</b>	<b>122</b>	<b>57</b>	<b>11</b>	<b>190</b>

However, the area of Stari Grad was the least explored (Figure 1), because of prevalence of small family houses, often surrounded by tall walls and fences. This also applies to northern parts of Novo Sarajevo and Centar, so it is likely that *Reynoutria japonica* is more common than this research has showed.



Fig. 1: Surveyed area and position of registered *Reynoutria japonica* stands

In Novi Grad municipality, most colonies of *Reynoutria japonica* were registered in Dobrinja, Švrakino selo, Alipašin Most and Otoka. In most cases the plant was found in disturbed places and construction sites, along roads (especially in Safeta Hadžića street in Švrakino selo and Džemala Bijedića street in Alipašin Most) and on the banks of Miljacka river, in sunny locations.

In Novo Sarajevo municipality the largest stands were found along Drinska street, on both sides of Vilsonovo šetalište: along Miljacka river (Ars Aevi bridge) and between Elektroprivreda and Hotel Bristol, although those plants were growing in shade under *Tilia cordata* Mill. trees.

Only 14 *Reynoutria japonica* stands were registered in Centar municipality, most of them around Miljacka river near Suada and Olga bridge and several in private gardens in Skenderija. The small number of *Reynoutria japonica* stands in this municipality is probably due to good maintenance of green spaces around public buildings.

In Stari Grad municipality, on the eastern part of the city, Japanese knotweed was found only in bank of Miljacka river near Bentbaša, but most properties in this municipality are small private gardens enclosed by tall walls, which were only partially surveyed.

Generally, it was observed that the plants were more developed (i.e. the stands were larger) in sloped grounds than in flat ones, probably because of underground water which hinders development of the rhizomes. The plant was frequently mowed in several locations in the city, but has quickly regenerated. However, no damages of urban infrastructure due to activity of *Reynoutria japonica* plants were observed.

During this research there were four sites in which the plants were not registered in 2014 but have appeared in 2015: in Zagrebačka street near Suada and Olga bridge in Centar municipality, in Botanical Garden of National Museum of Bosnia and Herzegovina, and in two locations in Dobrinja neighbourhood in Novi Grad municipality near public garage in Trg grada Prato street and in Park prijateljstva in Sulejmana Filipovića street. Since there were no construction or other soil-moving works performed in those locations during the past year, and the Botanical Garden has been closed for public since 2012, it can be assumed that those plants have developed from seeds. It may indicate either that the populations in Sarajevo do produce viable seeds, as Forman & Kesseli (2002) have showed that germination of *Reynoutria japonica* seeds does occur in and is not rare, or that those plants are in fact bohemian knotweed, *Polygonum × bohemicum* (Chrték & Chrtková) Zika & Jacobson, a fertile hybrid between *Reynoutria japonica* Houtt. and *Reynoutria sachalinensis* (F.S. Petrop.) Nakai in T. Mori. Bohemian knotweed was first described in Czech Republic in 1983, and it is currently present in number of European countries, but there is no data for Bosnia and Herzegovina or any other country in the region, except Croatia (EPPO Global Database, 2014).

In order to develop effective eradication measures of this invasive alien, it is necessary to conduct more detailed research and test seed germination of plants found in Sarajevo. If the seeds prove to be viable, the flowering plants should be cut before the formation of achenes, but the focus should still remain on removal of vegetative growth.

## CONCLUSIONS

- During the research conducted in urban green spaces of city of Sarajevo a total of 190 *Reynoutria japonica* stands were recorded, mostly in municipality of Novi Grad, followed by municipality of Novo Sarajevo, then Centar, and the smallest number of established stands was found in municipality of Stari Grad, which was the least explored, so it is likely that *Reynoutria japonica* is more common than this research shows.
- Most stands (122) were smaller than 10 m<sup>2</sup>, 57 of them covered an area of 10-50 m<sup>2</sup>, and 11 stands were larger than 50 m<sup>2</sup>.
- In most cases *Reynoutria japonica* was found in disturbed places and construction sites, along roads and on the banks of Miljacka river, in sunny locations, but some were also well developed in shade. The plants were more developed in sloped than in flat grounds.
- There were four sites in which the plants were not registered in 2014 but have appeared in 2015, which may indicate either that the populations in Sarajevo produce viable seeds, or that those plants are in fact *Polygonum × bohemicum*.
- In order to develop effective eradication measures of this invasive alien, it is necessary to conduct more detailed research and test seed germination of plants found in Sarajevo. If the seeds prove to be viable, the flowering plants should be cut before the formation of achenes, but the focus should still remain on removal of vegetative growth.

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## NUTRITIONAL CHARACTERISTICS OF KALE POPULATIONS FROM THE HERZEGOVINA REGION

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*Scientific paper*

### Summary

Kale is vegetable crop with high biological, nutritional and health values. Due to the high consumption of kale in Herzegovina region, where the large number of local populations are traditionally cultivated, chemical analyzes of leaf material have been carried out. To set up the field trial, the seed obtained from isolated self-fertilized inflorescence of collected original kale populations is used. Leaves were sampled in five harvests on five representative plants of each population. Analytical methods have been determined the dry matter content, ash, fat, protein, total and natural sugars and cellulose. Statistical analysis of the data showed that there are significant differences in certain researched quality characteristics between self fertilized generations in compare to original populations. The results certainly contribute to a better knowledge of the nutritional properties of our native species of vegetables.

*Key words: kale, nutritional characteristics, original populations, self fertilized generations*

### INTRODUCTION

Kale is considered as one of the highest-quality brassicas in terms of chemical composition. Younger and more tender leaves of kale are used for human consumption and older leaves as fodder crop (Cartea *et al.*, 2002). Kale has a long tradition of cultivation and has an important place in the diet of the population in the northern parts of Turkey (Ayaz *et al.*, 2006), on the Pyrenean Peninsula (Velasco *et al.*, 2007), southeast of the USA (Olson and Freeman, 2007), and insular and coastal parts of Croatia (Batelja *et al.*, 2009). Based on the levels of protein and calcium in leaf, this vegetable with the common name Galega kale was assumed to be a substitute of milk in the poorest rural areas of Portugal (Monteiro and Rosa, 2008). Vilar *et al.* (2008) selected two kale landraces with the highest vegetable yield and the best nutritional qualities for human consumption, which were significantly better than the best commercial varieties. Compared to other types of vegetables, kale has a higher content of beta carotene and lutein (USDA, 2002). Kale leaves are rich in amino acids (Lisiewska *et al.*, 2008). In the Herzegovina region, kale is certainly the best known brassica with many local populations and is an indispensable part of the traditional gastronomy. Previously described Herzegovinian kale populations showed a high variability in individual morphological and agronomic traits (Sefo *et al.*, 2011; Knezovic *et al.*, 2013).

### MATERIALS AND METHODS

A field trial was carried out by the method of complete random design at the Jasenica site near Mostar. Seeds of sixteen kale populations originating from different parts of the Herzegovina region were used as the material. Seeds of the populations are the progeny obtained from isolated self-fertilised inflorescences of the original populations collected in the previous year at the Buna site, where an analysis of morphological and agronomic properties was carried out. Fifteen plants of each population in the phase of 4-5 true leaves were transplanted at the inter-row spacing and inter-plant spacing within the row of 60 cm. Five harvests were carried out on five representative plants of each population. One most typical leaf was saved from each representative plant from each harvest, of which an average sample of five leaves was made for each plant. The following nutritional and chemical properties were analyzed on samples of plant material using the analytical methods: the content of dry matter (by drying in a thermostat at the temperature of 105°C), ash (by burning in a muffle furnace at the temperature of 600°C), proteins (using the Kjeldahl method), total and natural sugars (Luff-Schoorl method), fat (Weibull-Stoldt method) and cellulose (Gravimetric method). T-test was used to evaluate the significance of

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differences in qualitative properties between the average values of the tested initial populations and progeny produced by self-fertilization grown at two sites.

## RESULTS AND DISCUSSION

In order to contribute to the knowledge of the nutritional value of our native species *Brassica oleraceae* L. var. *acephala*, chemical analyses of leaves were carried out. The results are also the first exact indicators of chemical properties of Herzegovinian kale populations (Table 1).

Tab. 1. Chemical analyses of kale leaf (%)

Traits	Mean and range of 16 local populations (P1-P16)
Dry matter	11.85 (10.85-12.89)
Fat	1.65 (0.87-2.77)
Protein	20.62 (15.00-24.56)
Cellulose	13.00 (7.40-18.62)
Natural sugar	4.75 (3.45-6.88)
Total sugar	6.81 (3.98-10.18)
Ash	13.82 (12.69-14.81)

As shown in Table 1, the dry matter content was from 10.85% (P4) to 12.89% (P6) with the average of 11.85% for all populations. The average protein content in all populations was 20.62% and the values of protein content ranged from 15.00% (P5) to 24.56% (P12). The content of cellulose was from 7.40% (P8) to 18.62% (P6), while the average cellulose content for all the populations was 13.00%. The ash content was from 12.69% (P11) to 14.81% (P7) with the average of 13.82% for all populations. The lowest content of total sugars was 3.98% (P4), and the highest value was 10.18% (P12). The average content of total sugars for all populations was 6.81%. Natural sugars were represented from 3.45% (P4) to 6.88% (P5) with the average of 4.75% for all populations. The average content of fat was 1.65%, and the values ranged from 0.87% (P6) to 2.77% (P3). Comparison of the results obtained before and after self-fertilization was carried out i.e. data from the Buna site, where initial originally collected populations were grown in the previous year, and the Jasenica site, where the first generation of progeny after self-fertilization was grown, were compared (Table 2), and the significance of differences in qualitative properties between the average values of the populations at two locations was evaluated by a t-test. The results of t-test ( $p < 0.01$ ) showed that there were significant differences in the content of dry matter, ash, protein and total sugar (Table 3).

Lower average values of the content of protein, fat, natural and total sugars in the progeny obtained after self-fertilization can be explained by inbreeding depression because kale is an allogamous plant, but also by climatic conditions during the test. Similarly, significantly lower values of most of the studied agronomic and morphological properties in relation to the plants of original populations were determined on plants of the same populations grown from seeds produced by self-fertilization of isolated inflorescences (Sefo *et al.*, 2011).

Tab. 2. Mean values of chemical characteristics of kale leaf in original populations and self fertilized generations (%)

Chemical characteristics	Dry matter	Ash	Fat	Protein	Cellulose	Natural sugar	Total sugar
Original populations	10.50	12.78	1.69	22.36	12.80	5.43	13.97
Self fertilized generations	11.85	13.82	1.65	20.62	13.00	4.75	6.81

Chemical characteristics, primarily the content of dry matter, sugars and proteins, depend on climatic conditions. The amount of proteins in brassicas increases in years with higher precipitation, while the amount of sugars increases in dry conditions (Pavlek, 1978). Average values of the dry matter content (10.50%) were lower in kale leaves of the original material in relation to the dry matter content (11.85%) in the progeny produced by self-fertilization. Slightly higher average temperatures (16.1°C) and lower precipitation sum values (1167 l/m<sup>2</sup>) were measured during the test with progeny produced by self-fertilization, which lead to an increased content of dry matter.

Tab. 3: t-test for the studied traits of the original populations of kale (the Buna site) and self fertilized generations (the Jasenica site)

Trait	Dry Matter	Ash	Fats	Proteins	Cellulose	Total sugars	Natural sugars
t <sub>exp</sub>	8.18 **	3.27 **	0.47 n.s.	2.75 *	0.3 n.s.	11.35 **	1.78 n.s.
t <sub>tab</sub> 5%	2.13	2.13	2.13	2.13	2.13	2.13	2.13
t <sub>tab</sub> 1%	2.95	2.95	2.95	2.95	2.95	2.95	2.95
Sig.	0	0	0.65	0.015	0.77	0	0.09

It is the opposite with the content of proteins for which higher average values were established during the test with originally collected populations when higher precipitation was measured (1394.8 l/m<sup>2</sup>) i lower values average temperatures (15.2°C). Higher content of cellulose was found in all analyzed kale samples. Of all the brassicas, kale has the highest cellulose content, followed by cauliflower and cabbage (Pavlek, 1978). Two landraces of the *acephala* group had high content of protein (17.9% and 18.00%) (Vilar *et al.*, 2008). Also, a higher protein content in analyzed kale samples was established by Ayaz *et al.* (2006). According to the same authors, the content of total sugars, defined as the sum of fructose, glucose and sucrose, was 3.961 mg 100 g<sup>-1</sup> st.

### CONCLUSIONS

High content of proteins, cellulose, natural and total sugars was found in leaves of all Herzegovinian kale populations. Populations selected as the best are P3 with the highest fat content, P5 with the highest content of natural sugars, P6 with the highest content of cellulose, and P12 with the highest content of proteins and total sugars, which is considered positive in terms of nutrition quality.

Analyzing sixteen local kale populations that represent progeny produced by self-fertilization, lower mean values in relation to parent populations were found for most properties as a result of inbreeding depression and reduced vigor.

Selection of populations that are assessed as the best has a great practical significance for breeding procedures in order to obtain improved varieties of kale and other brassicas.

Certainly a significant contribution of the research is in raising awareness of the nutritional and health value of kale.

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## THE EFFECTS OF FERTILIZATION ON SUGAR CONTENT IN THE GRAPES AND ALCOHOL CONTENT IN THE WINES OF VRANAC VARIETY

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*Original scientific paper*

### Summary

Proper fertilization and nutrition of vines are among the important measures in modern viticulture that have impact on the quality of grapes and grape products. For the purpose of determining the effects of fertilization on sugar content in grapes and content of alcohol in wines made of *Vranac* variety of grape vine trial was set up at the site of Konjusi in the Mostar vineyard area. The trial was set in 4 variants with different fertilizer applications and doses of fertilizers and a control without fertilization. Production of experimental wines took place in a family winery in Stolac.

During the three-year research, sugar content in grapes was the highest in the variant III (22.00%) and the lowest in variant I (21.61%). These quantities of sugar in the grapes represented a good basis for the production of quality wines.

The averages by modalities of the experimental factor of fertilizing variant show that the largest content of alcohol in wines was found in variant IV (12.63 vol.%), and the smallest in variant II (12.16 vol.%). Based on the average amount of alcohol, wines made of the *Vranac* variety can be classified as high alcohol wines. The results obtained are consistent with the literature data where the main role in the content of alcohol in wine is played by the sugar content in grapes.

Based on the obtained results it can be concluded that the proper and well-balanced nutrition has a positive effect on the sugar content in the grapes and the content of alcohol in the wines of the *Vranac* variety.

Key words: *fertilisation, Vranac variety of grape, sugar content, alcohol content*

### INTRODUCTION

Grape vine in a perennial plant, which needs appropriate plant feeding for its proper growth, in order to give regular, high and quality yield of grape. Hanić *et al.* (2009) point out that proper fertilisation have influence on yield and grape quality, as well as on grape products. Jackson (1995) stated that quantity of sugar in mature grape might vary from 12% to 28%. The same author points out that variety of grape *Vitis vinifera* mostly reach sugar quantity from 20 % and more in the phase of their full mellowness. Average share of sugar in grape consists of glucose 47.6%, fructose 47.6%, saharose 3.1%, pentose 1.1%, pectin 0.6%. (Yair Margalit, 1997). Savić (2000) indicates that the sugar content in the must is one of the basic factor which has influence on higher or lower quantity of alcohol in future wine.

### MATERIALS AND METHODS

The experiment was established on the locality of Konjusi, in vineyard which had been planted in row growing system, with row distance of 2,8 m and distance between vines in the row of 1 m. Basic growing vine shape is Moser cordon, on which a short pruning applies in this vineyard. Research has been made on the *Vranac* variety, which had been ingrafted at the rootstock Berlandieri x Riparia Kober 5BB. Research has been performed for three years, in the field environment and laboratory conditions. Experiment has been set up on the basis of random sampling in 4 variants with doses of fertilizer and control variant without fertilizer application. Experiment comprised the following variants:

Variant I - conventional, standardised fertilisation – kemira 5 : 14 : 28= 210 g/vine plant (the month of January)

Variant II – fertirrigation (chrystal master fertilizer): in the growth phase of the shoots 10 - 15 cm master 20: 5: 10 = 7.5 g/m<sup>2</sup>, before flowering 13: 40: 13 = 20 g/m<sup>2</sup>, start developing berries 15: 11: 15 = 10 g/m<sup>2</sup>, discoloration berries 03: 37: 37 = 10 g/m<sup>2</sup>

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Variant III – conventional (kemira) + pholiar fertilisation (plantafol): plantafol 10: 54: 10 before flowering, 20: 20: 20 at the stage of development of the berries, 5: 15: 45 every 10 days to change color berries, 0: 25: 25 - phase color changes berries. Foliar fertilizers were applied at a concentration of 0.15%, and kemira 210 g/vine plant.

Variant IV – conventional (kemira) + pholiar (plantafol) + biometabolic fertilisation (megafol, benefit, kendal) kemira 5:14:28 = 210 g /vine plant, plantafol 10: 54: 10 before flowering, megafol and benefit - in the growth and development of the berries, kendal - during the growing season in order to lift the endogenous resistance of experimental plants. Foliar fertilizers were applied at a concentration of 0.15%.

Variant V – control option without application of fertilizer

While preparing wine out of grape variety *Vranac*, the classical approach in production of red wines with sunken cumin has been applied. The content of sugar in the must was determined by Oechsle's must-meter, and was calculated with Saleron's tables. Content of alcohol in wines, given in vol.%, was defined with method of picnometers. Calculation of variant analysis on the *Vranac* variety, as well as tests on differ importance (Tukey test) has been made in order to check the influence of analysed experimental factors on average sugar quantity in grape and quantity of alcohol in wine.

## RESULTS AND DISCUSSION

### Sugar content in must

Average sugar content in the must in *Vranac* variety was determined in years of research and with application of different options of fertilisation, presented in the table 1.

Tab. 1. Average sugar content in the must (%) in the years of research and the application of different variant

Variant	Year			
	2006	2007	2008	Average for the variant fertilization
I	21.15	21.28	22.40	21.61
II	20.93	22.15	22.00	21.69
III	21.08	22.80	22.13	22.00
IV	21.73	22.25	21.40	21.79
V-control	19.60	20.30	21.00	20.30
Average for the year	20.90	21.76	21.79	

According to data from the table 1., average content of sugar in the must of the variety *Vranac* range from 19.60% which was noted in 2006. in the control variant, until 22.80% which was noted in 2007. in the variant III. Averages per modalities of fertilisation variant experimental factor show that the highest content of sugar in the must was in the variant III (22.00%), and the lowest was in the variant I (21.61%).

Tab. 2. Analysis of variance of sugar content in the must during three years of research at different variant

VARIANT ANALYSIS						
Deviations		St.sl.	Variant	F <sub>exp</sub>	F <sub>teor 005</sub>	F <sub>teor 001</sub>
Source	Amount					
Year	10.217	2	5.109	<b>51.606**</b>	3.15	4.98
Variant	21.854	4	5.464	<b>55.192**</b>	2.52	3.65
Interaction	8.583	8	1.073	<b>10.838**</b>	2.10	2.82
Error	4.448	45	0.099			
Total	45.102	59				

Amounts of F indicators given in the table 2 shows that there is an influence of statistically high importance of analysed experimental factors in years of research and different fertilisation variants, but also their interaction on given different quantities of sugar in the must in the *Vranac* variety.

Tab. 3. Significance of difference between the average sugar content in expanding in the years of research and application of different variant (Tukey test)

Variant	Year			
	2006	2007	2008	Average for the variant fertilization
I	21.15 <sup>c</sup>	21.28 <sup>bc</sup>	22.40 <sup>ab</sup>	21.61 <sup>a</sup>
II	20.93 <sup>cd</sup>	22.15 <sup>ab</sup>	22.00 <sup>b</sup>	21.69 <sup>a</sup>
III	21.08 <sup>cd</sup>	22.80 <sup>a</sup>	22.13 <sup>ab</sup>	22.00 <sup>a</sup>
IV	21.73 <sup>bc</sup>	22.25 <sup>ab</sup>	21.40 <sup>bc</sup>	21.79 <sup>a</sup>
V-control	19.60 <sup>d</sup>	20.30 <sup>d</sup>	21.00 <sup>cd</sup>	20.30 <sup>b</sup>
Average for the year	20.90 <sup>b</sup>	21.76 <sup>a</sup>	21.79 <sup>a</sup>	
				W <sub>005 (V-G)</sub> =0.754
				W <sub>005 (G- )</sub> =0.579
				W <sub>005 (V- )</sub> =0.725

Test results given in the table 3. show that all applied fertilisation variants have obtained statistically significantly higher average content of sugar in the must compared to the control variant.

While analysing the results of the test of importance of modality combinations with experimental factors, the average content of sugar in the must of 22.80% from 2007. In the variant III should be distinguished, since it was statistically considerably higher compared to the average sugar content in the must obtained in all other combinations of modalities of experimental factors, except in comparison to the average sugar content from 2007. in the variants II and IV, and from 2008. in the variants I and III.

Results of the research are in correlation with researches of Avramov (1991), Savić (2000) and Cindrić *et al.* (2000), which that show the positive effects of fertilization on sugar content in grape. The *Vranac* variety, in respect to the sugar quantity in the must, belongs to the group of varieties suitable for production of quality and high quality wines. According to Radovanović (1986) varieties with sugar content in the must in amount of 20% to 23% are used for production of quality wines, while for the high quality wine varieties have the same sugar content, but also its balanced ratio with acids as well as pronounced organoleptic characteristics.

### Alcohol content

Grape of *Vranac* variety used for wine processing in experimental purposes had sugar in amount of 20.90% (2006) to maximum 21.79% (2008), which was a good basis for production of quality wine, taking into consideration the variety characteristics, locality and weather conditions during vegetation period. Average content of alcohol in wines from *Vranac* variety given in the years of research, and with application of different variants of fertilisation, is given in the table 4.

Tab. 4. Average content of alcohol (vol.%) in wines in years of research and with application of different variants of fertilisation

Variant	Year			
	2006	2007	2008	Average for the variant fertilization
I	12.89	11.84	12.36	12.36
II	12.27	12.10	12.10	12.16
III	12.71	12.62	12.18	12.50
IV	13.51	12.62	11.75	12.63
V-control	12.36	11.15	11.63	11.71
Average for the year	12.75	12.07	12.00	

According to the table 4, average content of alcohol in wines produced from the *Vranac* grape variety varied from minimum 11.75 vol.% as noted in 2008. in variant IV, up to maximum 13.51 vol.% as was noted in 2006 in the same variant.

Averages per modalities of experimental factors of fertilisation variants show that the highest content of alcohol in wine is given by the variant IV (12.63 vol.%), and the lowest is given by the variant II (12.16 vol.%). When comparison of all fertilisation variants with control variant without fertilisation is made, it could be concluded that there is higher content of alcohol is obtained in variants with fertilisation. Experimentally produced wines out of the *Vranac* variety, according to the table given by Nastev (1977) could be characterised as alcoholic strong wines with regard to average quantities of alcohol. Results obtained coincide with the data from the literature, where the quantity of sugar actually plays the main role in quantity of alcohol in wine.

Tab. 5. Analysis of variant of alcohol content in wines during three-years of research of different fertilisation variants

VARIANT ANALYSIS					
Deviations		St.sl.	Variants	F <sub>exp</sub>	F <sub>teor 005</sub>
Source	Amount				
Year	1.704	2	0.852	<b>5.164*</b>	4.46
Variant	1.539	4	0.385	2.335	3.84
Error	1.319	8	0.165		
Total	4.562	14			

Amounts of F indicators given in the table 5. shows that there is statistically considerable influence of analysed experimental factor of research years on given amounts of different content of alcohol in wines produced out of Vranac variety, while the influence of different fertilisation variants was not statistically significant.

Tab. 6. Testing of importance of differences between average content of alcohol in wines in years of research and by usage of different fertilisation variants (Tukey test)

Years of research	Average differences per years		
2006	0.74 <sup>x</sup>	0.68	0.00
2007	0.06	0.00	
2008	0.00		
W <sub>005(V-)</sub> =1.475			
W <sub>005(G-)</sub> =0.722			

As it could be seen from the table 6., wines from the 2006. harvest contain statistically considerably higher average amount of alcohol (12.75 vol%) compared to the wines from 2008. harvest (12.00 vol%), while other differences in average alcohol content per harvesting years were not statistically significant. In that sense, as the the analysis of variants showed, the variants of fertilisation did not have statistically considerable influence on given different average concentration of alcohol in wines. The results are consistent with literature data of similar experiments, where the main impact of the concentration of alcohol in the wine has a concentration of sugar in the grapes, which is determined by climate conditions in the years of research. (Zoecklein *et al.*, 2004).

## CONCLUSIONS

All applied fertilisation variants have obtained statistically considerable average sugar content in musts compared to the control variant without fertilisation. Content of sugar in must was the highest in the variant III (22.00%), and the lowest in the variant I (21.61%). These sugar amount in must represented a good basis for production of quality wines. Wines produced of the *Vranac* variety had a high content of alcohol and therefore belong to the group of alcoholic strong wines. In that respect, the highest quantity of alcohol of 12.63 vol.% was obtained from the variant IV, whilst the lowest quantity was from the variant II 12.16 vol,. On the basis of obtained results it could be concluded that the appropriate and well-balanced plant feeding has positive effects on sugar content in grape, and is considered as the primary factor which makes influence on the content of alcohol in the wine produced out of the *Vranac* variety.

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## COMPARATIVE RESEARCH OF NEWLY INTRODUCED STRAWBERRY CULTIVARS

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*Scientific paper*

### Summary

Strawberry production in Bosnia and Herzegovina in recent years has a tendency of high growth, with simultaneous introduction of a large number of new varieties. Thus, research of new genetic capacities of strawberry in local agro-ecological conditions is important. The aim of this study is to investigate and compare the newly introduced cultivars of strawberry: Alba, Asia, and Joly. Tests have been carried out in the area of Bihać, at the experimental plot of the Biotechnical Faculty. The following pomological characteristics have been examined: fruit weight (g), fruit width (cm), the thickness of fruit (cm), the height of the fruit (cm). Chemical properties of fruit have been investigated: the content of sugar, natural invert and total invert according to Luff-Schoorl, the content of total acidity by standard method (titration of NaOH), and the determination of water content in fruits and vegetables by drying method. Cultivar Joly had the highest average fruit weight (21.05 g), then Asia (19.95 g), and Alba (17.29 g). Cultivar Alba has the highest percentage of the total water (89.94%) and acidity (0.9%).

Cultivar Joly had the biggest quantity of dry matter (10.44%). Cultivar Asia stood out with the highest average content of natural invert (3.95%) and total invert sugars (5.67%). When it comes to morphometric characteristics, cultivar Joly proved to be the best, especially in the first round of the harvest. The most drastic decrease in morphometric values from I to III picking cycles was recorded in the cultivar Asia, and the lowest in cultivar Alba.

**Key words:** *strawberry, chemical properties, pomological characteristics*

### INTRODUCTION

Strawberry is a perennial herbaceous plant and the most important variety of berries. It is the first fresh fruit after winter, and is therefore extremely requested in the market and reaches a high price. Organoleptic properties of strawberry are specific, giving it a special significance as a savoury foodstuff in the modern diet. Strawberry fruit offers an abundance of antioxidant substances (Skender *et al.*, 2015). A strawberry is the fruit which is very successfully grown in a system of highly intensive technology, such as growth on foils, in greenhouses, vertical system in pots and hydroponics. Strawberry production is the only type of fruit production which is feasible in a fully controlled microclimate conditions (Skender *et al.*, 2012). The production of strawberry fruits in Bosnia and Herzegovina is increasing because it is highly profitable. Some old varieties are mainly used in the production, but new ones are being ever more introduced. It is very important to examine the morphological and chemical-technological properties of newly introduced varieties before their mass introduction into production. Therefore, the aim of this paper is to explore the most important properties of some new varieties of strawberry, that are being introduced in production in Bosnia and Herzegovina in the last few years, in order to offer a concrete recommendation to farmers who produce strawberry.

### MATERIALS AND METHODS

The three newly introduced strawberry cultivars—Alba, Asia and Joly—have been observed as a material for this paper. The paper examines the morphological parameters of the fruit of the three cultivars: fruit weight (g), fruit width (cm), the thickness of fruit (cm), as well as the height of the fruit (cm). Morphological analysis of fruit has been conducted for all three cycles of maturation. Thirty fresh strawberry fruits have been used for each cultivar and each maturation cycle. Furthermore, the chemical properties of fruit have been examined: sugar content, natural invert content, as well as the content of total invert according to Luff-Schoorl method. The content of total acidity has been analyzed using the standard method of titration using NaOH. The drying

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method has been used to determine water content in fruits (Skender *et al.*, 2012). The data obtained by measuring the morphological properties have been analyzed by ANOVA and Tukey test and chemical properties by ANOVA and Mann-Whitney test.

## RESULTS AND DISCUSSION

Tab.1. The results of analysing pomological properties of fruit of the studied strawberry cultivars

Cultivar/ the examined property	ALBA	ASIA	JOLY	ANOVA
Fruit weight (g)	17.29 <sup>a</sup> ± 6.94	19.95 <sup>ab</sup> ± 9.32	21.05 <sup>b</sup> ± 9.62	<b>P&lt;0.05</b>
Fruit height (cm)	4.04 <sup>a</sup> ± 0.61	4.33 <sup>b</sup> ± 0.82	4.02 <sup>a</sup> ± 0.54	<b>P&lt;0.05</b>
Fruit width (cm)	3.20 <sup>a</sup> ± 0.52	3.34 <sup>ab</sup> ± 0.70	3.43 <sup>b</sup> ± 0.69	<b>P≤0.05</b>
Fruit thickness (cm)	2.80 <sup>a</sup> ± 0.43	2.97 <sup>ab</sup> ± 0.52	3.10 <sup>b</sup> ± 0.66	<b>P&lt;0.05</b>

Average values marked with the same letter do not differ significantly according to Tukey test ( $P>0.05$ )

By analyzing the results using the Tukey test, a significant difference in terms of fruit weight, width and thickness has been noticed between Alba and Joly cultivars. We have not noticed significant differences neither between Alba and Asia cultivars, nor between Asia and Joly cultivars. In their two-year studies of different strawberry cultivars, Weissinger *et al.* (2010), registered a value of 15.87 g as the fruit weight of Alba cultivar, and 16.72 g for Asia cultivar, which are slightly lower values in relation to this research. Sylanaaj and Shala (2008) examined the newly introduced cultivars on the territory of Kosovo, and recorded the fruit weight of 18.54 g, the fruit height of 4.55 cm, and the fruit width 3.31 cm for Alba cultivar. Kiprijanovski *et al.* (2010) examined newly introduced cultivars in Macedonia, whereby they recorded the fruit weight of 18.1 g for Alba cultivar in the first year, and 14.5 g. in the second year of research, which are considerably lower values in relation to this research. In their studies of fruit weight in Asia cultivar, Virginie and Crespo (2010), recorded a value of 26.4 g, which is slightly higher value than the one in this research. In their three-year research of Alba cultivar, Mladin *et al.* (2008) recorded the average value of 3.74 cm when it comes to fruit height, and the average value of fruit width was 3.61 cm. Pešaković and Milivojević (2014) conducted studies of Joly cultivar in Serbia and recorded the following pomological results: fruit weight (29.46 g), fruit height (5.11 cm), and fruit width (4.36 cm).

Tab. 2. Descriptive statistics for the morphometric analysis of strawberry fruit

	A property	I CYCLE	II CYCLE	III CYCLE
Alba	Fruit weight (g)	20.05 ± 5.63	16.76 ± 7.84	15.08 ± 6.46
	Fruit height (cm)	4.33 ± 0.49	3.96 ± 0.72	3.84 ± 0.51
	Fruit width (cm)	3.43 ± 0.41	3.12 ± 0.55	3.05 ± 0.53
	Fruit thickness cm)	2.90 ± 0.35	2.72 ± 0.42	2.79 ± 0.50
	Asia	Fruit weight (g)	25.87 ± 8.39	21.85 ± 7.66
Fruit height (cm)		4.77 ± 0.45	4.65 ± 0.62	3.56 ± 0.73
Fruit width (cm)		3.80 ± 0.74	3.41 ± 0.44	2.82 ± 0.52
Fruit thickness cm)		3.29 ± 0.39	3.08 ± 0.35	2.55 ± 0.48
Joly	Fruit weight (g)	29.60 ± 10.36	18.04 ± 5.61	15.50 ± 5.29
	Fruit height (cm)	4.27 ± 0.49	4.07 ± 0.42	3.72 ± 0.56
	Fruit width (cm)	4.04 ± 0.68	3.19 ± 0.42	3.05 ± 0.48
	Fruit thickness (cm)	3.68 ± 0.49	2.86 ± 0.63	2.72 ± 0.37

According to the results obtained by the morphological analysis of strawberry fruit, we can see from Table 2 that Joly cultivar has the largest fruit weight (29.60 g) in the first picking cycle. Asia cultivar had the highest average fruit weight in the second picking cycle (21.85 g), and Joly cultivar showed the best results again in the third picking cycle (15.50 g). The most drastic decrease in morphometric values from I to III picking cycles was recorded in Asia cultivar, and the lowest in Alba cultivar.

Tab. 3. The results of analysing chemical composition of the studied strawberry cultivars

Cultivar/ the examined property	ALBA	ASIA	JOLY	ANOVA
Total water (%)	89.94 <sup>a</sup> ± 1.71	88.60 <sup>a</sup> ± 1.51	89.56 <sup>a</sup> ± 1.28	P>0.05
Dry matter (%)	10.06 <sup>a</sup> ± 1.71	10.24 <sup>a</sup> ± 1.51	10.44 <sup>a</sup> ± 1.28	P>0.05
Natural invert sugar(%)	3.69 <sup>a</sup> ± 0.13	3.95 <sup>b</sup> ± 0.10	3.73 <sup>a</sup> ± 0.17	<b>P&lt;0.05</b>
Total invert sugar (%)	5.30 <sup>a</sup> ± 0.16	5.67 <sup>b</sup> ± 0.16	5.56 <sup>c</sup> ± 0.17	<b>P&lt;0.05</b>
Acidity (%)	0.09 <sup>a</sup> ± 0.02	0.08 <sup>a</sup> ± 0.01	0.08 <sup>a</sup> ± 0.01	P>0.05

Average values marked with the same letter do not differ significantly according to the Mann-Whitney-test ( $P>0.05$ )

Asia cultivar has the highest percentage of natural invert sugar (3.95%), as well as total invert sugar (5.67%) (Table 3). The fruits of Alba cultivar had the highest acidity value (0.09%). According to Mann-Whitney-test, Alba, Asia and Joly cultivars do not differ significantly when it comes to these properties: total water, dry matter and acidity of the fruit ( $P>0.05$ ). When it comes to the observed parameter of natural invert sugar of strawberry fruit ( $P\leq 0.05$ ), there were significant differences between Asia, Alba and Joly cultivars. Test results for the parameter of total invert sugar indicate there was a significant difference between all three strawberry cultivars that have been studied ( $P\leq 0.05$ ), i.e. that the cultivar factor had a significant effect.

Studies of different strawberry cultivars have been conducted by Weissinger *et al.* (2010), whereby they recorded the value of 6.18% of total sugar for Alba variety, and 6.57% of total sugar content in Asia cultivar. Voća *et al.* (2008) examined the chemical composition of different strawberry cultivars in the area of Croatia and recorded the value of 8.22% of dry matter and 7.01% of total invert sugar in Alba cultivar. Similar studies have been conducted by Sylanaj and Shala (2008) in the area of Kosovo and they also obtained approximate values, dry matter (9.8%) and total inverts sugar (6.9%).

Kiprijanovski *et al.* (2010) examined the chemical composition of the newly introduced cultivars in the Republic of Macedonia, based on which they obtained results of total acidity of Alba cultivar, which was 8.4% in the first year of research, and was slightly lower (8.2%) in the second year. The values obtained by the aforementioned authors about acidity content are considerably higher than the results from this research. Virginie and Crespo (2010) examined the individual components of the chemical composition of new strawberry cultivars in the area of Switzerland and found that Asia cultivar had 11% of dry matter, which is slightly higher content from the results of this research.

Tab. 4. Descriptive statistics for chemical analysis of strawberry fruit

	The examined property	I CYCLE	II CYCLE	III CYCLE
	Alba	Total water (%)	91.45 ± 0.66	90.47 ± 0.93
Dry matter (%)		8.55 ± 0.66	9.53 ± 0.93	12.10 ± 0.62
Natural invert (%)		3.59 ± 0.11	3.71 ± 0.11	3.78 ± 0.11
Total invert (%)		5.09 ± 0.02	5.39 ± 0.11	5.40 ± 0.02
Acidity (%)		0.08 ± 0.01	0.11 ± 0	0.09 ± 0.01
Asia		Total water (%)	91.00 ± 0.49	90.45 ± 0.24
	Dry matter (%)	9.00 ± 0.49	9.55 ± 0.24	12.18 ± 0.39
	Natural invert (%)	3.84 ± 0	3.97 ± 0.11	4.03 ± 0
	Total invert (%)	5.60 ± 0.28	5.71 ± 0.08	5.71 ± 0.05
	Acidity (%)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Joly	Total water (%)	89.48 ± 0.34	90.74 ± 0.83	88.47 ± 1.37
	Dry matter (%)	10.52 ± 0.34	9.26 ± 0.83	11.53 ± 1.37
	Natural invert (%)	3.52 ± 0.11	3.84 ± 0	3.84 ± 0
	Total invert (%)	5.36 ± 0.14	5.68 ± 0.03	5.63 ± 0.05
	Acidity (%)	0.08 ± 0.01	0.10 ± 0.01	0.08 ± 0.01

## CONCLUSIONS

Joly cultivar has proved to be the best when it comes to morphometric properties, especially in the first picking cycle. The most drastic decrease in morphometric values from the first to the third picking cycle was recorded in Asia cultivar, and the lowest in Alba cultivar. When it comes to the chemical properties of the fruit, Joly cultivar proved to be the best, with the highest average content of dry matter, acid and sugar. Therefore, the main conclusion of this study is that Joly cultivar had the best results in both morphological and chemical properties of fruit, so it can be recommended to local strawberry growers.

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## GERMINATION OF JOHNSONGRASS (*SORGHUM HALEPENSE* L.) INFLUENCED BY VARIOUS DORMANCY BREAKING METHODS

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### Summary

Dormancy is defined as a period during which seeds are unable to germinate under a combination of environmental factors that are normally suitable for the germination of the non-dormant seeds. Johnsongrass (*Sorghum halepense* L.) is one of the weed species with proven seed germination issues. Seeds of different ages were grown in various light conditions and their germination was influenced by seven methods in order to determine the most effective one for dormancy breaking in Johnsongrass. Scarification with sulfuric acid, seed immersion in water for 24 hours, mechanical scarification and heating method showed the best results on older seeds germinated under a 16:8 light-dark regime in germination cabinets. When germinated under room conditions, only the sulfuric acid showed positive effects on seed germination. In average, better germination rates were achieved in germination cabinets.

Understanding the seed biology and ecological characteristics of weed species, as well as the various factors that influence germination and seed dormancy loss, is still very important in order to predict the time of weed seedling emergence and determine the best weed management strategies.

Key words: *Johnsongrass, weed germination, seed dormancy*

### INTRODUCTION

Seed dormancy is defined as a state in which seeds are prevented from germinating even under environmental conditions normally favorable for germination (Grbić, 2003; Janjić *et al.*, 2003). Freshly harvested seeds can be characterized by primary dormancy which is caused by different inhibitors in the seed-coat and the embryo (Šera *et al.*, 2009). Secondary dormancy can occur in some non-dormant and post dormant seeds that are exposed to conditions that are not favorable for germination (Baskin and Baskin, 2004). Dormancy is usually broken under environmental conditions favorable for seed germination, emergence and the normal development of the plant (Kojić and Šinžar, 1985).

Dormancy loss can be caused by temperature (Martinez-Ghersa *et al.*, 1997; Hoyle *et al.*, 2008; Lemić *et al.*, 2014), moisture (Yamasue *et al.*, 1992; Lemić *et al.*, 2014), light, carbon dioxide, oxygen (Gorai *et al.*, 2011; Hilhorst *et al.*, 2010), as well as different agrotechnical practices, such as soil cultivation (Janjić *et al.*, 2003).

Agricultural land contains millions of weed seeds and understanding the factors that influence the dynamics of weed seed bank can help in the development of integrated weed management principles (IWM) (Bares and Sardi, 1999). Efficient and sustainable weed management, focused on reducing levels of herbicide dependency, requires adequate knowledge about biology and ecology of weeds which is the key step in the IWM strategies development (Sonsnoskie, 2005; Loddo *et al.*, 2014). Taab and Andersson (2009) highlight that control measures application time in the integrated weed control should be synchronized with the emergence of weeds, because stimulation of germination of seeds can lead to rapid wear of weed seed bank if properly aligned with weeds control measures. The same authors state that this kind of suppression requires knowledge about changes in the dormancy of seeds and the conditions necessary for the germination of seeds. Soil cultivation in late spring and early summer can reduce the seed bank of many weed and decrease weed population to a desired level.

Johnsongrass (*Sorghum halepense* L.) is a perennial, xerophilous, heliophilous and thermophilic weed species (Dakić *et al.*, 2000). Dormancy of Johnsongrass seeds is the result of chemical inhibitors present in the pericarp as well as mechanical strength of the seed-coat (Warwick and Black, 1983; Šarić, 1987; Huang and Hsiao, 1987; Jian-shu *et al.*, 2001). Forecasting the occurrence of weed species on arable land is essential for planning of effective strategies for their control. Consequently, weed seed banks and factors influencing the emergence of weeds are studied intensely in agricultural science. Relying on this, the aim of this paper is to

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examine seed germination of Johnsongrass in different conditions of temperature and light, as well as to examine some of the treatments that cause loss of seed dormancy of this weed.

## MATERIALS AND METHODS

Johnsongrass seeds germination and seed dormancy examination was conducted in the Seed laboratory at the Faculty of Agricultural and Food Sciences in Sarajevo. This experiment included seeds of different ages (ones collected in 1999 and ones in 2008), which were dried, cleaned and stored in paper bags at a dark and dry place. For the purpose of germination and dormancy loss examination, the following treatments were applied:

- examining seed germination by a classical procedure (according to Regulations for the germination testing of cultural plants) – control;
- cold stratification (previous seed cooling at +4°C during 7 days);
- cold stratification (previous seed cooling at +4°C during 15 days);
- mechanical seed scarification (sandpaper);
- immersion in water during 24 hours;
- chemical treatment – using 0,2% KNO<sub>3</sub>;
- heating method (seeds were immersed in hot water (80°C), after which they are left to be cooled to room temperature);
- chemical treatment – using H<sub>2</sub>SO<sub>4</sub>.

Naked stratification method was applied for cold stratification lasting 7 and 15 days (Grbić, 2003), which implies that weed seeds are in plastic bags, slightly moistened and kept in a refrigerator at +4° C. All treatments were performed in four replications and under the influence of three different factors. *Temperature factor*: Tests in germination cabinet at day temperature of 27°C and night temperature of 17°C (12/12 hours). *Light/dark factor*: All treatments were performed in the presence and absence of light; testing in the dark was achieved by wrapping Petri dishes in aluminum foil. *Seed age factor*: The experiment examined wild sorghum seeds harvested in two different growing seasons, 1999 and 2008.

