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Araştırma Makalesi/*Research Article (Original Paper)*

Potential of *Rosmarinus officinalis* for Phytoremediation of Soil Contaminated with Cadmium

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Abstract: In this study, the use of *Rosmarinus officinalis* was investigated to clear soil contaminated with Cd. The soil sample for pot experiment is taken from the old municipal waste storage in Mardin. The pot experiment conducted in the greenhouse. Increasing dose of Cd (0-5-10-20 mg kg^{-1} as $3CdSO_4.7H_2O$ form) and 0.005 mM citric acid applied to the soil and all pots were incubated for 30 days. The fertilizer (200 mg N kg^{-1}) applied before seedling and then plant seedlings transferred to the pots. The plants were grown for 45 days in the greenhouse. After harvest Cd, N, P, K, Mn, Fe, Cu, and Zn concentrations of plant samples were determined in ICP-MS. The highest Cd content (8.31 µg $plant^{-1}$) was obtained in 20 mg Cd kg^{-1} treatments. The result of the experiment was shown that the *Rosmarinus officinalis* was not suitable for pyhtoextraction of Cd-contaminated soils.

Key words: *Rosmarinus officinalis*, phytoremediation, cadmium, contaminated soil, decontamination

Biberiye (*Rosmarinus Officinalis*) Bitkisinin Fitoremediasyon Amaçlı Kullanım Potansiyeli

Özet: Bu çalışmada, Cd ile kontamine olmuş toprağı temizlemek için *Rosmarinus officinalis* bitkisinin kullanım potansiyeli araştırılmıştır. Toprak deneyi için toprak örneği Mardin'deki eski belediye atık depolama alanından alınmıştır. Denemeler serada saksıda kontrollü koşullarda yapılmıştır. Artan dozlarda Cd (0-5-10-20 mg kg^{-1} Cd, $3CdSO_4.7H_2O$ formunda) ve 0.005 mM sitrik asit uygulanan tüm saksılar 30 gün boyunca inkübe edilmiştir. Çimlenmeden önce gübre (200 mg N kg^{-1}) uygulanmış ve daha sonra fideler saksılara aktarılmıştır. Bitkiler, serada 45 gün boyunca yetiştirilmiştir. Hasattan sonra bitki örneklerinin Cd, N, P, K, Mn, Fe, Cu ve Zn konsantrasyonları ICP-MS'de belirlenmiştir. En yüksek Cd içeriği (8.31 µg $bitki^{-1}$) sitrik asitli ortamda 20 mg Cd kg^{-1} uygulamalarından elde edilmiştir. Denenenin sonucu, *Rosmarinus officinalis* bitkisinin Cd ile kirlenmiş toprakların fitoekstraksiyonu için uygun olmadığını göstermiştir.

Anahtar kelimeler: *Rosmarinus officinalis*, fitoremediasyon, kadmiyum, kirlenmiş toprak, dekontaminasyon

Introduction

Heavy metals, especially cadmium, are widely being considered as responsible for soil pollution, soil infertility and decreased crop production. Due to high cost of traditional engineering methods (Glass 2000), in order to remediate the soils that are already being polluted by heavy metals, recent studies are offering new methods such as phytoremediation (Shi and Cai 2009), that are more friendly to the environment and less costly. The phytoremediation terminology was firstly introduced in 1991 (Etim 2012) and can be defined as the usage of plants to remove toxic metal ions from the soil as a biological cleaning method and it is reliable, environmentally friendly and a long-term technology to clean contaminated soils (Blaylock 2000). In our country, studies on removal of organic and inorganic pollutants from the soil are very limited. Compared to other already developed countries, our country is still having a lot to achieve in order to prevent soil pollution. On the other hand, there are only few studies reporting the pollution level of the soil. It takes a lot of investment and time to get rid of the polluters from soil, which is the biggest limitation of such works (Çiftçi 2016). Soil pollution can occur by various factors originated from both nature or human influence. Domestic, industrial and agricultural activities are the most soil polluting factors (Türkoğlu 2006). Aim of this study is to determine the ability of rosemary plant to accumulate cadmium (Cd) using flowerpots in order to be able to use the soil after refining from contaminants as well as assessing the growth and phytoremediation potential of the rosemary plant while it is absorbing the Cd within the contaminated soil.