Petri dishes lined with filter paper were used for examination of seed germination. 30 seeds of wild sorghum were placed into each Petri dish, which were moistened with distilled water. Water was optionally added during the test period. Germination lasted for 15 days. After 15 days of germination, germinated seeds were counted. To test the difference in the average values of the number of germinated seeds, first the analysis of variance was made in order to test the stability of impact of factors "treatment", "light mode" and "seed age", after which a *post-hoc* test was carried out. Since "treatment" factor implied the control variant as well, it was necessary to establish whether there is a difference between the average values of monitored parameters obtained by applying the control and all the other variants of the aforementioned factor. The Dunnett's test was conducted for that purpose. Given the number of observed factors, the temperature factor variants were analyzed in the end, based on which conclusions were made whether the seeds of the studied weed germinated better at a higher (27°C) or lower (room) temperature.

## RESULTS AND DISCUSSION

Tab. 1. The number of germinated seeds of Johnsongrass in the germination cabinet.

	Year: 2008						Year: 1999					
	light	%	darkness	%	average	%	light	%	darkness	%	average	%
CV	1.25	4.17	0.50	1.67	0.87	2.90	0.75	2.50	0.00	0.00	0.37	1.23
CS7	3.50	11.67	1.50	5.00	2.50	8.33	4.25	14.17	3.50	11.67	3.87	12.90
CS15	1.50	5.00	2.25	7.50	1.87	6.23	6.75	22.50	2.75	9.17	4.75	15.83
MS	5.25	17.50	1.25	4.17	3.25	10.83	6.75	22.50	2.00	6.67	4.37	14.57
IW	3.25	10.83	2.00	6.67	2.62	8.73	7.25	24.17	3.25	10.83	5.25	17.50
KNO <sub>3</sub>	1.25	4.17	1.00	3.33	1.12	3.73	0.25	0.83	0.00	0.00	0.12	0.40
HM	7.75	25.83	1.50	5.00	4.62	15.40	2.75	9.17	2.00	6.67	2.37	7.90
H <sub>2</sub> SO <sub>4</sub>	17.00	56.67	17.00	56.67	17.00	56.67	25.25	84.17	26.75	89.17	26.00	86.67
Average light	5.92*											
Average darkness	4.20											
Average 2008	4.23											
Average 1999	5.89											

CV - control, CS7 - cold stratification 7 days, CS15 - cold stratification 15 days, MS - mechanical scarification, IW - 24 hours immersion in water, KNO<sub>3</sub> - 0.2% KNO<sub>3</sub>, HM - heating method (80° C), H<sub>2</sub>SO<sub>4</sub> - chemical scarification sulfuric acid

Wild sorghum seeds in the control variant germinated very poorly, from 0 to 1.25 germinated seeds. However, treatment with sulfuric acid affected the germination of a large number of wild sorghum seeds. The seeds from 1999 gave 89% of germinated seeds in the darkness, and same seeds in the light had a germination rate of 84%. Seeds from 2008 under the same treatment germinated at 57% in both light and darkness. Joint analysis shows that all observed factors (treatment, light regime and seed age) affect the number of Johnsongrass germinated seeds at the level of statistical significance. In further analysis one can conclude that only H<sub>2</sub>SO<sub>4</sub> treatment has very highly significant effect on the number of wild sorghum germinated seeds, IW treatment has highly significant effect, and MS and HM treatments have significant effect. In average, older seeds (seeds from 1999) and seeds germinated under light conditions showed a higher germination percentage than those collected in 2008 and seeds that were germinated without light presence, respectively. The use of sulfuric acid successfully terminates the wild sorghum seed dormancy (Huang and Hsiao, 1987; Salimi and Termeh, 2002). Mechanical scarification is a method that gave the best results in termination of wild sorghum seeds dormancy according to Krenchinski *et al.* (2015). Heating method has proved to be effective in investigations by Đikić *et al.* (2011), wherein the percentage of germinated seeds was 34.2% compared to control (2.5%). Submerging the seeds in water enables increase of permeability of the seed cover layer in species with pronounced physical dormancy (Grbić, 2003). Whether the seed cover layer is removed mechanically or treated with sulfuric acid, significantly higher percentage of germinated seeds was achieved when the seed layer is damaged. Removal of the seed cover layer or treating seeds with sulfuric acid, and then subjecting the seeds to temperatures higher than 20°C results in germination of most wild sorghum seeds (Sastry *et al.*, 2006).

In all variants (light/darkness, 1999/2008) only sulfuric acid significantly affected the number of germinated wild sorghum seeds (Table 2). Influence of seed age and the light regime on the number of wild sorghum germinated seeds in seeds germinated at room temperature, was observed only by H<sub>2</sub>SO<sub>4</sub> treated seeds. That treatment had better effect on seeds from 2008 that were germinated in the dark.

Tab. 2. The number of germinated seeds of Johnsongrass at room temperature.

	Year: 2008						Year: 1999					
	light	%	darkness	%	average	%	light	%	darkness	%	average	%
CV	0.25	0.83	0.25	0.83	0.25	0.83	0.25	0.83	0.00	0.00	0.12	0.40
CS7	1.00	3.33	0.25	0.83	0.62	2.07	0.75	2.50	0.50	1.67	0.62	2.07
CS15	0.50	1.67	1.00	3.33	0.75	2.50	0.50	1.67	1.00	3.33	0.75	2.50
MS	1.00	3.33	0.25	0.83	0.62	2.07	0.50	1.67	0.75	2.50	0.62	2.07
IW	1.50	5.00	2.25	7.50	1.87	6.23	0.25	0.83	0.50	1.67	0.37	1.23
KNO <sub>3</sub>	0.00	0.00	1.25	4.17	0.62	2.07	0.00	0.00	0.00	0.00	0.00	0.00
HM	0.75	2.50	1.00	3.33	0.87	2.90	0.25	0.83	1.00	3.33	0.62	2.07
H <sub>2</sub> SO <sub>4</sub>	7.75	25.83	13.50	45.00	10.62	35.40	6.75	22.50	10.75	35.83	8.75	29.17
Average light	1.37											
Average darkness	2.14*											
Average 2008	2.03*											
Average 1999	1.48											

CV - control, CS7 - cold stratification 7 days, CS15 - cold stratification 15 days, MS - mechanical scarification, IW - 24 hours immersion in water, KNO<sub>3</sub> - 0.2% KNO<sub>3</sub>, HM - heating method (80° C), H<sub>2</sub>SO<sub>4</sub> - chemical scarification sulfuric acid

Significantly higher numbers of germinated wild sorghum seeds were obtained by germination of seeds in the germination cabinet, at the level of significance of 99.9%. An average of the room temperature germination was 1.77 germinated seeds, whereas the average number of germinated seeds in germination cabinets was 5.05. Similar results were reported by Huang and Hsiao (1987) who found that the germination percentage of Johnsongrass seeds was higher by 20-30% when the seeds were germinated at higher temperatures (from 28°C to 35°C) compared to germination rates achieved at temperatures from 10°C to 22°C.

## CONCLUSIONS

Due to the impermeable seed coat of the Johnsongrass seeds it is necessary to remove it mechanically (scarification) or chemically (sulfuric acid) in order to obtain a higher germination percentage. Better germination was achieved at higher temperatures.

Testing of biological and ecological characteristics of weed seeds will continue to be a current issue, because knowledge about the factors that affect the germination and dormancy loss of weeds may be relevant in predicting the germination time for some species, and consequently to take appropriate control measures.

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## THE EFFECT OF DIFFERENT DORMANCY BREAKING TREATMENTS ON GERMINATION OF *ALBIZIA JULIBRISIN* SEEDS

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*Original scientific paper*

### Summary

*Albizia julibrisin* Durazz. with common name Persian silk is an exotic, small deciduous tree growing up to 5–16 m that is native to tropical and subtropical Asia. *Albizia julibrisin* Durazz. seeds are dormant because of their hard seed coat and they need pre-treatment in order to germinate. This study was carried out with an aim of overcoming seeds dormancy. The seeds were treated with sulphuric acid, nitrate acid, two different concentrations of gibberellin acid, hot water, cold water as well as freezing and scarification treatment. The control group was immersed into plain water, at room temperature. The aim was to determine the best dormancy breaking treatment that resulted with successful and fast emergence. Statistical results showed significant difference among treatments for breaking of dormancy of *Albizia julibrisin* Durazz. seeds.

Key words: *Albizia julibrisin*, seed dormancy, dormancy breaking treatments, seed emergence

### INTRODUCTION

*Albizia julibrisin* Durazz with common name Persian silk is an exotic, small deciduous tree growing up to 5–16 m that is native to tropical and subtropical Asia. *A. julibrisin* is a hermaphroditic tree normally flowering from late spring to early summer, April to June in Nepal and from June to September in South Europe. Pods ripen in August-September and begin to disintegrate soon after, but remain on the trees into winter. Seeds are mostly dispersed below or around the parent plant, but can be dispersed further by water. The wood is used in furniture manufacture, while dried bark has got medicinal values and it is used in China for preparation of sedatives. It is leguminous plant, so it enhances soil fertility by nitrogen fixation. It is widely used as a landscape plant since it remains green till frosts. Silk tree seeds have impermeable seed coats that allow them to remain dormant for many years (Gogue & Emimo, 1979). Dormancy may be of several different types and sometimes more than one type occurs in the same seed. Seeds of some species have hard, cutinized seed coats that completely prevent the imbibitions of water and sometimes also the exchange of gases. Without imbibitions and gas exchange renewal of embryo growth and germination are impossible. Physical seedcoat dormancy of this kind occurs most frequently in species adapted to alternating dry and wet seasons, including several leguminous genera such as *Acacia*, *Prosopis*, *Ceratonia*, *Robinia*, *Albizia*, *Cassia*. The most often used methods for softening the hard seed coat are: (a) dry-heat; (b) chemical or mechanical scarification; and (c) soaking in warm water (Baskin and Baskin, 1989)

The aim of this research was to determine the best dormancy breaking treatment that resulted with successful and fast emergence of *Albizia julibrisin* Durazz seeds.

### MATERIALS AND METHODS

The experiment was conducted at the lab of Agromediterranean faculty Dzemal Bijedic University of Mostar, Bosnia and Herzegovina. Mature seeds of Persian silk (*Albizia julibrisin* Durazz.) were collected from three middle age trees in Mostar area, during autumn 2013. The seeds were manually separated from pods and numbered. The seeds were same size and same quality. Cleaned seeds were mixed thoroughly, packed in paper bags and stored at the room temperature until the time when tests were carried out. There were eight dormancy breaking treatments with 100 seeds in each treatment, with four replicates. The treatments were described in the Table 1.

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Tab. 1. Descriptions of treatments

Treatment sign	Treatment description
T1	seeds soaked in glass containers for 24 hrs in concentrated (98%) H <sub>2</sub> SO <sub>4</sub>
T2	seeds soaked in glass containers for 1 hrs in concentrated (98%) HNO <sub>3</sub>
T3	seeds soaked in glass containers for 24 hrs in gibberellin acid 1000 ppm
T4	seeds soaked in glass containers for 24 hrs in gibberellin acid 500 ppm
T5	seeds soaked in hot distilled water (90°C) which was allowed to cool gradually to room temperature and soaking period was 48 hrs.
T6	seeds soaked in cold distilled water and soaking period was 48 hrs.
T7	seeds wrapped into aluminium folic and kept for 24 hrs in the freezer at -18°C
T8	seeds scarified with a needle

After each treatment, seeds were given three surface washings with distilled water and dried closer to original moisture with forced air at room temperature. After drying, seeds were placed on two layers of filter paper in Petri dish (9 cm) containing 3 ml distilled water, three replicates. The Petri dishes were incubated at room temperature in 12 hrs fluctuated light (day and night), the plates were wet with 3 ml distilled water after 3 days to maintain moisten condition. Measurement of radicles started when the radicles were  $\geq$  1mm. The total germination percentage was counted as the average of the four replicates.

## RESULTS AND DISCUSSION

The petri dishes were observed every day and the numbers of germinated seeds were recorded. Within 3 days, after seed were set in Petri dishes, germination started. All the result were statistically analyzed (ANOVA and Lsd test) using MS Excell. Results from variance analyze showed that the effect all different treatments on seeds rate germination are statistically significant (Table 2). The first day of germination (FDG), the day when majority of treatments (all but one, T7 – freezing treatment) resulted with seed germination. The last day (LDG) when germination occurred was the seventh day. The main measuring was done on the LDG.

Tab. 2. Germination energy, speed of germination and germination percentage after seven days

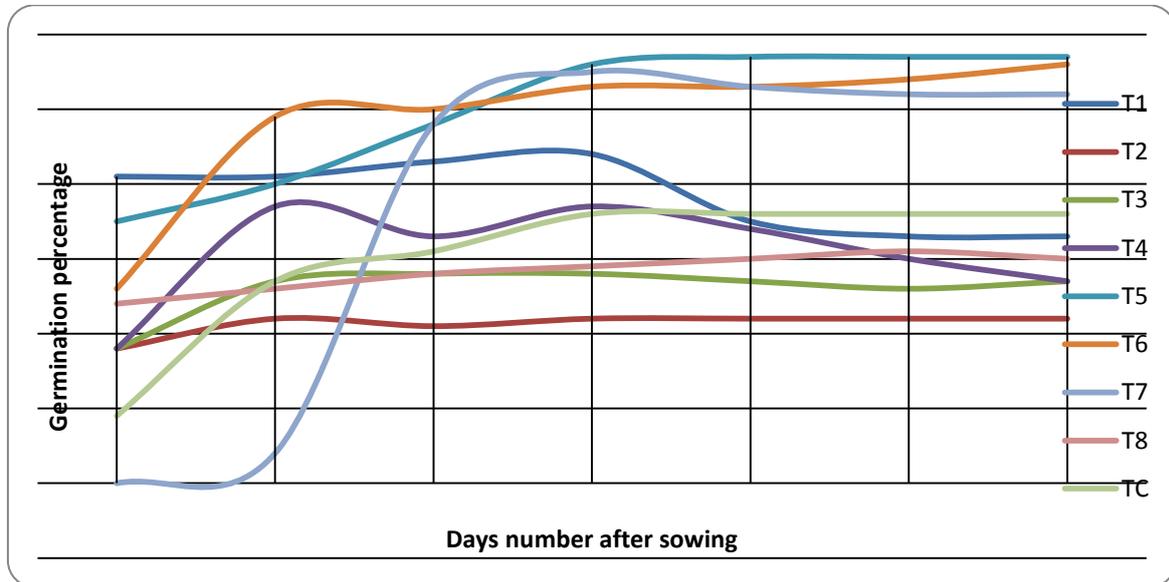
	T1	T2	T3	T4	T5	T6	T7	T8	TC
Germination energy%	47.86 <sup>c</sup>	29.36 <sup>f</sup>	35.06 <sup>g</sup>	40.85 <sup>de</sup>	23.99 <sup>h</sup>	70.31 <sup>a</sup>	63.91 <sup>b</sup>	40.48 <sup>df</sup>	41.28 <sup>d</sup>
Speed of germination%- Vigour index	48.98 <sup>ab</sup>	25.26 <sup>de</sup>	30.12 <sup>ce</sup>	36.62 <sup>c</sup>	55.87 <sup>a</sup>	54.07 <sup>a</sup>	33.84 <sup>ce</sup>	33.09 <sup>ce</sup>	31.59 <sup>ce</sup>
Germination%	33 <sup>d</sup>	22 <sup>g</sup>	27 <sup>f</sup>	27 <sup>f</sup>	57 <sup>a</sup>	56 <sup>a</sup>	52 <sup>b</sup>	30 <sup>e</sup>	36 <sup>c</sup>

Figures in a column with similar letter (s) do not differ significantly

Seed germination parameters varied depending on the treatment. The highest germination speed occurred for the seeds treated with hot water.

High values of germination vigour mean higher seedling vigour of one sample in relation to another. In seed technology this value is used to predict the relative vigour of samples, especially for cultivated species, since samples with the same quantity of seeds germinated can present different values for this index (Ranal and Santana, 2006). Seed vigour is defined as "seed property that determines potential for fast and uniform emergence, and development of seedlings under a wide range of field conditions" (AOSA, 2002). Best values of seed vigour were recorded for seeds soaked in warm water, plain water, and the worst in HNO<sub>3</sub> treated seeds. Although the seeds soaked in warm water showed the best germination percentage, their germination energy was the lowest. At the same time the best germination energy was observed for the seeds that were under freezing treatment. They were the only seeds that showed zero germination on the third day after sowing. Best germination percentage 57 and 56 % occurred in the seeds soaked in hot and water at room temperature.

Soaking in tap water for one or two days gave significantly higher germinability (67 and 73% respectively), and soaking in warm water (30°C) for one hour significantly increased the germination to 70% as compared to the control seeds, but increasing the soaking time (up to six hours) did not improve germination further (67%) (Merou *et al.*, 2011). The hot water treatment (100°C for 1 min) was also recommended for seed germination of *Albizia procera* in Bangladesh (Azad *et al.*, 2012). The lowest germination in this research 22% was observed in seeds treated with HNO<sub>3</sub>.



Graph 1. Cumulative germination curves through the days after sowing

Graph 1. presents that germination was not equally distributed. The most stable curve was the curve that represents the treatment T2 (HNO<sub>3</sub>) with no big difference between FDG and LDG. The largest difference between germination percentage on FDG and LDG was shown with the curve of T7 (freezing treatment). Treatment T1 (H<sub>2</sub>SO<sub>4</sub>) showed the biggest fall taking into consideration 41% at FDG, and ended with 31% on the LDG.

The peak of germination was reached 7 to 8 days after sowing, and after this date a large number of seeds started to terminate.

## CONCLUSIONS

At the end, as an overall conclusion it has to be noted that soaking of seeds in water should be considered as safe, the cheapest and practical method for breaking of dormancy of *Albizia julibrissin* Durazz seeds, especially in hot water.

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## EFFECTS OF ORGANIC AND MINERAL FERTILIZERS ON CHEMICAL COMPOSITION OF JERUSALEM ARTICHOKE TUBER (*HELIANTHUS TUBEROSUS* L.)

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*Original scientific paper*

### Summary

Jerusalem artichoke (*Helianthus tuberosus* L.) is an agricultural crop that can successfully be grown in almost all sowing periods. Plantation of Jerusalem artichokes was planted during the vegetation period in 2014 in two localities: Vrnograč locality (Velika Kladuša) and Čelinac locality (Banja Luka) and will be used in experimental part of this paper and for determination of fertilization effects on tuber chemical composition. Experiment was based on standard scientific methods and had to pass three repetitions without fertilizer application in Vrnograč locality, while the plantation was planted in three parcels in Čelinac locality: one parcel without fertilizer application, one fatten with mineral fertilizer (KAN) and one with Californian worm humus. Statistically significant difference ( $p < 0.05$ ) along with the statistical evaluation of gained results regarding their nutritive values were determined using chemical analysis of grown Jerusalem artichoke tubers of different fertilizer treatments. The highest percentage of dry substance was determined in Čelinac locality by fertilizing Californian worms with humus, and it was  $24.75 \pm 0.02$ , while in Vrnograč locality, without fertilizer application, statistically significant lower content of dry substance was recorded in the quantity of  $22.12 \pm 0.01$ . Significant difference ( $p < 0.05$ ) and significantly higher content of analyzed parameters in relation to other kinds of fertilization were determined by analyzing the content of mineral substance, natural invert, vitamin C, phenol and inulin of the tuber fatten with humus fertilizer.

Key words: *Jerusalem artichoke, tuber, plantation, fertilization.*

### INTRODUCTION

*Helianthus tuberosus* L (Jerusalem artichoke) belongs to the family *Asteraceae*, genus *Helianthus* and is several year lasting green plant. It is being grown as an annual crop which originates from Central America. (Terzic *et al.*, 2012). It is spread out all over the world, especially in the east parts of the USA, Central and East Europe. There it is usually considered to be weed (Kays & Nottingham, 2007). Jerusalem artichoke is grown in warm and continental climate, and most successfully near rivers and streams. Tuberosus in the soil can endure very low temperatures (down to  $-40$  °C). Due to its well-developed root system it has high resistance to drought (Lazić *et al.*, 2001). It can be well cultivated, also in sandy, poor soil where it is easier to dig the tubers out (Cieslik *et al.*, 2011). For this plant, its unique chemical composition is the main feature. The most valuable component is inulin, present from 8 to 21 % in total fresh tuber mass (Roberfroid, 1993; Van Loo *et al.*, 1995). Tuberosus part is used for nutrition, remarkably rich in carbohydrates, mostly inulin. Inulin is known as probiotic which transforms into fructose (the sweetest natural sugar, 16 times sweeter than sucrose), and such is believed to be ideal for diabetics). Positive inulin influence to human health includes less risk for cardiovascular diseases, prebiotic effect, prevents and/or diminishes osteoporosis (Kaur & Gupta, 2002; Tunland, 2003). Inulin can be found in other plant families: *Asteraceae*, *Agavaceae*, *Boraginaceae*, *Campanulaceae*, *Goodenaceae*, *Haemodoraceae*, *Iridaceae*, *Liliaceae* i *Stylidiaceae* (Kays & Nottingham, 2007). *Asteraceae* family species accumulate inulin in underground organs, but only in artichoke (*Helianthus tuberosus* L.) and chicory (*Cichorium intybus* L.), this amount is enough for the effective industrial extraction (Abou-Arab *et al.*, 2011). Tuberosus plants have high energy value (73 kcal/100g), high vitamin (Van Loo *et al.*, 1995) and mineral content (Cieslik, 1998) B group vitamins, vitamin C, D and provitamin A (beta caroten) (Antanaitis *et al.*, 2004; Cieslik *et al.*, 2011; Griffaut *et al.*, 2007; Kays & Nottingham, 2007). They are, also, significant source of fatty acids (Takeuchi & Nagashima, 2011). The main goal of experimental research is to establish the effect of fertilizers on chemical composition of Jerusalem artichoke tuber. The research hypothesis is set upon the planting of artichoke tuber on two sites. The fertilization with California earthworm humus will affect the chemical composition of tuber and rear more valuable artichoke when it comes to tested chemical features.

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## MATERIALS AND METHODS

Experimental research has been conducted in 2013/2014 in two sites: Vrnograč (Velika Kladuša) and Čelinac (Banja Luka). The trial is set in an open field by standard scientific methods with three repetitions and three treatments: T1 (mineral fertilizer-KAN), T2 (California earthworm humus) and T3 (test parcel- no fertilizers). The size of tested site is 20 m<sup>2</sup>, the soil is being prepared by standard and agrotechnical basic and supplementary measures. The tuber planting was conducted in October, spacing 50 x 50 cm and the depth of 6 cm (80 plants/experimental plot). The chemical analysis of grown Jerusalem artichoke tuber with different fertilizing treatments, is made in the lab in Biotechnical faculty. We used standard chemical analysis by AOAC (1995), with following parameters: dry matter, protein, fat content, cellulose, natural invert, inulin, as well as iron amount, magnesium, calcium, phosphorus, vitamin C and phenol. The research result is processed using the help of statistic program XL STAT, 2012.

## RESULTS AND DISCUSSION

Low fat content and high mineral composition, with inulin as dietetic fiber, makes Jerusalem artichoke nutritionally valuable food, desired in everyday human nourishment. The values of examined parameters often vary as a consequence of various factor effects, e.g. climate conditions, soil quality and nutritious matters (fertilization). The chemical composition of grown tuber in this experimental research, is not notably different than quantitative composition of artichoke tuber described in scientific publications. However, we noticed the difference in quantitative composition of protein, iron, magnesium, calcium, phosphorus, vitamin C, as well as in the phenol and inulin amount depending on climate conditions (Chart 1 & 2) and fertilization treatments (Table 1 & 2), (Chart 3).

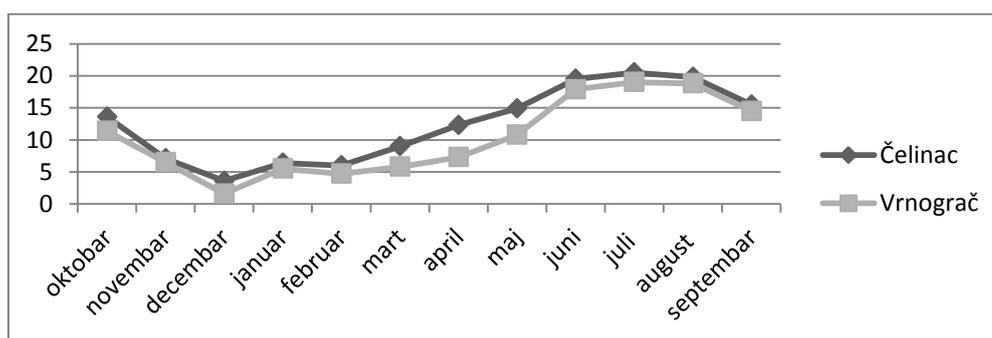


Chart 1. The average temperature of the air during experimental research (°C)

During the experimental research we recorded higher temperature than average (from 0,6 to 5,0°C) on Čelinac site, and also lower average precipitation (from 0,9 l/m<sup>2</sup> to 20 l/m<sup>2</sup>). Taking into consideration the publication data, that says that artichoke is grown better in sandy soil with lower percentage of humidity and higher temperature climate, the results are as expected. These mentioned parameters on Čelinac site had positive effect on examined chemical composition of reared artichoke tuber.

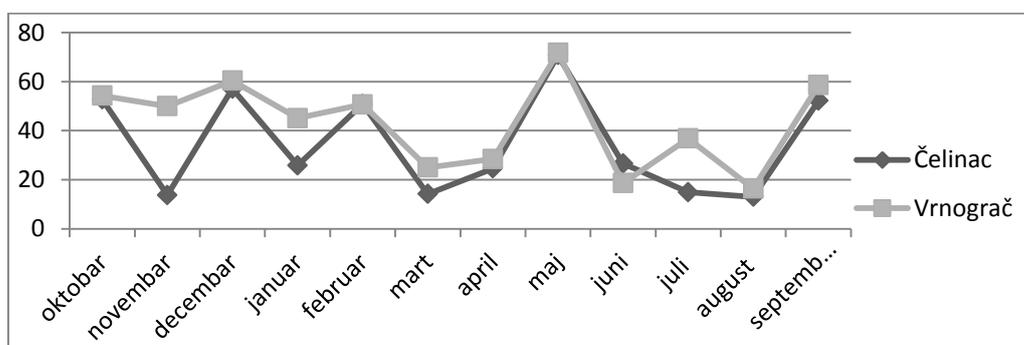


Chart 2. Average amounts of precipitation in the period of experimental research (l / m<sup>2</sup>)

In the laboratory in Biotechnical faculty the same chemical analysis of artichoke tuber has been done, depending on fertilization treatment, and the results are processed with statistic program XL STAT 2012, and shown in Table 1 & 2.

Tab. 1. Chemical composition of tuber in the Jerusalem artichoke with two test localities, the treatment of T3

Chemical composition of Jerusalem artichoke	units of measurement	Vrnograč BG - T3	Čelinac BG - T3
dry matter	%	22.12±0.01 <sup>b</sup>	23.50±0.02 <sup>a</sup>
protein	%	1.18±0.02 <sup>b</sup>	1.79±0.01 <sup>a</sup>
fat	%	0.18±0.02 <sup>a</sup>	0.17±0.01 <sup>a</sup>
ash	%	1.17±0.01 <sup>a</sup>	1.20±0.02 <sup>a</sup>
total acidity	%	0.20±0.01 <sup>b</sup>	0.28±0.01 <sup>a</sup>
crude fiber	%	1.72±0.02 <sup>a</sup>	1.69±0.02 <sup>a</sup>
natural invert	%	0.96±0.02	0.80±0.03 <sup>b</sup>
inulin	%	15.08±0.01 <sup>a</sup>	16.57±0.03 <sup>b</sup>
iron	mg/100g	4.12±0.02 <sup>a</sup>	4.01±0.01 <sup>b</sup>
magnesium	mg/100g	23.5±0.17 <sup>b</sup>	25.1±0.17 <sup>a</sup>
calcium	mg/100g	20±0.26 <sup>b</sup>	22.21±0.19 <sup>a</sup>
phosphor	mg/100g	85±0.95 <sup>a</sup>	80±0.83 <sup>b</sup>
vitamin C	mg/100g	3.6±0.86 <sup>b</sup>	4.15±0.13 <sup>a</sup>
phenol	mg/100g	36±0.86 <sup>b</sup>	45±0.45 <sup>a</sup>

Tab. 2. Chemical composition of tuber in the Jerusalem artichoke T1, T2 i T3, localities Čelinac

Chemical composition of Jerusalem artichoke	units of measurement	Čelinac BG - T3	Čelinac KAN - T1	Čelinac Humus - T2
dry matter	%	23.50±0.02 <sup>b</sup>	22.47±0.03 <sup>c</sup>	24.75±0.02 <sup>a</sup>
protein	%	1.79±0.0 <sup>c</sup>	2.09±0.03 <sup>a</sup>	1.84±0.01 <sup>b</sup>
fat	%	0.18±0.01 <sup>a</sup>	0.16±0.01 <sup>a</sup>	0.19±0.03 <sup>a</sup>
ash	%	1.2±0.02 <sup>b</sup>	1.30±0.02 <sup>a</sup>	1.35±0.03 <sup>a</sup>
total acidity	%	0.28±0.01 <sup>a</sup>	0.42±0.02 <sup>a</sup>	0.24±0.03 <sup>a</sup>
crude fiber	%	1.70±0.01 <sup>b</sup>	1.86±0.03 <sup>a</sup>	1.75±0.03 <sup>b</sup>
natural invert	%	0.8±0.03 <sup>b</sup>	0.75±0.01 <sup>c</sup>	1.12±0.02 <sup>a</sup>
inulin	%	16.57±0.02 <sup>b</sup>	14.95±0.01 <sup>c</sup>	17.26±0.01 <sup>a</sup>
iron	mg/100g	4.01±0.01 <sup>a</sup>	4.2±0.23 <sup>a</sup>	4.05±0.01 <sup>a</sup>
magnesium	mg/100g	25.1±0.17 <sup>b</sup>	20±0.26 <sup>c</sup>	31.25±0.07 <sup>a</sup>
calcium	mg/100g	22.21±0.19 <sup>c</sup>	23.18±0.17 <sup>b</sup>	24.15±0.02 <sup>a</sup>
phosphor	mg/100g	80±0.83 <sup>c</sup>	82.1±0.26 <sup>b</sup>	91±0.36 <sup>a</sup>
vitamin C	mg/100g	4.15±0.13 <sup>b</sup>	3.7±0.15 <sup>a</sup>	4.65±0.13 <sup>a</sup>
phenol	mg/100g	45±0.45 <sup>b</sup>	40±1.32 <sup>c</sup>	60±1.80 <sup>a</sup>

The scientific publications data of Jerusalem artichoke chemical composition differ because the values vary and depend on specific parameters, and as a consequence of various factor effects: climate conditions, rearing, digging the tubers out, cultivation and treating tubers after taking them out, etc. The chemical composition of grown tubers in this research on two sites, depending on fertilization treatment, is qualitatively contrastive than the Jerusalem artichoke tuber composition described in publications. Statistically, the most notable differences are found in the contents of protein, iron, magnesium, calcium, phosphorus, vitamin C, phenol and the most important component, inulin (Table 1 & 2). In already mentioned examined parameters significant statistical differences are found, in other words, significant value increase is found which had a positive effect on the tuber quality. In relation to controlled treatment (T3), the inulin content with humus treatment (T2) increased for

0.69% and with T1 treatment (KAN) at the same time inulin content decreased for 1.62%. When vitamin C was tested, it is noticed that its amount increased with T2 treatment for 0.50% and decreased with T2 treatment for 0.45%. Similar results are shown when other values were tested, which means that T2 treatment enhances tuber quality, and T1 treatment has the opposite effect. (Table 2)

## CONCLUSIONS

After the conduct of experimental research on two sites, identifying the climate factor effects, and also the fertilization of Jerusalem artichoke composition, we can conclude following:

- The differences are found in quantitative composition of protein, iron, magnesium, calcium, phosphorus, vitamin C, as well as phenol and inulin amount depending on climate conditions. Such higher values are found on Čelinac site, the one with sandy soil, lower percentage of humidity and higher temperatures which showed positive effect on examined chemical composition of reared artichoke tuber.
- The previously set hypothesis was proven, that fertilizing with T2 treatment (humus) will change the chemical composition of tubers and grow better quality tubers when it comes to chemical features. The increase of all examined values is noted: inulin content for 1.69%, vitamin C for 0.45%, magnesium for 6.15 mg/100g, calcium for 1.94 mg/100g, phosphorus for 11 mg/100g and phenol for 15mg/100g.
- Using artificial fertilizer T1 (KAN) showed significantly lower examined values in relation to the controlled treatment: inulin content decreased for 1.62%, vitamin C for 0.45%, magnesium for 5.10 mg/100g, slight increase in calcium for 0.97 mg/100g, phosphorus for 2.1 mg/100g and phenol decrease for 5 mg/100g.

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## TALL FESCUE (*Festuca arundinacea*) CULTIVARS ADAPTATION AND TURF PERFORMANCE UNDER TOKAT CLIMATIC CONDITIONS IN TURKEY

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*Scientific paper*

### Summary

In present study, we have targeted to determine the turf performances of newly introduced tall fescue cultivars, such as Firaces, Merida, Turbo, Apache, Eldorado and SR8600 under cool climatic conditions of Tokat/Turkey between the years of 2009-2012. Turf color, weed infestation, cover and turf quality characteristics of those cultivars were tested. Three years average results of experiment indicated statistically significant variations among genotypes of tall fescue tested and Merida and Turbo were prominent cultivars.

Keywords: *Turfgrass adaptability, tall fescue, turf quality.*

### INTRODUCTION

C3-cool season turfgrasses are widely used depending on very old data and tradition on turf sector in Mediterranean countries such as Turkey and similar regions of neighbouring countries and objection to the warm season turfgrasses which occurs generally in the Mediterranean region is the lack of green colour during the winter dormancy period (Kir *et al.*, 2011). It is a fact that cool season turfgrasses adaptability and physiologically enduring to cope with the conditions of Mediterranean environments are the major question of vital significance. Thus, knowledge on adaptability to weather conditions allows us to identify turfgrass species and genotypes better able to tolerate and exploit the resources of the environments (Beard, 1989; Shearman *et al.*, 2001). Genetic information on the environmental adaptability of the most popular varieties of cool season species may be determined by direct field comparison in targeted environments. Researches suggested that tall fescue (*Festuca arundinacea* Schreb.) was the most adaptable species for dry and hot ecological conditions (Van Huylenbroeck *et al.*, 1999; Yılmaz and Avcioglu, 2000; Russi *et al.*, 2004). In this study, considering tall fescue as most adaptable cool season turf species to Mediterranean environments, turf color, turf cover and visual turf quality traits of various tall fescue cultivars were tested in terms of adaptability under conditions of Tokat Region/Turkey.

### MATERIAL AND METHOD

The research study was carried out from March 2009 to January 2012 for three years at the Parks and Gardens Directorate of Tokat Municipality and six tall fescue turf type cultivars (Apache, Eldorado, Firaces, Merida, SR8600 and Turbo) were tested. Each cultivar turfs were established on loamy soil and top-dressed with sand. Mowing frequency ranged from once every two weeks to twice a week depending on growing seasons. A randomised complete block design with four replications was established, plots being 2 m<sup>2</sup>. Annual fertilization (250 kg ha<sup>-1</sup> N, 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 150 kg ha<sup>-1</sup> K<sub>2</sub>O) based on soil analysis was applied in seven applications from April to October. Irrigation was applied twice every week from May to September. For many years the average rainfall is 445 mm and the average annual temperature is 12.4°C in Tokat. The following parameters were usually estimated once a month and indicated as seasonal means; turf color (1=lightgreen, 9=darkgreen), turf cover (1=poor, 9=best) and visual turf quality (1=poor, 9=best). Seasonal data as three years average were subjected to TOTEMSTAT Statistical Program (Acikgoz *et al.*, 2004).

### RESULTS AND DISCUSSION

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Variations among the seasons and cultivars, and interaction were significant (Table 1). **Turf color** mean values of tall fescue cultivars were always higher than 8.5 in the three years average, except cv Apache (Table 1). Color values in summer and winter were higher than spring and fall, and cv Merida and Turbo had higher scores. These results were confirmed by Demiroglu *et al.* (2011) findings.

**Turf cover** score variations among the cultivars, seasons and interaction were significant (Table 1). Mean cover values were higher in summer and winter than spring and fall. Cv Merida and Turbo had higher mean cover scores than other cultivars.

**Visual turf quality** scores varied significantly among the cultivars and seasons and reflected similar distribution to other characteristics. In general, mean quality scores in winter and summer were higher than the other seasons. Cv Merida and Turbo had again higher mean quality scores than others. Yılmaz and Avcioglu (2000) also stated similar turf cover scores. Overall findings of turf color, turf cover and visual turf quality scores as the result of three years average indicated excellent adaptability of tall fescue cultivars to sub-Mediterranean conditions of transitional zones like Tokat, and cv Merida and Turbo performed better than other genotypes.

Tab. 1. Turf color, cover and visual turf quality characteristics of tall fescue cultivars

Turf Cultivars	3 Years Mean														
	Turf Colour					Turf Cover					Visual Turf Quality				
	Wi	Sp	Su	Fa	Me	Wi	Sp	Su	Fa	Me	Wi	Sp	Su	Fa	Me
Apache	7.7	7.5	7.7	7.3	7.5	8.1	8.4	8.4	8.4	8.4	8.2	7.5	7.6	8.1	7.5
Eldorado	8.6	8.5	9.0	8.4	8.6	8.2	8.5	8.6	8.6	8.6	8.7	8.3	8.3	8.7	8.4
Firaces	8.7	8.4	9.0	8.3	8.6	8.1	8.5	8.5	8.5	8.6	8.8	8.3	8.4	8.8	8.3
Merida	9.0	8.9	9.0	8.8	8.9	8.6	8.8	8.8	8.8	8.9	9.0	8.7	8.7	9.0	8.9
SR8600	8.9	8.5	9.0	8.5	8.7	8.4	8.7	8.7	8.7	8.8	8.9	8.2	8.4	8.9	8.4
Turbo	9.0	8.9	9.0	8.8	8.9	8.6	8.8	8.8	8.8	8.9	9.0	8.7	8.7	9.0	8.9
Mean	8.7	8.5	8.8	8.4	8.6	8.3	8.6	8.6	8.6	8.7	8.8	8.3	8.4	8.8	8.4
LSD %5	C: 0.03 S: 0.02 C x S: 0.03					C: 0.01 S: 0.03 C x S: 0.04					C: 0.01 S: 0.02 C x S: 0.04				

Abbreviations: Winter: Wi., Spring: Sp., Summer: Su., Fall: Fa., Mean: Me., Cultivar: C., Season: S.

## CONCLUSION

Three years average data of research study highlighted that turf type cultivars of tall fescue (*Festuca arundinacea* Schreb.) are most suitable to hot and dry weather conditions of sub-Mediterranean climatic conditions of Tokat region and to establish high quality and sustainable turfs. Although the all cultivars were in acceptable limits, Merida and Turbo cultivars of tall fescue species had higher scores than all other cultivars.

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## ECONOMIC ASPECTS OF GREENHOUSE TOMATO PRODUCTION IN TURKEY

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*Review article*

### Summary

Turkey is one of the major greenhouse tomato production countries in the world. In 2014, 3.02 million tons tomato was produced in 57% (21,734 ha) of total greenhouse areas in Turkey. Greenhouse tomato production of Turkey increased 62% in 2005-2014 period. The majority of the tomatoes produced are consumed domestically. But, Turkey also exports some of tomatoes to countries such as Russia, Germany, Bulgaria, Romania, Iraq, United Kingdom, and USA. In 2014, Turkey exported 591,831 tons tomatoes to some countries in the world. Total tomato export value of Turkey was 430 million dollars in 2014. The purpose of this study is to analyse economic aspects of greenhouse tomato production in Turkey. Statistical data for 2005-2014 used in the study have been obtained from Turkish Statistical Institute and The Turkish Ministry of Food, Agriculture, and Animal Husbandry.

Key words: *greenhouse, vegetable, tomato, marketing, economic analysis.*

### INTRODUCTION

Turkey is one of the major greenhouse production countries in the world with total of 37,963 ha glass and plastic greenhouse area. Turkey lies between 36 and 42° North and 26 and 45° East. The main advantage of Turkey in terms of wide spreading of greenhouse growing is mild winter climate which compared to the countries located at higher latitudes is characterized by relatively high radiation and mild temperatures during winter (Tuzel and Leonardi, 2009).

Greenhouse tomato growing plays an important role both from the perspective of the farm, as well as from that of the entrepreneur in Turkey. In recent years, many studies have been made on economic analysis of greenhouse tomato growing in Turkey (Engindeniz and Tuzel, 2002; Engindeniz, 2003; Ozkan *et al.*, 2004; Rad and Yarsi, 2005; Hatirli *et al.*, 2006; Karaman and Yilmaz, 2007; Kadanali *et al.*, 2008; Engindeniz *et al.*, 2009; Daka *et al.*, 2012). Though, there is still need for study, especially at farmers' level. The purpose of this study is to investigate recent developments in greenhouse tomato production and marketing of Turkey in 2005-2014 period. Statistical data used in the study have been obtained from TurkStat (Turkish Statistical Institute) and The Turkish Ministry of Food, Agriculture, and Animal Husbandry.

### GREENHOUSE TOMATO PRODUCTION IN TURKEY

In 2014, total protected cultivation area and greenhouse area were 64,912 ha and 37,963 ha in Turkey, respectively. 79% of total greenhouses in Turkey are covered with plastic and the rest with glass. Vegetable production is dominant in greenhouse production in Turkey, accounting for 95% of total greenhouse production, and that is followed by flower (4%) and fruit production (1%) (Tuzel *et al.*, 2015). Among the vegetables, tomato is the most prominent while cucumber, pepper, eggplant, squash, watermelon, melon and other vegetables are grown over rest of the area.

In Turkey, glass and plastic greenhouses which are generally used for tomato production are constructed without any project or planning criteria. For these kinds of greenhouses, the entrepreneurs go to local blacksmiths and construct greenhouses without taking any standard or engineering information into account (Emekli *et al.*, 2010). High-technology greenhouses are generally built with galvanised iron support structure and glass or plastic as covering material. More advanced growing technologies, including hydroponics, are used in those greenhouse (Tuzel, 2003).

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Tomatoes are grown in autumn (from August till January) and spring (from January/February till July) seasons as short cycle in order to avoid from the cold temperatures in winter time or as long cycle starting from September/October till July.

The sizes of the greenhouses are small in Turkey and they are mostly family business greenhouses. 64% of glass greenhouses and %56 of plastic greenhouses are 0.1-0.2 hectare range (Sevgican, 1999). 91% of glass greenhouses and 64% plastic greenhouses are less than 0.3 ha in Turkey (Sevgican *et al.*, 2000).