Material and Methods

Materials

Rosemary (*Rosmarinus officinalis*) plant was used as plant material in the study. As a Needle-tipped, small-leafed plant that belongs to *Lamiaceae* family. The aromatic plant, which is about 1-2 m in length, has a strong aroma such as *Camphor* or *Eucalyptus* odour, which does not foliage its leaves in winter (Çoban and Patır 2010). The rosemary plant used in the research was rooted for 3 months with the steel picking method and then the pot planting was done. The rosemaries used in the experiment were prepared in the greenhouse of Mardin Metropolitan Municipality Nursery and Green Areas Branch Directorate. The soil material for the pot experiment was taken from the old wild garbage landfill located in the Artuklu county, Mardin province, at a depth of 0-30 cm as reported by Jackson (1967). Test soil taken from a depth of 0-30 cm was passed through a 4 mm sieve after being dried and filled with a pot. The physical and chemical properties of the experimental soil taken from the solid waste landfill are given in Table 1.

Table 1. Some physical and chemical properties of test soil

Soil Properties	**Value**	**References**
Structure	Loam	(Bouyoucos, 1951)
pH	7.60	(Kacar, 1995)
Salt (%)	0.025	(Soil Survey Staff, 1951)
$CaCO_3$ (%)	33.1	(Loeppert and Suarez, 1996)
Organic substance (%)	1.94	(Kacar, 1995)
Organic Carbon (%)	1.13	(Kacar, 1995)
Total N (%)	0.10	(Bremner, 1965)
Available P (mg kg^{-1} P_2O_5)	4.12	(Olsen, 1954)
Available K (mg kg^{-1} K_2O)	30.9	(Sommers and Lindsay, 1979)
Extractable Cd with DTPA (mg kg^{-1})	3.02	(Sommers and Lindsay, 1979)
Extractable Fe with DTPA (mg kg^{-1})	34.7	(Sommers and Lindsay, 1979)
Extractable Cu with DTPA (mg kg^{-1})	33.1	(Sommers and Lindsay, 1979)
Extractable Mn with DTPA (mg kg^{-1})	17.7	(Sommers and Lindsay, 1979)
Extractable Zn with DTPA (mg kg^{-1})	97.1	(Sommers and Lindsay, 1979)

The Cd dose to be applied at the trial was determined by considering the averages and upper limits reported by (Lindsay 1979). In order to obtain a homogeneous distribution in the soil, 0.005 mM citric acid ($C_6H_8O_7$) was given in the form of Cd (0-5-10-20 mg kg^{-1}) $3CdSO_4.7H_2O$ at increasing doses prior to planting. Planting was successfully carried out covering the 60-65% of the soil capacity and they were left to be incubated under controlled conditions for a month.

Amount of the chlorophyll was determined according to the method reported by Wellburn (1994). 0.5g of fresh leaf was homogenized with 80% acetone and then centrifuged at 4^{o}C and 10.000 rpm for ten minutes. After that, chlorophyll-a, chlorophyll-b and total carotenoid levels were determined using a spectrophotometer by the wavelengths of 663.2 nm, 646.8 nm and 470 nm respectively.

As a result of the experiment, the plants that are being already grown for 45 days were harvested about 1 cm from the soil surface and the green parts (leaf and stem) were selected and washed with pure water, dried until reaching a constant weight at 65°C in the drying cabinet. The plant's dry weights were noted and then plants were milled in a grinding mill for plant analysis. Test results were evaluated as whole plant.

The total element concentrations (Cd, P, K, Fe, Mn, Cu and Zn) which will be evaluated after were determined by ICP-MS by dissolving the seeded plant samples in microwave (MarsXpress CEM) using nitric acid (HNO_3).

Data obtained as a result of the experiment were grouped by using Duncan test according to Bek (1986) using SPSS 22.0 statistical analysis program.

Results

It is observed that cadmium applications are statistically insignificant ($p \leq 0.01$) in terms of dry weight of plants, chlorophyll-a, chlorophyll-b and carotenoid amounts are not statistically significant (N.I.).

Table 2. The effect of different Cd applications on citric acid and citric acid-free environments on the mass of *Rosmarinus officinalis* (g), dry mass (g), chlorophyll-a (µg g^{-1}), chlorophyll-b (µg g^{-1}) and carotenoid (µg g^{-1}).

	Dose	Dry Mass	Chlorophyll-a	Chlorophyll-b	Carotenoid
(-) Citric Acid	Cd_0	1.63 a	25.7 a	15.0 a	-2.40 b
	Cd_5	1.54 ba	24.8 a	13.2 a	-1.53 ba
	Cd_{10}	1.51 cb	25.6 a	12.4 a	-1.26 ba
	Cd_{20}	1.44 dc	26.2 a	10.6 a	-0.77 ba
(+) Citric Acid	Cd_0	1.41 ed	27.3 a	13.5 a	-1.58 ba
	Cd_5	1.36 ed	25.8 a	10.5 a	-0.80 ba
	Cd_{10}	1.33 fe	27.2 a	10.6 a	-0.73 ba
	Cd_{20}	1.25 f	26.8 a	7.11 a	0.10 a
	F	19.3**	1.54 N.I..	1.43 N.I.	1.59 N.I.

(**): p≤0.01 statistically significant within error bounds
(N.I.): not statistically significant

The dry masses of citric acid with different doses of Cd were between 1.25-1.63 g $plant^{-1}$ and the highest amount with 1.63 g $plant^{-1}$ were observed in the group of Cd_0, which is a citric acid-free application, and the dry masses exhibited a decrease with respect to the Cd doses as compared to the control group (Table 2). Kalınbacak et al. (2012) found that the plants cultivated in the pots with addition of 0, 5, 15, 30 and 45 mg Cd kg^{-1} to soil were negatively affected by Cd toxicity and decreased dry mass in wheat as Cd amount increased. Daghan et al. (2012) reported that when transgenic tobacco plants were used to treat soil contaminated with Cd (0, 0.2, 0.4, 0.6, and 1.6 mg Cd kg^{-1}) with phytoextraction, growing and dry mass of plant will be reduced with increased dose of Cd.

It was determined that the highest value (27.3) on the application of citric acid with various cadmium doses varied between 24.8-27.3 for chlorophyll-a in the Cd_0 group with citric acid application and the highest value (15.0) with various cadmium doses varied between 7.11-15.0 for chlorophyll-b in the Cd_0 group with citric acid-free application (Table 2).

Eren and Mert (2017) reported that chlorophyll amounts in the leaves of *Inula helenium*, *Physalis angulata* and *Verbascum thapsus* plants were reduced compared to control plants in the soil which Cd had been applied at increasing doses (0-5-10-20 and 40 mg Cd kg^{-1}). Amirjani (2012) reported that wheat was adversely affected in general and decreased the amount of chlorophyll (chlorophyll-a, b and a + b) in studies of increased Cd doses on wheat plant effects. Zengin and Kirbag (2007) reported that decreasing the chlorophyll-a, chlorophyll-b, total pigment I and II amounts of Cd (0, 0.05, 0.06 and 0.08 mM Cd) applied bean seedling compared to the control groups with investigating the effects of Cd, Cu, Hg and Pb on the pigment amounts of bean seedling.

It is stated by Zengin and Munzuroglu (2005) that heavy metals led to the formation of free radicals, leading to the oxidative destruction of lipids of the tilacoid membrane, thereby increasing the degradation of chlorophyll and inhibiting its synthesis. It was also found that cadmium applications were important at the level of p≤0.01 in terms of N%, P% and K% in *Rosmarinus officinalis* plant (Table 3).

Table 3. Effects of N%, P% and K% on rosemary plants with different Cd applications in citric acid and citric acid-free environments.

	Dose	% N	% P	% K
(-) Citric Acid	Cd_0	2.21 a	0.19 e	4.02 d
	Cd_5	2.19 a	0.25 ed	6.80 c
	Cd_{10}	1.97 cb	0.30 dc	7.45 c
	Cd_{20}	1.90 c	0.30 dc	7.44 c
(+) Citric Acid	Cd_0	2.09 ba	0.41 ba	8.42 cb
	Cd_5	1.91 c	0.25 ed	10.1 ba
	Cd_{10}	1.98 cb	0.43 a	6.77 c
	Cd_{20}	1.84 c	0.35 cb	11.2 a
	F	9.74**	16.6**	19.5**

(**): p≤0.01 statistically significant within error bounds

The effect of citlric acid application on the amount of N by different Cd applications was highest with 2.21% N in the Cd_0 group in which citric acid-free treatment, was highest with 0.43% P in the Cd_{10} group in which citric acid treatment, and was highest with 11.2% K in the Cd_{20} group in which citric acid treatment was applied (Table 3).