Tomatoes were grown in 57% of total greenhouse area in 2014. 75% of greenhouse tomatoes was produced in plastic greenhouses. Greenhouse tomato production increased 62% in 2005-2014 period (Table 1).

Tab. 1. Development of greenhouse tomato growing areas and production in Turkey

Years	Greenhouse tomato growing Area (ha)			Greenhouse tomato production (ton)		
	Glass greenhouse	Plastic greenhouse	Total	Glass greenhouse	Plastic greenhouse	Total
2005	5,222	11,024	16,246	736,160	1,133,538	1,869,698
2006	5,293	11,065	16,358	751,196	1,150,055	1,901,251
2007	5,331	11,477	16,808	779,183	1,387,532	2,166,715
2008	6,122	12,482	18,604	831,188	1,292,792	2,123,980
2009	5,834	13,829	19,663	842,132	1,578,853	2,420,985
2010	5,885	14,664	20,549	818,074	1,769,075	2,587,149
2011	5,348	15,874	21,222	737,453	2,006,602	2,744,055
2012	5,455	17,004	22,459	705,618	2,132,540	2,838,158
2013	5,424	17,156	22,580	739,738	2,215,191	2,954,929
2014	5,473	16,261	21,734	758,951	2,261,956	3,020,907

Source: Turkish Statistical Institute (<http://www.tuik.gov.tr>)

In Turkey, greenhouse production is generally located on the coastal regions. Beside the Mediterranean coastal line, greenhouse practices are also expanded to Aegean, Marmara, Black Sea and South Eastern Anatolia regions. In 2014, about 81% of the greenhouse tomato production area in Turkey is concentrated in the Mediterranean region (Table 2).

Tab. 2. Regional distribution of greenhouse tomato growing in Turkey

Regions	Tomato growing area (ha)	%	Tomato production (ton)	%
Mediterranean	17,639	81.16	2,391,535	79.17
Aegean	3,595	16.54	564,706	18.69
East Marmara	263	1.21	28,954	0.96
South Eastern Anatolia	87	0.40	12,399	0.41
Middle Anatolia	32	0.15	6,845	0.23
Others	118	0.54	16,478	0.54
Total	21,734	100.00	3,020,917	100.00

Source: Turkish Statistical Institute (<http://www.tuik.gov.tr>)

The majority of the greenhouses in the Mediterranean region are located in province of Antalya. Kumluca district of Antalya is an important plain with its intensive agricultural activities employing greenhouses. This climate zone allows unheated greenhouse production most of the time due to abundant solar radiation during the winter season. The greenhouse area usually heated only for frost prevention (Kacira *et al.*, 2004).

## GOVERNMENT SUPPORTS FOR GREENHOUSE TOMATO GROWING IN TURKEY

There is no direct government support policy for greenhouse tomato production in Turkey. However, growers benefit from the general support policies pursued in agricultural production (Yilmaz *et al.*, 2005). Greenhouse procedures are supported by agricultural investments, and input and export support within the context of government support policies. The Agricultural Bank of Turkey has provided investment and special project credits to support growers. Furthermore, short-term credit support is provided to growers by Agriculture Credit Cooperatives. Further In 2014, subsidy practises by the Turkish Ministry of Food, Agriculture, and Animal

Husbandry for growers are presented in Table 3. Further, technical adviser support is also given to greenhouses which are 0.3 ha and more than 0.3 ha.

Tab. 3. Subsidy practises for greenhouse growers in Turkey (2014)

Subsidy practises	Unit subsidy (€/ha)
Soil analysis	8.83
Fuel	16.25
Fertilizer	21.20
Beneficial insects for biological control	1236.75
Bumble bees (*)	21.20
Organic production	247.35
Good agricultural practices	530.03

(\*) Subsidy is given per bumble bee colony.

Source: The Turkish Ministry of Food, Agriculture, and Animal Husbandry (<http://www.tarim.gov.tr>)

### MARKETING OF GREENHOUSE TOMATOES IN TURKEY

Wholesale markets are the main point of sale for tomatoes in Turkey. Growers deliver their products to brokers in the wholesale centres who in turn sell the product on the grower's behalf. In this marketing system, growers do not have any marketing power. Sometimes it is possible to sell products outside this structure, e.g., selling of limited products directly to consumers by growers, exporting of products, and selling by cooperative (Yucel Engindeniz, 2013).

Tomato prices in domestic markets of Turkey are presented in Table 4. Tomato production increases can decrease domestic prices and growers are disadvantaged as a result for some years.

The majority of the tomatoes produced are consumed domestically. But, Turkey also exports some tomatoes to countries such as Russia, Germany, Bulgaria, Romania, Irak, United Kingdom, and USA. In 2014, Turkey exported to different countries 591,831 tons tomato. Total tomato export value of Turkey was 354 million € in 2014 and tomato export value formed 61% of total fresh vegetable export value of Turkey (AKIB, 2015).

Tab. 4. Tomato prices in domestic market of Turkey (€/kg)

Years	Tomato prices received by growers (1)	Retail price for consumer (2)	Marketing margin (2-1)
2005	0.27	0.64	0.37
2006	0.26	0.55	0.29
2007	0.33	0.84	0.51
2008	0.29	0.58	0.29
2009	0.29	0.59	0.30
2010	0.51	0.84	0.33
2011	0.33	0.61	0.28
2012	0.35	0.70	0.35
2013	0.39	0.64	0.25
2014	0.40	0.69	0.29

Source: Turkish Statistical Institute (<http://www.tuik.gov.tr>)

### PROFITABILITY ANALYSIS OF GREENHOUSE TOMATOES IN TURKEY

Greenhouse production is a highly intensive enterprise requiring substantial labor and capital inputs. Because of this, potential growers should carefully consider all of the factors necessary for better crop yields, higher labor productivity, energy efficiency and a successful enterprise (Kacira *et al.*, 2004).

One of the purposes in a greenhouse enterprise is to provide and maintain the environment that will result in an optimum crop production or maximum profit (Emekli *et al.*, 2010). The likely reason for the higher growth rate of the plastic greenhouses is due to their lower investment costs per unit area compared to glass houses (Yilmaz *et al.*, 2005).

Variable and fixed cost form total production costs of greenhouse tomatoes. The variable costs include costs for labor, fertilizer, seed, electricity, certification, marketing, transportation, and interest on total variable cost.

Variable costs are calculated by using current input prices and labor costs. The fixed costs include interest on total initial investment, annual initial investment costs, costs, administrative costs, and land rent. Total costs are subtracted from total gross return to calculate the net return (Engindeniz and Tuzel, 2002; Engindeniz *et al.*, 2009). The results of conducted studies in different regions on profitability of greenhouse tomatoes in Turkey are presented in Table 5.

Tab. 5. Results of previous studies on profitability of greenhouse tomatoes in Turkey

Region	Production period	Cover type	Yield (kg/m <sup>2</sup> )	Price (€/kg)	Total gross return (€/m <sup>2</sup> )	Total cost (€/m <sup>2</sup> )	Net return (€/m <sup>2</sup> )
Izmir *	Autumn	Plastic	11.31	0.35	3.96	3.31	0.65
Mugla **	One-Crop	Glass	15.93	0.26	4.14	3.70	0.44
Antalya **	Spring	Glass	14.75	0.24	3.54	3.30	0.24
Izmir ***	Autumn (organic)	Plastic	7.29	0.67	4.88	3.46	1.42

Source: \* Engindeniz, 2003; \*\* Engindeniz *et al.*, 2009; \*\*\* Engindeniz and Tuzel, 2002.

### SWOT ANALYSIS OF GREENHOUSE TOMATO GROWING IN TURKEY

The results of swot analysis of greenhouse tomato growing in Turkey are presented in Table 6. Strengths and opportunities of greenhouse tomato growing are severely. But, the needs development for greenhouse tomato growing of Turkey are: product innovation, production cost reduction, yield increase, production qualification, record keeping of growers, forming technical and economical co-operation and international relations, subsidy for some inputs, training of growers, production planning, and environmental impact reduction (Tuzel and Duzyaman, 2010).

Tab. 6. Swot analysis of greenhouse tomato growing in Turkey

<p><b>Strengths</b></p> <ul style="list-style-type: none"> <li>- Introduction of modern production facilities</li> <li>- Availability of cheap labour</li> <li>- Capacity for high product quality</li> <li>- Convenient of environment conditions</li> <li>- Water availability</li> </ul>	<p><b>Weaknesses</b></p> <ul style="list-style-type: none"> <li>- High cost of energy</li> <li>- Inadequate financing capabilities</li> <li>- Small size farms</li> <li>- Lack of training of growers</li> </ul>
<p><b>Opportunities</b></p> <ul style="list-style-type: none"> <li>- Enter new markets</li> <li>- Close relationship with EU</li> <li>- Development in the demands of the domestic market</li> <li>- To take into consideration of different consumer demand</li> <li>- Being organized as farm commodity groups</li> </ul>	<p><b>Threats</b></p> <ul style="list-style-type: none"> <li>- Likely entry of new competitors</li> <li>-Crisis of regional and global impact</li> </ul>

### CONCLUSION

According to this study, greenhouse tomato production may be profitable. But growers should gather all the economic data about the greenhouse vegetable production, and market conditions before building a greenhouse. Also producers should make investigations on other greenhouse enterprises and determine if greenhouse tomato can be profitable. Although, cost and return estimates are believed to be typical and realistic, individual growers should adjust these values to their own specific situations and circumstances.

Turkey possesses some advantages in terms of climatic conditions, geothermal sources, cheap labor and as a consequence the chance exportation is increased. But, there are several problem in greenhouse production of Turkey. Over fragmented structure of production areas and small farm size is one of the most important problem. Thus production is realized in low-tech greenhouses resulting in intensive use of chemicals. Further, growers are also facing serious problems such as declining crop prices, price fluctuations based on over-supply, a poor market system and sales uncertainty, and a lack of grower cooperatives.

Many problems and challenges faced by growers could be solved by grower organisations. Within the context of the organisation, cooperatives and growers' associations could play an important role in solving, in particular, the marketing problems of growers. The success of organisations will likely depend on active participation of growers' and on government support.

Growers and exporters should be meet international standards for food safety, environmental protection, and product quality to overcome the current problems and to meet consumer expectations.

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## THE INFLUENCE OF ANTI-HAIL NETS ON THE FRUIT QUALITY OF RED APPLE CULTIVARS

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*Original scientific paper*

### Summary

The production of high quality fruit is of vital importance to the fruit industry related to international standards. The anti-hail net is a physical barrier installed over an orchard to protect plants against damage by hail. Anti-hail nets may change microclimate of orchards and modify the physiological and sensory characteristics of fruits. This study was carried out to evaluate the influence of anti-hail nets on fruit quality of red apple cultivars. The experiment was conducted during one growing season (2014). 'Idared' and 'Jonagold' apple trees (grafted on M9 rootstock, trained as slender spindles with a planting distance 3.8 x 1.2 m) were covered with a black net as well as left uncovered (control trees). After harvest, on fruit samples were examined following fruit quality parameters: fruit firmness, fruit skin colour (using L\*a\*b\* system), total soluble solids, titrable acidity and contents of anthocyanins. Also, samples of both cultivars were putted in cold storage (under conditions of normal atmosphere) for seventy days to determine the influence of storage on fruit quality of covered and control fruits. It was found that selected cultivars reacted differently to the altered microclimate under the netting for some of fruit quality parameters such as content of anthocyanins and chroma of fruit skin colour of 'Jonagold' apple.

Key words: *anti-hail nets, apple cultivars, fruit quality, storage, anthocyanins, L\*a\*b\* colour system*

### INTRODUCTION

Installation of anti-hail nets is an important practice in apple orchards to insure sustainable fruit production and fruit quality. Apple orchards are covered with nets to protect fruit and plants from hail damage in areas with high frequency of hailstorm. Covering apple trees with nets can result with multiple vegetative and reproductive plant responses, fruit colour and sugar content, modifying tree vigour, flower induction etc. Most of these plant responses are related to changes in micro-environmental conditions such as air temperature and relative humidity, but mainly in light conditions. Light conditions play a fundamental role on different physiological responses in apple trees, including direct effects on leaf photosynthetic capacity, fruit colour, carbohydrate partitioning, flower induction and changes in the growth pattern of leaves, shoots and fruit. Therefore, the light environment of an orchard influences fruit quality, including red pigmentation, soluble solids concentration, and fruit size and weight as well as maturity development. The uncertainty about effects of netting on fruit quality is concerns that have made producers reluctant to install netting over orchard. Under protected cultivation, the interaction between plants and environment occur differently than they do under the open sky due to changes in a various microclimatic variables that may influence the fruit quality (Tanny and Cohen, 2003).

This study presents the influence of black nets on the fruit quality of two red apple cultivars. Except their protection effect, nets should not have any others negative effects on the quality parameters, because producing apples with high quality that meet international standards are of high importance for fruit industry.

### MATERIALS AND METHODS

This study was carried out with an apple orchard located in Gradačac, at 129 m above sea level. In this study two apple cultivars: 'Jonagold' and 'Idared' grafted on M9 rootstock were included. The apple trees were trained as slender spindle and planting distance was 3.8 x 1.2 m. The experiment encompassed the following treatments: (1) Trees covered with black net and (2) Uncovered (control) trees. Apples were harvested from uncovered and covered trees on the same day (corresponding to commercial maturity of fruits from uncovered trees). Each treatment was comprised of four replications (totaling 30 fruits per plant from each environment

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and cultivar). Fruit samples from control and anti-hail nets covered trees fruits were left in air cold storage. The apples were stored under conditions of normal atmosphere (at 0°C of temperature and 90 ± 5% of relative air humidity).

The variable analyzed in the laboratory included skin color, pulp firmness (kg/cm<sup>2</sup>), total soluble solids content (TSS; °Brix), total titratable acidity (TTA; %) and anthocyanins content. The skin color of each sampled apple was randomly measured using a Konika/Minolta CR-400 colorimeter according to the CIELAB system proposed by Commission Internationale de L'Eclairage in L\*a\*b\*. In this study, a skin color representation is given by three different parameters: lightness luminosity (L), hue angle (H) and chromacity. The hue angle indicates the location of the color on a diagram where angle of 0° corresponds to pure red, 90° to pure yellow, 180° to pure green and 270° to pure blue. The hue angle was calculated using the equation  $H = \arctan(b/a) \times 57.3$ . The chroma indicates the color intensity (saturation) and it describes degree of departure from the gray of the same lightness and it was calculated by the equation  $C = (a^2 + b^2)^{0.5}$ .

Pulp firmness was measured with a digital penetrometer (model 53205, T.R. Turoni SRL, Forli, Italy) with an 11-mm-diameter plunger (measured on four sides of each fruit). Total soluble solids contents were determined in the juice of each sample using a refractometer (model Atago, Tokyo, Japan) at 20°C and titratable acidity was determined by titrating apple juice diluted with 0.1 M NaOH until reaching pH 8.1, with a phenolphthalein as indicator, and expressed as percentage of malic acid. Total anthocyanin content was measured with the pH differential absorbance method. The absorbance of the mixture was then measured at 510 and 700 nm. Briefly, absorbance of the extract was measured at 420 and 700 nm in buffers at pH 1.0 (potassium chloride, 0.025 M), and 4.5 (sodium acetate, 0.4 M). Results were expressed in mg cyanidin 3- glucoside equivalents 100 g<sup>-1</sup> fresh weight.

Fruit quality parameters were determined before storage and after seventy days of cold storage. The analysis of variance (ANOVA) for a completely randomized design was performed for each variable. Statistical computer software SPSS 22, was applied for computing ANOVA.

## RESULTS AND DISCUSSION

Tab. 1. Pulp firmness and total soluble solids (TSS) of 'Idared' and 'Jonagold' fruit under the open sky and anti-hail net related to storage term

Term	Total soluble solids (TSS)						Pulp firmness					
	'Idared'		AHN Sig.	'Jonagold'		AHN Sig.	'Idared'		AHN Sig.	'Jonagold'		AHN Sig.
	Control	AHN*		Control	AHN		Control	AHN		Control	AH N	
Before storage	13.3a	13.2 a*	NS	14.1 a	15.2 a	NS	6.8 a	6.9 a	NS	5.3 a	5.7 a	NS
After storage	13.0 a	12.8 a	NS	14.8 a	15.0 a	NS	5.1 b	5.0 b	NS	4.4 b	4.5 b	NS
Mean	13.2 a	13.1 a	NS	14.3 a	15.11 a	NS	6.0 a	6.0 a	NS	5.0 a	5.3 a	NS
Mean of cultivar	13.1 b			14.7 a			6.21 a			5,03 b		
AHN: Anti-hail nets; * Mean values with similar letters are not significantly different (P=0.05)												

Regarding the presented data in Table 1 there was no significant influence of anti-hail nets and storage condition on pulp firmness. Control fruit had the same results as fruit under anti-hail net in terms of pulp firmness loss. This result is in accordance with Iglesias and Algere (2006), Borin and Saoncella (2000), Crété (2000), Scott (1989) as well as Wagenmakers and Callesen (1995). Widmer (2001), Gardner and Fletcher (1990) reported that the fruit under net had slightly greater pulp firmness then fruit grown under the open sky. The analysis of variance showed a statistically significant effect of cold storage on the fruit flesh firmness. However, stored fruit had significant loss of pulp firmness after 70 days of storage.

The use of anti-hail nets during the fruit growing period would not be beneficial to change of total soluble solids. The obtained results are not in accordance with Coreau *et al.* (1997), Crété *et al.* (2001), Peano *et al.* (2002), Iglesias and Algere (2006) who reported that enviromental under anti-hail nets reduced content of soluble solids in 'Gala' apple. 'Jonagold' apple cultivar was recorded significant greater total soluble solids compared to observed results in 'Idared' fruits.

Tab. 2. Total titratable acidity (TTA) and Anthocyanin content of 'Idared' and 'Jonagold' fruit under the open sky and anti-hail net related to storage term

Term	Total titratable acidity (TTA)						Anthocyanin content					
	'Idared'		AHN Sig.	'Jonagold'		AHN Sig.	'Idared'		AHN Sig.	'Jonagold'		AHN Sig.
	Control	AHN*		Control	AHN		Control	AHN		Control	AHN	
Before storage	0.72 a	0.78 a	NS	0.46 a	0.49 a	NS	136.4 a	76.4 a	***	100.9 a	65.1 a	***
After storage	0.63 b	0.66 b	NS	0.41 a	0.42 b	NS	63.8 b	55.8 b	**	54.5b	46.6 b	**
Mean	0.68 a	0.72 a	NS	0.44 a	0.46 a	NS	100.1 a	66.1 b	***	77.7 a	55.8 b	**
Mean of cultivar	0.70 a			0.46 b			83.10 a			66.75 b		
AHN: Anti-hail nets; * Mean values with similar letters are not significantly different (P=0.05)												

A significant decrease in total acidity was recorded during the cold storage of 'Idared' cultivar.

There was no significant decrease of the total acidity during storage period and there were no significant differences in titratable acidity between control fruits and fruits under anti-hail net. Bosco *et al.* (2015) noted that there were differences in the acidity between the environments under anti-hail nets and under sky. 'Idared' apple cultivar was recorded significant greater titratable acidity (0.70%) compared to 0.46% observed in 'Jonagold' fruits.

The results from table 2 presented significantly higher concentration of anthocyanins ( $p < 0.001$ ) in fruits of 'Idared' apple cultivar than in 'Jonagold'. According to presented study, after the storage there was a significant decrease of the anthocyanins content. Significant difference in anthocyanins content between the apple under anti-hail net and in open sky was found. The apples grown under anti-hail nets had lower anthocyanins content than control apples. The decrease of anthocyanin content between apple grown under anti-hail nets and without nets was greater in 'Idared' apple cultivar than in 'Jonagold'.

Tab. 3. Skin color of 'Idared' and 'Jonagold' fruit under the open sky and anti-hail net related to storage term

Term	Lightness (L)					
	'Idared'		AHN Sig.	'Jonagold'		AHN Sig.
	Control	AHN*		Control	AHN	
Before storage	42.89 a	43.67 a	NS	55.90 a	53.89 a	NS
After storage	43.47 a	44.01 a	NS	50.40 b	49.98 b	NS
Mean	43.08 a	43.78 a	NS	52.23 a	51.40 a	NS
Mean of cultivar	43.43 b			51.83 a		
Chromacity (C)						
Before storage	34.97 a	33.45 a	NS	37.75 a	33.34 a	*
After storage	35.57 a	34.09 a	NS	43.26 b	36.86 b	**
Mean	35.17 a	33.67 a	NS	40.51 a	35.10 b	**
Mean of cultivar	34.42 a			38.35 b		
Hue angle (H)						
Before storage	33.06 a	37.03 a	NS	70.53 a	68.35a	NS
After storage	34.33 a	37.31 a	NS	53.25 b	50.52 b	NS
Mean	33.49 a	37.13 a	NS	59.01a	57.01a	NS
Mean of cultivar	35.31b			58.05 a		
AHN: Anti-hail nets; * Mean values with similar letters are not significantly different (P=0.05)						

The fruit skin colour differs between the environment under anti-hail net and under open sky only in accordance to chroma of cultivar 'Jonagold'. The chroma of 'Jonagold' fruits under the open sky was significant greater than under the anti-hail net. The results suggest that anti-hail net did not influence the hue angle and lightness, even with microclimatic changes under the net (Table 3). This result does not in accordance to results of Bosco *et al.* (2015) who reported that anti-hail net did not influence the any parameter of fruit color.

In the case of color lightness and hue angle the results in Table 3 indicates that storage caused significant decrease of this parameters in the skin of 'Jonagold' fruit. Storage of fruit caused decline of chroma in the skin

of 'Jonagold' cultivar. The fruit color parameters of 'Jonagold' were significant greater than the skin color of 'Idared' cultivar.

## CONCLUSIONS

The pulp firmness, total soluble solids and total titratable acids of 'Idared' and 'Jonagold' apples are not altered by the use of black anti-hail nets over the orchard. Coverage by anti-hail nets had significant influence on the anthocyanin's content and chroma of 'Jonagold' apple. The use of anti-hail nets over the apple orchards caused only small changes in fruit quality and it could be recommended as a suitable alternative for protecting apple trees against hail.

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## EFFECT OF POST HARVEST TREATMENTS ON PHYSICOCHEMICAL PROPERTIES OF CANTALOUPE

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*Original scientific paper*

### Summary

Cantaloupes (*Cucumis melo* L. subsp. *melo* var. *cantalupensins* Naudin) cv. 'Chianti' were evaluated for quality traits during 14 days of storage at three different temperatures i.e., 2, 10 and 18°C with 85-95% relative humidity. The weight loss of fruits slightly increased during the stored period studied, as well as insignificant differences between the temperatures. Whereas significant texture loss was more rapidly in the samples stored at 18°C and 10°C than those stored at 2°C. TSS was also affected by storage time and temperature. The TSS (total soluble solids) content of fruit at 2°C increased and then remained constant over storage. At higher temperatures and at every stage of storage time TSS increased as storage time increased. The predominant carotenoid in all samples was  $\beta$ -carotene. The carotenoids components were increased and then decreased with the time; however, the decrease processes were delayed by low temperature. The alpha form was the predominant tocopherol fraction. The level of tocopherol isomers significantly ( $\alpha$ - tocopherol) and gradually ( $\gamma$ - and  $\delta$ - tocopherol) increased during the 7 days, but after 1st week of storing for all isomers a significant decrease was measured. High temperature storage at 18°C in comparison to 10°C and 2°C promoted  $\gamma$ - and  $\delta$ - tocopherol level.

Key words: *Cantaloupe; storage; weight loss; texture; soluble solids; carotenoids; tocopherols*

### INTRODUCTION

Cantaloupe (*Cucumis melo* L. subsp. *melo* var. *cantalupensins* Naudin) is one of the most important horticultural crops in the world of agriculture (Nuñez-Paleniús *et al.*, 2008). According to Ban *et al.* (2011) the global consumption of these fruits are greater than that of any other species in the Cucurbitace family. Cantaloupes are a popular delicacy and exclusively consumed as fresh or minimally processed products such as fresh cut pieces of watermelon with rind or as cubes without rind.

Some researchers suggest that extract of cantaloupe melon may serve as a potential source of natural phytochemicals for food and nutraceutical application (Lester, 2006). Over the last decade, there has been increased interest in plant foods that are rich in health-protecting source of minerals, vitamins and phytochemicals (Veberič *et al.*, 2009). Among these, carotenoids and tocopherols constitute important groups in human diet (Landrum and Bone, 2001). The protection provided by fruits and vegetables against a wide range of several degenerative diseases, including cancer, cardiovascular diseases and other chronic diseases, has been attributed to the antioxidants they contain (Sangeetha and Baskaran, 2010). The reasons for this relationship appear to be multi-faceted and include components in plants that are used as a free radical scavengers, singlet and triplet oxygen quenchers, detoxification agents, or as plant defense response components (Perkins-Veazie *et al.*, 2003).

One of the most important factors in the production and marketing of cantaloupes is the postharvest quality. Cantaloupe fruits in general have limited storage time and become soft and shriveled after about two weeks because the open, netted epidermis favors high transpiration rates (Fallik *et al.*, 2005). The recommended storage temperature for whole cantaloupe is varied between 2 (Flores *et al.*, 2007) to 18°C (Radulović *et al.*, 2007).

The development of new cultivars of cantaloupe melons with high added value and which are well adapted to the Slovenian growing conditions has stimulated the interest in the commercial cantaloupes production. During the 2012 season, study was conducted to evaluate the effectiveness of holding fresh cantaloupe fruits in different storage temperatures of 2, 10 and 18°C to extend their post harvest life and maintain fruit quality parameters.

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Further, research was focused on the degradation of carotenoids of cantaloupe fruits, which data are missing in the literature.

## MATERIALS AND METHODS

Cantaloupes cv. 'Chianti' (Semenarna d. o. o., Ljubljana) were grown at the Experimental Station of the Biotechnical Faculty in Ljubljana. Standard agricultural practices were adopted uniformly according to crop requirements. Thirty of the tagged fruits per plot were harvested on the same day when they reached the horticultural maturity (i.e. at the 3/4 to full-slip stage). After harvest fruits were held overnight at about 10-15°C and transferred the following day to the laboratory. Fruits were randomly divided into three groups, each group containing 30 fruits in four replicates. Fruits were then placed in the chambers 14 days at 85-95 relative humidity to simulate a commercial storehouse situation. The first group was stored at 2°C, the second one at 10°C and the third one at the 18°C. The fruits were analysed three times during storage from August 28<sup>th</sup> to September 10<sup>th</sup> 2012.

Weight loss was evaluated by the difference in fruit weight on the first day of the experiment and each sampling. The weight of fruits was recorded using a Mettler balance model Toledo PB 602.

Texture (firmness) was measured in the pulp, by removing 1 cm<sup>2</sup> of the fruit skin, on opposite sides using a Chatillion penetrometer (model DFG 50), equipped with an 11 mm diameter round stainless steel probe with flat end (John Chatillion & Sons, U.S.A.). The crosshead speed during the firmness testing was 10 mm/min. Two measurements of the force needed to penetrate the mesocarp tissue were taken for each fruit, and an average was calculated as a puncture force (kg cm<sup>-2</sup>) recorded during penetration.

Total soluble solids concentration (Brix was noted in percentage) of the expressed juice was determined using a hand-held Atago PR1 refractometer (brix range 0-20% at 20°C).

When weight, texture and soluble solids measurements had been performed, sample of fruits flesh were chopped, frozen in liquid nitrogen and stored at -20°C. For detection of dry weight (DW), 2 g of the frozen sample was freeze-dried for 22 h in a Gamma 2-20 lyophilizer (Christ, Germany). Water content (%) was calculated from the difference between the masses before and after the lyophilization.

Carotenoid components and isomers' composition of tocopherols were determined using an improved method described in Kacjan-Maršič *et al.* (2010). Carotenoids and isomers' composition of tocopherols were extracted from the dry fruit powder with ice-cold acetone. All extraction procedures were performed in dim light. Acetone extracts were subjected to HPLC gradient analysis (column Spherisorb S5 ODS-2 250 x 4.6 mm with precolumn S5 ODS-2 50 x 4.6 mm). Tocopherols were separated using methanol as solvent. Tocopherols were detected directly by fluorometry (excitation 295, emission 325). Carotenoids were separated using the following solvents: solvent A, acetonitrile/methanol/water (100/10/5, v/v/v); solvent B, acetone/ethyl acetate (2/1, v/v), at a flow rate of 1 mL3 min<sup>-1</sup>, linear gradient from 10% solvent B to 70% solvent B in 18 min was applied, run time 30 min, photometric detection at 440 nm.

All measurements were performed in triplicates (n = 3). Statistical analysis was performed using the Statgraphics programme, version 4.0. Results were compared by Tukey HSD test.

## RESULTS AND DISCUSSION

The average weight of randomly chosen cantaloupe fruits measured after harvesting was 1142 g. It was found that weight loss is quite uniform irrespective of storage temperatures. Effects of storage conditions on weight loss of stored fruits are listed in Figure 1. Maximum weight loss occurred at 18°C (7.6%), while lowest loss was recorded at 10°C (0.9%), after the first week of storage, and 13.6% (at 18°C) and 3.5% (at 2°C) for fruits stored for two weeks. However, the difference was not statistically significant.

The weight loss experienced for other fruits and vegetables, during recommended storage conditions and storage period varies between 2 and 10%, due to moisture losses (Elkashif *et al.*, 1989). Minimal economical loss (weight loss) for cantaloupe melon during storage is due to the rind thickness and wax layer on the surface of the fruit, and for the cantaloupe melons are reported to show no climacteric activity (Karakurt and Huber, 2002).

The textural quality of fruits is influenced by flesh firmness. The development of pericarp firmness, i.e. the softening of the fruits, was significantly affected by storage time and temperature (Figure 2). Firmness decreased notably at all temperatures during the storage period. It was observed from the present research intervention that firmness decreased as a function of storage time (Požrl *et al.*, 2010). However, firmness decreased slowly in fruits stored at 2°C. At the higher temperatures, the decrease in firmness was more

noticeable. This was in agreement with the slow maturation expected at low temperatures (Lainez and Krarup, 2008). A close relationship between the softening of the fruits, higher temperature and extension of storage time was described in previous studies (Beaulieu and Lea, 2007). It was suggested by Žnidarčič *et al.* (2010) that a postharvest change in texture can occur due to the loss of moisture through transpiration, as well as enzymatic changes. In addition, hemicelluloses and pectin become more soluble, which results in disruption and loosening of the cell wall structure and composition (Paul *et al.*, 1999).

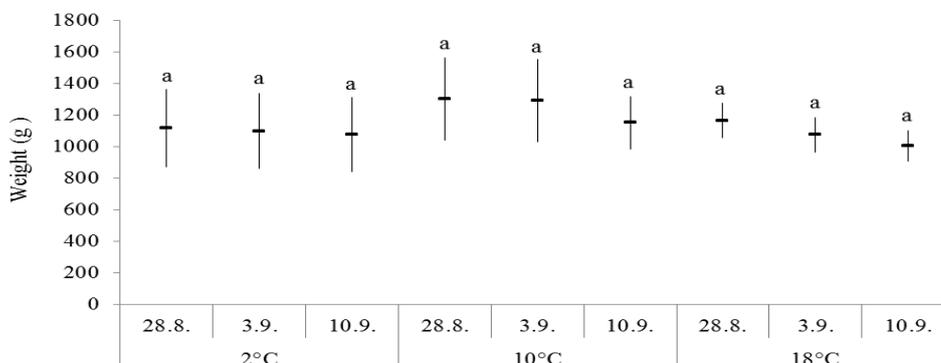


Fig. 1. Changes in the weight loss (%) during 14 days storage at 2, 10 and 18°C (vertical bars represent standard errors of the means).

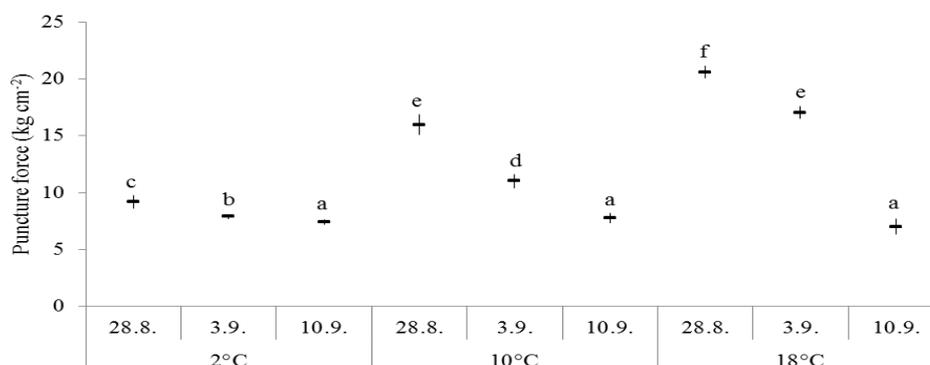


Fig. 2. Changes in the puncture force ( $\text{kg cm}^{-2}$ ) during 14 days storage at 2, 10 and 18°C (vertical bars represent standard errors of the means).

TSS content is related to the balance of sugars and acids and it has a major impact on the flavour of the vegetable (Guilen-Rios *et al.*, 2006). Figure 3 show that the storage period has a significant effect on TSS. It is clear from the obtained data that TSS has a significant rise through fruit development regardless of temperature and the maximum is observed in the end of the storage period. The gradual increase of TSS up to 21 days of storage might be due to conversion of insoluble to soluble forms of sugars and the least utilization of organic acid.

According to Rathore *et al.* (2007), the increment of TSS might be due to the alteration in cell wall structure and breakdown of complex carbohydrates into simple sugars during storage.

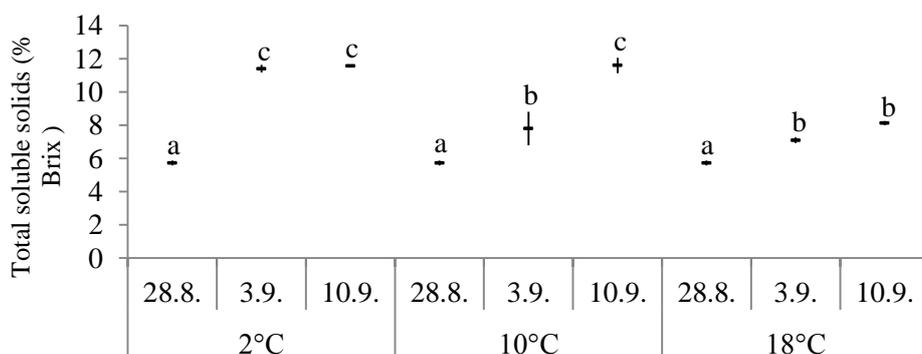


Fig. 3. Changes in the total soluble solids (% Brix) 14 days storage at 2, 10 and 18°C (vertical bars represent standard errors of the means).

Carotenoid levels appears to vary by species, variety, cultivar, biochemical attributes, irrigation, degree of maturity at harvest, growing site, climate, soil fertilization (van den Berg *et al.*, 2000). Some authors have observed that operation carried out during the post-harvest storage is also very important (Kidmosea *et al.*, 2006). For example, some authors (Mencarelli and Salveit, 1988) mention that fruits biosynthesize carotenoids during ripening through-out the storage time. In our study, the major carotenoid found in the melon fruits was  $\beta$ -carotene, making up on average 90% of the total carotenoids.  $\beta$ -carotene have been widely reported as being one of the major carotenoids found in vegetables (Calvo, 2005).

Changes in carotenoid components of fruits are presented in Table 1. No significant differences in  $\beta$ -carotene content were found between fruits stored at 2°C and 10°C. On the contrary,  $\beta$ -carotene content showed significant increase in fruits stored at 18°C. A steady loss in  $\beta$ -carotene was observed during storage. Initial  $\beta$ -carotene content of fruits was  $90.41 \pm 28.19$  mg/100 g, respectively, on dry weight basis. The lowest value was registered in fruits within three weeks of storage ( $51.25 \pm 3.88$  mg/100 g).

In general, lutein content progressively increased with increasing temperatures. The initial level of lutein content was about  $0.82 \pm 0.24$  mg/100 g dry weight. During the storage the lutein content was increasing slightly in the first seven days and afterward the content of lutein started to decrease. As far as we know, there are no published works quantifying lutein content throughout storage of melon fruits.

The lycopene content was found to increase from a low value ( $2.74 \pm 0.16$  mg/100 g) at 2°C g to a maximum ( $6.01 \pm 1.34$  mg/100 g) at 18°C. Lycopene concentrations were also affected by storage time. Maximum lycopene accumulation ( $3.71 \pm 1.18$  to  $4.81 \pm 1.93$  mg/100 g) occurred in fruits that were 0-7 days in age, while the lowest lycopene concentration ( $1.91 \pm 0.52$  mg/100 g) was in fruits at the end of storage.

Some authors (Lee and Chen, 2002) are generally in agreement that lycopene remains relatively stable during typical food processing procedures, except at extreme conditions. On the other hand, Tonucci *et al.* (1995) reported that lycopene content of vegetables increased by thermal process. For example, Shi *et al.* (2002) explained that this change might be caused by an increased release of lycopene from the cell, skin and insoluble fiber of tomato.

Tab. 1. Changes in the carotenoid components (mg/100g) during 14 days storage at 2, 10 and 18°C

	$\beta$ -carotene	Lutein	Lycopene	Total carotenoids
<i>Temperature</i>				
2 °C	69.06 $\pm$ 7.05	0.56 $\pm$ 0.03	2.74 $\pm$ 0.16	72.35 $\pm$ 7.23
10 °C	69.58 $\pm$ 20.02	1.02 $\pm$ 0.49	3.42 $\pm$ 1.67	74.02 $\pm$ 22.18
18 °C	145.31 $\pm$ 15.51	1.31 $\pm$ 0.38	6.01 $\pm$ 1.34	152.64 $\pm$ 32.33
<i>Days</i>				
0	90.41 $\pm$ 28.19	0.82 $\pm$ 0.24	3.71 $\pm$ 1.18	94.92 $\pm$ 20.27
7	93.76 $\pm$ 15.79	1.31 $\pm$ 0.71	4.81 $\pm$ 1.93	99.87 $\pm$ 26.14
21	51.25 $\pm$ 3.88	0.48 $\pm$ 0.02	1.91 $\pm$ 0.52	53.63 $\pm$ 4.25
<i>Significance</i>				
Temperature (T)	*	*	*	*
Days (D)	*	*	*	*
T x D	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at  $P < 0.01$

Tocopherols, collectively known as vitamin E, are one of the most important components of cellular antioxidant systems. Although tocopherols appear to be universal constituents of all higher plants (Threlfall and Whistance, 1971), to our knowledge, there have been no reports of the qualitative composition of tocopherols in cantaloupe fruits. In our study, three isomers of tocopherols were detected ( $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol), and the results are shown in Table 2.

Among the tocopherols we determined after harvest (at 0 day)  $\alpha$ -tocopherol was predominant at the level of  $4.82 \pm 0.87$  mg/100 g. The contents of  $\gamma$ - and  $\delta$ -tocopherol were  $1.42 \pm 0.61$  mg/100 g and  $0.17 \pm 0.08$  mg/100 g, respectively.

Tab. 2. Changes in the isomers' composition of tocopherols (mg/100g) during 14 days storage at 2, 10 and 18°C

	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	Total tocopherols
<i>Temperature (°C)</i>				
2	4.32±0.97	1.23±0.07	0.08±0.04	5.63±0.89
10	4.92±0.95	1.24±0.68	0.27±0.17	6.43±0.96
18	7.06±1.48	2.61±0.44	0.48±0.38	10.15±1.52
<i>Days</i>				
0	4.82±0.87	1.42±0.61	0.17±0.08	6.41±0.87
7	8.86±0.64	1.98±0.62	0.41±0.12	11.25±2.07
21	3.44±1.59	0.89±0.33	0.06±0.02	4.39±1.92
<i>Significance</i>				
Temperature (T)	*	*	NS	*
Days (D)	*	*	*	*
T x D	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at  $P < 0.01$

During 21 days of post harvest the higher the storage temperature, the higher the content of tocopherols. Only exception is  $\delta$ -tocopherol which concentration showed no substantial variations due to temperature conditions. As shown in Table 2 content of tocopherols grew and peaked after 1<sup>st</sup> week of storage. Decrease of tocopherols after seven days was almost linear in all cases although the rate of decrease was slightly higher in  $\alpha$ - and  $\delta$ -tocopherol. According to Abramovič *et al.* (2007), the lower stability of  $\alpha$ -tocopherol in comparison to  $\gamma$ - and  $\delta$ -tocopherol is due to the fact that tocopherols reacts faster with peroxy radicals formed in the process of autooxidation.

## CONCLUSIONS

As consumers become increasingly aware of the nutritional value of foods and interested in a healthier lifestyle, the positive nutritional information on cantaloupe melons will help consumer to make informed decision on consuming this nutritious food. The current data demonstrated the importance of not only temperature but also time of storage for the cantaloupe fruits quality parameters. To preserve fruits bioactive compounds it is recommended to keep them in storage for maximum of 7 days at 18°C.

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## SOIL-WATER CHARACTERISTIC CURVE AND RETENTION OF WATER FOR DIFFERENT TYPES OF AGRICULTURAL SOILS IN TUZLA CANTON

Jasminka Žurovec<sup>1</sup>, Sabrija Čadro<sup>1</sup>

*Original scientific paper*

### Summary

About 78% of soil in Tuzla Canton predominantly belongs to IVa, IVb, V and VI quality group. These soils are mostly heavy-textured, with poor soil water and physical properties. Knowledge of their soil-water characteristics and possibility for water retention plays a critical role in water management of these soils, especially within agriculture. Construction of soil-water characteristic curve (SWCC) and determination of water retention capacities for three most important agricultural soil types in the area of Tuzla canton (Luvisol, Pseudogley, Fluvisol) was a main objective of this paper. In order to fulfill this objective, the following analyses were carried out: basic soil water and physical characteristics; soil water retention capacity at maximum water capacity (MC), field water capacity (FWC), lentic-capillary point (LCP) and wilting point (WP); Readily (RAW) and total available water (TAW). For conducting previously mentioned analysis, undisturbed and disturbed soil samples were taken from nine soil profiles in three replicates. Soil in each profile was divided into two separate layers: anthropogenic surface layer (depth from 0 - 25 cm) and subsurface layer (depth from 25 – 60 cm). The highest amount of water in the soil (mm), over the entire water potential range (pF), is in Luvisol (81 - 186 mm), while the lowest in Fluvisol (60 - 168 mm). Pseudogley have the best water characteristics and the highest capacity for readily available water (146 mm), this is primarily the result of favorable conditions in its subsurface horizon (25 – 60 cm) compared to other two analyzed soil types.

**Key words:** *Soil water characteristic curve, retention, soil water capacity, agriculture soil*

### INTRODUCTION

The water in the soil can be absorbed, adsorbed, capillary, gravitational, and in the form of vapor or ice. Absorbed water enters into the composition of the solid soil particles as chemically bound or crystallization water in minerals, rocks, or organic matter. Adsorbed water is physically attached to soil particles due to adhesion force of water and soil particles. Capillary water is held in the small soil pores with capillary forces or surface tension forces and represents the plant most available form of water in the soil. Together adsorbed and capillary water are called soil retention water (Resulović *et al.*, 2002). The joint effect of the adsorption and capillary forces, or forces which hold the soil retention water, is called the soil matrix potential -  $\Psi_m$  (Dugalić *et al.*, 2012).