Gouia et al. (2000) reported that the activity of nitrate reductase and nitrite reductase, enzymes of N metabolism, decreased nitrate assimilation of plants under Cd stress. Daghan et al. (2013) noted a significant reduction in N, P and K concentrations of green parts of plants with increasing doses (0, 5 and 10 mg Cd L^{-1}) of cadmium. Ciećko et al. (2004) stated that using different doses of Cd (0, 10, 20, 30, and 40 mg Cd kg^{-1}) led to a decrease in the K concentration of the corn plant for 10 and 20 mg Cd kg^{-1} applications compared with the control plant that increased in 30 and 40 mg Cd kg^{-1} usages, a decrease in the K concentration in the hay section of oat plant compared to the control group and a decrease in the K concentration of the bean plant was for 0, 20 and 40 mg Cd kg^{-1} applications compared with the control plant increased in 30 mg Cd kg^{-1} usage. As can be seen on Table 4, application of cadmium is statistically significant ($p \leq 0.01$) in terms of the amounts of Mn, Fe, Cu and Zn in the plants.

Table 4. Effects of different Cd applications on the amount of Mn, Fe, Cu and Zn in rosemary plants in citric acid and citric acid-free environments.

	Dose	**Mn**	**Fe**	**Cu**	**Zn**
		(mg kg^{-1})			
(-) Citric Acid	Cd_0	7.09 d	224 e	4.86 c	17.7 d
	Cd_5	13.4 b	439 a	6.13 b	21.8 c
	Cd_{10}	11.7 cb	344 c	4.83 c	16.8 ed
	Cd_{20}	18.2 a	390 b	7.19 a	28.7 a
(+) Citric Acid	Cd_0	16.5 a	408 ba	6.16 b	24.2 cb
	Cd_5	11.3 cb	375 cb	3.70 d	10.9 f
	Cd_{10}	10.3 c	287 d	6.39 b	25.2 b
	Cd_{20}	12.5 cb	397 ba	4.80 c	13.9 fe
	F	22.7**	33.0**	35.1**	41.1**

(**): $p \leq 0.01$ statistically significant within error bounds

It was determined that the effect of citric acid usage on the amount of Mn in different Cd applications was the highest in the control Cd_0 group with 16.5 mg Mn kg^{-1} and usage on the amount of Fe in different Cd applications was the highest as 439 mg Fe kg^{-1} for the Cd_5 group, which is the one has citric acid-free application (Table 4).

Hernández et al. (1998) reported that 10 and 50 µM Cd applied plants showed a decrease in concentrations of Mn and Fe in plant roots and shoots according to their concentrations in the control group of their studies with pea plants. It was determined that the amount of Cu was increased to 7.19 mg Cu kg^{-1} and the effect on Zn amount was highest as 28.7 mg Zn kg^{-1} for Cd_{20} group which has citric acid-free application with the effect of citric acid application with increasing usage of Cd (Table 4). Wu et al. (2004) reported that the concentration of Fe, Cu and Zn in the suprasellar area of the plant decreased with the application of Cd (1 and 10 mM Cd) on the cotton plant. Cadmium applications seem to be statistically significant at $p \leq 0.01$ in terms of Cd concentration and Cd content in plants (Table 5).

Table 5. Effects of cadmium applications on the concentration of Cd in plants and the amount of Cd content in plants

	Dose	**Cd (mg kg^{-1})**	**Cd (µg $plant^{-1}$)**
(-) Citric Acid	Cd_0	0.58 e	0.95 d
	Cd_5	2.02 d	3.11 c
	Cd_{10}	4.99 b	7.52 a
	Cd_{20}	5.32 b	7.62 a
(+) Citric Acid	Cd_0	1.05 e	1.49 d
	Cd_5	3.34 c	4.56 b
	Cd_{10}	5.83 ba	7.73 a
	Cd_{20}	6.63 a	8.31 a
	F	87.9**	63.9**

(**): $p \leq 0.01$ statistically significant within error bounds

In Table 5, influences of the difference Cd and citric acid applications on the Cd concentration were demonstrated. They were changed in the range of 0.58-6.63 mg Cd kg^{-1}. Highest value was determined as 6.63 mg Cd kg^{-1} for Cd_{20} group which was treated with citric acid application. On the other hand, lowest value was obtained as 0.58 mg Cd kg^{-1} by Cd_0 control group, which has citric acid-free application.