Water in the soil has a free energy that is a measure of its potential for movement and change. In soils with a high moisture content, adhesive force of water to soil particles is weak and free energy is high. With the decrease in moisture, adhesive force becomes higher and the free energy decreases. State of free energy in the soil determines water behavior and the availability for plants.

The total water potential ( $\Psi$ ), and the force that causes the energy state of water in the soil is the sum of gravitational ( $\Psi_g$ ), matrix ( $\Psi_m$ ) and osmotic ( $\Psi_o$ ) potentials. In addition to the potential, we can find synonyms in the literature, like tension or suction of soil water (Resulović *et al.*, 2002; Dugalić *et al.*, 2012; Žurovec, 2012; Šimunić, 2013). The water potential of the soil is expressed as a negative pressure (bar, psi, Pascal, cm of water) or pF units. In the soil there is a direct relationship between the strength of the negative water potential ( $\Psi$ ), or pF value and quantity of water. This value of water quantity depending on the water potential ( $\Psi$ ) varies from soil to soil and mainly depends of the texture, organic matter content and soil differential porosity (Resulović 2002; Dugalić *et al.*, 2012).

Of the total Tuzla Canton area (2,650 km<sup>2</sup>), agricultural land represents 68.4%. About 78% of soil in this Canton predominantly belongs to IVa, IVb, V and VI quality group, according to methodology for the classification of agricultural land of Federation of BiH (Off. Gazette of FBiH, No. 52/09). These soils are mostly heavy-textured, with poor soil water and physical properties (Resulović *et al.*, 2000, Žurovec *et al.*, 1987). Knowledge of their soil-water characteristics (SWC) and possibility for water retention, especially at readily available water

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capacity (RAW) plays a critical role in water management of these lands, particularly within the field of agriculture.

## MATERIALS AND METHODS

According to Soil map of Bosnia and Herzegovina, scale 1:50.000 (1990), most important soils for agricultural production in the research area are Luvisol, Pseudogley and Fluvisol. Three locations across Tuzla Canton were selected for each soil type. Nine soil profiles (up to 1 m deep) were opened on these locations. Undisturbed and disturbed soil samples were taken from each soil profile and each horizon in three replicates, 81 samples in total. Analyses of soil physical and water characteristics were carried out in laboratories of Institute for Pedology, Agrochemistry and Melioration at the Faculty of Agriculture and Food-science and the Federal Institute for Agropedology, Sarajevo.

The following analyzes had been carried out: Soil physical characteristics through analysis of soil texture (*international pipette B method*) bulk density (*gravimetric method in Kopecky cylinders*) and absolute density (*Air pycnometer*) according to Manual for Soil Testing (Resulović *et al.*, 1971); Soil water characteristics through soil water retention ( $\Theta_v$ ) at a given matric potentials using a pressure-membrane extraction apparatus according to Richards method (1941). Following negative potentials ( $\Psi$ ) were used: -0.33 bar (pF 2.54: field water capacity), -1 bar, -6.25 bar (pF 3.8: lento-capillarity point) and -15.5 bar (pF 4.2: permanent wilting point). The relationship between water potential ( $\Psi$ ) and soil water content ( $\theta$ ) were graphically presented in form of soil water characteristic curve (SWCC) or pF curve. Totally (TAW) and readily (RAW) available water capacity of soil was calculated from following relation between field water capacity (FWC), lentocapillary point (LCP) and permanent wilting point (WP): TAW = FWC – WP; RAW = FWC – LCP. Maximum water capacity (MC) was divided from bulk and absolute density (Resulović *et al.*, 1971). Soil in each profile was divided into two separate layers: anthropogenic surface layer depth from 0 - 25 cm and subsurface layer depth from 25 – 60 cm.

## RESULTS AND DISCUSSION

After the insight into soil map of B&H (1:50.000) and reviewing the situation in terms of spatial distribution and the importance for agricultural production, three soil types in the area of Tuzla canton were selected for this research: Luvisol, Pseudogley and Fluvisol. These are soils that are often used in agricultural production, occupying significant areas, mostly in the lowland part of the Canton and require a certain degree of melioration improvement. The soil samples were taken from agricultural plots that were used for many years, so the soil on this plots through the influence of anthropogenization lost its original type characteristics. After taking soil samples from the field and the laboratory analysis, the following average soil physical characteristics (Table 1) for these three soil types are determined.

Tab. 1. Average physical characteristics of analyzed soils

Soil type	Horizon	Depth (cm)	Skeleton (%)	Texture			Class	Bulk density (g/cm <sup>3</sup> )	Absolute density (g/cm <sup>3</sup> )
				Sand (%)	Silt (%)	Clay (%)			
Pseudogley	Ap	0 - 23	0.21	25.74	46.05	28.21	Clay loam	1.15	2.69
	Sw	23 - 55		24.73	40.81	34.46	Clay loam	1.34	2.78
	Sd	55 - 100		20.95	36.40	42.64	Clay	1.41	2.79
Luvisol	Ap	0 - 20	0.13	27.45	41.55	31.00	Clay loam	1.22	2.60
	E	20 - 45	0.97	25.71	39.02	35.27	Clay loam	1.36	2.71
	Bt	45 - 100	0.18	15.66	25.79	59.55	Clay	1.25	2.73
Fluvisol	Ap	0 - 20	3.18	43.27	29.92	29.28	Clay loam	1.34	2.72
	I	20 - 60	6.86	45.20	26.62	29.82	Clay loam	1.41	2.69
	II	60 - 100	5.95	41.31	28.57	31.39	Clay loam	1.43	2.68

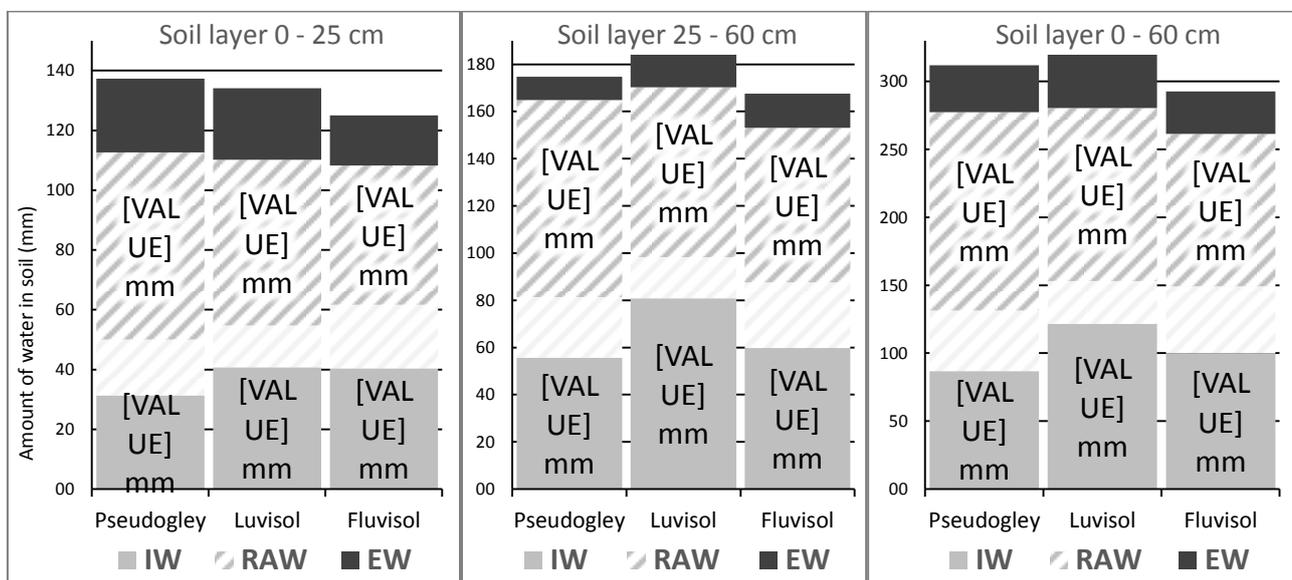
All three soils in general are heavier textured, with significant clay content (28.21 - 59.55%) which increases with depth. Fluvisol has a slightly higher sand content and the presence of skeleton compared to the other two soil types. Bulk density is within the limits of normal agricultural production. However, higher values in the second horizon (1.34 to 1.41 g/cm<sup>3</sup>) in all the three soils, and a high content of clay (29.82 - 35.27%) indicate near surface presence of impermeable soil layer. Table 2 shows average soil water content for different retention

capacity points and layers. For the surface horizon (0 - 25) the highest average value of a water content at maximum water capacity (MC), field water capacity (FWC) and 3 bar point is in Pseudogley, respectively 137, 113 and 83 mm. Lento-capillarity point (LCP) was highest is in Fluvisol (62 mm) and wilting point (WP) in Luvisol (41 mm). In sub-surface horizon (25 – 60 cm) Luvisol has highest soil water content for all retention capacity points. Same situation is evident when the entire researched depth (0 - 60 cm) is considered in total.

Tab. 2. Average soil water content (mm) for different water retention capacities

Soil type	Depth (cm)	$\Psi$ pF (bar)	MC	FWC	-	LCP	WP	TAW	RAW
			-	2.54 (-0.33)	3 (-1)	3.8 (-6.25)	4.2 (-15.5)	FWC - WP	FWC - LCP
Pseudogley	0 - 25		137	113	83	50	31	81	63
	25 - 60		175	165	129	81	56	109	84
	0 - 60		312	277	212	131	87	191	146
Luvisol	0 - 25		134	110	82	55	41	69	55
	25 - 60		186	170	137	98	81	89	72
	0 - 60		320	280	219	153	121	159	127
Fluvisol	0 - 25		125	108	82	62	40	68	46
	25 - 60		168	153	113	87	60	94	66
	0 - 60		292	261	195	149	100	161	112

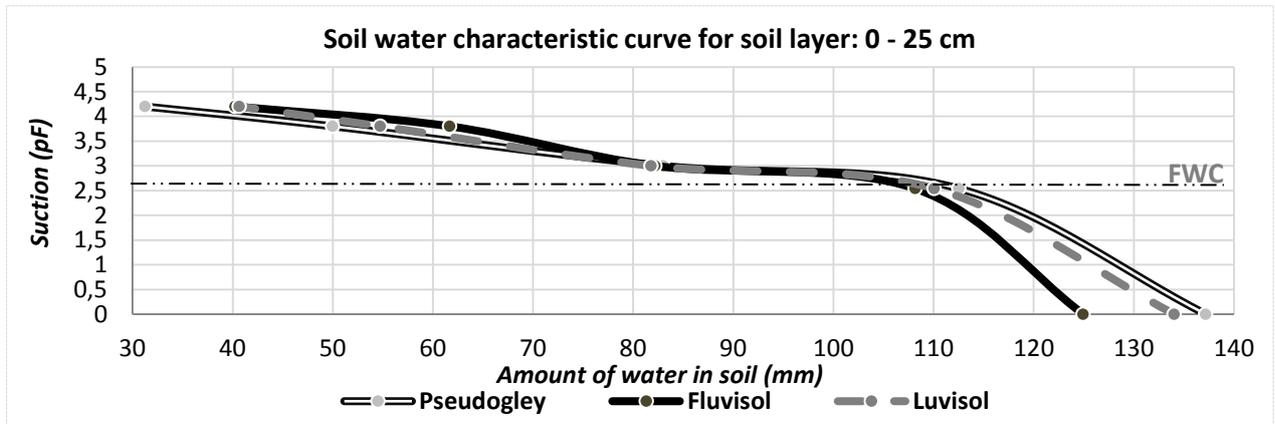
The soil water retention at excess water (EW), readily available water (RAW) and inaccessible water (IW) capacity for three analyzed soils and all layers is shown in Graph 1. Excess water (EW) represents a plant adverse water, which is found in large pores (diameter > 10  $\mu\text{m}$ ), and moves under the influence of gravity force. In the surface layer, the biggest values of EW are in Pseudogley and Luvisol (24 mm), while Fluvisol has lowest values (17 mm). The amount of water at RAW (FWC – LCP) capacity represents the most accessible form of soil water for plants and it is regarding agriculture main indicator of its good retention characteristics. In both layers, surface (0 – 25 cm) and sub-surface (25 – 60), as well as in whole profile (0 – 60 cm), Pseudogley has the highest RAW or in other words the best retention properties from three analyzed soils.



Graph 1. The soil water retention at excess water (EW), readily available water (RAW) and inaccessible water (IW) capacities

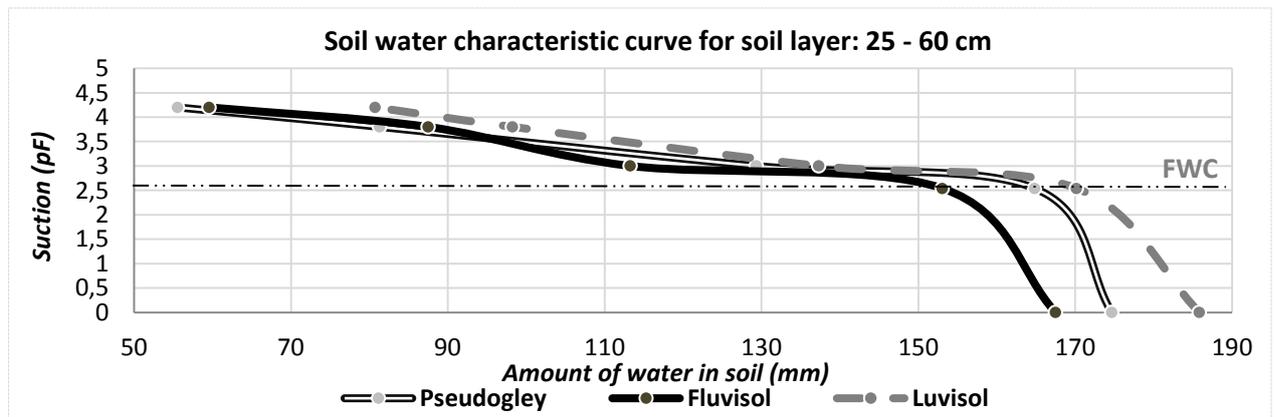
Inaccessible water (IW) in the soil is located in fine - micro pores (diameter <0.2  $\mu\text{m}$ ). This water is holding on soil particles with forces higher than roots average absorption force (15.5 bar), therefore the plants cannot use it. For all the three analyzed soils, the maximum value of IW is in Luvisol. As a result of IW high value, this soil remains wet and cold for a longer time during spring, tillage is difficult and sowing is often delayed. The Dutch experience shows that each day of spring sowing delay reduces programmed yield for 1% (Vlahinić, 2004). This statement is confirmed by research in US (Seymour *et al.*, 1992). Although Fluvisols are usually

considered as soils with good water characteristics, they have a low value of RAW and high IW in this case. This is mainly due to heavier textural composition (Clay loam) of this soil type in Tuzla Canton. The relationships between water potential ( $\Psi$ ) in pF unities and soil water content ( $\Theta_v$ ) in mm for three analyzed soil types in the area of Tuzla Canton are graphically presented as soil water characteristic curve (SWCC).



Graph 2. Soil water characteristic curve (pF curve) for soil layer: 0 – 25 cm

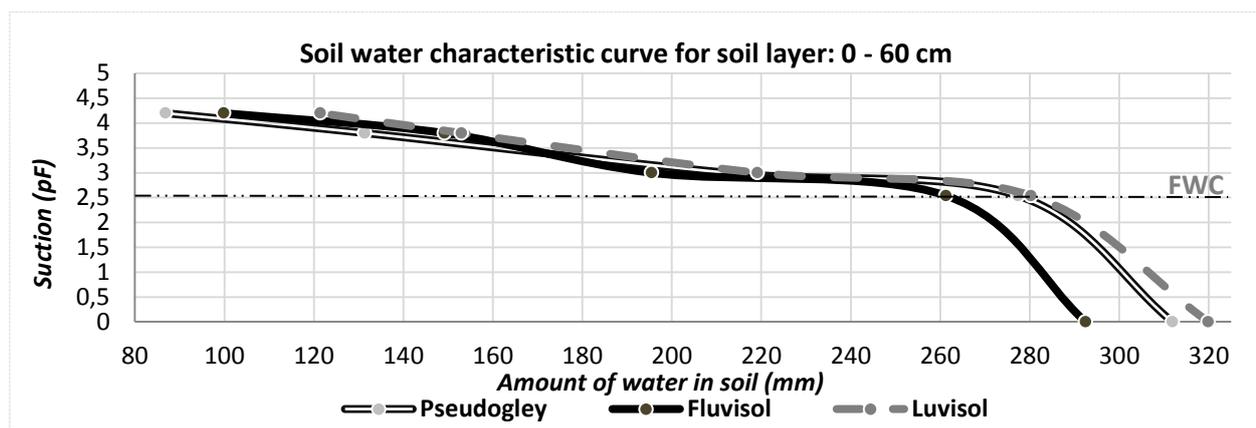
Graph 2 shows SWCC for surface soil layer (0 – 25 cm) for each of three analyzed soils. One can notice an uniform movement of curves for all three types of soil, especially in the middle part of the soil water potential (2.5 - 3.5 pF). Larger differences are on the lower potentials (higher water content in the soil), in particular it relates to Fluvisol which maximum water capacity ( $\Psi = 0$ ) is significantly lower (124 mm) compared to Pseudogley (137 mm) and Luvisol (134 mm).



Graph 3. Soil water characteristic curve (pF curve) for soil layer: 25 - 60 cm

Graph 3 shows the SWCC for sub-surface soil layer (25 - 60 cm), in which significant differences in the three analyzed soil types can be seen. The highest amount of water in the soil (mm) over the entire water potential range (pF) is found in Luvisol (81 - 186 mm), while Fluvisol has lowest values (60 - 168 mm). Pseudogley water characteristics for sub-surface horizon are between Luvisol and Fluvisol, this can be seen through SWCC which lies among the curves of these two types of soil.

Previously, we found that among three analyzed types of soil Pseudogley has the best water characteristics and the highest capacity for RAW (146 mm), this is primarily the result of favorable conditions in its sub-surface horizon (25 – 60 cm) comparing to other two soil types.



Graph 4. Soil water characteristic curve (pF curve) for soil layer: 0 - 60 cm

If we take into account both researched horizons, Luvisol in this case also has the highest amount of water over the entire water potential range (Graph 4). At higher potentials (4.2 pF), Fluvisol curve is getting close and values for LCP (3.8 pF) are similar, 153 mm for Luvisol and 149 mm for Fluvisol. At the lower potentials (2.54 pF) Pseudogley SWCC has similar movement like Luvisol curve and for FWC (2.54 pF) amounts 280 mm for Luvisol and 277 mm for Pseudogley.

Luvisol has the highest water capacities, and most importantly the highest value of FWC (280 mm). However, due to the high value of excess water (EW) and inaccessible water (IW) capacities, values of TAW and RAW are considerable low.

## CONCLUSIONS

Three soil types in the area of Tuzla canton were selected for this research: Luvisol, Pseudogley and Fluvisol. These are soils that are often used in agricultural production, occupying significant areas, mostly in the lowland part of the Canton and require a certain degree of melioration improvement. As result of anthropogenization and long-term use, researched soil types lost their original type characteristics, especially in the soil layer up to 60 cm. All three soils in general are heavier textured, with significant clay content (28.21 - 59.55%) which increases with depth. Fluvisol has a slightly higher sand content and the presence of skeleton compared to the other two soil types. Higher values of bulk density in the second horizon (1.34 to 1.41 g/cm<sup>3</sup>) in all the three soils, and a high content of clay (29.82 - 35.27%) indicate near surface presence of impermeable soil layer. For the surface horizon (0 - 25) the highest average value of a water content at maximum water capacity, field water capacity and 3 bar point is in Pseudogley (137, 113 and 83 mm). Lento-capillarity point is highest in Fluvisol (62 mm) and wilting point in Luvisol (41 mm). In sub-surface horizon (25 - 60 cm), Luvisol has highest soil water content for all retention capacity points. Same situation is if the entire research depth (0 - 60 cm) is considered. In the surface layer, the biggest values of excess water are in Pseudogley and Luvisol (24 mm), while Fluvisol has lowest values (17 mm). In both layers, surface (0 - 25 cm) and sub-surface (25 - 60), as well as in the entire researched depth (0 - 60 cm) Pseudogley has the highest readily available water capacity or in other words the best retention properties from the three analyzed soils. For all the three analyzed soils, the maximum value of inaccessible water is in Luvisol. As a result, this soil remains wet and cold for a longer time during spring, tillage is more difficult and sowing is often delayed. Although Fluvisols are usually considered as soils with good water characteristics, they have a low value of readily available water and high value of inaccessible water in this case. This is mainly due to heavier textural composition (Clay loam) of this soil type in Tuzla Canton. For the surface layer (0 - 25 cm), soil water characteristic curves for all three types of soil have uniform movement, while significant differences can be noted in the sub-surface layer (25 - 60 cm). The highest amount of water in the soil (mm) over the entire water potential range (pF) is in Luvisol (81 - 186 mm), while Fluvisol has lowest values (60 - 168 mm). Pseudogley has the best water characteristics and the highest capacity for readily available water (146 mm), this is primarily the result of favorable conditions in its sub-surface horizon (25 - 60 cm) compared to other two soils.

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## TEMPORAL DROUGHT AND SOIL MOISTURE VARIABILITY IN THE ARABLE LAND OF SPREČA VALLEY

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*Original scientific paper*

### Summary

The sustainable use of water in agriculture is one of a priority questions for not only arid and drought prone areas, but also in regions where rainfall is relatively abundant, as is the case of the Spreča valley region. This paper deals with drought severity and soil moisture temporal variability in the three main arable soils (Luvisol, Pseudogley and Fluvisol) of this region.

For determination of physical and soil-water characteristics, including soil texture, bulk density and available water content (AWC) of three analyzed arable soil types, disturbed and undisturbed soil samples were taken from each horizon in nine opened soil profiles. Potential evapotranspiration (PET) calculated by Thornthwaite method (1948) was used in Palmer Soil Water Balance for required elements of scPDSI and water deficit calculations. The analysis of temporal drought and soil moisture variability was performed using the monthly mean precipitation and temperature datasets from Tuzla meteorological station (305 m.a.s.l.), situated nearby Spreča valley, for the time period of 54 years (1961 – 2014), which was for better representation of climate change divided into two equal periods (at 27 years old), the first from 1961 to 1987 and the second from 1988 to 2014.

The longest drought (scPDSI <-1) for first period (1961 – 1987) lasted for 27 months, from July 1962 until September 1964. The Longest drought in second period (1988 – 2014) lasted more than 3 years (37 months), from August 2011 to August 2014. Drought most sensitive soil is Fluvisol with 41.67% (270) of drought months in whole analyzed period. The highest soil moisture deficit in analyzed period was in 2012, ranging up to 315 mm for Fluvisol, 299 mm in Luvisol and 280 mm in Pseudogley.

**Key words:** *soil moisture deficit, scPDSI, agricultural drought, palmer Z-index, arable soil*

### INTRODUCTION

In general, all the definitions of drought clearly state that drought occurs mainly due to break down of rainfall regime, which causes a series of consequences, including agricultural and hydrological hazards, which result from severity and duration of the lack of rainfall in certain area. As drought affects different sectors with varying frequency, duration and magnitude, operationally it is often categorized into four types: meteorological, agricultural, hydrological and socio-economic (Subrahmanyam, 1967).

Agricultural drought occurs when the soil water availability for a specific crop is reduced to such a level that it adversely affects the agricultural production and therefore the corresponding profit (Panu and Sharma, 2002). The severity of drought may be quantified using a drought index. A drought index integrates all meteorological, hydrological and agricultural information typically into a number and gives a comprehensive picture on drought conditions for decision making (Narasimhan and Srinivasan, 2005).

The Palmer Drought Severity Index (PDSI) has been used for 40 years to quantify the long-term drought conditions on specific time and location. The PDSI was created by Palmer (1965) with the intent to measure cumulative departure in atmospheric moisture supply and demand at the surface (Dai *et al.*, 2004). Palmer used two-layer bucket-type model for soil moisture computations and made certain assumptions relating to field water-holding capacity and transfer of moisture to and from the layers based on limited data from the central United States (Heim, 2002). There have been many criticism of the PDSI over the years, but perhaps one of the most common complaints is that PDSI values are not comparable between diverse climatological regions (Wells *et al.* 2004). To improve PDSI and solve this problem, N. Wells and S. Goddard in 2004, have created self-calibrated Palmer Drought Severity Index (scPDSI). The scPDSI automatically calibrates the behavior of the index at any location by replacing empirical constants in the index computation with dynamically calculated values (Wells *et al.*, 2004).

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Bosnia and Herzegovina, as well as many other countries in the region, is faced with the consequences of climate change and more frequent occurrence of drought periods, whose influence is mainly manifested in agricultural production, which in these circumstances becomes impossible without irrigation (Žurovec *et al.*, 2010). It should be noted that most previous studies of drought in B&H were conducted for the period until the end of the eighties. Stated by many authors in the nineties, the first decades of the twentieth century can be characterized as distinct periods of extremes in terms of excessive humidity, severe droughts and heat waves (Žurovec *et al.*, 2011).

Spreča valley is located in the north-eastern part of B&H (Canton Tuzla) and with its climate and soil for resources represents one of the most attractive areas for development of agricultural production in the Federation B&H.

Soils in this area are mostly heavy-textured, with poor soil water and physical properties (Resulović *et al.*, 2000, Žurovec *et al.*, 1987) and as such subject to the negative excess water effect. On the other hand, the lack of rainfall which in the area of the northeast B&H is approximately 100 mm (Vlahinić, 2000) and more frequent occurrence of prolonged dry periods during a summer throughout whole B&H, including its northeastern part, result in increased agriculture crop yield reductions (Žurovec *et al.*, 2010).

Temporal drought and soil moisture variability for agricultural production the most important arable soils of Spreča Valley was determined by computing soil water deficit, scPDSI and Palmer Z-index. Used indices can also quantify the wet spells and periods of extreme rainfall and floods, but in this paper we are focusing mainly on drought.

## MATERIALS AND METHODS

According to *Soil map of Bosnia and Herzegovina*, scale 1:50.000 (1990), most important soils for agricultural production in the research area are Luvisol, Pseudogley and Fluvisol. Three locations across Tuzla Canton were selected for each soil type. Nine soil profiles (up to 1 m deep) were opened on these locations. Undisturbed and disturbed soil samples were taken from each soil profile and each horizon in three replicates, 81 samples in total. Analyses of soil physical and water characteristics were carried out in laboratories of *Institute for Pedology, Agrochemistry and Melioration* at the *Faculty of Agriculture and Food Science* and the *Federal Institute for Agropedology*, Sarajevo. The following analyzes had been carried out: Soil physical characteristics through analysis of soil texture (*international pipette B method*) bulk density (*gravimetric method in Kopecky cylinders*) and absolute density (*Air pycnometer*) according to *Manual for Soil Testing* (Resulović *et al.*, 1971); Soil water retention using a pressure-membrane extraction apparatus according to Richards method (1941). Following negative potentials ( $\Psi$ ) were used: -0.33 bar (pF 2.54: field water capacity), -6.25 bar (pF 3.8: lento-capillarity point) and -15.5 bar (pF 4.2: permanent wilting point). Available water content (AWC) of each soil and layer was calculated from following relation between field water capacity (FWC) and lentocapillary point (LCP):  $AWC = FWC - LCP$ . Maximum water capacity (MC) was divided from bulk and absolute density (Resulović *et al.*, 1971).

In Palmer water balance method, each soil was divided into 2 layers: the surface layer where available water content (AWC) is 25 mm, and underlying layer where AWC depends upon determined soil characteristics.

The analysis of temporal drought and soil moisture variability was performed on the monthly mean precipitation and temperature datasets from Tuzla meteorological station (305 m a.s.l.), situated nearby Spreča valley, for the period from 1961 to 2014. The datasets were provided by *Federal Hydrometeorological Institute BH*.

Potential evapotranspiration (PET) calculated by Thornthwaite method (1948) was used in Palmer Soil Water Balance (1965) for required elements of scPDSI and water deficit calculations. The computation of scPDSI was done using the program offered by *National Agricultural Decision Support System* (NADSS), through the site <http://nadss.unl.edu>. The classification of scPDSI (X) and Palmer Z-index values is done according to Table 1.

Tab. 1. Classification of Palmer drought index, PDSI (Palmer, 1965)

scPDSI or Z-index	Class
-1.00 to -1.99	Mild drought
-2.00 to -2.99	Moderate drought
-3.00 to -3.99	Severe drought
$\leq -4.00$	Extreme drought

## RESULTS AND DISCUSSION

After the insight into soil map of B&H (1:50.000) and reviewing the situation in terms of spatial distribution and the importance for agricultural production, three soil types in the area of Tuzla canton were selected for this research: Luvisol, Pseudogley and Fluvisol. These are soils that are often used in agricultural production, occupying significant areas, mostly in the lowland part of the Canton and require a certain degree of melioration improvement. After taking soil samples from the field and the laboratory analysis, the following average soil physical characteristics (Table 2) for these three soil types are determined.

Tab. 2. Average physical characteristics of analyzed soils

Soil type	Horizon	Depth (cm)	Skeleton (%)	Texture			Class	Bulk density (g/cm <sup>3</sup> )	Absolute density (g/cm <sup>3</sup> )
				Sand (%)	Silt (%)	Clay (%)			
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	Sw	23 - 55		24.73	40.81	34.46	Clay loam	1.34	2.78
	Sd	55 - 100		20.95	36.40	42.64	Clay	1.41	2.79
Luvisol	Ap	0 - 20	0.13	27.45	41.55	31.00	Clay loam	1.22	2.60
	E	20 - 45	0.97	25.71	39.02	35.27	Clay loam	1.36	2.71
	Bt	45 - 100	0.18	15.66	25.79	59.55	Clay	1.25	2.73
Fluvisol	Ap	0 - 20	3.18	43.27	29.92	29.28	Clay loam	1.34	2.72
	I	20 - 60	6.86	45.20	26.62	29.82	Clay loam	1.41	2.69
	II	60 - 100	5.95	41.31	28.57	31.39	Clay loam	1.43	2.68

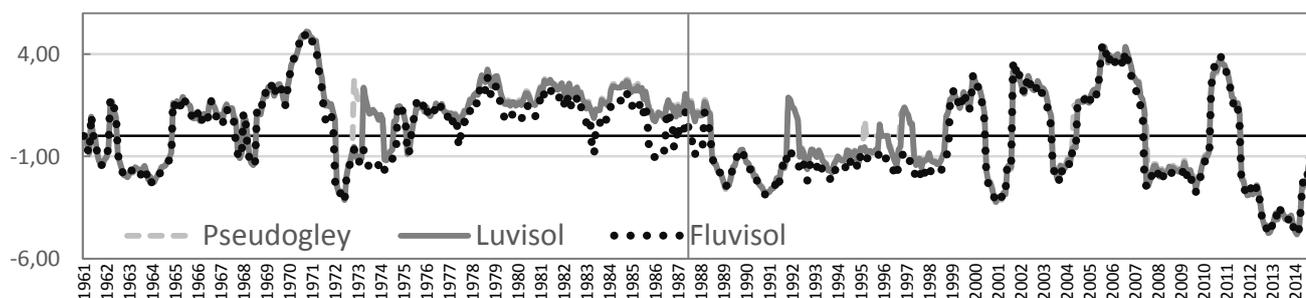
All three soils in general are heavier textured, with significant clay content (28.21 - 59.55%) which increases with depth. Fluvisol has a slightly higher sand content and the presence of skeleton compared to the other two soil types. Bulk density is within the limits of normal agricultural production. However, higher values in the second horizon (1.34 to 1.41 g/cm<sup>3</sup>) in all the three soils, and a high content of clay (29.82 - 35.27%) indicate near surface presence of impermeable soil layer.

Table 3 shows average soil water content for 60 cm soil layer and different retention capacities, as well as for Palmer water balance used available water contents (AWC). From all three analyzed soils, Luvisol has highest soil water content for all retention capacity points, while Pseudogley has biggest AWC (146 mm). For Palmer's water balance calculations it is assumed that top layer of all three soils contain 25 mm (Palmer, 1965) of water, while amount of water in underlying sub-surface layer of each soil is result of relation: AWC – 25 mm.

Tab. 3. Average soil water content (mm) for different water retention capacities and layers

Soil type	Depth (cm)	$\Psi$ pF (bar)	MC	FWC	LCP	WP	AWC		
							Total (0 – 60)	Surface layer (0 – 20)	Sub-surface layer (21 – 60)
Pseudogley	0 - 60		312	277	131	87	146		121
Luvisol			320	280	153	121	127	25	102
Fluvisol			292	261	149	100	111		86

In Graph 1 values of scPDSI for three analyzed soils in Speča valley are shown. Total analyzed period of time (54 years), for better representation of climate change, is divided into two equal periods (at 27 years old), the first from 1961 to 1987 and the second from 1988 to 2014.



Graph 1. scPDSI Spreča Valley, for three analyzed soils, period 1961 - 2014

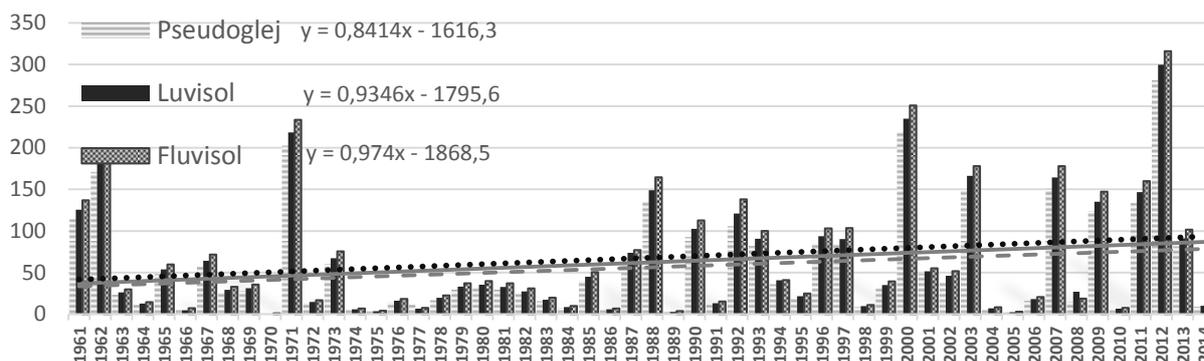
The longest drought (scPDSI <-1) for first time period (1961 – 1987) lasted for 27 months, from July 1962 to September 1964, with highest scPDSI value of -3.10 for Pseudogley, -3.14 Luvisol and -3.25 Fluvisol on April 1972. The Longest drought in second time period (1988 – 2014) lasted for more than 3 years (37 months), from August 2011 to August 2014. This drought period ended between 13<sup>th</sup> and 18<sup>th</sup> of May 2014, when a low-pressure cyclone affected a large area of Southeastern and Central Europe, causing floods and landslides. The rain in south and eastern part of B&H was the heaviest in 120 years of recorded weather measurements (FHMZ, 2014). Highest scPDSI value was calculated for January 2014, ranging from -4.82 for Pseudogley and Luvisol up to -4.87 for Fluvisol. Other droughts worth mentioning are drought from July 1962 to September 1964 (27 months), from November 1989 to September 1991 (23 months) and from April 2007 to February 2010 (34 months).

Tab. 4. Number of occurrences and percentage of drought months (scPDSI <-1) in Spreča valley, 1961 - 2014

scPDSI	1961 - 1987			1988 - 2014			1961 - 2014					
	P <sup>2</sup>	L	F	P	L	F	P		L		F	
							No.	%	No.	%	No.	%
Mild drought (-1 to -1.99)	36	40	54	96	97	120	132	20.37	137	21.14	174	26.85
Moderate drought (-2 to -2.99)	10	10	12	40	47	59	50	7.72	57	8.80	71	10.96
Severe drought (-3 to -3.99)	1	1	1	13	11	9	14	2.16	12	1.85	10	1.54
Extreme drought ( $\leq -4$ )	0	0	0	13	14	15	13	2.01	14	2.16	15	2.31
Total	47	51	67	162	169	203	209	32.25	220	33.95	270	41.67

By comparing drought severity, significant change between two periods can be noted (Table 4). If we consider the soil most sensitive to drought, in our case Fluvisol, there is no Extreme drought month ( $\leq -4$ ) in first period while second period has 15 Extreme drought months, or 4.63%. Similar situation is for other drought severity levels. Between soils, the difference is not too big. Fluvisol is most sensitive to drought with 41.67% (270) of drought months in the entire analyzed period. Pseudogley is most resilient with 32.25% (209) and close to it is Luvisol with 33.25% (220) of drought months.

It is common that agronomists use the word drought to define water stress condition affecting crop growth and yield (Maracchi, 2000). Short duration dry periods in agriculture are not considered droughts, but are defined as dry spells. These are characteristics of sub-humid climate and may cause relatively long periods of low soil moisture that limits agricultural activities (Pereira *et al.*, 2004). A soil moisture deficit occurs when the demand for water (PET) exceeds that which is actually available from the rain or in the soil. As result of temporal soil moisture variability, soil moisture deficit occurs in the study area for all three soils, (Graph 2).



Graph 2. Annual soil moisture deficit (mm) for Pseudogley, Luvisol and Luvisol (1961 – 2014)

The highest soil moisture deficit in analyzed period was in 2012, ranging up to 315 mm for Fluvisol, 299 mm in Luvisol and 280 mm in Pseudogley. In this year, In order to achieve full plant potential, these 315 mm (3.150 m<sup>3</sup>/ha) of water had to be given to plants through irrigation. A positive linear trend of the water deficit for all three soils can be seen throughout the entire analyzed period. The highest average deficit for analyzed period is in Fluvisol (67.3 mm), followed by Luvisol (61.9 mm) and Pseudogley (55.9 mm).

<sup>2</sup> P – Pseudogley; L – Luvisol; F – Fluvisol

Tab. 5. Number of occurrences and percentage of drought months (Z-index &lt;-1) in vegetation period

Z-index April - September	1961 - 1987			1988 - 2014			1961 – 2014					
	P	L	F	P	L	F	P		L		F	
							No.	%	No.	%	No.	%
Mild drought (-1 to -1.99)	23	23	24	33	35	34	56	17.28	58	17.90	58	17.90
Moderate drought (-2 to -2.99)	17	17	16	20	19	20	37	11.42	36	11.11	36	11.11
Severe drought (-3 to -3.99)	2	2	2	11	11	11	13	4.01	13	4.01	13	4.01
Extreme drought ( $\leq -4$ )	0	0	0	2	2	2	2	0.62	2	0.62	2	0.62
Total	42	42	42	66	67	67	108	33.33	109	33.64	109	33.64

The Palmer model also computes, as an intermediate term in the computation of scPDSI, the Palmer moisture anomaly index (Z-index), which is a measure of surface moisture anomaly for the current month without the consideration of the antecedent conditions (Dai, 2004). The Z-index can track agricultural drought, as it responds quickly to changes in soil moisture (Karl, 1986).

Number of months when palmer Z-index had drought character, for two analyzed periods, as well as for three soil types, can be seen in Table 5. For this analysis, period from April to September was considered, which in Spreča valley represents vegetation period for majority of agricultural crops. Like in case of scPDSI, significant change of drought severity and number of drought months between two time periods can be noted. In the first period (1961 – 1987), Fluvisol had 25.6% or 42 drought months, while in second period this number rises to 41.3% or 67 months. In total, there were 33.6% of drought months, with 2 months (0.62%) in which Z index had extreme values ( $\leq -4$ ). There is no significant difference in drought sensitivity between analyzed soils.

## CONCLUSIONS

Soils of Spreča valley are differently sensitive to drought. Pseudogley has the best soil-water characteristics, and the highest value of AWC resulting in the lowest sensitivity to drought. For the analysis of scPDSI and soil moisture deficit different soils were represented through different AWC. On the basis of the results, we can conclude that with increase of AWC of soil it is possible to mitigate the negative impact of drought.

As a result of climate change, dry periods that last for a longer period of time occur more frequently in the area of Spreča valley. Also, there is a positive linear trend of soil moisture deficit that have recently had its record value (315 mm in 2012). These results indicate the urgent need to start with climate change adaptation, otherwise, agriculture of the area and therefore the people engaged in it, will face significant negative consequences.

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**SUSTAINABLE DEVELOPMENT OF AGRO-INDUSTRY  
AND RURAL AREAS**

## LANDMARKS IN PERCEPTIONS OF ORNAMENTAL TREES BY USERS OF GREEN AREAS

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*Original scientific paper*

### Summary

Selecting the ornamental trees is very important in the process of planning green areas because the trees determine the appearance of open space a lot more powerful than the other elements. Effective and successful selection of trees obtained by the election process detailing defines the difference between potentially suitable trees based on their design characteristics: functions, spatial forms and visual character. Evaluation of the differences in the characteristics of the experience of ornamental trees has conducted by the method of Semantic Differential composed of 13 pairs of bipolar adjectives. For each pair of bipolar adjectives scale had continuity from -3 to +3 with zero in the middle of scale. The respondents evaluated the 13 tree species on photos in colour. The goal was to see how the same tree species experienced by different users of space: the student population who researches plants in the future will deal with any breeding or by applying plants in the area and users of open spaces selected at random. The calculation of the mean values for each type allowed us to determine the order of attractiveness of the survey included species. In reviewing the results of the survey, we see that the plant habitus and flowers played an important role in the ranking of the trees. If we look at the trees with regard to the height we see that it did not affect the ranking, as well as identifying trees. Of the total of 105 surveyed, 49 patients were men and 56 women participants. The average age of patients was 29 years, of which the youngest participant was 16 and the oldest 60 years.

Keywords: *ornamental trees, perception, survey*

### INTRODUCTION

Whether the user will be attracted to green area is mainly influenced by the choice of plants. Regarding visual perception in our research, we were led by the fact that different plant species were used in arranging garden spaces. Regarding the methodology, we also accepted the goals of previous research on landscape visual perception, which used method of Semantic Differential made up of different bipolar scales that were adjusted to aspects and goals of the research (Aničić, 1997; Cifrić & Trako, 2008; Echelberger, 1979; Kravanja, 1989; Marković *et al.*, 2002a; Mehrabian & Russel, 1974; Nohl, 1974; Russel & Carroll, 1999). Though some researchers were alerted about bad sides of using photography in perception research, most of the researches show that photography is valuable replacement to physical experience (Coeterier, 1983). Similar disagreement exists on usage of instrument for semantic differential, but insight into many previously mentioned researches points to value of the method and the need to use it prudently.

### MATERIAL AND METHODS

Evaluation of the differences in the characteristics of the experience of ornamental trees by individual approach, surveyed evaluated 13 tree species shown on photos in colour, on a scale of Semantic Differential composed of 13 pairs of bipolar adjectives. For each pair of bipolar adjectives the scale had continuity from -3 to +3. Zero was in the middle of scale. Numbers on the left presented positive meaning, while numbers on the right side presented negative meaning of the adjectives on scale. In descriptive sense, zero meant 'neither little nor much', numbers 1 on left and right side – 'little', 2 – 'quite', 3 – 'very'. Out of 103 surveyed, 49 were men, 54 women. The average age of surveyed was 29. Methods of univariate statistics were used in processing results (percentage, arithmetic mean, modal values and ANOVA). Contingency tables were made for each significant crossing of categorical variables; statistic importance was tested by  $X^2$  test. Profiles were made for semantic differential scales, based on appropriate central tendency measure. To determine the existence of statistically significant differences in the perception of different species of trees, ANOVA were carried out for all of these

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properties at the level of risk  $p < 0.01$ . Statistically significant differences were shown in all the variables. After significant F test was carried out t-test that was to determine the differences between some types of ornamental trees. Significant differences were shown to be the most variable.

### RESULTS AND DISCUSSION

All photos of ornamental trees had identical scales. For each photo that was evaluated, the total average was calculated including all elements, as shown in Table 1.

Tab.1. Overview of the results per arithmetic mean and variability coefficient

Species	Order	Arithmetic mean	Variability coefficient (%)
<i>Celtis australis</i> L.	1	5.84	6.5%
<i>Tilia tomentosa</i> Moench	2	5.69	9.13%
<i>Cupressus arizonica</i> Greene	3	5.60	8.75%
<i>Lagerstroemia indica</i> L.	4	5.52	18.47%
<i>Pinus halepensis</i> Mill.	5	5.40	10.18%
<i>Robinia pseudoacacia</i> L. 'Umbraculifera'	6	5.33	4.5%
<i>Melia azedarach</i> L.	7	5.31	10.54%
<i>Platanus x hispanica</i> Münchh.	8	5.12	10.15%
<i>Acer negundo</i> L.	8	5.12	10.35%
<i>Tracycarpus fortunei</i> (Hook.) H. Wendl.	10	5.08	14.96%
<i>Cupressus sempervirens</i> L.	11	4.86	18.51%
<i>Celtis tournefortii</i> Lam	12	4.67	10.7%
<i>Sophora japonica</i> L. 'Pendula'	13	4.16	16.82%

The photo of *Celtis australis* L. prove as best valued, which average value of total elements is  $\bar{x} = 5.84$  with variability coefficient of 6.5%, while *Sophora japonica* L. 'Pendula' took last place on the scale with average value of total elements  $\bar{x} = 4.16$  and variability coefficient of 16.82%. This species took last place on perception scale due to the fact that it is less known to surveyed and it is mostly recognised as neither striking nor average, and therefore less likeable.

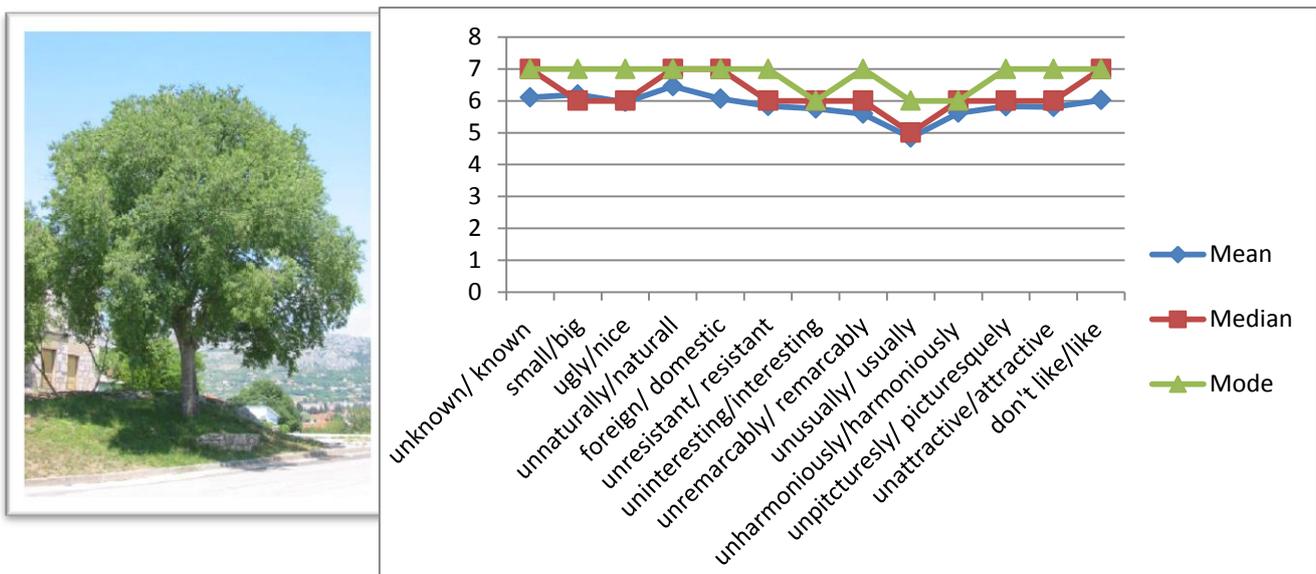


Fig. 1. Polarization profile - *Celtis australis* L.

After examining total average calculated per all elements, all photos were experienced positively, without negative values. Only three species on the scale of semantic differential were experienced neutrally, but still with plus sign. All abovementioned shows relatively uniform experience of all ornamental trees, but also shows that most of surveyed find ornamental trees attractive in all, or they experience them little to quite acceptable.

Impression of a single photo caused by its observation is the fastest noticed from the graphic of surveyed scores. Therefore, the results of this research on the example of *Celtis australis* L. and *Sophora japonica* L. 'Pendula', presented in the graph, using profiles incurred on basis of arithmetic mean, median and mode, and shown on figures 1. and 2.

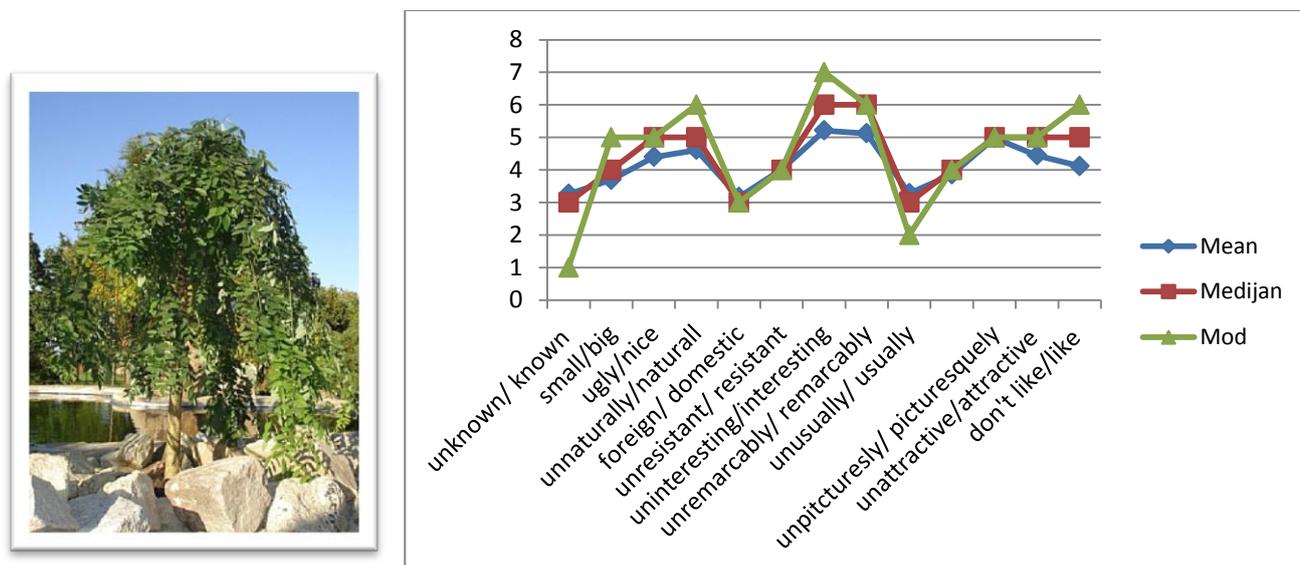


Fig. 2. Polarization profile - *Sophora japonica* L. 'Pendula'

Reviewing the overall average calculated on the basis of all elements, we see that all the photos, by the respondents, perceived as positive. There are no negative values. Only three species experienced on a scale of semantic differential in the neutral zone, but still a positive sign. Most respondents like ornamental trees and see them a little to quite acceptable.

In Table 2 appealing of trees, we can see that the majority of respondents as the three most beautiful trees chose *Lagerstroemia indica* L., *Celtis australis* L. and *Tilia tomentosa* Moench. As at three least beautiful trees there were chosen *Cupressus sempervirens* L., *Sophora japonica* L. 'Pendula' and *Platanus x hispanica* Münchh. Frequency response shows that 53 respondents (50.5%) in the group that they like allocated two species: *Celtis australis* L. and *Lagerstroemia indica* L. (Table 3). Also only two species are isolated in the category of trees that they less like them: *Cupressus sempervirens* L. for which chose 71 respondents (67.5%) and *Sophora japonica* L. 'Pendula' which was decided 54 of respondents (51.5%). The most respondents are accommodated the remaining trees in a neutral category. On the other hand *Cupressus sempervirens* L. them know the kind but he was also ranked among the trees that they are less like, that is the last place to the appeal.

Tab. 2. The rank of appealing trees

Species	Order	Mean value $\bar{x}$	Variability coefficient Cv (%)
<i>Lagerstroemia indica</i> L.	1	3.65	26.30
<i>Celtis australis</i> L.	2	3.60	26.11
<i>Tilia tomentosa</i> Moench	3	3.36	27.67
<i>Melia azedarach</i> L.	4	3.29	22.79
<i>Tracycarpus fortunei</i> (Hook.) H. Wendl.	5	3.06	29.41
<i>Robinia pseudoacacia</i> L. 'Umbraculifera'	6	3.06	20.26
<i>Acer negundo</i> L.	7	3.04	22.69
<i>Cupressus arizonica</i> Greene	8	2.94	25.17
<i>Pinus halepensis</i> Mill.	9	2.89	33.21
<i>Celtis tournefortii</i> Lam	10	2.89	25.95
<i>Platanus x hispanica</i> Münchh.	11	2.79	30.10
<i>Sophora japonica</i> L. 'Pendula'	12	2.35	37.87
<i>Cupressus sempervirens</i> L.	13	2.01	51.24

Habitus of plants and flowers have played a vital role in the ranking of trees. However, if we look at the colour of leaves, we see that those species that had cultivars with yellow leaves and bluish needles ranked 4<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> position. If we look at the trees with regard to the height we see that it did not affect the ranking, even the first three and the last three positions selected tree species that belong to the group of small to large trees. Better or worse knowledge of plant species by respondents, not noticeably affect the ranking. Thus respondents are not familiar with *Sophora japonica* L. 'Pendula' and it is considered artificial and kind of tune, and is ranked in a group of trees that they are less like. On the other hand *Cupressus sempervirens* L. them know the kind but it was also ranked among the trees that they are less like, that is the last place to the appeal.

Tab. 3. Frequency response for appealing trees

Species	At least like Category 1	Don't like Category 2	Neutrally Category 3	Like Category 4	Most like Category 5
<i>Sophora japonica</i> L. 'Pendula'	<u>22</u>	<u>32</u>	43	8	0
<i>Cupressus arizonica</i> Greene	5	15	<u>68</u>	15	2
<i>Platanus x hispanica</i> Münchh	7	27	<u>54</u>	15	2
<i>Celtis australis</i> L.	3	4	45	<u>32</u>	<u>21</u>
<i>Pinus halepensis</i> Mill.	7	24	<u>56</u>	9	9
<i>Tilia tomentosa</i> Moench	2	11	<u>55</u>	21	16
<i>Celtis tournefortii</i> Lam	5	17	<u>71</u>	8	4
<i>Cupressus sempervirens</i> L.	<u>43</u>	<u>28</u>	24	9	1
<i>Melia azedarach</i> L.	0	8	<u>69</u>	17	11
<i>Trachycarpus fortunei</i> (Hook.) H. Wendl.	4	20	<u>53</u>	21	7
<i>Lagerstroemia indica</i> L.	3	3	46	<u>28</u>	<u>25</u>
<i>Robinia pseudoacacia</i> L. 'Umbraculifera'	3	4	<u>85</u>	9	4
<i>Acer negundo</i> L.	1	17	<u>66</u>	18	3

It is also interesting which ornamental trees respondents had gave more or less unique answers. Already during the survey noted that they are more easily decide on the plants that they fancied. The most common their comment was that they all presented beautiful plants and therefore can not choose which one they like less. The heterogeneity of the whole group of respondents, the differences in the professional orientation and education were provided for high coefficients of variability assessment in some species, especially those that have taken place in the last third of scale.

## CONCLUSIONS

This study has established the following:

1. the knowing and unfamiliarity of the species had no effect on ranking in the scale of attractiveness,
2. respondents opted for the easier types that they like from those they like less,
3. certain morphological characteristics of the plants themselves are not decisive for the ranking of the plant, but the decisive combination of morphological features.

If landscape architecture aims to create livable and effective environments for people in the community, people's perception and interpretation of environments must be investigated.

The results can not be uncritically generalized, because it is recognized that these results are based on verbal reactions and that they may have been subject to preconceived viewer biases.

Also, the study results may be useful advice to designers of open space, because the uses of plants that people like them better maximize the satisfaction and well-being of visitors parks and other public green spaces.

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## ASSESSMENT OF CURRENT AND FUTURE STATUS OF SUSTAINABLE AND FOSSIL ENERGY RESOURCES IN TURKEY AND BOSNIA AND HERZEGOVINA WITH ECOLOGICAL ECONOMICS PERSPECTIVE

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*Scientific paper*

### Summary

Energy sector causes %57 of CO<sub>2</sub> emissions all over the world. This situation creates mankind to more effective use the "green energy" resources. Fossil energy resources provides %61 of energy production all over the world. Scarce and expensive resources encourages human for use alternative energy resources. This study aims to explain current and future status of energy resources in Turkey and Bosnia and Herzegovina and aims to find relationship between CO<sub>2</sub> emissions and energy production with an econometric analyses. The basis of this study is econometric forecasts.

Keywords: *CO<sub>2</sub> emissions, Energy sectors, Green economy*

### INTRODUCTION

Ecological economics is a new transdisciplinary field of study that addresses the relationship between ecosystems and economic systems in broadest sense (Costanza, 1991). Environmental problems span a continuously growing range of pollutants, hazards and ecosystem degradation over ever wider areas (Dincer, 1999). Climate change is a significant change in the average weather conditions, or in the distribution of weather around the average conditions (Kovačević *et al.*, 2013). Sustainable access to natural resources, particularly those related to food, has been a crucial factor in human development throughout history. In different time periods, new production methods and technologies have allowed for a more efficient use and greater availability. However, at the same time, population growth and changing consumption patterns have increased the demand for these resources (Detlef van Vuuren *et al.*, 2005). One solution to the impending energy shortage is to make much more use of renewable energy sources and technologies (Dincer, 2000). Renewable energy is the energy generated from natural resources such as sunlight, wind, rain, tides and geothermal heat which are renewable (Kahraman, 2009).

A nation's economic geography can have an enormous impact on its development. Turkey's geographical location makes it a natural bridge between the energy-rich Middle East and Central Asian regions. Energy is one of Turkey's most important development priorities. Energy is essential to economic and social development and improved quality of life in Turkey as in other countries (Kaygusuz, 2009). Population of Turkey is about 65 million. Although Turkey has a wide range of energy resources, these resources are limited. With Total Primary Energy Supply (TPES) growth rates of 4% to over 5% per annum and Total Final Consumption (TFC) growth of around 4% over the last three decades. Turkey is among the fastest growing energy markets in the World (IEA, 2001). According to Investment Support and Promotion Agency of Turkey, The Turkish electricity market is one of the fastest growing in the world, with an average of approx. 9% annual growth in 2010 and 2011. Bosnia and Herzegovina is a country of Southeast Europa, located in the western part of Balkan Peninsula. Population of Bosnia and Herzegovina is about 3,8 million and on 51,197 km<sup>2</sup> area. The Gross Domestic Product in Bosnia and Herzegovina was worth 18.34 billion US dollars in 2014 (Worldbank, 2014). Bosnia and Herzegovina's primary energy was 0.938 toe/USD 2000, which is 2.5 times more than the primary energy intensity in the EU 27 countries and more than in almost all countries in the region. Bosnia & Herzegovina has abundant resources of two traditional energy sources: coal and water. Some 55% of generated power is thermo-electric, while the remaining 45% is hydro-electric, according to the European Association for Coal and Lignite. Coal is the main source of energy in Bosnia, including dark coal, lignite and treset (Pasic, 2011). However, the country has significant potential of renewable resources in terms of additional small hydro, wind, solar and

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biomass (Karakostan *et al.*, 2012). No progress has been made in terms of renewable energy in Bosnia and Herzegovina however Transposition of the Habitats and wild birds Directives, including aspects required under the Energy Community Treaty, is at an early stage. There has been limited progress in the field of nature protection (Mihajlov *et al.*, 2010).

### MATERIALS AND METHODS

A time series is a set of numbers that measures the status of some activity over time. Time-series forecasting is a technique that helps to predict what will occur in the future if trends do not change. In this study, we used the Autoregressive Integrated Moving Average -ARIMA methods to estimate the future sustainable and fossil energy resources in Turkey and Bosnia & Herzegovina from 2014 to 2050. The popularity of the ARIMA model is due to its statistical properties as well as the well-known Box–Jenkins methodology in the model building process (Zhang, 2003). Box-Jenkins forecasting models are based on statistical concepts and principles and are able to model a wide spectrum of time series behavior. Given a certain time-series, we try to ascertain which ARIMA model is capable of generating said series, that is to say, which model adequately represents its behavior, to thus calculate the forecasts of the time-series under study. For this, four stages are performed in the order: identification, estimation, diagnostic checking and forecasting. (Gonzales *et al.*, 1999). In the ARIMA analysis, an identified underlying process is generated based on observations to a time series for generating a good model that shows the process-generating mechanism precisely (Box and Jenkins, 1976). The ARIMA, which is one of the most popular models for time series forecasting analysis, has been originated from the autoregressive model (AR), the moving average model (MA) and the combination of the AR and MA, the ARMA models (Volkan *et al.*, 2007). The time series of data of Worldbank obtained is analyzed using ARIMA technique. A general ARIMA model of order (p,d,q) representing the time series can be written as

$$\phi(B)\nabla^d x_t = \theta(B)e_t$$

According to the statistical computer software EViews-9. Automatic ARIMA forecasting is a method of forecasting values for a single series based upon an ARIMA model. EViews offers an automatic ARIMA forecasting series procedure that allows the user to quickly determine an appropriate ARIMAX specification and use it to forecast the series into the future. The series follows an ARIMAX model if:

$$D(y_t, d) = \beta X_t + V_t$$

$$V_t = p_1 v_{t-1} + p_2 v_{t-2} + \dots + p_p v_{t-p} + \theta_2 \varepsilon_{t-2} + \dots + \theta_q \varepsilon_{t-q}$$

ARIMAX models may be estimated through a number of different methods, including transforming the model into a non-linear least squares specification, or using GLS or maximum likelihood estimation. Since maximum likelihood estimation does not require dropping observations from the start of the sample, or backcasting to create observations, it lends itself nicely to model selection/comparison algorithms. In the present study the time series has been analyzed using statistical computer software EViews-9. We use GDP data from the World Bank Cross Country Data. Any missing data are extrapolated from other sources.

### RESULTS

Electricity production from oil, gas and coal sources and renewable sources in BIH and Turkey has been given below in figure 1/A-B and figure 2/A-B.

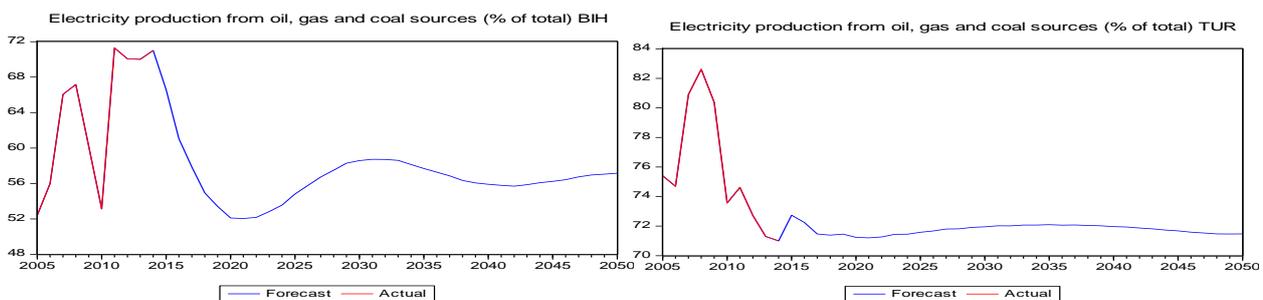


Fig. 1/A-B

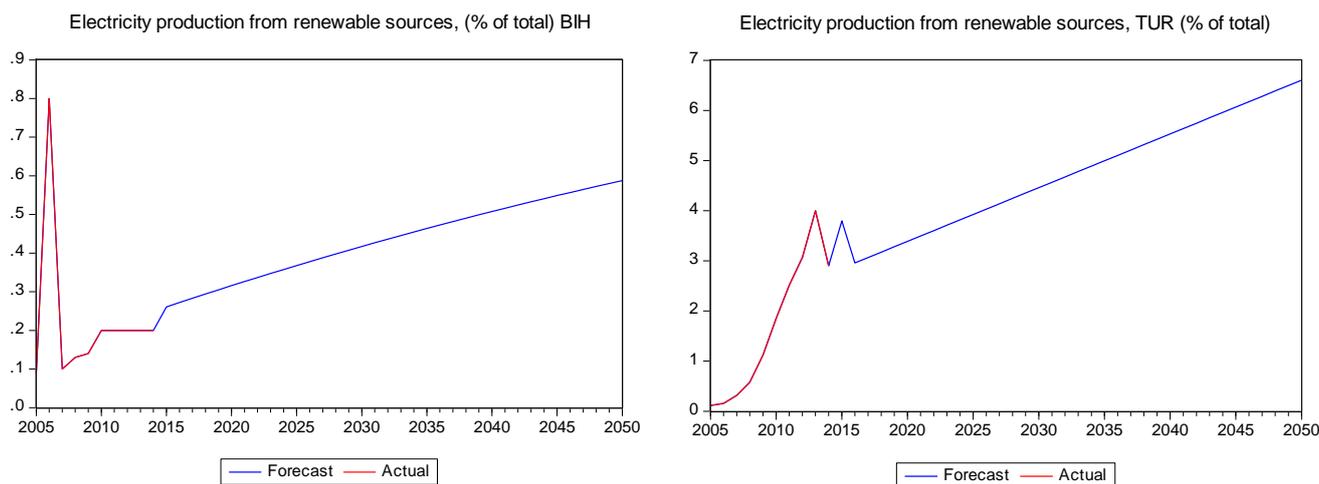


Fig. 2/A-B

### CONCLUSIONS

Table 1 Shows statistics which are obtained from ARIMA analysis.

Tab. 1.

	<b>R<sup>2</sup></b>	<b>Akaike Information Criteria</b>	<b>F Statistic</b>	<b>Probability</b>	<b>Selected Model</b>
Electricity production from oil, gas and coal sources (% of total) BIH	0.75	7.33	21.70	0.000	ARMA (2,0)
Electricity production from oil, gas and coal sources (% of total) TUR	0.82	5.85	9.89	0.013	ARMA (0,2)
Electricity production from renewable sources, (% of total) BIH	0.85	-0.26	34.81	0.000	ARMA (0,2)
Electricity production from renewable sources, (% of total) TUR	0.76	0.92	470	0.012	ARMA (0,2)

“Electricity production from oil, gas and coal sources” as percentage in Bosnia-Herzegovina reached maximum level in 2005-2010 period. In 2010, it dropped to about 50% level. In subsequent years, resource use reached about 70% and is expected to drop minimum levels in 2020’s and remain in 50-60% range in the following 30 years (until 2050).

Electricity production from fossil fuels in Turkey reached maximum in 2005-2010 period and then rapidly fell until 2015. Estimations made for the future until 2050 indicate that it will have a steady trend and remain around 70%.

Energy production from renewable resources in Bosnia-Herzegovina reached maximum levels of 0.8% in 2006 and then fell rapidly. It is estimated that energy production from renewable resources will be around 0.5-0.6% in the country.

Energy production from renewable resources in Turkey increased from 1990 and reached to the maximum level of 4% in 2014. It had a steady increase trend and is estimated to reach 7-8% level in 2050.

In conclusion, energy production from fossil fuels is higher than the world average in both countries. Since the energy production from fossil fuels is not environmentally friendly, it leads to increases in CO<sub>2</sub> emission. The use of fossil fuels for energy production contributes to air and water pollution. Therefore, transition to renewable resources, which appear to be an expensive investment, should be hastened.

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## COMPARATIVE ADVANTAGES AND EXPORTS COMPETITIVENESS OF FRESH FRUITS PRODUCTION IN BOSNIA AND HERZEGOVINA

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### Summary

Production of fresh fruit in Bosnia and Herzegovina (BH) has been recording significant expansion over the past decade. Taking into account the structure of Bosnia and Herzegovina exports of agricultural products in the period 2009-2013, fresh fruit production shows a marked positive trends and capabilities of further increase both in the physical (quantity) and financial (value of production) aspects. This paper aimed to determine the exports characteristics of fresh fruits produced in Bosnia and Herzegovina and of the achieved level of export to EU-27 and CEFTA countries, both at the level of groups of countries and at the level of individual countries, Serbia, Croatia and Macedonia. Grubel-Lloyd index (intra-industry type trade GLIIT) and RCA (Revealed Comparative Advantage) indicator were used to determine these characteristics. The results showed that the Grubel Lloyd index of the fresh fruit sector for EU 27 is relatively high, on the average 0.608 over the 2009-2013 period. Intra-industry character of fresh fruit trade and Grubel Lloyd index above 0.7 were observed in relations with the CEFTA countries, as well as with Serbia and Croatia individually. The average value of the GL index for the fruit trade between BH and CEFTA countries over 2009-2013 period was 0.796, i.e. it shows strong intra-industry trade tendency. Values of RCA indicator of BH trade of fresh fruit with EU-27 and CEFTA countries shows that BH has strong export capabilities since the RCA value is above 1.00. The average value of the RCA at the trade of fresh fruit with the EU-27 amounted to 3.524 and 2.213 with CEFTA countries. This means that this sub-sector is more capable than the rest of the agro-food sector, and that there are possibilities for its further development.

*Key words: Bosnia and Herzegovina, fresh fruits, exports, Grubel Lloyd index, RCA indicator*

### INTRODUCTION

Bosnia and Herzegovina has a favourable climate for production of continental fruit and of a certain number of Mediterranean species (Kurtovic *et al.*, 2004). After the great destruction of orchards during the 1992-95 war, the production of fresh fruits in BH has experienced a significant expansion in the previous decade. This is primarily related to the production of apples, plums, peaches and especially berries - raspberries and strawberries, which have made a significant step forward when it comes to export performance. Although the value of fresh fruit exports significantly increased in the last five years, the share of this group in total sector's export was not pronounced accordingly<sup>2</sup>.

The main limitation for exports of fresh fruit is its inability to meet three requirements: quality - quantity and continuity (Bajramovic *et al.*, 2006, USAID, 2014). Produced quantities are insufficient, quality is often insufficient (due to varietal composition) and the continuity in the delivery is not provided. Restriction related to the pre-harvest phase is pronounced lack of storage capacities in Bosnia and Herzegovina. In addition, system of control of imported plants and their certification is not efficient, there is the lack of modern breeding and selection services with the network of identification registries, as well as institutions of plant health protection. In the phase related to the post-harvest of fruit, limiting factor in BH is so-called poor finalization of the products, referring to the lack of marketing standards and of equipment for products' calibration, sorting, packaging and labelling.

The aim of this paper was to quantify the current exports level of competitiveness of fresh fruit sector in Bosnia and Herzegovina. Analysis was done on the basis of available information and data from Chamber of Foreign Trade of BH, Agency for Statistics of BH and entities' statistics offices using the relevant scientific method.

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<sup>2</sup> Source: Federalno ministarstvo poljoprivrede, vodoprivrede i šumarstva (2014)

## MATERIALS AND METHODS

Revealed Comparative Advantage – RCA indicator was used for evaluation of competitive performance. This index developed by Balassa in 1965 represents a classic trade index for measurement of comparative advantage. It reflects the differences between the countries that produce a given product in relation to its relative price (acceptable prices – higher market share) and in relation to non-price characteristics of the product. This index, in essence, shows the ratio of the relative share of the value of product' export in the total exports of Bosnia and Herzegovina to certain market, and the relative share of product' imports in total import of Bosnia and Herzegovina from that market (Buturac, 2008; Nikolic *et al.*, 2010-b). The indicator is calculated using following formula:

$$RCA_{ij} = \frac{x_{ik}/X_{it}}{m_{jk}/M_{jt}}$$

$x_{ik}$  – total product' export to market j  
 $X_{it}$  - total BH export to market j  
 $m_{jk}$  – total product' import from market j  
 $M_{jt}$  - Total BH import from market j

RCA indicator was chosen because it indicates the market position of the sector and it is consistent with the changes in the relative level of availability of factors for production and productivity. It indicates the trade pattern of the global exchange of goods and services, so the change of RCA is influenced by changes in the structure of the economy, by demand characteristics at the global market and by the level of trade specialization (Ferto and Hinbard, 2002, Bojnec and Ferto, 2006). This factor reflects the benefits of the sector that are associated both with the pricing/cost, and with non-pricing characteristics of products, such as quality (how well it meets the demand by a set of additional services offered to customers). Production of apples, plums and raspberries, as the most promising export fruit species, were chosen for determination of the sector's competitiveness at the level of individual products (IFC,2014). Aggregated Grubel Lloyd index was used for the analysis of the level of the specialization in intra-industry exchange of the fresh fruit sector. According to Nikolic *et al.* (2010) Grubel-Lloyd Intra Industry Trade is considered as indicator of economic integration among countries with similar factors' availability. In all modern economies significant amounts of similar products are the subject of trade at the same time and this explains the level of intensity of trade between countries of the same development level. Trade with the same group of products is intra-industry trade, while trade with various commodity' groups is inter-industry trade. Grubel-Lloyd index indicates whether the trade flows between countries are inter or intra trade type. The index is calculated according to the following formula:

$$GL = 1 - \frac{|x - m|}{x + m}$$

GL = Grubel-Lloyd index , x = value of sector' import, m = value of sector 'export

Respectively,

$$GL_{sector\ i} = 1 - \left( \frac{|export_{sector\ i} - import_{sector\ i}|}{export_{sector\ i} + import_{sector\ i}} \right)$$

The index ranges from 0 to 1. The higher index value indicates the higher level of specialization in intra-industry trade.

## RESULTS AND DISCUSSION

Results of the research are given as characteristics of fresh fruit sector's foreign trade, in the context of the competitive performances' assessment of the analysed sector and selected products within it, as well as the determination of the level of specialization in the sector, intra-industry and inter-industry trade of Bosnia and Herzegovina with EU-27 and CEFTA countries as the whole and individually with selected countries. Trade indicators of BH fresh fruit subsector on EU-27 and CEFTA market expressed by GL index shows good performance and integration of the subsector (GL index is over 0.5).

Tab. 1. Grubel-Lloyd index of the fresh fruit sector by group of countries and individual countries from the Region (2009-2013)

Country/Group of countries	2009	2010	2011	2012	2013	Average 2009-2013
EU - 27	0.558	0.792	0.593	0.407	0.692	0.608
CEFTA	0.781	0.908	0.743	0.734	0.679	0.769
Serbia	0.843	0.960	0.625	0.506	0.587	0.704
Croatia	0.985	0.987	0.590	0.634	0.492	0.737
Macedonia	0.033	0.007	0.073	0.002	0.008	0.025

Source: Authors calculations on the basis of database of the Chamber of Foreign Trade of BH

GL index of the fresh fruit sector for EU-27 is relatively high, on the average 0.608 over the 2009-2013 period. It varied over the period, so it was the lowest in 2012 (0.407), as total fruit production in BH, due to unfavourable climatic conditions was significantly reduced in 2012, thus, export opportunities were reduced as well. The highest GL index was in 2010, 0.792, when the value of BH export was the closest to fresh fruit import from EU-27 countries.

Intra-industry character of fresh fruit trade and Grubel Lloyd index above 0.7 were observed in relations with the CEFTA countries, as well as with Serbia and Croatia individually. The average value of the GL index for the fruit trade between BH and CEFTA countries over 2009-2013 period was 0.796, i.e. it shows strong intra-industry trade tendency. It also means that there is a marked tendency of approaching the values of imports by exports between BH and CEFTA countries. Similar relations and Grubel - Lloyd indexes were observed in fresh fruit trade exchange of BH with Serbia and Croatia. As far as relations between Macedonia and Bosnia and GL index for the fruit are concerned, it was very low and almost approached 0. The average value this index over the analysed period was 0.025 which shows that BH is expressed net importer of fruit from Macedonia.

Values of RCA indicators of BH trade with fresh fruit to EU-27 and CEFTA countries (Table 2) show that BH has strong export capabilities to these markets, since the RCA value is above 1.00. On the average it amounted to 3.524 for trade with EU-27 and 2.213 with CEFTA countries. These figures also show that, in this segment of agro-food, BH used advantages of trade agreements (CEFTA and the Stabilization and Association Agreement with the EU) with both regional and EU partners.

Tab. 2. RCA indicator of BH fresh fruit' trade with countries and groups of countries (2009-2013)

Country/Group of countries	2009	2010	2011	2012	2013	Average 2009-2013
EU - 27	2.950	4.116	3.291	2.401	4.863	3.524
CEFTA	2.670	3.234	1.954	1.846	1.362	2.213
Serbia	4.001	6.503	14.356	21.068	16.219	12.430
Croatia	4.801	4.022	1.633	1.746	1.264	2.693
Macedonia	0.046	0.011	0.048	0.001	0.004	0.022

Source: Authors calculations on the basis of database of the Chamber of Foreign Trade of BH

While RCA of BH fresh fruit trade with the EU-27 varied from year to year, ranging from 2.401 to 4.863, the negative tendency of RCA value with CEFTA countries is the issue of concern and worry. In 2010, the value of RCA amounted to 3.234, and in 2013 it decreased to only 1.362. This indicates reduction+ of competitive capabilities of the subsector in relation to CEFTA countries and imposes the need for response and implementation of agricultural policy measures which would increase export competitive advantage. RCA indicators of BH fresh fruit trade with Serbia, Croatia and Macedonia shows different levels of trade relations

and competitiveness. Fruit production of BH is significantly competitive on Serbian (RCA = 12.430) and Croatian (RCA = 2.693) market, and absolutely uncompetitive on the market of Macedonia (RCA = 0.022). When trends of RCA indicators for these countries in the period 2009-2013 are concerned, RCA of fresh fruit trade between Bosnia and Serbia has trend of significant increase, between BH and Croatian it shows pronounced tendency to decline, and for Bosnia and Herzegovina and Macedonia it shows no pronounced changes. The high value of the RCA trade of fresh fruits BH to the EU-27 and CEFTA countries, particularly with Serbia, indicates satisfactory price/costs characteristics of the product, and therefore its quality. Positive values of the RCA indicator of fresh fruit show that this group of BH agricultural and food commodities has competitive advantage foreign trade and export to the international market. Finally, the aim within research was to analyse (to confirm assumptions) if selected individual products, apple, plum and raspberry are competitive on markets of EU-27 and CEFTA countries, and to compare this competitiveness with the competitiveness of the fruit sector as a whole (Table 3).

Tab. 3. RCA indicators of apple, plum and raspberry by groups of countries and countries from the Region (fruit sector)

Country/Group of countries	2009	2010	2011	2012	2013	Average 2009-2013
<b>Apple</b>						
EU - 27	0.009	0.190	0.215	0.121	0.500	0.207
CEFTA	0.543	0.996	2.689	1.057	0.729	1.203
Serbia	1.506	3.084	9.089	1.801	0.886	3.273
Croatia	0.303	0.230	0.668	1.503	3.916	1.324
Macedonia	0.000	0.000	0.000	0.000	0.000	0.000
<b>Plum</b>						
EU - 27	41.269	155.586	42.269	218.474	232.001	137.920
CEFTA	0.436	0.158	0.824	1.591	0.766	0.755
Serbia	0.269	0.045	0.135	0.073	0.120	0.128
Croatia	1794.345	12.707	26.767	293.458	3.613	426.178
Macedonia	0.000	0.000	0.000	0.000	17.089	3.418
<b>Raspberry</b>						
EU - 27	102.688	3373.226	306.712	5821.917	84.002	1937.709
CEFTA	2.430	0.765	1.487	35.460	17.741	11.577
Serbia	1.816	0.537	0.278	13.812	3.378	3.964
Croatia	2.519	0.000	471.650	9.632	0.000	96.760
Macedonia	0.000	0.000	0.000	0.000	0.000	0.000

Source: Authors calculations on the basis of database of the Chamber of Foreign Trade of BH

The Table 3 clearly shows that BH apple production, compared to total production of fruits at EU-27 and CEFTA countries' markets, is uncompetitive at EU-27 (the average value of the RCA 0.207) market, and only slightly competitive in CEFTA countries (1.203). BH' trade of apple with Croatia and Serbia was characterized with noticeable variations, so in some years the value of RCA was below 1.00, and in some other years it was very high (9.089, Serbia, 2011). Compared to entire fruit production, plum production and plum trade with EU-27 shows a high level of competitiveness (average RCA = 137.920). On the other hand, RCA value below 1.00 indicates that plum is not competitive in CEFTA countries (RCA 0.755), particularly in Serbia (0.128), as expected, since plum is traditionally produced in most of CEFTA countries with significantly high share in their total fruit production. Unlike Serbia, BH plum production has strong competitive advantage in Croatia (RCA = 426.178) and in Macedonia in 2012 and 2013 (RCA = 3.418). Finally, obtained results confirmed the assumption that production of raspberries is one of the most competitive production within fruit sector in Bosnia and Herzegovina. Very high RCA values were calculated for BH raspberry' production with EU-27 (1937.709), and high value with CEFTA countries (RCA = 11.577), in particular with Croatia (RCA = 96.760). Although Serbia is one of the leading producers of raspberries in both Europe and in the world, raspberry' trade between BH and Serbia shows that BH' raspberry was significantly competitive in some years (especially in 2013 and 2012), and uncompetitive in some others (2010 and 2011). This happens as consequences of primarily climate conditions, but also of elements of grey economy, re-export, and export opportunities Serbian producers to some countries (e.g. Russian Federation).

## CONCLUSIONS

Trade indicators of BH fresh fruit sub sector expressed with GL index shows good performance and integration of subsector on EU-27 and CEFTA market, as the average value of the index between BH and both EU-27 and CEFTA countries over 0.5 indicate intra-industry trends. Values of RCA indicators of BH trade with fresh fruit to the EU-27 and CEFTA countries shows that BH has strong export capabilities, since RCA value is above 1.00. The average value of RCA in trade of fresh fruit with the EU-27 amounted to 3,524, and 2,213 with CEFTA countries. This means that this subsector is more capable than the rest of the agro-food sector, and that there are possibilities for its further development. Besides, these figures show that, in this trade segment of agro-food sector, BH used advantages of trade agreements (CEFTA and the Stabilization and Association Agreement with the EU) with regional and EU partners. Analysis of competitiveness of the fruit sector and of individual products within it, apples, plums and raspberries on the EU-27 and CEFTA countries market using RCA indicators confirmed assumptions about their competitiveness. Except in apples trade of Bosnia and Herzegovina to the EU-27 and plums to CEFTA countries, high level of competitive capabilities in target markets for all other products was observed, which was, in some cases, expressed with four-figures values of RCA indicators. In other words, further specialization in plum, and particularly in raspberries production should be supported.

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## **ECONOMIC EFFICIENCY OF THE GREEN HOUSES' VEGETABLE PRODUCTION IN THE CONDITIONS OF CONTINENTAL AND MEDITERRANEAN CLIMATE IN BOSNIA AND HERZEGOVINA**

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*Original scientific paper*

### **Summary**

Vegetable production in the greenhouses in Bosnia and Herzegovina has been recording strong expansion over the last decade. The main reason for introduction of this type of production on farms is high profitability on relative small land units, which is very important for small and fragmented farms in B&H. Regardless increasing importance, there are still no significant and detailed research on economics of these production units. Bosnia and Herzegovina is characterised by numerous climate types, with two dominant types: Mediterranean climate in the southern part, and continental climate in central and northern parts. This causes varied conditions for production among agricultural regions and further leads to different economic results of vegetable production in greenhouses. This was the motive for this research, which aimed to analyse current situation and economic results achieved in greenhouse production of vegetables in two regions with different climate.

The main source for the research was the questionnaire. To ensure relevance of the sample, 20 farms operating in different climate conditions (10 from Mediterranean and 10 from continental region) in Bosnia and Herzegovina were selected and interviewed. Results are presented by gross margin of three dominant productions (tomato, pepper and spinach) and by total gross margin on the farm from greenhouse production. Farms in continental part achieved higher gross margin per 100 m<sup>2</sup> of greenhouse tomato production by 221 EUR, and by 197 EURO in pepper production than these ones in Mediterranean regions. But, as far as spinach production is concerned, gross margin per land unit was achieved by farms from Mediterranean area (157 EUR/100 m<sup>2</sup>) than farms from continental area (122 EUR/100 m<sup>2</sup>).

*Key words: Bosnia and Hercegovina, greenhouse, gross margin, net income*

### **INTRODUCTION**

The development of agricultural production is defined as one of the priorities of economic growth of Bosnia and Herzegovina in almost all policy documents developed in the post war period at the various levels of government (Ognjenovic, 2009). Relatively favourable climate, low cost of land and other factors of production, in relation to the conditions in the region, as well as over 20% of the population who are officially involved in agricultural production, could be the initial catalysts for rural development of Bosnia and Herzegovina (BH Union of Cooperatives). Within agriculture, alongside livestock and fruit production, production of vegetables, with a specific focus on greenhouse production, should be at the forefront of development. Bosnia and Herzegovina possesses relatively good natural potentials for the development of vegetable production. However, it occasionally faces aggravating factors, such as adverse weather conditions (high or low temperatures, late spring or early autumn frosts, deficit or surplus of rainfall). In this context, greenhouse production, as a form of plant production with partially controlled conditions of production, may be one of the solutions to overcome these issues.

Greenhouse production in Bosnia and Herzegovina in the last twenty years is experiencing significant expansion. Limited available land resources are the main reason why family farms turn to high income production per unit of area, such as the production of berries and, certainly, cultivation of vegetables in greenhouses. If we take into account the existence of specific support measures in certain cantons (in the Federation) through provisions of stimulations for this production, as well as extremely limited employment opportunities in other industries, it is expected to see an increase in number of farms which turn to greenhouse production as the primary economic activity and the main source of household income.

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Bosnia and Herzegovina is characterised by numerous climate types, with two dominant types: Mediterranean climate in the southern part, and continental climate in central and northern parts. This causes varied conditions for production among agricultural regions and further leads to different economic results of vegetable production in greenhouses. This was the motive for this research, which aimed to analyse current situation and economic results achieved in greenhouse production of vegetables in two regions with different climate.