The effect of increasing Cd during citric acid application, on the amount of Cd was measured 0.95-8.31 μg plant^{-1}, while the highest amount was in the Cd_{20} group with citric acid application, resulted with 8.31 μg plant^{-1}. On the contrary, the lowest value was determined as 0.95 μg plant^{-1} for the Cd_0 group, which had citric acid application (Table 5).

Jiang et al. (2003) reported that different applications of Cd-containing soil at different doses reduced the accumulation of Cd in the roots of the mustard plant but increased it in the part above the soil, and for some applications, especially EDTA, it has reduced the amount of Cd deposition at the roots. This phenomenon has been explained by the fact that the movement of Cd from the root of the plant was increasingly facilitated to the parts above the soil.

Zhang et al. (2014), investigated the application of different ratios of Cd (15,30,60,100 mg Cd kg^{-1}) to two different grass plants and reported high amount of Cd accumulation within the roots and green parts of the plants.

Conclusion and Suggestions

The most pronounced response of plants to heavy metals in increasing doses was observed as reduction in the amount of biomass they produce. Additionally, the biomass was found to be decreased for the plant pots treated with citric acid application compared to ones which have citric acid-free application. In terms of body mass and dry mass, among the rosemary plant that is being treated with cadmium and citric acid application along with the Cd_{20} application, the lowest dry mass was measured as 1.25 g plant^{-1}.

Chlorophyll levels during the increased doses of Cd applications were found to be decreasing compared to the control groups in few applications such as citric acid, Cd (except for chlorophyll-a in Cd application without citric acid) and Cd without citric acid. It has been also found that the concentration for the Cd_{20} and citric acid applications of cadmium was 5.32 and 6.63 mg Cd kg^{-1}, respectively. Additionally, for the plants which were treated with Cd_{20} applications were found to possess 7.62 μg Cd plant^{-1} content while the other pots that are treated by citric acid including 8.31 μg Cd of plant^{-1}.

As a conclusion, it has been observed that the increased Cd content had a bad influence on the chlorophyll and biomass amount which were resulted with a diminished in terms of growth rate for rosemary plant. It has been also concluded that the heavy metal application has different effects on the intake of macro and micro nutrients of plants depending on types of the elements. Therefore, rosemary plants have no potential to be used in phytoremediation treatment of Cd-contaminated soils. Future studies are required to examine extended period of time and also reveal the accumulation of Cd within the roots.

The results obtained with citric acid application were also determined by this study and indicating that the rosemary plant was more abundant in soil with heavy metal (Cd) pollution. Since the rosemary plant used in the study is also used as a spice, the metal content of the rosemary plant, especially for the ones growing in dirty soil, should not be consumed as spice. Additionally, since the rosemary plant can be used as medical aromatic plant or as a spice, it's oil also needs to be controlled in order to determine any kind of possible toxic metal content.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of Excess Cadmium on Growth, Tolerance and Physiological Characteristics of Purslane Varieties

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Abstract: This study was designed to understand the effects of excess cadmium (Cd) on plant growth and physiological characteristics as well as the accumulation and distribution of some metal nutrient ions with two purslane (*Portulaca oleracea* L.) varieties (cv. Istanbul and wild variety) in greenhouse at natural light conditions. For this purpose, six levels of Cd (0, 50, 100, 200, 400, and 800 µM $CdCl_2$) were treated to the soil. The results indicated that Cd stress gradually depressed plant growth and caused the decreases in photosynthetic pigment contents (chlorophyll *a, b, a+b,* and carotenoids), relative water content (RWC), bio-concentration factor (BCF), and proline accumulation in shoot for both varieties. The reductions in biomass production and photosynthetic pigments contents in wild variety were higher than in cv. Istanbul. Moreover, Cd exposure increased the concentrations of Cd, zinc (Zn), and sodium (Na) and the uptakes of Cd in shoot and root, total accumulation rate (TAR) of Cd and net accumulation of Cd *via* roots, the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in both varieties. The changes in root length, the concentrations of potassium (K) and calcium (Ca) in shoot and root, translocation factor (TF) of Cd, and membrane permeability (MP) of the leaves were shown differences in varieties. According to these results obtained, it was concluded that the effect of Cd exposure was more evident in wild variety (Cd-sensitive) than in cv. Istanbul (Cd-tolerant).