The aim of this paper is a comparative analysis of the economic efficiency of greenhouse cultivation of vegetables in the Mediterranean and continental climates of Bosnia and Herzegovina, in such manner to determine how the climate conditions and regional distribution of the studied family farms affect the production and economic results in their absolute and relative terms. The hypothesis is that the farms in the Mediterranean region of Bosnia and Herzegovina should achieve better average yield of crops cultivated in greenhouses, and have a higher gross margin. The hypothesis is based on the fact that the Mediterranean part of Bosnia and Herzegovina has a much more favourable climate for greenhouse cultivation, as observed by the earlier arrival of produce on the market when the prices of vegetable produce are higher.

Studies of agricultural farms aimed at greenhouse vegetable production in the post-war period are virtually non-existent, and the information on vegetable production is based mainly on statistics regarding the total and average yields of certain crops, while further studies on achieved economic results and their complex analysis are lacking. Given the abovementioned, as well as the importance of vegetable production in greenhouses, the initial motive in defining the subject of research in the framework of this study was precisely to analyse and determine the current economic success of these production units.

## MATERIALS AND METHODS

The main source of research material for determining the production and economic results of family farms were questionnaires. For the purpose of this paper, the questionnaire contained sections on the average yield, revenue, variable and fixed costs, leading up to the total profit of the farm.

When selecting the units for observation, care was taken to make the sample as representative as possible, in order to realistically reflect the actual state of the research areas. This involved the selection of individual farms on various territories and with different climate characteristics (Mediterranean and continental climate) in Bosnia and Herzegovina. A total of 20 family farms were selected, of which ten were in the area with Mediterranean, and ten in the area with continental climate. Research was carried out in 2012.

All data in this paper were obtained through interviews with managers (owners) of family farms and were entered into the questionnaire. Once the questionnaires were completed, gathered data was processed in order to obtain respective values and indicators. All numerical data was processed in Microsoft Excel 2010.

This study used indicators relating to 100 m<sup>2</sup> floor area of a greenhouse, observing the yield, revenue, and costs per unit of production. Revenue includes all revenue from greenhouse production, while the costs are calculated with regards to conduct in relation to yield, and were separated into two groups: variable and fixed costs. The final profit of the farm includes all income and expenses of the farm in terms of plant and livestock production.

One of the manners of demonstrating the obtained results is the gross margin realized through individual production. According to Kay and Edwards (1999), gross margin represents the difference between revenues and variable costs of all individual production lines of a farm, and essentially serves to cover fixed costs and provide profit after variable costs are paid. Gross margin is observed for the two most important and most commonly cultivated crops in the summer (tomatoes and peppers), as well as for spinach grown in the winter. Gross margin achieved in total greenhouses production on analysed farms is presented too, as well as gross margin of other productions that were present on the farms but with minor share and significance.

## RESULTS AND DISCUSSION

In both regions, average yields for the surface of 100 m<sup>2</sup> were observed for tomatoes, peppers, and spinach. Looking at Figure 1, we see that farms in the continental part of BH achieve significantly higher average yields (1,687 kg) in the production of tomato, in comparison to farms with Mediterranean climate (1,217 kg). The results are contrary to expectations, but the research also showed that continental farms retain tomato production for a longer period of time in comparison to farms in the Mediterranean part of Bosnia and Herzegovina, which terminate this production earlier in the year in order to start the first batch of winter production as early as possible. Additionally, research has found that farms in the continental part of the country, in most cases, start their sowing and planting of all observed lines of cultivation on time, and apply a more intensive agricultural technology, which is not the case with farms in the Mediterranean parts of BH. When it comes to the production

of peppers, there is no significant difference between the observed areas; the average yield per 100m<sup>2</sup> in the Mediterranean part of BiH amounted to 1,077 kg, while the yield in the continental climate was 1,061 kg. Unexpectedly, the average yield of spinach per 100 m<sup>2</sup> in both observed regions of Bosnia and Herzegovina was uniform, amounting to 248 kg in the Mediterranean area, and 244kg in the continental area. Even though the farms in the Mediterranean regions have more favourable conditions for winter production inside the greenhouse, according to this survey, they fail to utilize this to their advantage in the production of spinach, according to average obtained yield.

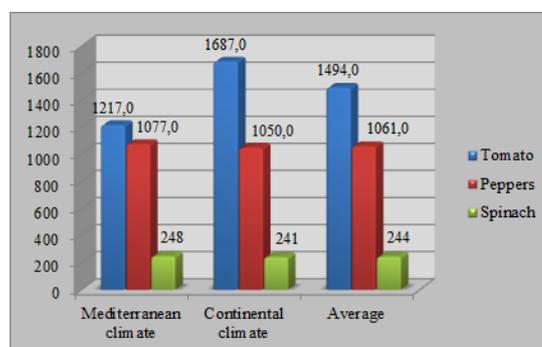


Fig. 1: Average yield of significant vegetable cultures in greenhouses by the observed areas (in kg/100 m<sup>2</sup>)

Table 1 shows the obtained gross margin in tomato production per 100 m<sup>2</sup>. On average, revenues for all farms amounted to 769 EUR, variable costs were 179 EUR, while the gross margin was 590 EUR. Higher income was observed in the areas with continental climate (874 EUR), in comparison to areas with Mediterranean climate (621 EUR). Similarly, variable costs were higher in the continental area (192) than in the Mediterranean area (160 EUR).

Tab. 1. Gross margin in tomato production at the studied farms, depending on the climate conditions of production (EUR /100 m<sup>2</sup>)

Description	Studied farms according to production conditions		Average
	Mediterranean	Continental	
<b>A) REVENUE</b>	<b>621</b>	<b>874</b>	<b>769</b>
<b>B) VARIABLE COSTS</b>			
Seed	66	86	78
Fertilizer	39	39	39
Protective agents	16	18	17
Leased mechanical work	0	5	3
Other costs	38	44	42
<b>Total (B)</b>	<b>160</b>	<b>192</b>	<b>179</b>
<b>C) GROSS MARGIN (A-B)</b>	<b>461</b>	<b>682</b>	<b>590</b>

Source: Private research

Table 2 shows the obtained gross margin in the production of peppers per 100 m<sup>2</sup>. Although it was previously stated that the average yields of this crop per 100 m<sup>2</sup> were similar in both regions, obtained revenues per the same surface area are significantly higher for farms that are located in the territory of the continental climate, amounting to 697 EUR, while in they amount to 483 EUR in the territory with Mediterranean climate. There are no significant differences in variable cost per 100 m<sup>2</sup>, but the gross margin for 100 m<sup>2</sup> of peppers is higher by 197 EUR for continental farms. Such a difference in gross margin occurs as a result of the difference in the sale price of peppers in these different areas. On the one hand, farms in the Mediterranean part sell 81% of their produce in the wholesale market, where the sale price is considerably lower compared to other distribution channels, which are mainly used at continental farms (households 34.7%, farmer's markets 29.3%, cooperative 14.9%). Therefore, despite the fact that the farms in the territories with Mediterranean climate push their produce in the market at an earlier date than those in the continental areas, they still do not achieve the expected advantage in the amount of the sales of their products.

Tab. 2. Gross margin in the production of peppers at the studied farms, depending on the climate conditions of production (EUR /100 m<sup>2</sup>)

Description	Studied farms according to production conditions		Average
	Mediterranean	Continental	
<b>A) REVENUE</b>	<b>483</b>	<b>697</b>	<b>612</b>
<b>B) VARIABLE COSTS</b>			
Seed	94	96	95
Fertilizer	46	38	41
Protective agents	9	9	9
Leased mechanical work	0	4	3
Other costs	26	46	38
<b>Total (B)</b>	<b>173</b>	<b>192</b>	<b>185</b>
<b>C) GROSS MARGIN (A-B)</b>	<b>309</b>	<b>506</b>	<b>427</b>

**Source:** Private research

An examination of Table 3 shows that the average studied farm had revenue of 174 EUR/100 m<sup>2</sup> in the production of spinach, while its costs were 38 EUR/100 m<sup>2</sup>, which means that the gross margin on these farms was 135 EUR/100 m<sup>2</sup>. Gross margin, 157 EU/100 m<sup>2</sup>, was observed at the farms in the Mediterranean area, compared to 122 EUR/100 m<sup>2</sup> in the continental area. In contrast, the costs per 100 m<sup>2</sup> are slightly lower in Mediterranean farms, which ultimately leads to higher gross margin on these farms, which is by 35 EUR/100 m<sup>2</sup> more than on the continental farms.

Tab. 3. Gross margin in spinach production at the studied farms, depending on the climate conditions of production (EUR /100 m<sup>2</sup>)

Description	Studied farms according to production conditions		Average
	Mediterranean	Continental	
<b>A) REVENUE</b>	<b>189</b>	<b>164</b>	<b>174</b>
<b>B) VARIABLE COSTS</b>			
Seed	6	12	10
Fertilizer	20	16	18
Protective agents	0	0	0
Leased mechanical work	0	2	1
Other costs	6	12	10
<b>Total (B)</b>	<b>32</b>	<b>42</b>	<b>38</b>
<b>C) GROSS MARGIN (A-B)</b>	<b>157</b>	<b>122</b>	<b>135</b>

**Source:** Private research

Table 4 shows average farm gross margin and individual gross margins achieved in individual vegetable productions in greenhouses. Average total gross margin on analysed farms was 4,697 EUR, and it was higher in continental part of BH (5,357 EUR), compared to the one in Mediterranean part (4.036 EUR). Tomato production, with 1,280 EUR, has the highest contribution to gross margin, then paper production with 1,280 EUR, and spinach production with 725 EUR. Higher gross margin in tomato production was achieved by farms in continental (2,115 EUR) than in Mediterranean part of BH (1,737 EUR). In paper production farms from continental part gained higher gross margin (1,673 EUR) than farms from Mediterranean part (886 EUR) of BH as well. Significant difference was observed in cucumber production as well, as farms in continental region achieved gross margin that amounted to 604 EUR, compared to 299 in Mediterranean region. But, gross margin achieved in spinach production was somewhat higher in Mediterranean region (821 EUR) than in continental part of BH (628 EUR). Spring onion production was registered only in farms that operate under the Mediterranean climate conditions and average realized gross margin was 142 EUR. On the other hand, mangel production was registered only by farms in the part with Mediterranean climate, and the average achieved gross margin was 139 EUR.

Tab. 4. Overall gross margin at the studied farms, depending on the climate conditions of production (average per farm in EUR)

Description	Studied farms according to production conditions		Average
	Mediterranean	Continental	
<b>GROSS MARGIN FROM:</b>			
1. Tomato	1,737	2,115	1,926
2. Peppers	886	1,673	1,280
3. Cucumber	299	604	451
4. Spinach	821	628	725
5. Green salad	154	196	175
6. Spring onion	0	142	71
7. Mangel	139	0	69
<b>OVERALL GROSS MARGIN</b>	<b>4,036</b>	<b>5,357</b>	<b>4,697</b>

Source: Private research

## CONCLUSIONS

Contrary to expectations, it was shown that higher average yields in the production of tomato were achieved at the farms in continental BH, where they were by 470 kg higher compared to the yields at the farms of the Mediterranean area, while there were no significant differences in average yields of peppers and spinach. The farms in the continental areas achieved a greater gross margin per 100 m<sup>2</sup> in the production of tomato (higher by 221 EUR), and peppers (by 197 EUR), while the farms in the Mediterranean parts achieved greater gross margin in the production of spinach (by 35 EUR). Average total gross margin on analysed farms was 4,697 EUR, and it was higher in continental part of BH (5,357 EUR) than in Mediterranean part (4,036 EUR). In both groups, tomato production had the highest share in gross margin, and it was slightly higher in continental (2,115 EUR) than in Mediterranean part (1,737 EUR). Spinach was the only of all analysed production that gained higher gross margin in surveyed farms from Mediterranean part (821 EUR) than in those from continental part (628 EUR).

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## PANEL DATA ANALYSIS FOR ENVIRONMENT IN BOSNIA AND HERZEGOVINA AND EU COUNTRIES

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*Scientific paper*

### Summary

The aim of this study is to test the Environmental Kuznets Curve (EKC) hypothesis for 13 European countries and Bosnia and Herzegovina which are participated EU after 2004 and spanning the period 1990 - 2014. We focused on how both income and policies in these countries affect the income emissions (environment) relationship. The methodology is using panel data is employed in a multivariate framework to test the EKC hypothesis. The multivariate framework includes: CO<sub>2</sub> emissions, GDP per capita, population density, and three indicators that measure the quality of institutions. In terms of the presence of an inverted U - shape association between emissions and income per capita, support to the presence of an Environmental Kuznets Curve hypothesis.

Key words: *Carbon dioxide emissions, Environmental Kuznets Curve, Panel data European countries*

### INTRODUCTION

In this study, long run was made by using data on CO<sub>2</sub> emissions and GDP (1990-2014) caused by Bosnia-Herzegovina and European Union to outcome.

The environmental effects of economic growth have been receiving increasing attention of economists in recent years. One particular aspect, the linkage of environment with economic growth/development, evoked much discussion in the last decade and a sizeable literature on the pollution–income growth relationship has grown in recent period (Dinda, 2004). In the early stages of economic growth degradation and pollution increase, but beyond some level of income per capita, which will vary for different indicators, the trend reverses, so that at high income levels economic growth leads to environmental improvement (Stern, 2004). Carbon dioxide emissions are the consequence of economic activity and the technologies which we use to produce and consume energy (Pielke, 2013). The scientific community has reached a consensus that global warming is occurring; increasing carbon dioxide (CO<sub>2</sub>) emissions have produced a massive build-up of greenhouse gases, giving rise to higher temperatures (IPCC, 1995; Watson, 1998).

### MATERIALS AND METHODS

Recent studies have identified inverted-U relationships between pollution and economic development. (De Bruyn *et al.*, 1998). We were used panel of data on emissions of carbon dioxide per capita and GDP per capita of EU and Bosnia-Herzegovina. Data on GDP and CO<sub>2</sub> emission are obtained from the World Bank. In this study, environmental Kuznets curves have been estimated using a simple reduced-form model that gives no information on the mechanisms underlying the estimated inverted U-shaped relationship between some pollutants and income (Sander, M., De Bruyn, 1997). Much of the income-environment literature assumes the relationship is homogeneous of degree one, i.e. population does not matter to emissions per capita independently of income per capita. The existence of public goods may result in both income per capita and total income having an effect on the demand of polluting goods; in other words, a higher population would result in higher emissions for given income per capita, and population growth would result in growth of emissions independently of the growth in per capita incomes (Panayotou, 2000).

EKC literature finds that as incomes rise, there is a domain of incomes over which per capita measures of environmental degradation, pollution, or energy use declines. In general, neither trade nor energy prices have

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been considered to be important explanatory factors in most of the empirical work to date (Agras, 1999). Most of the data used in these studies are panel data.

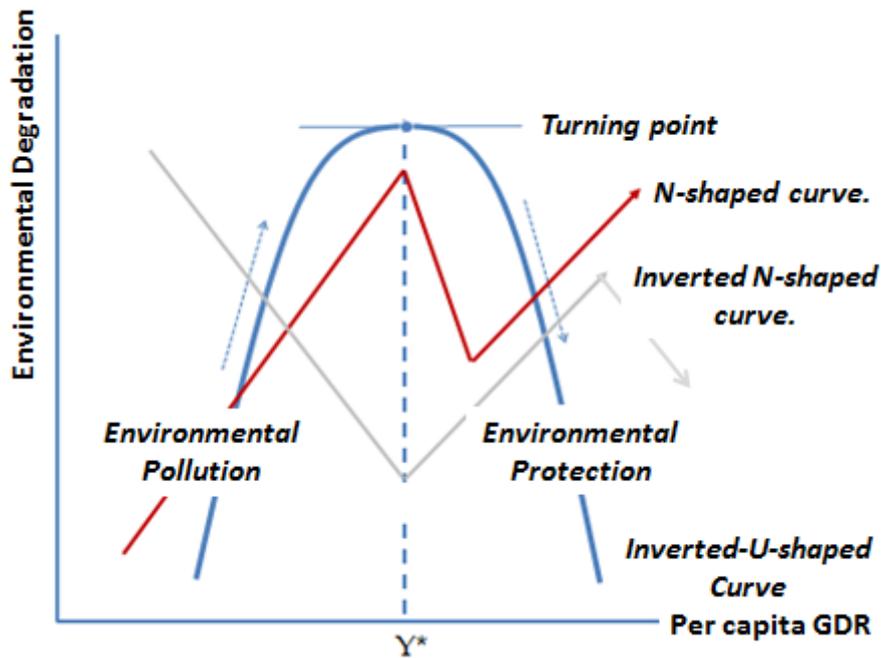
The following reduced form model is used to test the various possible relationships between pollution level/environmental pressure and income: A large number of econometric studies have used the model:

$$y_{it} = \alpha_i + \beta_1 x_{it} + \beta_2 x_{it}^2 + \beta_3 x_{it}^3 + \beta_4 z_{it} + \varepsilon_i$$

The most simple model specification shows a relationship between an environmental indicator “y” where “y” is CO<sub>2</sub>, “x” is GDP per capita (PPP) and “z” relates to other variables (population) of influence on environmental degradation. Several models on the EKC hypothesis include “population density” instead of population. Here, the subscript “i” is a country, “t” is time, “α” is constant, “β”<sub>k</sub> is the coefficient of the “k” explanatory variables. Model provides us to test several forms of environment–economic development relationships (Dinda, 2004):

- (i)  $\beta_1 = \beta_2 = \beta_3 = 0$ . A flat pattern or no relationship between “x” and “y”.
- (ii)  $\beta_1 > 0$  and  $\beta_2 = \beta_3 = 0$ . A monotonic increasing relationship or a linear relationship between “x” and “y”.
- (iii)  $\beta_1 < 0$  and  $\beta_2 = \beta_3 = 0$ . A monotonic decreasing relationship between “x” and “y”.
- (iv)  $\beta_1 > 0$ ,  $\beta_2 < 0$  and  $\beta_3 = 0$ . An inverted-U-shaped relationship, i.e., EKC.
- (v)  $\beta_1 < 0$ ,  $\beta_2 > 0$  and  $\beta_3 = 0$ . A U-shaped relationship.
- (vi)  $\beta_1 > 0$ ,  $\beta_2 < 0$  and  $\beta_3 > 0$ . A cubic polynomial or N-shaped figure.
- (vii)  $\beta_1 < 0$ ,  $\beta_2 > 0$  and  $\beta_3 < 0$ . Opposite to the N-shaped curve.

We can show different models of the environmental Kuznets curves in figure 1.



Source: *The Environmental Kuznets Curve, (EKC)- Developing countries and SD*  
<http://sdca.wordpress.com>

Fig. 1. Different models of the EKC

## RESULTS

For the 13 EU countries as a whole, CO<sub>2</sub> emissions approximately decreased by 9% from 1990 to 2014; over the same period, GDP per capita (PPP) increased by 3.30 times. For the BIH CO<sub>2</sub> emissions approximately increased by 6 times and GDP per capita increased by 13.99 times. On the other hand, in order of population of EU and BIH decreased 6.11% and 15.50% from 1990 to 2014.

When viewed from this perspective, the changes in carbon dioxide emissions and population appear to be a coincided. This is why we can say that there is an impact on environmental pollution of economic growth and population. The results found by the According to the Panel Least Squares analysis for EU and BIH are given in table 1 and table 2.

Tab. 1. Panel Least Squares Analysis For selected 13 EU  
Dependent Variable: CO<sub>2</sub>, Sample: 1990 2014 Periods included: 25 Cross-sections  
included: 13, Total panel (balanced) observations: 975

Variable	Coefficient	Std. Error	t-Statistic	Prob.
GDP	-8.81E-06	7.87E-06	-1.118787	0.0041
POPULATION	5.23E-07	1.19E-07	4.406646	0.0000
C	0.000000	1.007026	2.676246	0.0078
Effects Specification				
Cross-section fixed (dummy variables)				
R-squared	0.933109	Mean dependent var		6.938843
Adjusted R-squared	0.930088	S.D. dependent var		2.737054
S.E. of regression	0.723700	Akaike info criterion		2.236176
Sum squared resid	162.3601	Schwarz criterion		2.410814
Log likelihood	-348.3786	Hannan-Quinn criter.		2.305874
F-statistic	308.8862	Durbin-Watson stat		0.590972
Prob(F-statistic)	0.000000			

Tab. 2. Panel Least Squares Analysis For BIH

Dependent Variable: CO<sub>2</sub>, Method: Least Squares., Sample: 1990 2014,  
Included observations: 75

Variable	Coefficient	Std. Error	t-Statistic	Prob.
GDP	0.001046	6.65E-05	15.72449	0.0000
POPULATION	-2.05E-07	5.08E-07	-0.403545	0.0004
C	1.841130	1.985364	0.927351	0.0038
R-squared	0.919494	Mean dependent var		3.474919
Adjusted R-squared	0.912175	S.D. dependent var		1.913411
S.E. of regression	0.567044	Akaike info criterion		1.815407
Sum squared resid	7.073853	Schwarz criterion		1.961672
Log likelihood	-19.69258	Hannan-Quinn criter.		1.855974
F-statistic	125.6357	Durbin-Watson stat		0.450463
Prob(F-statistic)	0.000000			

The income-environment relationship specified and tested in much of the literature is a reduced form function that aims to capture the “net effect” of income on the environment. The income variable captures the locus of the equilibrium abatement levels, where demand and supply, both income dependent, are equal. Hence, the abatement effect is expected to be a monotonically decreasing function of income. Figure 2/a-b-c below depicts these three effects (Panayotou *et al.* 1999):

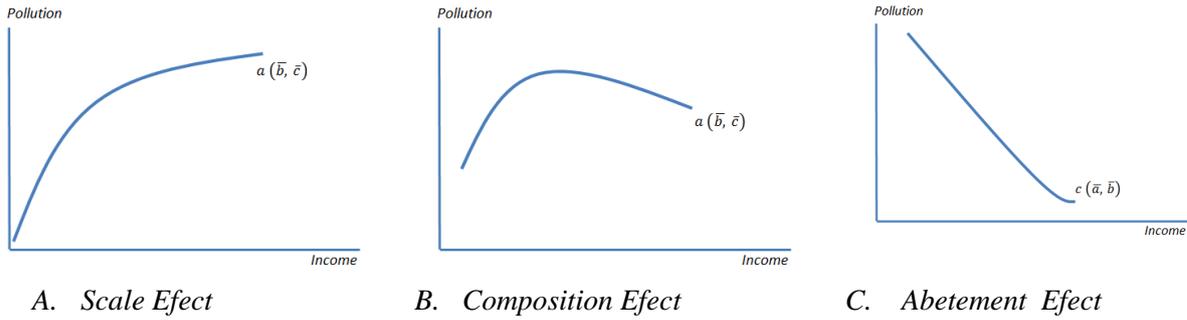


Fig. 2/A-B-C : The Income-Environment Relationship

In this study, we found out 2 important relationships for environment–economic development relationships.

For selected 14 UE countries :  $\beta_1 < 0, \beta_2 > 0$  and  $\beta_3 = 0$ .  
 For BIH :  $\beta_1 > 0, \beta_2 < 0$  and  $\beta_3 > 0$ .

### CONCLUSIONS

In the present study, following formulae were produced from the analysis of 1050 panel data from 14 selected EU countries and BIH

Formula 1 is for 14 selected EU countries.

$$CO_{2it} = -\beta_1 8,8168306 + \beta_2 [7,25 (10)^7]^2 + \beta_3 + \beta_4 5,23 (10)^6 + \varepsilon_i \quad (1)$$

$i=1, \dots, 1050$  ve  $t=1990, \dots, 2014$

Formula 2 is for BIH.

$$CO_{2it} = 1,841130 - \beta_1 0,001046 + \beta_2 1,19 \cdot 10^6 + \beta_3 4,89^{11} + \beta_4 2,05 (10)^6 + \varepsilon_i \quad (2)$$

$i=1, \dots, 1050$  ve  $t=1990, \dots, 2014$

There were decreases in CO<sub>2</sub> emission in countries which joined to EU after 2004. Unlike this trend, emission rates in 1990 increased by six-fold in 2014 in BIH. According to PPP, GDP per capita increased in all countries considered in the present study. GDP increase in BIH, on the other hand, is higher than in 13 EU countries covered in the study. An U-shaped association was found between CO<sub>2</sub> emission and GDP in EU countries. In BIH, this association was in N-shape.

There were decreases in CO<sub>2</sub> emissions along with the increasing income in EU countries. Nevertheless, in high income levels, increases in CO<sub>2</sub> emission were higher than increases in incomes.

In BIH, CO<sub>2</sub> emissions increased along with increasing income levels.

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## **GENERATING THE LAND INFORMATION SYSTEM OF ULUDAG UNIVERSITY, AGRICULTURE FACULTY, AGRICULTURAL EXPERIMENT AND RESEARCH CENTER (AFAERC-LIS)**

Ekin Ulaş Karaata<sup>1</sup>, Ertuğrul Aksoy<sup>1</sup>, Gökhan Özsoy<sup>1</sup>

*Original scientific paper*

### **Summary**

Developing the land database system of Uludag University, Agriculture Faculty, Agricultural Experiment and Research Center (AF-AERC) by using the remote sensing and geographic information systems, providing the ability of updating same data fields in following years and easing up verification and evaluation and compassion of perennial data are main objectives of the study. For this aim, the current land cover / land use map, soil series map, digital elevation model, slope map and database of the AF-AERC have been created with the attribute table.

With using the method of screen digitizing, boundaries of land cover / land use types from satellite image of Worldview-2 and aerial photographs were digitized thus detecting the current land usage based on area and updating attribute data belongs to each of the mapping units has become available. Soil maps and digital elevation model were produced by the digitizing and clipping procedure from previously produced soil maps and contour lines of ortho-photo maps of the Uludag University campus area. In addition, statistical information of the produced maps were gathered from attribute tables for the AF-AERC's areas using table and map calculation tools of ArcGIS program.

As a result, the coverage of AF-AERC lands were was determined as 314.5 ha and the most common land use type is agricultural land parcels with the rate of 72.5%. In addition, the largest three soil series of AF-AERC were determined as Çiftlik series (28.5%, 89.55 ha), Açma series (14.2%, 444.9 da) and Taşköprü series (12.0%, 377.7 da). Studies showed that lands of AF-AERC lie on the areas whose altitude ranges between 54.6 and 143.9 m asll, and also that flat / nearly flat areas were covered by 60.6% (192.55 ha).

**Key words:** *GIS, Land Information System, Remote Sensing*

### **INTRODUCTION**

Increasing environmental awareness therewithal various peripheral activities caused the necessity of generating massive amount of soil based information recently. Also, agricultural and other anthropic influences on soil gave rise to the provision new actslaws. In order to conduct the aforementioned acts and consideration of the generated data, there is an important demand for an elaborate and easily accessible soil information system. This kind of soil information system is also considerably useful for monitoring and evaluating the vegetative production within the body of Uludag University Agriculture Faculty, Agricultural Research and Experiment Center.

The most basic necessary component to make sustainable and effective management plans is generating correct and actual qualitative and quantitative data of natural resources. Nowadays technologies for obtaining, processing and modeling information from environment are rapidly improving. Corresponding improvement in technics like Geographic Information Systems (GIS) also makes it easier to integrate with environmental data resources (Sezgin, 2006).

### **MATERIAL AND METHODS**

Fields of Agriculture Faculty, Agricultural Research and Experiment Center within the borders of Uludag University Gorukle campus were selected for study area.

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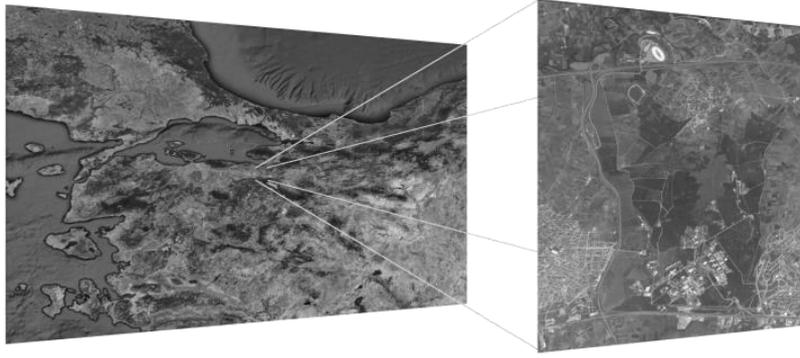


Fig. 1. Uludag University Gorukle Campus location in Marmara Region of Turkey

As a part of the study, some physical, chemical and mineralogical analyses made on 114 different soil samples taken from the area. Analyses showed that salinity and alkalinity problems were not mentioned, vast majority has clay texture, low organic matter, high cation exchange capacity with generally  $Ca^{++}$  and  $Mg^{++}$  (Özsoy, 2001).

Furthermore, soil series due to soil taxonomy rated in Entisol, Inceptisol, Mollisol and Vertisol. Due to FAO/Unesco World Soil Map legend majority of them classified into Eutric vertisol and eutric leptosol, calcaric regesol, calcaric fulvisol, eutric cambisol, calcaric cambisol and calcaric phaeozem, respectively (Özsoy, 2001).

Base maps have been derived from 50x50 cm resolution 2010 dated digital aerial photos and Worldview-2 satellite images belong to same date. These images were processed by on-screen visual interpretation in ArcGIS 10.0 software and supported by area visits and observations in order to generate database, result output, data analysis and digital data input.

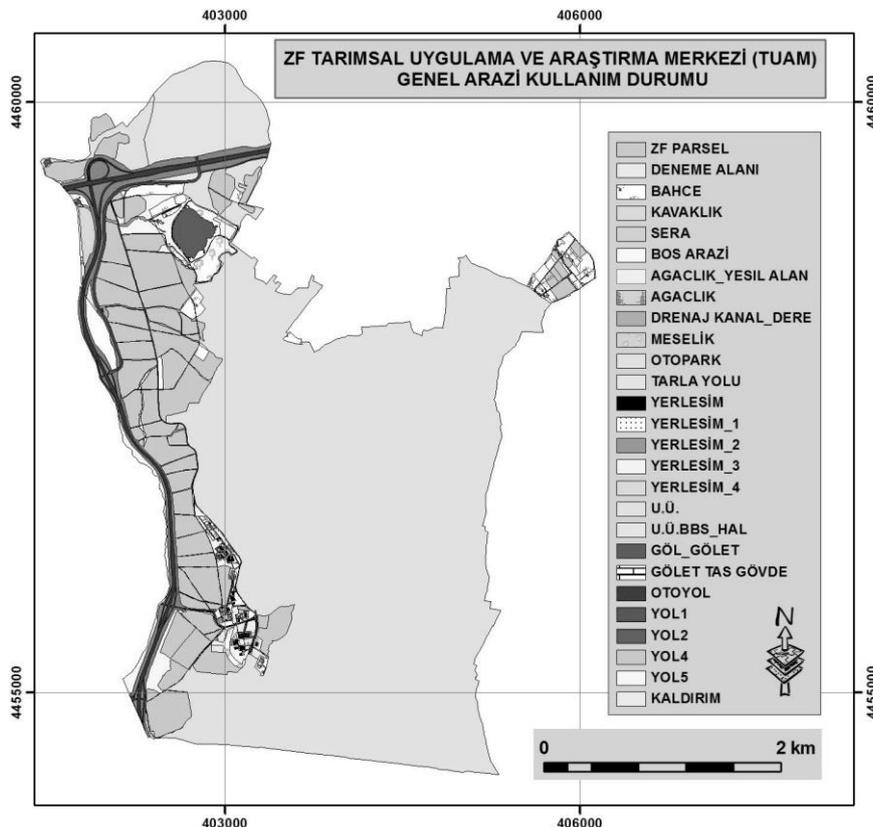


Fig. 2. Classification map of land coverage / land usage types of land of AFAERC-LIS.

## RESULT AND DISCUSSION

As a result of analyses and classification process, available distribution values are given in the table 1. Within this scope it has been found that the subject study area takes up 22% of all campus area with 3144,98 decaire.

Tab. 1. Areal and proportional distribution of Uludag University campus area.

Uludag University Land Usage	Area (decaire)	Ratio (%)
<b>AFAERC-LIS areas</b>	3144.98	22.0
<b>Irrigation pond</b>	298.64	2.09
<b>Wholesale vegetation market hall</b>	658.04	4.61
<b>Highway and access roads</b>	672.2	4.71
<b>Uludag University Campus (others)</b>	9509.11	66.59
<b>Total</b>	14282.97	100

In order to generate land usage and land coverage types, alteration in color tone, shape, size, pattern and texture has been considered and analyzed in coloured base maps. Due to consideration and analyses for 2011 a total of 118 different land usage/land cover types were identified in 399 polygonal area, they were mapped and attribute tables created. This map used ArcGIS area table calculation module and obtained areal and proportional values. Also within the scope of the study, recent land cover and usage of whole campus, digital soil map and database of AFAERC soils, soil series map, soil aptitude map, availability for irrigated farming map, soil depth classes map, digital elevation model, slope index map and its database have been generated.

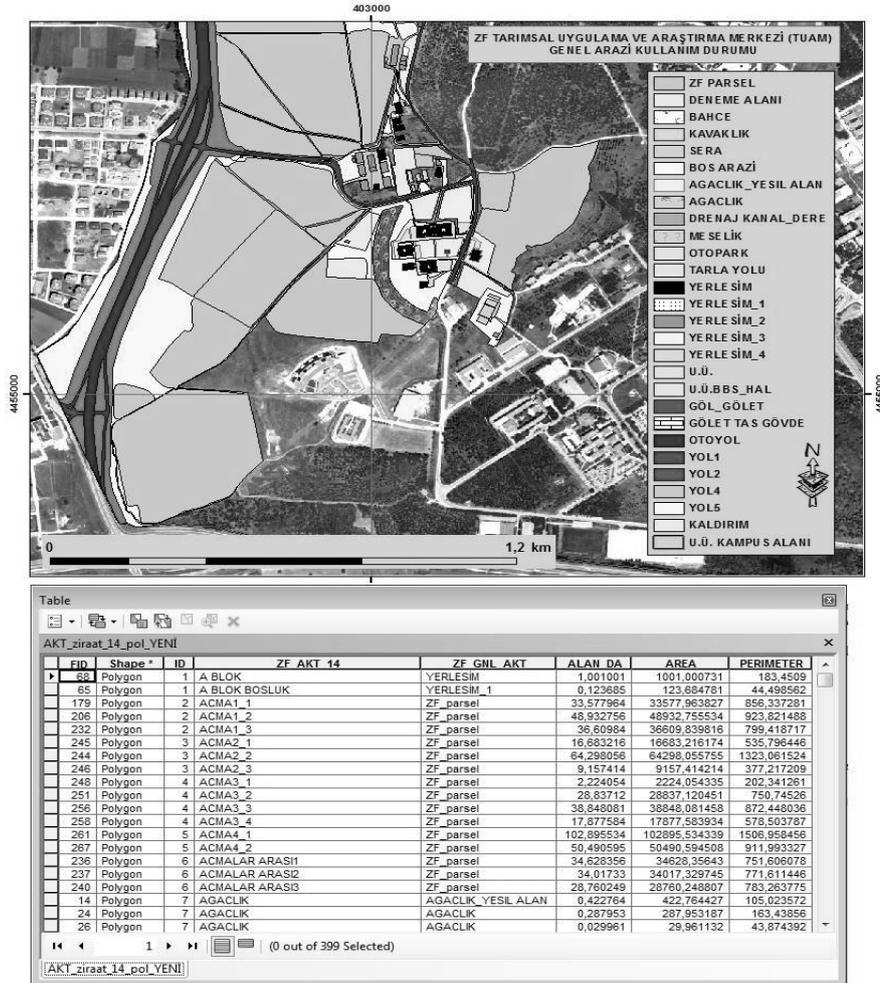


Fig. 3. 2011 land cover and land use map and attribute table of AFAERC-LIS fields.

Uludağ University campus digital soil map has clipped via ArcGIS software in order to obtain vector based soil map belongs to study area. Soil series data from previous reports (Özsoy, 2001) merged with each polygon and edited attribute table to add extra features.

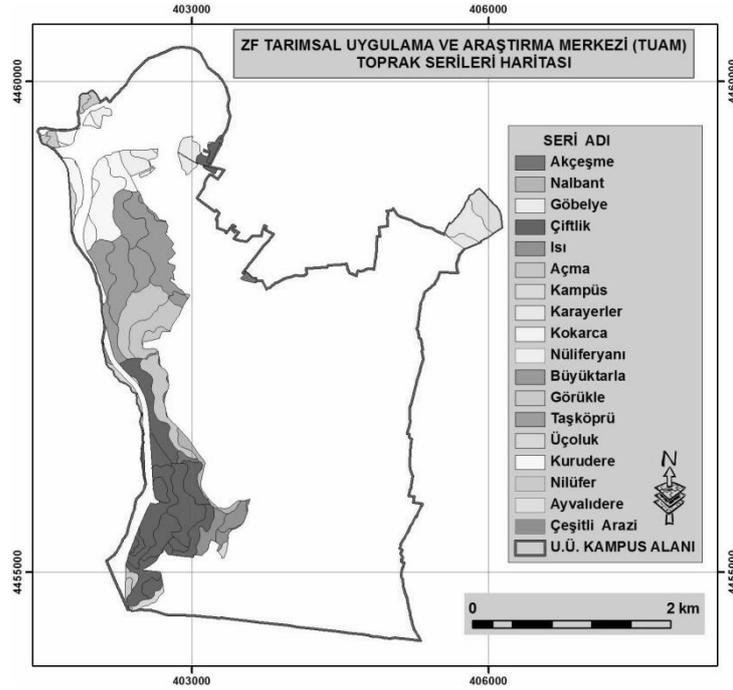


Fig. 4. AFAERC-LIS digital soil series map.

As a result of classification 18 different soil series have been placed in study area. Çiftlik series takes the majority with 28.5% (89.5 ha) of total 314.5 ha study area. Other series, their percentage and areas are given in Table 2.

Tab. 2. Soil series distribution of study area.

Soil Serie Name	Area ( ha )	Ratio ( % )
Açma	44.488	14.15
Akçeşme	2.562	0.81
Ayvaldere	7.763	2.47
Büyüktarla	35.158	11.18
Çeşitli Arazi	0.21	0.07
Çiftlik	89.548	28.47
Göbelye	5.982	1.90
Görükle	2.62	0.83
Isı	12.583	4.00
Kampüs	3.054	0.97
Karayerler	11.833	3.76
Kokarca	0.06	0.02
Kurudere	36.955	11.75
Nalbant	5.002	1.59
Nilüfer	5.012	1.59
Nülferyanı	9.812	3.12
Taşköprü	37.768	12.01
Üçoluk	4.085	1.30

In order to ensure for providing in-database layer compatibility and enhance the query diversity from database work flow, the soil depth classification, digital elevation model and slope index map with slope classification tables of study area have been added to attribute table.

Soil depth data obtained from field analyses have been used to generate digital soil depth map belongs to study area. Soil depth classes which originated from Soil Survey Manual (1993) and calculated areal values and ratios with ArcGIS software modules have been presented in figure 5 and table 3.

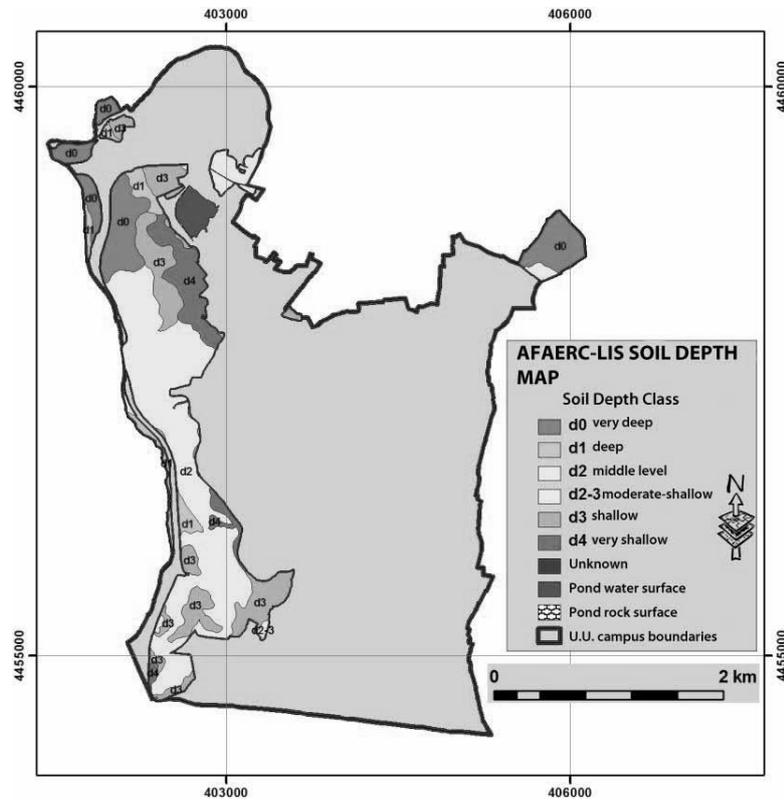


Fig. 5. AFAERC-LIS soil depth classification map

Tab. 3. Areal distribution of soil depth classes.

Soil Depth Classes (cm)	Area (ha)	Ratio (%)
<b>d<sub>0</sub>: Very deep (150 +)</b>	52.811	<b>16.79</b>
<b>d<sub>1</sub>: Deep (100-150)</b>	14.928	<b>4.75</b>
<b>d<sub>2</sub>: Middle level (50-100)</b>	154.278	<b>49.05</b>
<b>d<sub>2,3</sub>: Moderate-shallow</b>	0.896	<b>0.28</b>
<b>d<sub>3</sub>: Shallow (25-50)</b>	61.476	<b>19.55</b>
<b>d<sub>4</sub>: Very shallow (10-25)</b>	29.894	<b>9.51</b>
<b>Unknown</b>	<b>0.218</b>	<b>0.07</b>

As presented in table 3, it has been founded that 67.73 ha wide fields are able to consider as suitable for irrigated farming which are able be productive for all agricultural crops as long as having suitable seasonal conditions while almost 50% of study area has been placed on moderately shallow soils.

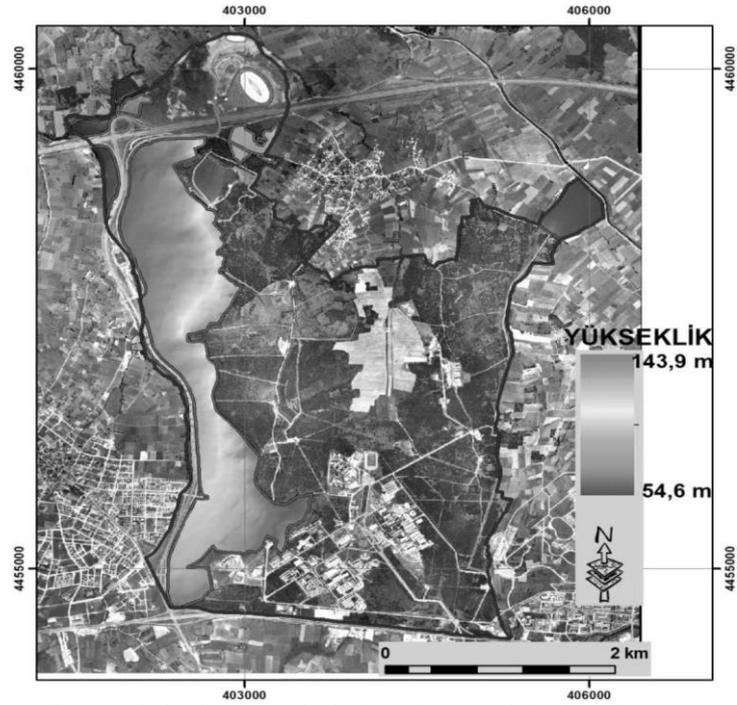


Fig. 6. Calculated digital elevation model of study area.