Key words: Bio-concentration, translocation, metal nutrient ions, photosynthetic pigments

Aşırı Kadmiyumun Semizotu Varyetelerinin Büyüme, Tolerans ve Fizyolojik Karakteristiklerine Etkisi

Özet: Bu çalışma, serada ve doğal ışık koşulları altında aşırı kadmiyumun (Cd) iki semizotu (*Portulaca oleracea* L.) varyetesinin (İstanbul çeşidi ve yabani varyete) bitki gelişimi ve fizyolojik karakteristikleri ile bazı metal besin iyonlarının bitkideki akümülasyonu ve dağılımı üzerine etkisini belirlemek amacıyla planlanmıştır. Bu amaçla, toprağa altı düzeyde Cd (0, 50, 100, 200, 400 ve 800 µM $CdCl_2$) uygulanmıştır. Sonuçlara göre, Cd stresi her iki varyetede de doz artışına bağlı olarak bitki gelişimini kademeli olarak azaltmış ve varyetelerin fotosentetik pigment içerikleri (klorofil *a, b, a+b* ve karotenoid), nispi nem içeriği, biyokonsantrasyon faktörü ve prolin akümülasyonunda azalmaya neden olmuştur. İstanbul çeşidine göre yabani varyetede biokütle üretimi ve fotosentetik pigment içeriklerindeki azalmalar daha fazla olmuştur. Bununla birlikte, Cd uygulamaları sonucu her iki varyetede gövdede ve kökte Cd, çinko (Zn) ve sodyum (Na) kapsamları ile Cd alımları, toplam Cd akümülasyon oranı, kökler aracılığıyla alınan net Cd akümülasyonu, malondialdehit (MDA) ve hidrojen peroksit (H_2O_2) içerikleri artmıştır. Kök uzunluğu, gövdede ve kökte potasyum (K) ve (Ca) kapsamları, Cd'un translokasyon faktörü (TF) ve yaprakların membran geçirgenliğindeki (MP) değişimler çeşitlere göre farklılıklar göstermiştir. Elde edilen sonuçlara göre; yetişme ortamındaki aşırı Cd'un etkilerinin İstanbul çeşidine (Cd'a dayanıklı) göre yabani semizotu varyetesinde (Cd'a hassas) daha belirgin olduğu kanaatine varılmıştır.

Anahtar Kelimeler: Biokonsantrasyon, translokasyon, metal besin maddesi iyonlar, fotosentetik pigmentler

Introduction

Cadmium (Cd), is one of the most highly toxic non-essential elements for humans, animals and plants, has been a major pollutant in both terrestrial and aquatic environments for last decades. Although Cd occurs naturally in soils, recent advances in industry and agriculture have led to an increment of Cd level in agricultural soils. Cd enters agricultural soil mainly through several anthropogenic resources, such as the intensive use of phosphate fertilizers, waste water, sewage sludge, and manure, burning of fossil fuels, mining activities, metallurgical and cement industry and urban traffic emissions (Sanitá di-Toppi and Gabbrielli 1999; Singh and Agrawal 2007).

Increasing Cd concentrations in the terrestrial and aquatic environment have given rise to serious concern, because in the form of Cd^{2+} it is highly mobile in soil and toxic to plants, animals and humans (Zembala et al. 2010). In consequence of its high mobility and water solubility, Cd quickly enters the roots through the cortical tissue and can reach the xylem via an apoplastic and/or symplastic pathway, complexed to organic acids or phytochelatins (Nazar et al. 2012). Cd is easily taken up into plant cells in different plant parts by membrane transporters of essential elements (Clemens 2006), and competes with the uptake, transport and physiological function of macro- and micronutrients (Rivetta et al. 1997). Accumulated Cd ions in plants compete for the same transmembrane carriers with most nutrients such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and nickel (Ni) across the same trans-membrane carriers (Rivetta et al. 1997; Sanitá di-Toppi and Gabbrielli 1999).

Cd-induced negative effects can result in the inhibition of photosynthesis and transpiration, the reduction of root growth and biomass production, plant growth retardation, restriction of chlorophyll biosynthesis, imbalance of water and mineral nutrition, induction of oxidative stress, inhibition of enzyme activities, and effects on membrane structure and permeability (Tran and Popova 2013; Nazar et al. 2012; Shamsi et al. 2010; Benavides et al. 2005; Sandalio et al. 2001; Sanitá di-Toppi and Gabbrielli 1999). At cellular level, Cd generates oxidative stress by interfering with the antioxidant defense system by virtue of stimulating the production of reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) (Tran and Popova 2013; Benavides et al. 2005). Many studies documented an increment in lipid peroxidation, proline and H_2O_2 content, and membrane damage in plant species as Cd-induced oxidative stress indicators (Ahmad et al. 2016; Moradi and Ehsanzadeh 2015; Xu et al. 2015; Shamsi et al. 2010; Singh and Agrawal 2007).

Plant species and their genotypes show variable ability to Cd accumulation, and remarkably differ in uptake of and tolerance to Cd and other heavy metals. Among *Solanaceae* plants, tomato are high tolerant to Cd stress than pepper, eggplant and goldenberry, and the classified by translocation of Cd have been determined as goldenberry < pepper < eggplant < tomato (Çikili et al. 2016). Cereals (maize, oat, barley, and rice) are exhibited greater tolerance to Cd^{2+} (100 µM) than Adzuki bean, cucumber, lettuce, pea, radish, sesame and tomato (10-30 µM), and the restriction of biomass production in cereals are lesser than the others (Inouhe et al. 1994). Also, variation in Cd accumulation has been demonstrated among wheat genotypes (Zembala et al. 2010), safflower (Shi et al. 2010), soybean (Shamsi et al. 2010), and barley (Guo et al. 2007).

Purslane (*Portulacca oleracea* L.), which is a member of *Portulacaceae* and a succulent and robust medicinal plant, is one of the most widespread weed species in temperate and tropical regions world-wide. The "Wild" purslane which is represented 25 genera of succulent herbs and shrubs in *Portulacaceae* family is a widely distributed throughout all over the world where it grows all in cultivated fields, lawn and waste places up to 3850 m elevated (Mitich 1997). Purslane has high nutritive value and noteworthy bioactive compounds such as ω-3 fatty acid, α-linolenic acid and some antioxidants (α-tocopherol, β-carotenes, ascorbic acid, glutathione, betalain, and anthocyanins), and has the highest amount of α-linolenic acid among terrestrial sources (Gonnella et al. 2010). Many researchers explained that purslane possesses competitive advantages over many other cultivated crops due to highly adaptable to various stress environments (Gonnella et al. 2010; Tiwari et al. 2008). Tiwari et al. (2008) reported that purslane could be grown at sites contaminated with multiple metals of industrial origin and might produce high biomass, because of good growth, short life cycle and its high regeneration potential. Also, they stated that it shown potentiality to hyperaccumulate Cd, chromium (Cr) and arsenic (As), especially in roots. The objective of this study is to understand the effects of Cd exposure on plant growth, photosynthetic pigments, the bioaccumulation and translocation of Cd, and the accumulation of some metal nutrient ions in two varieties of purslane, as well various components being an indicator in phytotoxicity of heavy metal (HM) stress.

Materials and Methods

Growth Condition and Treatments

Two different purslane (*Portulaca oleracea* L.) varieties were used for the experiment. To investigate the effects of Cd on two purslane varieties (cv. Istanbul and wild variety), the experiment was performed using pots in a greenhouse under natural light conditions at an ambient temperature in summer season at Düzce University, Çilimli Campus (lat 40°89'46"N, long 31°04'89"E). The climatic conditions in the greenhouse during the experiment were average air temperature 27/18 °C (day/night) and average relative humidity 63%.