AFAERC-LIS area's elevation from sea level varies between 54.6 m with 143.9 m as seen in figure 6 while main dip direction lays on north-east and south-west direction. With digital elevation data, it has been possible to derive slope surfaces and slope classification maps with reclassification commands. Slope classification process has been interacted with limit slope values given in Soil Survey Manual (1993), results and ratios are presented in figure 7 and table 4.

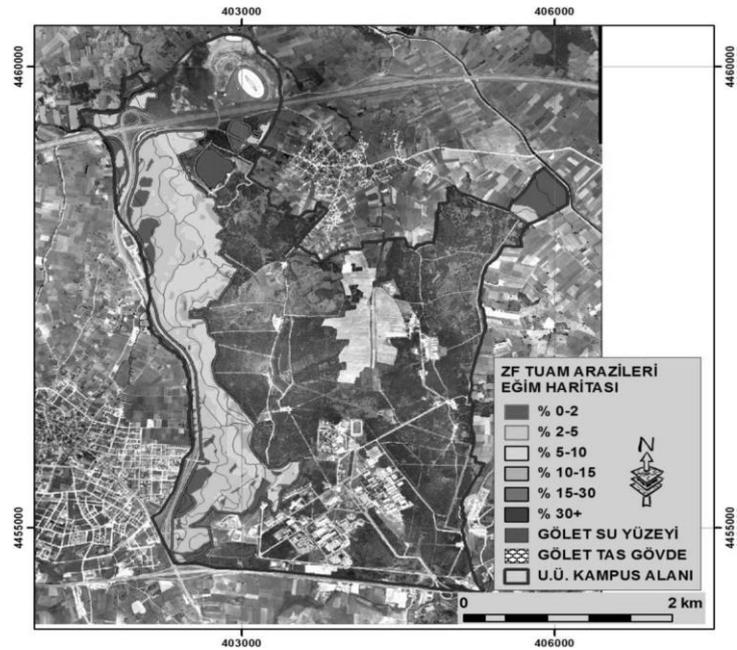


Fig. 7. Slope classes map for AFAERC-LIS area

Tab. 4 . Slope classes distribution and ratios of study area.

Slope ( % )	Slope Classes	Area (ha)	Ratio (%)
<b>0-2</b>	Nearly level	62.95	<b>19.80</b>
<b>2-5</b>	Gently sloping	129.64	<b>40.78</b>
<b>5-10</b>	Rolling	104.87	<b>32.99</b>
<b>10-15</b>	Moderately steep	18.84	<b>5.93</b>
<b>15-30</b>	Steep	1.56	<b>0.49</b>
<b>30+</b>	<b>Very steep</b>	<b>0.03</b>	<b>0.01</b>

## CONCLUSIONS

Some important advantages of remote sensing and geographical information systems enable importing of topographic or non-topographic data into digital environment, create association with each other or digital satellite images, analyses and produce the output.

As explained in previous paragraphs, generating sub-thematic maps, obtaining statistical values and updating with reclassification due to individual or grouped cover / use is made much faster and with high accuracy in GIS environment, while these processes are time consuming and compelling with classic methods.

AFAERC-LIS geographic database has an extraordinary potential for optimizing future academic agricultural researches and conventional agricultural production within the body of Uludağ University via having ability to acquisition of knowledge belongs to soil, climate and areal information. In process of planning new researches, compare, edit and create new data compositions and generating connected data have become much easier and more precisely.

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## LIQUIDITY OF AGRO-FOOD SECTOR IN BOSNIA AND HERZEGOVINA IN FUNCTION OF SUSTAINABLE BUSINESS

Berin Kulelija<sup>1</sup>, Dragana Ognjenović<sup>1</sup>, Sabahudin Bajramović<sup>1</sup>, Emir Bećirović<sup>1</sup>

*Original scientific paper*

### Summary

Liquidity as one of the key indicators of business analysis. It essentially measures the ability of the company to timely and fully meet all of its current obligations and it indicates the state of financial stability as well. This paper aims to determine trend of these indicators in food industry in Bosnia and Herzegovina, and on the basis of these results, to assess financial stability and, thus, economic viability of companies within three main food processing sectors in BH, dairy, meat and fruit and vegetable. The analysis encompassed all 416 registered companies in Bosnia and Herzegovina operating within the mentioned branches. Data source were collected from financial reports of companies, i.e. data had been evaluated and liquidity ratios: the coefficient of current ratio and coefficient of financial stability were calculated.

Key words: *liquidity, milk processing, meat processing, fruit processing*

### INTRODUCTION

In the time of globalization of markets and increased competitiveness in all types of business, analysis of operations is necessary, in order to identify the strengths and weaknesses of a given business and to take appropriate measures for improvement. Although the state of a business can be favorable, because of the dynamics of the market and competition that "do not sleeps", continuous improvement is necessary, and again this does not guarantee success, but increases chance for success. This applies both at the level of a company, and the level of entire industry or agriculture. For this reason, this paper relates to the testing of liquidity as one of the most important indicators of business analysis which indicates how much the observed subjects are able to settle their obligations on time and thus maintain good relations with suppliers and avoid obligations to pay interest for late payment.

Vujević and Balen (2006, p. 40) defines liquidity as the ability of undisturbed flow of essential factors in the business process, and convert the material in the form of cash or cash into material forms. Sorić (2004, p. 557) states that liquidity expresses several indicators in which cash and its more or less cashable equivalents primarily take place in relation to current liabilities. Van Horne and Wachowicz (1995, p.128) state that liquidity has two dimensions and that are: the first -time required to convert assets into cash, and the second-security of realized price. Aquila and Ebohi (2012, p. 75) state that an unexpected liquidity problems of one company can be transferred to the suppliers, but with mentioned above it indicates the importance of this indicator.

The three strongest sectors of the food industry in B&H in the period 2008-2014 were included in the analysis of liquidity indicators and interesting results were obtained.

### METHODOLOGICAL APPROACH

The paper is based on an analysis of the financial statements of 416 companies that operated during the period 2008-2014 as part of three sectors, meat processing, dairy processing and fruit and vegetables processing. Data source were the Agency for financial, informatics and Intermediary Services (abbreviated AFIP) and the Agency for Mediation, Information and Financial Services (abbreviated APIF). Companies which, in at least one year of the period, did not have a single employee were excluded from the sample. There were 263 such firms, which means that 153 firms were included in the further analysis. A joint balance sheet and income statement was made for these companies, obtained by calculating the average values of balance sheet positions of all 153 firms. Liquidity indicators and indicators of current liquidity and financial stability were calculated. The coefficient of current ratio was calculated as the ratio of current assets and current liabilities. According to

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Kulelija (2012, p. 81) with the coefficient of current ratio in the recent literature, the optimal value is considered to be 2 because the stocks are considered less liquid assets. Also, any excess liquidity is not desirable because it loses the opportunity to reproduce the capital. The coefficient of financial stability places into relation the fixed assets with the capital and long-term obligations. According to Ognjenovic (2009, p. 93), it is important that the value of this indicator is below 1. Liquidity ratio were calculated and presented for different groups firms such as: the total for all companies from all three sectors at the state and entity level and particularly for each of the three sectors. The following methods were used in the paper: parsing, comparison and the calculation of average size and balance sheet.

## RESULTS AND DISCUSSION

The state of meat processing sector, milk processing and processing of fruits and vegetables in terms of liquidity was analyzed on the basis of the collected financial statements i.e. balance sheet and income statement. Some of the liquidity ratios could not be calculated because of the form of available data, but current ratio and indicators of financial stability enabled quality judgment on the situation in all three sectors.

Calculated value of the coefficient of current liquidity of firms in the period 2008-2014 is presented in the following table.

Tab. 1. Coefficient of current liquidity of the companies of meat, milk and fruit and vegetables processing in B&H (2008-2014)

Model grouping	Year							
	2008	2009	2010	2011	2012	2013	2014	Index 2008=100
The total processors	1.16	1.18	1.16	1.24	1.39	1.40	1.39	119.38
Processors in FB&H	1.24	1.27	1.19	1.32	1.51	1.50	1.49	119.95
Processors in RS	0.97	0.98	1.08	1.07	1.18	1.21	1.20	122.76
Meat processors	1.04	1.12	1.24	1.35	1.31	1.43	1.33	128.56
Milk processors	1.47	1.54	1.21	1.27	1.70	1.51	1.49	101.99
Fruit and vegetable processors	1.20	1.12	1.03	1.06	1.37	1.27	1.51	125.56

Source: Own calculations based on data from AFIP and APIF

It is evident that the liquidity of processors in B&H is unsatisfactory especially in the beginning of the analyzed period, when referring to a recent literature that suggests that the coefficient of current ratio should be two (2) in order to facilitate maintenance of liquidity. However, it is positive that the coefficient is still above 1, and the tendency of growth in the value of the coefficient of current liquidity is evident, and even better condition can be expected in the future. Processors from FB&H recorded a better current ratio than processors from the RS. Processors of milk in average had a better coefficient of current ratio than other sectors, except in 2014, when fruit and vegetable producers recorded the highest current ratio. It is encouraging that all three sectors recorded the tendency of growth of the coefficient of current liquidity. Table 2. presents calculated values of financial stability coefficient of companies over the 2008-2014.

Tab. 2. Coefficient of financial stability of companies of meat, milk and fruit and vegetables processing in B&H (2008-2014)

Model grouping	Year							
	2008	2009	2010	2011	2012	2013	2014	Index 2008=100
The total processors	0.90	0.89	0.91	0.88	0.83	0.82	0.82	91.77
Processors in FB&H	0.85	0.84	0.90	0.85	0.80	0.80	0.80	93.62
Processors in RS	1.01	1.01	0.95	0.95	0.90	0.89	0.89	88.22
Meat processors	0.96	0.90	0.86	0.83	0.84	0.80	0.83	86.91
Milk processors	0.81	0.78	0.92	0.87	0.77	0.82	0.82	101.17
Fruit and vegetable processors	0.89	0.94	0.98	0.95	0.85	0.86	0.81	90.45

Source: Own calculations based on data from AFIP and APIF

Analyzing the state level, it is evident that processors in B&H have a slightly lower coefficient of financial stability than 1, which indicates that the long-term sources of funding of processors is still higher than the value of fixed assets, or that the part of the current assets are financed by long-term liabilities. Besides, it means that there is an excess of current assets over current liabilities, which confirms the liquidity ratio. The downward trend in the value of the coefficient of financial stability indicates that the improvement of liquidity and financial stability of the sector can be expected in the future.

The processors of milk and fruits and vegetables recorded downward trend in the value of the coefficient of financial stability in contrast to milk processors. However, the processors of milk on average had better financial stability than the other two sectors over the analyzed period.

## CONCLUSION

Based on the conducted research of the liquidity sectors of meat processing, milk processing and fruit and vegetable processing in the period 2008-2014 it can be concluded that these sectors are not in an enviable position, but favorable tendencies indicate that liquidity and financial stability of these sectors can be expected in the future. The most successful sector in terms of liquidity and financial stability in the analyzed period was the milk processing sector. These statements are confirmed by following data:

- The value of the coefficient of current ratio of processors in B&H was below recommended value, but the calculated value of the coefficient of liquidity ratio was above 1, and it indicates a slight excess of current assets over current liabilities. There is an evident tendency of growth in the value of the coefficient of current liquidity.
- Processors from FB&H recorded a better coefficient of current ratio than processors from the RS. In addition, the processors from both entities recorded the tendency of improvement of current liquidity.
- Milk processors in the period 2008-2014 on average had the highest coefficient of current ratio but the processors of fruit and vegetables in 2014 recorded the highest coefficient. It is significant that all three sectors recorded the growing tendency of the coefficient of current ratio.
- Long-term sources of funding in processors in B&H were slightly higher than the value of fixed assets and a part of current assets was financed from long-term liabilities what ensures the relative financial stability. The tendency of the value of the coefficient of financial stability is going in the direction of additional improvements.
- Processors from FB&H had better financial stability than processors from the RS.
- Processors of milk and fruits and vegetables recorded the downward trend coefficient of financial stability in contrast to processors of milk processing sectors, which on average have better financial stability than other two sectors in the period 2008-2014.

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## INPUT/OUTPUT PRICE PARITY OF MAIZE AND WHEAT IN BOSNIA AND HERZEGOVINA

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*Scientific paper*

### Summary

This paper examines the input/output price parity of wheat and maize, being two strategic agricultural products during two time periods (2002/07 and 2008/13). The comparison of price parities allows us to define the status of producers before and after the global economic crisis. The research results indicate variation of the farmers' economic position in both studied periods which did not contribute to a better quality of life of rural population. All this indicates a necessity for introducing proper mechanisms of B&H agrarian policy which will improve economic efficiency and reproductive capabilities of the overall sector as well as the living conditions of farmers.

Key words: *input, output, price parities, farmers' economic position*

### INTRODUCTION

In recent years there has been a trend of steady increase in food prices with occasional short intervals in which abrupt price hikes occurred, for example in 2008 and 2010/11. This situation on the global food market, and particularly its impact on the quality of life of the poor, draws attention of not only the international developmental institutions and academic community but also the public policy makers at national and global levels (Dorward, 2013). Price instability is especially characteristic for the developing countries that are dependent on the import and/or export of food. Bearing in mind that nearly a third of the overall foreign trade deficit of B&H is accounted for by the deficit of agricultural and food sector, and that 17.9% of B&H population lives below the poverty line (Agency for Statistics B&H, 2011) it becomes clear that changes in food prices, especially the abrupt ones, have a direct effect on the quality of life of all citizens of Bosnia and Herzegovina as well as on the value of aggregate demand, and thus on the development potential of B&H economy. Therefore, information on price trends, especially on input/output price parities indicate a change in the position of farmers as well as the development potential of rural areas which is of great importance for the creation of adequate public sectoral and public policies. This is why the general objective of this study is to provide information on the trends of input/output price parity index for two strategic products (wheat and maize) in order to determine the change of status of producers of these crops, and based on it to assess the development potential of the sector and point out problems that could significantly slow down the strengthening of the sector and hence the recovery of rural areas.

### MATERIALS AND METHOD

According to Vlahović *et al.* (2010), the concept of parity - *paritas* implies uniformity, equality, equity, i.e. the equivalent or established relationship between the value of certain products – wheat and maize in this case, and their inputs in agricultural production. Through such price parity it is possible to examine the position of agriculture and its financial status in the primary distribution, i.e. the development potential of the sector. To make it easier to assess the impact of price change on the development potential of the sector we selected two strategic products which are very important for other sub-sectors of agribusiness as well. This paper examined the price parity for wheat and maize in relation to their input prices (seeds, mineral fertilizers and protective chemicals) in two time intervals (from 2002 to 2007 and from 2008 to 2013). This was carried out in order to determine if there was a change in the position of producers and to determine if the second time interval, which was characterized by unstable economical conditions, led to exacerbation of the producers' position. For the purposes of this study, we used the data for purchase prices for wheat and maize from Federal Institute of Statistics and of the Statistical Office of Republic of Srpska, while the prices of inputs are received from the local sellers. Price of oil, being a significant input, was not taken into account as its impact is already

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incorporated in the price of these inputs. The arithmetic mean, coefficient of variation and Average annual growth rate (AAGR) for both periods were calculated for each parity ratio, as well as for each individual product for both researched periods. Coefficient of variation was obtained from the relation between standard deviation and arithmetic mean, while the Average Annual Growth Rate is calculated with the formula

$$AAGR = \left[ \frac{\text{Ending Value}}{\text{Beginning Value}} \right]^{\frac{1}{\# \text{ of the years}}} - 1$$

### RESULTS AND DISCUSSION

The paper investigates the price parities of wheat and maize and their inputs within two periods. The first period is from 2002 to 2007 and the second from 2008 to 2013, which are characterized by climate extremes, climate variability and the global crisis. The status of these products has indicated changes that are reflected mainly in the deterioration of the producers of wheat and maize, particularly in the second period of monitoring. AAGR of wheat price in the first observed period was 2.2%, while the same value was negative in the second period (-6.51%). At the same time, the prices of inputs show the different AAGR – Urea had the highest AAGR (12%), while Dursban E48 achieved slight rate of decline. AAGR of parity ratio of wheat and its inputs in the first period resulted in the favorable conditions for producers compared to seeds and pesticides and unfavorable conditions when it comes to mineral fertilizers (Table 2). According to Todorović and Filipović (2010), the most significant element in the cost of wheat was the price of fertilizers which is a result of high sale prices.

Tab. 1. Average price, Average Annual Growth Rate and coefficient of variation of wheat and maize prices in 2002-2013

Price	Average price in BAM		AAGR %		Coefficient of variation in %	
	2002-2007	2008-2013	2007/02	2013/08	2002-2007	2008-2013
Wheat	0.28	0.37	2.20	-6.51	7.14	22.16
Wheat seeds	0.65	0.91	0.00	7.08	0.00	14.62
Maize	0.28	0.35	11.64	1.74	25.71	17.42
Maize seeds	3.80	7.63	0.44	14.42	1.32	32.69
KAN	0.26	0.59	8.06	2.75	23.08	22.71
UREA	0.36	0.82	12.09	0.74	33.33	29.02
NPK	0.34	0.89	10.48	-4.33	26.47	19.66
Deherban A I	5.90	11.55	0.57	16.37	1.69	24.50
Dursban E 48	24.30	27.72	-0.34	2.97	2.02	6.49

Recorded price drop (6.5%) in the second time period caused the unfavorable situation for producers relative to all inputs, in case of Deherban in particular, where the AAGR was highest (16.4%). This growth is mostly caused by the increase in price of oil, as the increase in oil prices causes reciprocal increase in prices of inputs.



Fig. 1. Average Annual Growth Rate of wheat and maize prices in 2002-2013

However, when the price of oil decreases, the decrease of input prices is not reciprocal, which is concerning. In addition, the inputs market is underdeveloped and nearly a 100% dependence on imports increases the risk for producers. High prices of raw materials are mainly a result of reliance on a few dominant retailers of raw materials combined with a high level of regulation of import markets. This situation leads to a decrease in productivity and competitiveness, as a limited level of availability of modern raw materials makes it difficult for farmers to export their products and participate in modern supply chains.<sup>2</sup> Present increase in prices of inputs that coincides with variable prices of outputs results in the uncertainty for producers to continue production.

Tab. 2. Average value, Average Annual Growth Rate and coefficient of variation in price parity of wheat and maize and their inputs, 2002-2013

Parity price	Average price in BAM		AAGR %		Coefficient of variation in %	
	2002-2007	2008-2013	2007/02	2013/08	2002-2007	2008-2013
Wheat/seeds	0.44	0.42	2.20	-12.69	7.23	39.76
Wheat/KAN	1.16	0.64	-5.42	-9.01	25.43	65.00
Wheat/UREA	0.87	0.46	-8.82	-7.20	35.17	18.26
Wheat/NPK	0.89	0.42	-7.49	-2.28	26.29	17.38
Wheat/Deherban A I	0.05	0.04	1.63	-19.66	7.50	61.11
Wheat/Dursban E 48	0.012	0.013	2.55	-9.21	8.33	23.08
Maize/seeds	0.07	0.05	11.15	-11.08	26.57	30.00
Maize/KAN	1.10	0.61	3.31	-0.98	20.44	54.66
Maize/UREA	0.82	0.45	-0.41	-0.99	25.86	26.12
Maize/NPK	0.84	0.40	3.31	-0.98	17.00	27.00
Maize/Deherban A I	0.05	0.03	11.01	-12.57	25.42	34.27
Maize/Dursban E 48	0.01	0.01	12.01	-1.19	26.10	16.10

AAGR of maize price, which was 12% in the first observed period, caused the better conditions for agricultural producers, as the AAGR of input prices was lower. The conditions for maize producers worsened in the second period, due to more significant increase in prices of inputs and AAGR of parity prices was negative compared to almost every input. Additionally, pronounced variation of parity of maize and inputs indicates a high level of uncertainty, which can certainly cause producers to abandon this production.

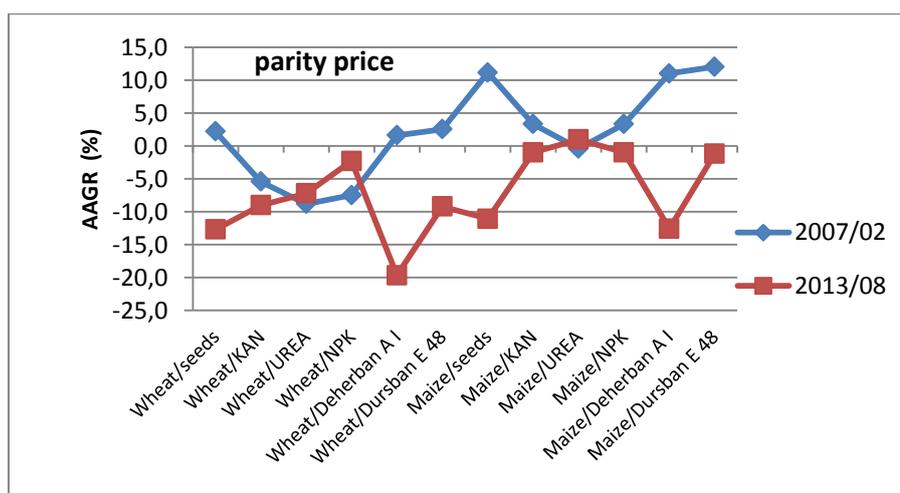


Fig. 2. Average Annual Growth Rate in price parity of wheat and maize and their inputs 2002-2013

<sup>2</sup> Study on the agricultural sector in Bosnia and Herzegovina, 2010.

Such situation can be reflected in reduced livestock production, especially in cattle breeding, where negative trends have already been noticed.<sup>3</sup> Price varying or volatility discourages producers from credit commitments or making new investments and using more quality but more expensive technologies which enable increased productivity (Jurčić, 2012).

## CONCLUSIONS

The obtained results show that changes in parity relations indicate deterioration in the position of wheat and maize producers in the second observed period compared to the first one. These changes are shaped up by a significant increase in prices of inputs in relation to the outputs. Constant fluctuations in the price of wheat and maize, as well as farmers being compelled to buy more expensive inputs, put them in a position of abandoning production. This attitude can certainly lead to the reduction of livestock production, particularly in cattle breeding, where negative trends have already been noticed. Therefore, we can say that the development potential is threatened and agriculture cannot be expected to serve as an effective buffer to mitigate the negative socio-economic and political developments in B&H. Bearing in mind the low productivity for these crops in relation to the EU, where it is more than two times higher than in B&H, public policies must create mechanisms that will help to increase the yield. Viewed through the higher total revenues, this could mitigate the possible adverse effects of unfavorable parities and encourage the continuation of production and development of the sector. Ultimately, only a functional value chain can develop the sector. Farmers, processors and retail facilities need to consolidate and integrate in order to improve quality standards, reduce costs and boost competitiveness. In this regard, the agrarian policy measures should be aimed at strengthening the system which will enable, primarily, the traceability of the entire system of production, processing and distribution, as well as the application of additional quality standards. This would raise confidence in domestic products, increase competitiveness and strengthen the sector.

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<sup>3</sup>Agency for statistics of B&H, First release 2014

## RISK MANAGEMENT BASED ON REGIONAL INDEX AS FINANCIAL INSTRUMENT IN CROP INSURANCE\*

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*Scientific paper*

### Summary

Insurance based on the regional index is one of the newer instruments for reducing losses in crop production. The regional index indicates the average yield or average value of production in a region, and it represents the basis for the calculation of premiums and benefits of insurance. The main advantage of this insurance model is that it does not require the assessment of damage, and which is one of the major problems in the relationship between the insured and insurer. In the case of corn, wheat and sunflower production as the most important crops in the region of Ada municipality, the authors describe the methodology of application of the analyzed insurance system. Organized steps taken by the state (government) should create an opportunity for the implementation of this contemporary form of insurance in Serbia, in order to further improve this area. This would reduce the negative financial consequences in agricultural production and it would also protect farmers from different production risks. Above-mentioned model of insurance can represent significant alternative to conventional insurance, and by establishing an effective risk management in crop production, the number of insured will be increased, as well as insured area, and trust and confidence in insurance companies will also be restored.

**Key words:** *crop production, financial risk, insurance, regional index*

### INTRODUCTION

In recent years, we have witnessed accelerated climate changes around the world. Unpredictable climatic events and natural disasters affect in a large extent on the lives of all people on our planet, as well as the various economic activities. Based on the research it is estimated that 80% of the world economy, depends on the fluctuations of the time factor (Auer, 2003). Climate changes particularly affect the results achieved in agriculture. Since it is a sector of the economy that is directly affected by weather conditions, the risk of occurrence of harmful events increases. For these reasons, the issue of reducing weather risks has become increasingly popular in contemporary agro-economic literature.

Undoubtedly, the best possible way to manage risk in the agricultural production is crop insurance. During the previous period, some of the classic insurance model showed many disadvantages. Risk management in the agricultural production, especially crop production has not been fully resolved, and there is always a constant need to develop new models of insurance. Constant development of crop insurance has contributed to the creation of a large number of the insurance system. Depending on the criteria, systems of crop insurance can be divided in different ways, and one of the most comprehensive classification differs insurance against loss of yield, insurance indicators of success, insurance of the entire farm and the so-called index (parametric) insurance (Marković and Jovanović, 2011). Of all the listed systems, index insurance is in a group of the latest models of insurance, and its deeper implementation for managing risk in agricultural production is certainly expected in the near future. This group of insurance includes insurance based on the regional index and weather derivatives. Insurance based on the regional index presents alternative to traditional insurance of yield from a number of risks, and as a parameter, this form of insurance takes an average yield or the average realized value of production in the specific region (Marković, 2014). In this paper, special attention will be paid to the insurance based on the regional index. In this model, insurance premium is realised if the regional average yield is below the expected long-term average yield (Skees *et al.*, 1997). This type of insurance was created in the mid of the last century as an alternative to individual insurance yield, with the aim of reducing moral-hazard and adverse selection (Halcrow, 1949), but a lot more attention was paid in the 90s when this model of insurance

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came again into the focus of scientific interest (Miranda, 1991). The condition for the implementation of this concept is the creation of geographic regions that are homogeneous with regards to climatic conditions. This assumes that all the farmers of the region pay the same insurance premium and during the occurrence of the insured event are also compensated in equal amounts (Ebneht, 2003). A significant advantage of this model compared to the classical, is reflected in the reduction of moral hazard, since individual insured have no influence on the amount of realized regional index, as well as on the level of compensation (Chambers and Quiggin, 2002). Also, the insurance based on the amount of regional index, is less susceptible to asymmetric information, and does not require the determination of expected yields and/or value of production for each individual farm (Deng *et al.*, 2008). In addition, the mentioned model enables farmers to better ensure comprehensive protection against all risks in relation to the certain individual insurances (Schlieper, 1997). Among the other advantages of insurance based on regional index should be single out elimination of adverse selection, reducing transaction costs, lower risk premium, lower franchise and a higher level of coverage (Wolf, 1998; Ebneht 2003; Hirschauer and Mußhoff, 2008).

In our country, modern systems of crop insurance are not available yet, although some empirical data indicate a real need for the mentioned models. Insurance based on regional index is certainly one of those whose implementation in Serbia would be desirable. For these reasons, the emphasis of the research effort in this paper, is placed on the practical examples of the functioning of the analyzed insurance system, whose characteristics are not fully known to domestic agro-economical public.

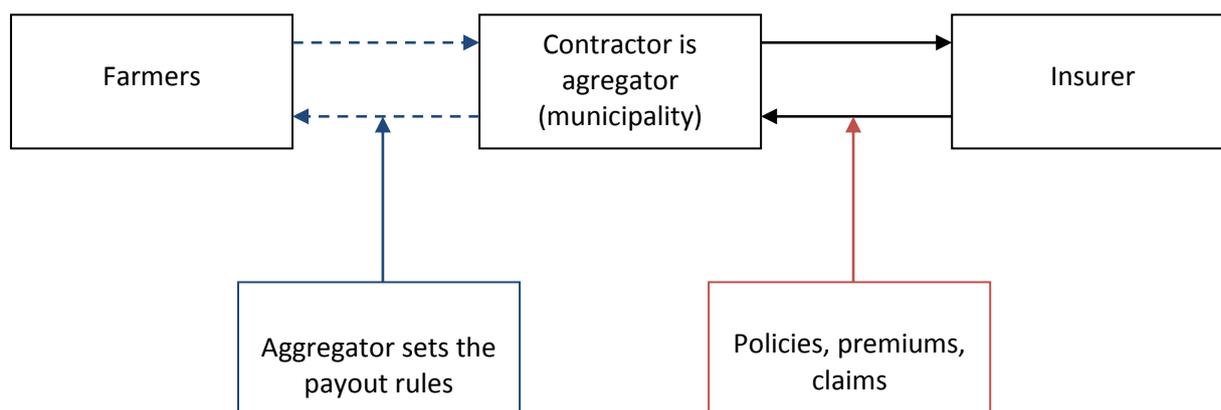
## MATERIALS AND METHODS

Data from Statistical Office of the Republic of Serbia of the average area and yield of corn, wheat and sunflower in the Ada municipality is used for this study. Data were analyzed during the period of five years (2009-2013). The application of insurance based on regional index is shown in the case of the mentioned municipality. When using this model as regional index, we used the average yield in the analyzed period, and insured area refers to the average area under individual crops in the observed region. Assuming the lower yields than average are achieved, the simulation of application of the analyzed insurance system at various guaranteed yields is performed. Guaranteed or insured yield represents the option of 90%, 80% and 70% of expected municipal yield. Insured value of certain crops per yield unit is taken from the Commodity Exchange in Novi Sad. The sum insured per area unit is calculated as the product of the two values or guaranteed (insured) yield and the contracted value per yield unit and the total sum insured was obtained as a product of the sum insured per area unit and total insured surface. Finally, simulation leads to the possible amount of loss, which can be compensated by using the analyzed model.

## RESULTS AND DISCUSSION

During the research, the model of insurance was analyzed, and which includes insurance loss of yield in certain crops in the region. In the specific case of the production of corn, wheat and sunflower, an example of index insurance at the municipal level is given, within which provides protection against the reduction of agricultural production through the insurance and reinsurance of the consequences of natural and climate risks (drought, floods, storms, excessive rainfall *et al.*), which can lead to a significant reduction in yield and production value. There are several key differences between insurance, which are concluded at the level of individual farmer and insurances that are concluded at the so-called. Meso-level, i.e. at the level of local government/municipality.

Scheme 1. Meso-level of program for insurance loss of yield in Serbia



The differences lie in the fact that at the Meso-level local government, namely municipality, appears in the function of policy holder and insured, whereby it is responsible for paying the entire insurance premium. The municipality needs to decide which farmers want to ensure, what crops will be covered by insurance, then it should decide on the width of the yield protection program that wants to buy i.e. total area that wishes to insure, also the level of insurance coverage, yield, as well as the sum insured per area unit. In the event that the actual yield on a municipal level is below the agreed yield, the right to compensation is acquired. Compensation is calculated based on the percentage reduction of the agreed yields. Insurance yields on municipal level, allows concluding guaranteed (insured) yield and yield coverage of at least 60% but less than 90% of municipal expected yield. Below, there is an example of the functioning of this new mechanism of insurance, i.e. example of calculation of insurance indemnity in the case of contracted insurance of a decrease in yield of corn, wheat and sunflower, in the amount of 10%, 20% and 30% of the average municipal yield.

Tab. 1. The calculation of insurance benefit by using model based on regional index

Corn			
Contracted level of insurance cover (insured yield in %)	90%	80%	70%
Guaranteed (insured) yield (kg/ha)	4.957	4.406	3.856
Contracted price (RSD/kg)	16	16	16
Sum insured per ha (RSD/ha)	79.312	70.496	61.696
Insured area (ha)	11.773	11.773	11.773
Total sum insured (RSD)	933.740.176	829.949.408	726.347.008
Achieved yield in 2015. (hypothetically)	3.540	3.540	3.540
Achieved loss (insured yield - achieved yield / insured yield)	0,28586	0,19655	0,08195
Achieved loss in %	28,59	19,66	8,20
Insurance payout in RSD (achieved loss * total sum insurance)	266.956.316	163.168.054	59.560.455
Wheat			
Contracted level of insurance cover (insured yield in %)	90%	80%	70%
Guaranteed (insured) yield (kg/ha)	3.685	3.275	2.866
Contracted price (RSD/kg)	18	18	18
Sum insured per ha (RSD/ha)	66.330	58.950	51.588
Insured area (ha)	2.508	2.508	2.508
Total sum insured (RSD)	166.355.640	147.846.600	129.382.704
Achieved yield in 2015. (hypothetically)	2.720	2.720	2.720
Achieved loss (insured yield - achieved yield / insured yield)	0,26187	0,16947	0,05094
Achieved loss in %	26,19	16,95	5,09
Insurance payout in RSD (achieved loss * total sum insurance)	43.568.542	25.059.999	6.585.580
Sunflower			
Contracted level of insurance cover (insured yield in %)	90%	80%	70%
Guaranteed (insured) yield (kg/ha)	2.084	1.852	1.621
Contracted price (RSD/kg)	36	36	36
Sum insured per ha (RSD/ha)	75.024	66.672	58.356
Insured area (ha)	1.095	1.095	1.095
Total sum insured (RSD)	82.151.280	73.005.840	63.899.820
Achieved yield in 2015. (hypothetically)	1.569	1.569	1.569
Achieved loss (insured yield - achieved yield / insured yield)	0,24712	0,15281	0,03208
Achieved loss in %	24,71	15,28	3,21
Insurance payout in RSD (achieved loss * total sum insurance)	20.299.581	11.155.292	2.051.184

In the five-year period at the level of analyzed municipality, without extreme values, in corn production an average yield of 5,508 kg / ha is achieved, in wheat production 4,094 kg / ha, while in sunflower production an average yield of 2,315 kg / ha is achieved. Mentioned crops were cultivated on 11,773 hectares (corn), 2,508 ha (wheat) and 1,095 ha (sunflower). Based on average data of areas and actual yields, values of guaranteed yield and insurance amount per area unit are obtained, as well as ultimately the total sum insured. In 2015 certain yields are assumed which are lower than the average for all three analyzed crops (3,540 kg / ha of maize; 2,720 kg / ha of wheat, 1,569 kg / ha of sunflower). In this potential situation, the insurer would be obliged to cover

the following damages in maize (267 million RSD - 90% level of coverage; RSD 163 million - 80%; RSD 60 million - 70%). For wheat, it would be the following amounts: £ 44 million - 90% level of coverage; RSD 25 million - 80%; RSD 7 million - 70% and sunflower 20 million RSD - 90% level of coverage; RSD 11 million - 80%; RSD 2 million - 70%. Seen in total for all three analyzed crops: 331 million RSD - 90% level of coverage; RSD 199 million - 80%; RSD 69 million - 70%.

An important issue in the analyzed insurance model is the methodology of allocation of funds in case of realization of an insured event, where in the specific case Ada municipality would appear in the function of the policy holder and the insured, but it would be also responsible for paying the entire insurance premium. Also, the municipality should settle the rules of distribution of insurance indemnity to the farmers. The two options are used for this purpose. The first option assumes that municipality uses the insurance of yield in agriculture as a simple instrument of financing consequences of natural disasters, according to the available budget to eliminate the consequences of natural disasters for agricultural crops. In this case, the municipality must base its decision on the issue "what area will be insured and how much insurance protection will buy", and based on the actual yield losses which the municipality has experienced in the worst drought years in the past at least the five years and the value of the actual payment of compensation to farmers that were carried out in those years. These parameters direct the municipality in the process of making a decision on the amount of the insured yield and the amount of the sum insured. If, after the harvest, comes to the realization of the insured event and the insurance indemnity is activated, the insurer will pay the municipality the entire sum calculated in the insurance contract (insurance policy).

For other options municipality use the insurance of yield loss as the official financial instrument for the protection from a disaster for all the farmers who grow analyzed crops at the municipal level, and aims to insure 100% of sown area of a particular crop in the municipality. The main difference relative to first option is that in this case the municipality must prepare an accurate list of all users of the insurance program and also must obtain accurate data on planned sown areas. The municipality shall decide what level of insurance protection purchased on behalf of the beneficiaries of the program and negotiates the value of the sum insured per unit, and on this basis has to determine the exact sum insured for each beneficiary, for each crop, according to the reported planned sown areas. In this case with the insurance policy, the exact list of beneficiaries for each crop, for precisely specified area in hectares and sum insured in RSD are stated, but it is important to emphasize that the municipality is the one who pays the insurance premium for all farmers, who would be beneficiaries of the protection program against loss of yield. For determining the final allocation mechanism, it can be started from the existing procedure provided at the municipal level in case of natural disasters, but still it has to improve with the aim of achieving a more efficient mechanism for allocation.

## CONCLUSIONS

Agricultural production, as one of the most important sectors of the economy, meets with many production risks, which specificity causes to be quality managed. Their specificity is reflected in direct dependence on the achieved results and present weather (none) conditions. To manage these specific risks, the best solution is offered by various insurance systems. One of the newer models is the insurance on the basis of realized regional yields, which use can greatly reduce the significant damages that frequently affect particular regions. In the case of the analyzed municipality, observing the three most common crops, 331 million RSD can be compensated at the level of yield coverage of 90%, 199 million RSD if we take the level of 80% coverage and 69 million RSD for the level of 70% yield coverage. A clear strategy on the state level, establishing a legal framework and financial incentives, analyzed insurance model could be successfully implemented in Serbia, and therefore many farmers will be insured, who perform their activity in climatic unstable regions.

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## THE DEVELOPMENT OF STORE BRANDED PRODUCTS IN RETAILING SECTOR

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*Professional paper*

### Summary

Store brands are a line of products which is branded by a retailer within a single brand identity. This is same with house brands, private label brands, own brands and home brands and generic brands. They are distinct in that a store brand is managed solely by the retailer for sale in only a specific chain of store. The retailer will design the manufacturing, packaging and marketing of the goods in order to build on the relationship between the products and the store's customer base. Store-brand goods are generally cheaper than national-brand goods, because the retailer can optimize the production to suit consumer demand and reduce advertising costs.

The biggest potential for store branded products is European market which has been already reached to the 1000 billion \$ sales income in the World wide. The 1 to 3 \$ which has purchased for the products packed is made for the store branded products. The biggest market share of the store branded products is exist in Switzerland with 45 % (ACNielsen, 2014). Turkey has some significant development with the 14-16 % on the store branded products which is close to World wide average and it shows fast expansion (PLMA, 2011). It foreseens that Turkey will perform significant take off in the next stages.

This study will analyse the latest situation and challenges of store branded products, generally in the World-wide and specifically in Turkey. And also, the reasons why retailer sectors diverge from producer brands to store branded products will be given. In addition, the study will be also focused on the positive and negative effects of such change in regard to the national economy, the consumer preferences and behaviours, etc.

Key words: *Store brand, marketing, retailers, World, Turkey*

### INTRODUCTION

The consumers have faced with the different prices and brands when they get decision on buying products. Consumers have widely faced with store branded besides the producer branded products.

Store brands are a line of products which is branded by a retailer within a single brand identity. This is same with house brands, private label brands, own brands and home brands and generic brands. They are distinct in that a store brand is managed solely by the retailer for sale in only a specific chain of store. The retailer will design the manufacturing, packaging and marketing of the goods in order to build on the relationship between the products and the store's customer base. Store branding is not a new approach. First store branded products was sold in USA, the date of 1863 made by the Company of "Great Atlantic and Pasific Tea". This company is known as A&P and now sells their products under the brand of "American's Choice" and "Master's Choice". The first store branded products was sold in Europa by Carrefour under the brand of "Champion" in 1969. The first store brand in Turkey was sold by Migros under the "Migros" in 1957 (Savaşçı, 2003).

### THE POSITIONING OF STORE BRANDS IN THE GLOBAL MARKET

The private label industry is approaching US\$1 trillion in sales annually and, as the recession of 2008-09 deepened, experienced spikes in sales and product introductions. The largest markets for private label food and beverage products are found primarily in North America and Europe. In 2007, private label spending in the United States (U.S.) reached just over US\$94 billion and European Union (E.U.) spending reached over US\$365 billion. The 1 to 3 \$ which has purchased for the products packed is made for the store branded products (ACNielsen, 2014).

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The research findings made by AcNielsen (2014), the rate of store branded products in the total sales of the Chain Markets is 16.5%, in the global World. The store branded products has a great share in Europe which is 27% in the retailing sectors and 18% in the North America (Figure 1).

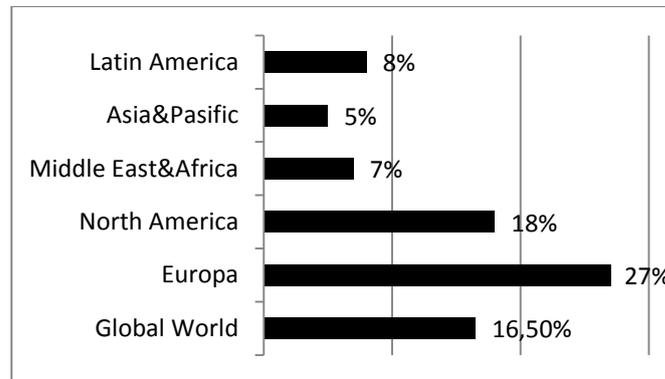


Fig. 1. Market Share of Store Brands (AcNielsen, 2014)

The biggest market share of the store branded products is exist in Switzerland (53%). Turkey has some significant development with the 17% on the store branded product which is close to World wide average and it performs fast expansion (PLMA, 2011). It foreseens that Turkey will perform significant take off in the next stages.

Tab. 1. Market share of store brands in European countries

Country	Market Share (%)	Country	Market Share (%)
Switzerland	53	Finland	29
UK	47	Denmark	28
Slovakia	44	Hungary	28
Spain	42	Sweden	27
Germany	41	Holland	26
Austria	38	Norway	26
Belgium	38	Greece	20
Çheck Rep.	35	Italy	17
France	35	Turkey	17
Portugal	34		

Source: PLMA, 2011

There are some reasons on the development of the store brands. These are; increasing of the consumer's perception of reliability, the ability of retailer's which is to overcome all activities on brand management, the developments of production technologies, the differences on prices between store and market brands, strong position of retailers in the distribution channels, well educated consumers, changes of consumer behaviours, the development of self-service marketing.

The reason why store brands are generally cheaper than national-brands (producer brands) is to optimize the retailer's capability of production according to the consumer demand and reduce advertising costs. The increasing demand of store brands and increasing marketing share is effected by the cost of promotion and advertisements and marketing tax of the producer brands given to the store. The retailers do not have to pay branding cost for the own private brand. All these activities like brand development, stocks, advertisement, promotion are done by retailers alone. The store chains do not have to pay fix cost of production, R&D, the cost of stocks. So, the final costs of store branded products are lower for 25% than the producer branded products (Retailing Institute, 2001).

Tab. 2. The Cost differentiations between producer and store brands

Cost Items	Producer Brands	Store Brands
Raw Materials	35	35
Packing	12	12
Production Cost		
- Variable	9	9
- Fix	5	-
R&D	3	-
Resource Planning	4	-
Advt. & Prom.	9	5
Logistics	5	2
Others	10	10
Profit	8	2
Selling Price	100	75

Source: Retailing Institute, 2001.

There is triple effect of store brands which is on consumers, retailers and producers (see Table 3).

Tab. 3. The Effects of the store brands into the different stages

For retailers		For producers		For consumers
Positive effects	Negative effects	Positive effects	Negative effects	Positive effects
*More freedom and flexibility in pricing *more control on product attributes and quality *higher margins(or lower selling price) *eliminating manufacturer's promotional costs	*negative perception of consumers *the risks on the quality which is the same for all product categories *quality awareness by the cheapest price *the problems on logistics by producers *the cost of brand promotion *technical& financial support to producers *reflection of the other producers	*the usage of utilised capacity *selling guarantee *expansion of production capacity *easy access to market *getting advantage through the price differentiation *easy competition with the other brands	*declining on selling and profit of the own brand *declining on resources for own products, shifting own market share to the other brands *declining profitability	*more convenient price *broad range of choices *easy access to products *post selling assurance

Sources: Pala and Saygı, 2004; Kahveci, 2007; Savaşçı, 2003; Orel, 2006; Özgül, 2004.

### THE MARKETS ON STORE BRANDED PRODUCTS IN TURKEY

The first store brand in Turkey was sold by Migros under the "Migros" in 1957 (Sapmaz, 2014). It has been started to be well known and preferred by consumers at the beginning of 2000's. The share of the store brands in the total purchase of the consumers is only 8% while private brands is 57%, in Turkey. The no name product selling is generally very common way to purchase by consumers which is 34% (See Table 4).

Tab. 4. The share of purchases in total consumer budget by brands and region of Turkey (%)

	Private Brands	Store Brands	No-name brands
General	57	8	34
Marmara	61	11	28
Ege	62	8	30
Central Anatolia	66	6	28
Mediterranean	53	6	41
Black Sea	56	11	33
East and Sout-east	43	4	53

Source:Ipsos KMG 2010.

According to the research findings, 65% of the consumers interviewed generally buy store branded products in the researched area of Turkey. And the frequency of the consumers which they sometimes buy store brands.

They are mostly rice, legumes and milk. No one of them buy store brands food as very often and always (Sapmaz, 2014).

Consumers who buy the store brands said that the most effective reasons to buy the store branded product are existing promotions and sales and the price which is cheapest than the private brands. And, consumers who do not buy store brands also said that I never give up producer brands and to buy producer brand is my habits-choices which are very important (Sapmaz, 2014).

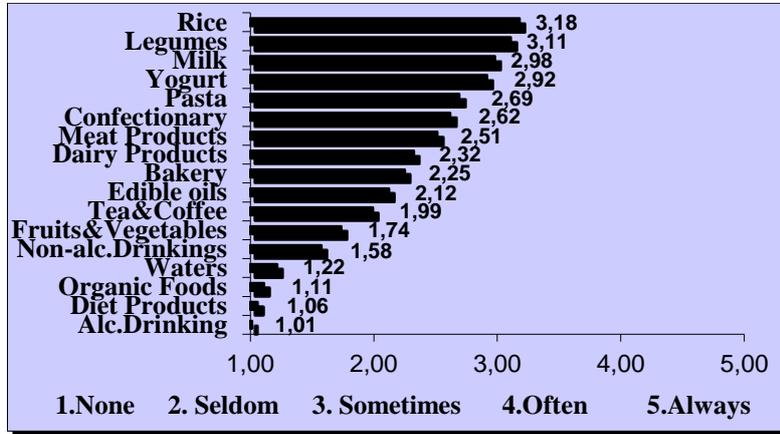


Fig. 2. Frequency bought of the store branded foods (Sapmaz, 2014)



Fig. 3. The reasons why consumers buy store branded foods (Sapmaz, 2014).

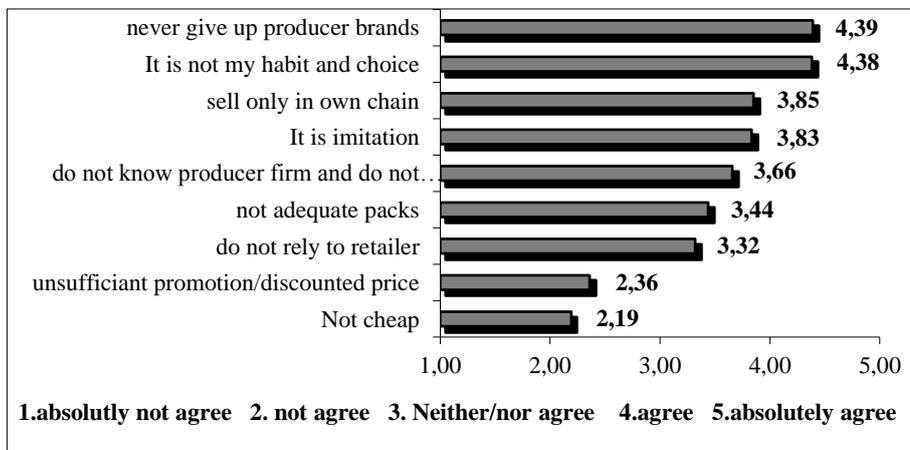


Fig. 4. The reasons why consumers don't buy store branded foods (Sapmaz, 2014).

## CONCLUSION

The retailer will design the manufacturing, packaging and marketing of the goods in order to build on the relationship between the products and the store's customer base. Store-brand goods are generally cheaper than national-brand goods, because the retailer can optimize the production through the consumer demand and reduce advertising costs. Goods sold under a store brand are subject to the same regulatory oversight as goods sold under a national brand. Consumer demand for store brands might be related to individual characteristics such as demographics and socioeconomic variables.

The sales of store branded products is 400 billion \$ in the global market while it is around 1 billion \$ in Turkey. They have 700 mil. \$ market share in domestic market and 300 mil. \$ for export market in Turkey (Para Dergisi, 2008). But, In Turkey, Store brands has just 8% in the household total purchases. Store branding performs so big expansion both in the Worldwide and in the Turkey. The Chain Markets like Migros, BİM, CarrefourSA, Metro Market, Kipa, Kiler, DiaSA, A101, Pehlivanoglu, Gürmar are the lead stores in Turkey which have a great share of their sales of store brands. They have established their brands like “M Selection”, “M Life”, Migros”, Tansaş”, “Şok” for Migros Turk Company; “Kipa”, Kipa Pazar” for Kipa; “Birşah”, “Peynes”, “Ustasından” for A101. Kipa is the lead company which has 4200 different products under the “Kipa” and “Kipa Pazar”. Then, BİM comes behind it with the 3103 different products categories.

It is observed that there is big producer companies behind the store brands which has already have own companies brands. The general idea for the store brands, the consumers are better off because of the price relatively cheaper with the almost the same quality assurance.

Moreover, The effects for the producers are very vital important behind the sense of the store brands which they have the possibilities of the the usage of utilised capacity, selling quarantine, expansion of production capacity, easy access to market, getting advantage through the price differentiation, easy competition with the other brands. All these positive results will effect also to the farmers with the same advantages like given above. This is called as “domino effects” in which one event causes a series of similar events to happen one after another.

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## SOCIO-ECONOMIC STATUS OF PARENTS OF ADOLESCENTS ACCORDING TO THE PLACE OF RESIDENCE

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*Original scientific paper*

### Summary

The concept of socio-economic status (SES) and socio-economic position (SEP) refers to the fact that social and economic factors influence the position of an individual or group within the social structure. If connected with the other fact saying that lots of issues among them also SES, depend upon the place of residence and that adolescence is a very sensitive period towards growing up conditions, that made the reason to do this kind of research. The aim was to investigate socio-economic status among adolescents living in rural and urban area of Canton Sarajevo as a pre-study of a larger research. The study included 630 adolescents. Purpose-designed SES questionnaire was used as a method. Results showed that the place of residence is correlated with socio-economic indicators, except for girls and the monthly income: in the urban area are more educated mothers (boys:  $p < 0,005$ ,  $\rho = 0,279$ ; girls:  $\rho = 0,383$ ,  $p < 0,0005$ ) and fathers (boys:  $p < 0,0005$ ,  $\rho = 0,250$ ; girls:  $\rho = 0,273$ ,  $p < 0,0005$ ); employment of mothers (boys:  $p = 0,02$ ,  $\chi^2 = 5,452$ ; girls:  $\rho = 0,250$ ,  $p < 0,0005$ ) and fathers (boys:  $p = 0,017$ ,  $\chi^2 = 5,658$ ; girls:  $\rho = 0,129$ ,  $p = 0,027$ ) is higher in the urban area; the families of adolescents from urban areas have a higher monthly income (boys:  $p = 0,049$ ,  $\chi^2 = 5,958$ ; girls:  $\rho = 0,132$ ,  $p = 0,70$ ). It can be concluded that the place of residence has a great impact to socio-economic status of individuals, in this research conditions.

**Key words:** *adolescents, place of residence, socio-economic status, education, employment, income.*

### INTRODUCTION

In order to assess adolescents' dietary habits and factors influencing them as much accurately as possible, socio-economic status (SES) was analysed before mentioned research. SES has an important role in the formation of dietary habits of people. The most important are the education of the individual, or in this case the parents' education, employment and income (Galobardes *et al.*, 2001; Groth *et al.*, 2001; Turrell *et al.*, 2003) which directly affects the purchasing power, and consequently form dietary habits. There are critical periods during childhood, when children are more sensitive to adverse physical, psychological and social circumstances. According to Salonen *et al.* (2009) the key indicators of socio-economic status associated with adult obesity are parents' occupation, own occupation, level of education and household income. These results also showed that men are more susceptible to adverse circumstances during childhood than women. Income was not associated with obesity in men, while they were and stayed statistically significant in women also after adjustment.

The place of residence has been used as a variable in many studies about food habits because of people's preferences and food availability. It is considered as a variable in this study because it directly affects SES.

### SUBJECTS AND METHODS

The study included 630 adolescents, 60 boys and 73 girls from rural area and 264 boys and 233 girls from urban area of Canton Sarajevo. Distribution of participants is in accordance to the demographic data (Federalni zavod za statistiku Kantona Sarajevo, 2013). Place of residence is regarded as urban and rural upon current Urbanistic plan of Canton Sarajevo (Zavod za planiranje razvoja Kantona Sarajevo, 2006). Participants from urban area are living in city municipalities (Stari grad, Centar, Novo Sarajevo, Novi grad), municipality Ilidža and municipality Vogošća. Many rural areas are multifunctional and many are under the influence of metropolitan areas and large cities as a result of improved transport and communication (CEMAT, 2007). Participants from rural area of Canton are living in secondary centers and villages, and they are as follows: Zovik, Gornji Zovik, Lokve, Sejdanovići, Kamenica, Homar, Dragoradi, Moševići, Donje Selo,

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Srednje, Crna Rijeka, Nišići, Vrutci, Solakovići, Ravne, Ljubina, Medojevići. Research protocol was approved by Ministry of Education and Sciences of Canton Sarajevo. Participants were recruited in schools and voluntarily participated. They filled out purpose-designed questionnaire about socio-economic status which was used as a method and it included: level of education and employment of each parent and family income. Statistical analysis was performed using the statistical software package SPSS 19.0. (SPSS Inc, Chicago, Illinois, USA). Significant difference was considered at the level of  $p < 0.005$ .

## RESULTS AND DISCUSSION

Data was collected and analysed in the context of participants' sex and place of residence. It has been summarized and presented by the asked questions.

Tab. 1. Level of Parents' Education

Sex	Place of Residence	Education	Primary	High school	College	University	Mr. sc.	Dr. sc.
Girls	Rural	Mothers' Education	65.80%	26.00%	4.10%	4.10%	0.00%	0.00%
	Urban		20.30%	56.70%	1.70%	2.20%	0.00%	0.00%
Boys	Rural	Fathers' Education	60.30%	34.50%	0.00%	5.20%	0.00%	0.00%
	Urban		26.50%	53.70%	0.80%	18.30%	0.00%	0.80%
Girls	Rural	Mothers' Education	33.80%	52.90%	7.40%	5.90%	0.00%	0.00%
	Urban		9.50%	62.40%	3.20%	24.00%	0.50%	0.50%
Boys	Rural	Fathers' Education	36.70%	50.00%	1.70%	11.70%	0.00%	0.00%
	Urban		9.50%	64.70%	2.00%	22.20%	0.80%	0.80%

Regarding girls, mothers' education and the place of residence are correlated ( $\rho=0.383$ ,  $p < 0.0005$ ). The highest percentage of mothers from rural areas have primary education (65.80%) and the highest percentage of mothers from urban areas have secondary education (56.70%), followed by higher education (21.20%). A very small percentage of mothers from rural area have a university degree (4.10%).

Regarding boys, mothers' education and the place of residence are correlated ( $p < 0.005$ ,  $\rho=0.279$ ). There is dominance of mothers with primary education (60.30%), followed by secondary education (34.50%) in rural area. Mothers are more educated in urban area, there is lower percentage of primary education (26.50%) and higher with secondary education (53.70%). There is also 18.30% of mothers with a university degree and 0.80% with a doctorate.

Fathers' education and the place of residence of girls are correlated ( $\rho=0.273$ ,  $p < 0.0005$ ). In rural areas secondary education is prevalent (52.90%) and followed by primary (33.80%). In urban areas secondary education is also prevalent (62.40%), followed by university education (24.00%), only 0.50% have masters and doctorate degrees.

Fathers' education and the place of residence of adolescent boys are correlated ( $p < 0.0005$ ,  $\rho=0.250$ ). In rural areas 50.00% of fathers have a secondary education, 36.70% have a primary education, and 11.70% university degree. In urban areas fathers are more educated. The lower the percentage of primary education (9.50%), and higher of secondary (64.70%) and university education (22.20%). There are also Master's degree and doctorate represented with 0.80%.

According to Hakeem *et al.* (2002) relatively more parents from urban area are educated (fathers: 64.00-100.00%, mothers: 55.00-89.00%) compared to the parents from rural area (fathers: 48.00%; mothers: 11.00%). In the research made by Hodžić and Smajić (2012) in the Canton Sarajevo (not regarded as rural vs. urban area, but it's data from the same Canton and it included participants of the same age) most of the fathers (43.30%) and mothers (53.90%) have secondary education and there is high percentage of parents with university degree (father: 24.10%; mothers: 34.4%).

Tab. 2. Parents' Employment Status

Sex	Place of Residence		Mothers' Employment	
			Unemployed	Employed
Girls	Rural	%	75.30	24.70
	Urban	%	46.10	53.90
Boys	Rural	%	63.79	36.21
	Urban	%	45.80	54.20
			Fathers' Employment	
			Unemployed	Employed
Girls	Rural	%	27.90	72.10
	Urban	%	16.00	84.00
Boys	Rural	%	33.33	18.36
	Urban	%	66.67	81.64

Mothers' employment and place of residence of girls are correlated ( $\rho=0.250$ ,  $p<0.0005$ ) pointing that most of the mothers in rural areas are unemployed (75.30%), and slightly more than half of mothers in urban areas are employed (53.90%). Regarding girls, fathers' employment and place of residence of girls are also correlated ( $\rho=0.129$ ,  $p=0.027$ ). Most of the fathers in both areas are employed (rural vs. urbano: 72.10%: 84.00%).

Place of residence of boys and employment of mothers are correlated ( $p=0.02$ ,  $\chi^2=5.452$ ). Employment is higher in the urban area (54.20%) as compared to rural (36.21%). Regarding boys, place of residence and employment of fathers are also correlated ( $p=0.017$ ,  $\chi^2=5.658$ ). Employment is higher in urban area (81.64%) as compared to rural (66.67%).

According to Hodžić and Smajić (2012) most of the parents have a job, 86.50% of fathers and 87.10% of mothers are employed.

Tab. 3. Level of Family Income

Sex	Place of Residence	Income per Month (BAM) <sup>3</sup>		
		<1,000	1,000-2,000	>2,000
Girls	Rural	42 57.50%	25 34.20%	6 8.20%
	Urban	104 44.60%	88 37.80%	41 17.60%
Boys	Rural	36 60.00%	30 18.00%	6 10.00%
	Urban	113 42.97%	105 39.92%	45 17.11%

Family income and place of residence of adolescent girls are not correlated ( $\phi=0.132$ ,  $p=0.70$ ). In both areas the most represented income is the one from 1,000 to 2,000 BAM (rural vs. urbano: 34.20%: 37.80%).

Family income and place of residence of adolescent boys are correlated ( $p=0.049$ ,  $\chi^2=5.958$ ). There is a lot more rural adolescents from families whose monthly income is less than 1,000 BAM (rural vs. urban: 60.00%: 42.97%). Also there's higher percentage of urban adolescents whose family income is higher than 1,000 BAM.

According to previous study made in Canton Sarajevo, almost half of adolescents (42.20%) live in families whose monthly income is between 1,000-2,000 BAM, followed by 31.90% of those whose monthly income is below 1,000 BAM, and 25.50% of adolescents living in families whose total monthly income is over 2,000 BAM.

<sup>3</sup> ISO 4217 Code for the Bosnian Convertible Mark is BAM. The Convertible Mark, is the official currency of the Bosnia&Herzegovina. Available at: <http://bosnian.currencyname.com/>

## CONCLUSIONS

The place of residence of adolescent boys and girls is correlated with socio-economic indicators used in this study, except for girls' place of residence and the family income. Adolescents living in the urban area of Canton Sarajevo have higher socio-economic status reflected through three examined indicators:

- In the urban area are more educated mothers and fathers;
- Employment of mothers and fathers is higher in the urban area;
- The families of adolescents from urban areas have a higher monthly income.

Because of given results, socio-economic status should be considered as an essential factor in nutrition studies (dietary habits and nutritional status) of adolescents.

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## ASSESSMENT OF FOOD SECURITY IN THE WORLD AND TURKEY

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*Review paper*

### Summary

Priority objectives of this study are to identify food security and its standard, to assess the current situation in the world and Turkey according to the food security index and to make some suggestions. Identification of food security is on the way to be developed continually over time in the world. Features of health and safety are included to definition of adequate nutrition along with economic and physical access. Improvements in food security are pointed out when interregional developments in the world is analyzed. Nevertheless, food security score of Sub Saharan-Africa region has not reached yet even to one half of North America's food security score where it is the highest. On the other hand, interregional differences on food security standards are pointed. Turkey is 39<sup>th</sup> of the world ranking on food security. Favorable developments on food security are associated with national and international economic and social policies, and will be provided by directly agricultural policy objectives and tools.

*Key words: dietary energy supply, food security, developed and developing countries, Turkey*

### INTRODUCTION

Even undernourishment of the human population is in decrease in recent ten years, food security is one of the upsetting issues in international arena. According to FAO data, in 2050, the world population is expected to reach to 9.6 billion. Increase in population and income growth rate are expected to be in developing regions. In this respect, national and international efforts are needed to increase production for fulfillment of increase on food demand (EUI, 2015). Access to large number of studies related to food security is possible. Some of them are focused on the topics of food security, conceptual framework, food policies and developments (Rosegrant, Cline, 2003; Andersen, 2009; Erbas and Arslan, FAO; 2014, Koç, Uzmay, 2015), food security measurement (Barret, 2010, EUI, 2015, FAO), urban agriculture, poverty, geography as developing countries (Smith, 2000; Zezza, Tasciatti, 2010), post-modern approach to food security Maxwell, 1996), especially climate change and food security recently (Kendall *et al.*, 2004; Wheeler and Braun, 2013; Lobel *et al.*, 2008; Schmidhuber, Tubiello, 2007; Brown and Funk, 2008). Analyzing this issue as a whole is important to follow and take measures at regional and national level. In this regard, objectives of this study is to identify food security and its criteria, to reveal assessments according to food security index in the regions and Turkey, and also to make some suggestions in Turkey.

### FOOD SECURITY AND FOOD SECURITY CRITERIA

Food security is to access of a person to adequate and healthy food in terms of economically and physically in any moment to sustain a healthy and also active life. Even firstly economically and physically access to adequate food were only required features for food security definition, lately health and safety have been added to features of food security.

While analyzing of meetings and declarations in the world about food security is taken into consideration, the following developments are noteworthy (Kıymaz and Şahin, 2010; Koç and Uzmay, 2015): United Nations (UN) Universal Declaration of Human Rights in 1948, which define the right of access to food as "basic human right", World Food Conference in 1974, UN Food and Agriculture Organization (FAO) in 1983, World Bank in 1986, World Food Program in 1989, International Conference on Nutrition organized by FAO and World Health Organization (WHO) in 1992, World Food Summit organized by FAO in 1996.

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Food security is unobtainable because of the issues such as climate change, water reduction, agricultural land drought, infertile and salinization of the soil, gain resistance of product diseases and pests, getting smaller of agricultural land parcels through inheritance sharing, rapid population growth, increase on rural to urban migration, political and economic instability, expansion of biofuel consumption, increase on demand for organic products, the storage of food in large quantities, unstable and high food prices and waste (Erbaş and Arslan, 2015). Thus, food security standards are identified to reveal the current situation in international arena to prevent food insecurity; Food security standards are on the way to be developed over time (FAO, 2014). Food security standards and the factors are set as follows; Food availability standard: average dietary energy supply adequate, average value of food production, share of dietary energy from cereals, roots and tubers, average protein supply, average supply of protein of animal origin. Food access standard (affordability): percentage of paved roads over total roads, road density, rail road density, prevalence of undernourishment, share of expenditure of the poor, depth of the food deficit, prevalence of food inadequacy, domestic food price index, gross domestic product per capita. Food stability: political stability and absence of violence/terrorism, domestic food price volatility, per capita food production variability, per capita food supply variability, cereal import dependency ratio, and percent of arable land equipped for irrigation, value of food imports over total merchandise exports. Food usage standard: access to improved water sources, access to improved sanitation facilities, percentage of children under 5 years of age affected by wasting, percentage of children under 5 years of age who are stunted, percentage of children under 5 years of age who are underweight, percentage of adults who are underweight, prevalence of pregnant women, prevalence of anaemia among children under 5 years of age, prevalence of vitamin A deficiency in the population, prevalence of iodine deficiency in the population. Global Food Security Index (GFSI) is developed to compare food security at interregional level and among the countries, and food security has been compared via GFSI since 2012. Particularly food safety standard is included in the food security standard recently (EIU, 2015). However food access (affordability), availability and quality and safety standards are considered primarily in food security analysis of EIU.

### INTERREGIONAL DEVELOPMENTS AND LEVEL ON FOOD SECURITY

Dietary Energy Supply (DES) is one of the important standards of food security which is defined as food of used for human consumption, per caput per day measured in kilocalories. It is calculated at the country level as the rest of food consumed by human after removal of export, industrial use, animal feed, seed, waste and changes in the stocks of the food. The average global calorie in the world was 2 193 kcal/day in 1961 which was increased to 2 868 kcal/day in 2011. Undernourishment population in 2013 was 842 million in the world. 295 million of them are from Southern Asia, 223 million of them are from Sub Saharan Africa, 167 million of them are from Eastern Asia (FAO, 2014). According to FAO data (2015), the highest average of DES is recorded in Austria, Belgium, Turkey, USA, Montenegro, Ireland, Luxembourg, Italy, France and Germany. Ten countries of lowest average of DES are Botswana, Mozambique Bolivia, Zimbabwe, Yemen, Kenya, Congo, Central African Republic, Rwanda and Tanzania (FAO, 2015). Dietary energy supply of Turkey was 3717 kcal/cap/day in 2014 while it was 2769 kcal/cap/day in the world. However average usage of amount of supply animal protein in Turkey (29 g/cap/day) was below the world's average (25 g/cap/day) (FAO, 2014). Thus, increase on price index of animal products reflects the consumption negatively. Indeed, while crop prices were increased by %28-67, livestock product prices were increased by %87-97 according to average prices between 2004-2006 (FAO, 2015).

Tab. 1. Global Food Security Index Scores (GFSI)

	Overall score	Affordability score	Availability score	Quality & Safety score
Asia & Pacific	57.3	56.4	58.5	56.6
Central & South America	58.0	58.6	56.7	59.9
Europe	75.7	79.2	71.5	78.5
Middle East & North Africa	61.0	62.0	60.2	60.7
North America	80.6	82.9	78.3	81.8
Sub-Saharan Africa	37.8	29.6	45.2	38.1
Turkey	66.0	62.9	67.7	69.1

Source: Quelle. EIU, 2015.

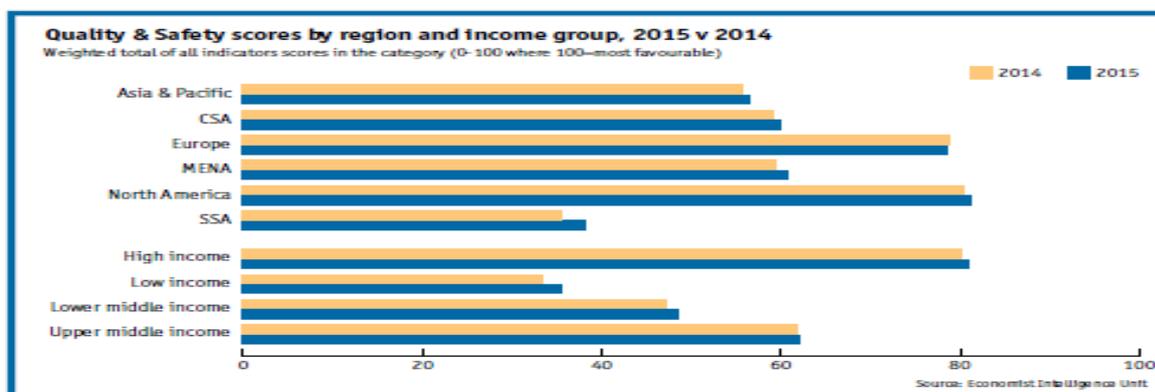
On the other hand, while food security is assessed, developed countries (America, Singapore, Ireland, Austria, Netherlands, Switzerland, Canada, Germany, Australia, and France) were ranked as in the first 10 top countries

according to GFSI in 2015. Considering food security scores of regions (Table 1), the highest score is observed at North America with 80.6 score, while Europe region is ranked as second with 75.7 score. The lowest score is at Sub-Saharan Africa (EUI, 2015). Turkey was 39<sup>th</sup> of GFSI index ranking in 2014 and 2015 (EIU, 2014; EIU, 2015), and was 47<sup>th</sup> by 62.5 score in terms of food access standard (affordability), 33<sup>rd</sup> by 67.7 score in terms of availability standard, and 40<sup>th</sup> by 69.1 score in terms of quality and safety standards (EUI, 2015).

When development level of the countries is examined, food security index score is between 63.8-89 in high income countries (US\$12,616 per capita or more) and between 35-71 in upper middle income countries (US\$4,086-12,615 per capita). Food security index score is 50 and over in this group except Angola. On the other hand, food security index score is between 33-61.8 in lower middle income (US\$1,036-4,085 per capita) while between 25.1-44 in low income countries (US\$1,035 per capita or less). These figures show that there is a relation between food security and income level. GDP per capita affects food affordability calculations by %22.2. Accordingly, it is an expected outcome that Turkey gets behind of the countries included in the high-income group especially EU countries (Dağdur ve Orhan, 2015).

The content of the average diet of a person and the legislation have been created in a country on diet are taken into account while assessing the nutritional quality of the food security. On the other hand, whether there are standards or institutions is taken into consideration for food safety assessment. Particularly, it is pointed out that food quality and safety score is over the average score (78.5) in European region. This situation can be explained within the effective functions of institutions such as The European Food Safety Authority (EFSA) and The Food and Veterinary Office (FVO) in European Union (EU) among others. Besides, EU has spent 3.3 billion Euros for food security policies in recent ten years. On the other hand, developed countries managed to achieve food security by applying policy tools such as gross domestic product, low political instability, improved agricultural infrastructure within effective food security (Koç, Uzmay, 2015). However, it is pointed out that the gap has been narrowed between Western countries which are improved in terms of food security and Sub-Saharan Countries taken place in the bottom rows. In general, reasons behind improvements of food security are increasing of storage capacity; reduction of food loses at the production and consumption stages; improvement of dietary habits and increased access to high-quality protein sources. Reasons of recorded improvements in low and middle level income countries are reduction of political instability and focusing on policies according to provide food security and safety of the countries. Particularly, Middle East and North Africa countries (MENA) are the biggest breakthrough capturing countries in terms of food security. Despite all these improvements, GFSI score has decreased in %85 of European countries which is the only worsening region in this regard (EUI, 2015). When assessed in terms of food quality and safety, scores in high income countries are more than twice of lower income countries (Figure. 1). On the other hand, food quality and safety score in America and Africa is well ahead (EIU, 2015). When Turkey is examined in studies related to food safety so far, significant developments that occurred in legislation in recent years and getting close score (99.5) to a full score of food safety standard that created by relevant institutions are remarkable developments (EIU, 2014, Dagbur, Olhan, 2015). However, creating legislation, determining standards or whether there are relevant institutions are not the main issues but deficiencies on implementation of legislation literally and supervision sufficiently cause not to achieve at the desired level of food safety in Turkey (Koç, Uzmay 2015).

Fig.1. Quality and Safety Scores by Region and Income Group (2014-2015)



While considering food security in developed and developing countries; preventing conditions of food security and safety are generally found on origin of producers in developing countries, but of consumers in developed countries. Consumers' low level of knowledge and education in developing countries cannot be impulsion for

providing food security and safety however conversely in developed countries. Rapid population growth, low productivity, lack of infrastructure and support on food sector directly or indirectly are the major problems in developing countries. On the other side, even agricultural sector is supported in these countries; these problems cause rural population to be used for interests easier than urban population due to economic and political instability or various political purposes, also cause wrong policy tools to be used in developing countries.

In conclusion, food security issue has gained importance at international level and also food security standards have been developed allowing comparison among the regions and countries. In this regard, future threats in least developed and developing regions continue, even these regions draw attention with their positive developments. Nevertheless, it is important to achieve rapid economic growth based on human capital and provide development for ensuring the sustainability of food access primarily through domestic or imports in the countries where food security level is the lowest. Another prominent issue is to initiate sustainable efforts that will be freed themselves from poverty in these countries (Smith *et al.*, 2000). FAO emphasize on the requirements about political leadership and management activities, preparation of effective policies, improving the macroeconomic environment for food and agriculture and inclusive economic growth in especially sub-Saharan Africa where the food security level is low (FAO, 2015). Although regional impacts of climate change on food security is not determined completely, it is indicated as an adversely impact especially for the countries where hunger and undernourishment is exists (Wheeler and Braun, 2015). In this context, food systems and policies are required that reduce the effects of climate change and provide adaptations in this direction. Furthermore, existing different parameters in terms of food security measurements according to various institutions and lack of national database lead to make wrong assessments for comparisons at interregional level (Barett, 2010, Koç, Uzmay, 2015). In this regard, the countries and international organizations must take measures. There are many factors threatening food security in Turkey also. Negative reflection of high input prices to output prices and speculative increases on price also bring about instability and disadvantages in Turkey. Insufficient farmer based organizations on the basis of agricultural products cause fluctuations in supply prices. On the other side, excess input use in agriculture, unconsciously usage of water resources, and destruction of forests, pastures and meadows block sustainable agriculture and sustainable food safety provision. The use of agricultural land for urbanization is another significant factor that creates risk for food security. Agriculture and food policies are required to deal with macro economy and social policies together and also policy goals and tools should match each other. Long term policies that provide added value within usage of local resources should be implemented instead of temporary short-term policies in the countries such as Turkey that most of the population lives is in rural area.

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## IN MEMORIAM

### Prof. dr Osman Sarić (1925-2016)



Rođen je 1.3.1925. godine u Gacku, a već 1930. preselio je u Mostar. U Mostaru je završio osnovnu školu i gimnaziju 1947. godine. Nakon završene gimnazije u Mostaru upisuje Poljoprivredni fakultet u Zagrebu i završava ga 1952.

Nakon završetka studija zaposlio se u Zavodu za poljoprivredna istraživanja u Sarajevu kao asistent na istraživačkim projektima krmnog bilja, gdje je radio od 1953. do 1959. godine. Od 1959. do 1962. godine radi kao asistent na predmetu "Proizvodnja krmnog bilja" na Poljoprivrednom fakultetu u Sarajevu. Profesor Sarić je 1962. godine izabran u zvanje docenta, a 1967. godine u zvanje vanrednog profesora. Godine 1972. izabran je u zvanje redovnog profesora na predmetu "Proizvodnja stočne hrane" (krmnog bilja). Profesor odlazi u mirovinu 1990. godine. Godine 2002. profesor dr. Osman Sarić je promovisan u počasno zvanje "Profesor emeritus".

U toku rata 1941.-1945. učestvovao je u NOB-u. U tom periodu zbog ilegalnog rada proveo je u zatvoru i koncentracionom logoru od kraja 1942. do septembra 1943. godine. Iz logora odlazi u partizane, gdje je ranjavan. Po završetku rata profesor Sarić je nastavio pohađanje gimnazije u Mostaru.

Prof. dr. Osman Sarić je odlikovan sa "Ordenom za hrabrost" i "Ordenom za zasluge za narod" 1945. godine. Pored ratnih odlikovanja, profesor Osman Sarić je dobitnik i: najvećeg odličja u SR Bosni i Hercegovini, "27. julske nagrade", "Ordena rada sa zlatnim vijencem" i niza nagrada i priznanja u našoj zemlji i van nje.

U toku radnog perioda pored nastavnih, naučnih i stručnih aktivnosti obavljao je i niz odgovornih društveno-političkih funkcija kao što su:

- Dekan Poljoprivrednog fakulteta u Sarajevu, od 1971. do 1973. godine,
- Direktor Instituta za ratarstvo Poljoprivrednog fakulteta u dva mandata,
- Predsjednik Zbora zaposlenih Fakulteta,
- Predsjednik Odbora za nastavni i naučni rad Univerziteta u Sarajevu,
- Član Skupštine grada Sarajeva,
- Osnivač i prvi predsjednik Udruženja za krmno bilje Bosne i Hercegovine,
- Predsjednik Udruženja za krmno bilje Jugoslavije,
- Član i predstavnik Jugoslavije u Evropskom udruženju za travnjake (European Grassland Federation),
- Član jugoslovenskog udruženja za proizvodnju sjemena krmnog bilja (Agrozajednica),
- Član Savezne sortne komisije
- i niza drugih.

Osim redovne nastave studentima prvog ciklusa i studentima na različitim postdiplomskim studijima, profesor Sarić je sa posebnim zadovoljstvom stručnjake na terenu (farmama i poljima) upoznavao sa savremenim dostignućima u proizvodnji i korištenju stočne hrane. Osim na Poljoprivrednom fakultetu u Sarajevu, profesor Sarić je kao gostujući profesor držao nastavu i u Zagrebu, Beogradu i Osijeku.

Podsticao je i pomagao mlađim kolegama da se usavršavaju u proizvodnji stočne hrane (krmnog bilja), a kao rezultat su mentorstva na doktoratima (3 na Poljoprivrednom fakultetu u Sarajevu, jednom na Agronomskom fakultetu u Zagrebu i jednom na Poljoprivrednom fakultetu u Osijeku), magistarskim radovima (6) i na desetina mentorstava na diplomskim radovima. Osim kao mentor učestvovao je u velikom broju odbrana kao član ili predsjednik komisije.

Osim nastavne djelatnosti, profesor Sarić se sa velikim uspjehom bavio naučnom problematikom iz oblasti proizvodnje stočne hrane (krmnog bilja), a rezultate svojih istraživanja izlagao je na svjetskim kongresima u SAD, Švedskoj, Španiji, Belgiji i bivšoj Jugoslaviji.

Naučno i stručno obrazovanje usavršavao je u Italiji, Švicarskoj, Holandiji, Francuskoj, Češkoj, Poljskoj i Mađarskoj.

Kao rezultat naučne i stručne aktivnosti objavio je preko 100 naučnih i oko 70 stručnih radova, 9 knjiga i prevoda, te kao rukovodilac ili učesnik u 17 projekata.

Podstaknut saznanjima o selekciji višegodišnjih krmnih biljaka, a boravkom u Velškoj stanici (Aberistwith V. Britanija) za selekciju bilja, profesor Sarić u saradnji sa profesorom dr. Vinkom Milinkovićem kao autor i koautor stvorio je 4 nove sorte krmnih kultura i to: novu sortu lucerke (Olimpik-84), dvije sorte slatke lupine (Bosna i Modriča) i sortu smiljkite (Butmirka).

Profesor Sarić je svojim radom na unapređenju stočarstva stekao ugled jednog od najvećih stručnjaka iz ove oblasti u Bosni i Hercegovini i Jugoslaviji, pa i šire. Profesor Sarić nije bio samo odličan predavač, već i veliki inovator i praktičar. Svoja znanja i tehnološke inovacije prof. dr. Osman Sarić je registrovao i neke uspješno aplicirao u proizvodni proces, a najčešće preko poljoprivrednih kombinata.

Životni put i privatni razvojni opus svjedoči da se radi o visokim moralnim kvalitetima našeg dragog profesora.

Poštovani profesore, znam da ste od svih Vaših nabrojanih i nenabrojanih uspjeha, priznanja, nagrada, svojim najvećim uspjehom i nagradom uvijek smatrali Vaše kćerke Jasnu i Sanju, sina Zlatana, te unučad Miju, Ranka i Enu, te praunuka Julijus-Zlatana. Oni su zajedno, a zaslužujući Vama i Vašoj pokojnoj supruzi nastavili da doprinose opšte društvenoj zajednici svojim radom, ne samo u Bosni i Hercegovini, već i znatno šire. Oni su bili Vaš ponos i dika, Vaše najveće ostvarenje ovog svijeta.

Dragi profesore, ovako Velikog, a tako jednostavnog i skromnog iznjedrila je zemlja Hercegovine, a pokriva tako draga i toliko voljena zemlja bosanska.

Pa neka Vam je lahka zemlja bosanska.

Prof. dr Ševal Muminović

## IN MEMORIAM

### Prof. dr Natalija Dozet (1923-2016)



Svaki čovjek jednom se rađa i jednom odlazi zauvijek.

Između toga dvoga stoji život kojeg je on živio i njegova djela koja o njemu svjedoče.

Takav jedan život, život najstarije generacije uposlenika i nastavnika ovog fakulteta, živjela je do ovih dana i prof. dr. Natalija Dozet.

Natalija Dozet rođena je u Sarajevu 26.7.1923. godine, gdje je završila osnovnu školu i gimnaziju.

Poljoprivredno-šumarski fakultet započela je da studira 1945. u Zemunu, da bi se 1947. godine upisala i diplomirala na Saveznoj visokoj školi za planinsko gazdovanje u Sarajevu.

U periodu 1950-1952. godine počela je pisati svoju radnu biografiju i to kao rukovodilac stočarstva na PD u Bijeljini.

U vremenu 1952-1953. radila je u Stočarskom zavodu u Sarajevu.

Godine 1953. izabrana je za asistenta na predmetu Mljekarstvo na Poljoprivredno-šumarskom fakultetu u Sarajevu.

Poslije habilitacije 1961. godine izabrana je u zvanje docenta.

Doktorsku disertaciju odbranila je 1963. godine.

U zvanje vanrednog profesora izabrana je 1966. godine.

Redovni profesor postala je 1972. godine.

Tokom rada obavila je dvije specijalizacije: 1952/1953. u Sarajevu, te 1954/1955. u Engleskoj.

Svoja studijska usavršavanja provela je u tri zemlje: Bugarskoj, Danskoj i Francuskoj.

Učestvovala je na mljekarskim kongresima 1956. u Rimu i 1978. u Parizu.

Kao veliki korak u budućoj historiji fakulteta, prof. Dozet utemeljila je Odsjek za preradu i kontrolu poljoprivrednih proizvoda.

Rukovodilac je bila na postdiplomskom studiju "Tehnologija, standardizacija i kontrola mlijeka i mliječnih proizvoda".

Rukovođila je sa izradom više magistarskih radova i doktorskih disertacija u Sarajevu, te Zagrebu i Skoplju.

Do 1991. godine objavila je preko 140 naučnih i stručnih radova, napisala tri knjige, više praktikuma i skripata, te rukovođila i radila u 22 elaborata i 12 projekata.

Bila je prodekan za nastavu 1983/1984. godine, odmah poslije toga dekan fakulteta, dugo vremena upravnik Instituta za preradu i kontrolu poljoprivrednih proizvoda i predsjednik Savjeta Univerziteta.

Izvan fakulteta bila je delegat u Skupštini Grada Sarajeva u dva mandata.

Odlikovana je Ordenom zasluga za narod sa srebrnim zracima.

Dobitnik je 27. julske i 6. aprilske nagrade.

Penzionisana je 1988.

U Sarajevu bila je do novembra 1993. godine, kada je prešla da živi u Beogradu.

Nakon penzionisanja, bila je angažirana na Poljoprivrednom fakultetu u Istočnom Sarajevu do 2001. godine.

Kao univerzitetski naučnik i profesor, prof. Dozet odgojila je i osposobila brojne generacije studenata našeg fakulteta, svoje ime ugradila je u povijest mljekarstva BiH, izučavajući i čuvajući od zaborava autohtonu

porodičnu preradu ovčjeg i kozjeg, pa potom i kravljeg mlijeka na područjima dinarskog sistema i drugih domaćih geografskih regija, vjerovala je i stalno negovala neposrednu povezanost domaće nauke sa praksom, bila je energična osoba i krajnje disciplinirano odana svom plemenitom i teškom profesionalnom pozivu, po tome, ako Britanci jesu imali svoju željeznu lady, i mi smo imali našu lady od željeza i to lady sa velikim i srcem i odlučnom voljom da učini i pomogne onima kojima je njena pomoć bila potrebna.

Kao takvu njeni suradnici su je priznavali, slijedili i veoma cijenili.

Kao njen mlađi kolega, iz druge polovine 1980-ih godina, ja je u sebi osobito nosim kao izuzetno pouzdanog i odgovornog koordinatora u PTO Mljekarstvo, TO Stočarstvo multidisciplinarnog naučnog projekta Društvenog cilja X – Poljoprivreda.

Osobito je se sjećam da je ona bila jedna od onih osoba koje su meni došle dati podršku prilikom odbrane Izvedbenog projekta tog Društvenog cilja pred komisijom vanjskih eksperata u ANU BiH.

Za to je ispred ukupne bih. poljoprivredne i napose njene mljekarske prerađivačke nauke i struke danas, ovdje možemo ispratiti sa jednim velikim HVALA.

Prof. dr Vjekoslav Selak

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