Wild purslane variety seeds were collected from nature at the end of the previous summer season at Mahirağa district in Çilimli (lat 40°87'89"N, long 31°05'13"E) and the other purslane seed (*P. oleracea* L., cv. İstanbul)

was supplied from the seed market. Uniform seeds were soaked in de-ionized water for 4 h at room temperature, and from both varieties 20 seeds have been sown into plastic pots containing 2 kg of air-dried soil. After good stands of the plants, young plants were thinned to 10 plants per pot.

Some properties of the experimental soil were as follows: loam texture (sand/clay, 35.8/21.7 by dry weight); pH (1/2.5 soil/water) 7.34; EC, 508 μS cm^{-1} (saturation extract); calcium carbonate ($CaCO_3$), 17.29 g kg^{-1}; organic carbon (modified Walkley-Black), 6.25 g kg^{-1} and total nitrogen (Kjeldahl method), 0.86 g kg^{-1}. Sodium bicarbonate ($NaHCO_3$)-available phosphorus (P) concentration was 12.43 mg kg^{-1} and hot water extractable-B was 1.64 mg kg^{-1}. Ammonium acetate (CH_3COONH_4)-extractable K, Ca, Mg and Na with were 0.256, 2151, 124 and 64 mg kg^{-1}, respectively. Diethylene triamine penta acetic acid (DTPA)-extractable Fe, Cu, Zn, Mn, and Cd were 24.28, 2.09, 1.17, 65.27, and 0.04 mg kg^{-1}, respectively. The soil properties were determined according to methods detailed in Page et al. (1982).

In a factorial (Cd levels and purslane variety) pot experiment, six levels of Cd (0, 50, 100, 200, 400 and 800 μM) as cadmium chloride ($CdCl_2$) were applied to the soil and designed as complete randomized design with three replications. For basal fertilization; N, P and K, as ammonium nitrate (NH_4NO_3), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and potassium sulfate (K_2SO_4) were applied to the soil at 150, 75 and 150 mg kg^{-1}, respectively. All the supplementary ($CdCl_2$, NH_4NO_3, $NH_4H_2PO_4$, and K_2SO_4) were incorporated into the soil by spraying the solutions before the planting and mixing them into the soil. During the experimental period, soil was kept at approximately 70% of the field capacity with tap water.

Sampling and Harvest Procedure

A fresh leaf sample was taken from the youngest fully expanded leaf for membrane permeability (MP, EC %), relative water content (RWC), and photosynthetic pigments analysis before harvest. After 24 days of Cd treatments, plants were harvested properly and separated into shoots and roots for determining fresh and dry matter biomass. The shoots were weighted and the roots were carefully separated from the soil and dipped into an aerated 0.5 mM calcium chloride ($CaCl_2$) solution for 15 minutes in order to eliminate adsorbed nutrients from the root surface. The roots were quickly washed with running tap water and then three-times rinsed with de-ionized water to remove any soil particles attached to the plant surfaces. The shoot and root samples were dried at 70ºC in oven for at least three days, quickly measured for dry weights (DW) and separately grinded and kept for nutrient ions analyses.

Chemical Analyses

For the measurement of nutrient ion concentrations, after weighing, 500 mg of each of the dry tissues were incinerated by the dry-ash method in a muffle furnace at 550±25ºC f or 6 h (Miller 1998). The cooled ash was then dissolved in 2 mL, 10 M nitric acid (HNO_3) solution. The nutrient ions were analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV; Waltham, MA).

The ion distribution, the ion uptake, bioconcentration factor (BCF) of ion, translocation factor (TF), total accumulation rate (TAR) of ion and net accumulation of Cd via roots (Net Acc) were calculated by the Equation 1, 2, 3, 4, 5 and 6, respectively (Çikili et al. 2016; Moradi and Ehsanzadeh 2015; Shi et al. 2010; Ait Ali et al. 2002).

$$\text{Ion distribution (\%)} = 100 \times ([\text{ion}]_{\text{shoot or root}} / ([\text{ion}]_{\text{shoot}} + [\text{ion}]_{\text{root}})) \quad (1)$$

$$\text{Ion uptake } (\mu\text{g plant}^{-1}) = DW_{\text{shoot or root}} \times [\text{ion}]_{\text{shoot or root}} \quad (2)$$

$$BCF = [\text{ion}]_{\text{shoot or root}} / [\text{total ion}]_{\text{growth media}} \quad (3)$$

where; $[\text{total ion}]_{\text{growth media}}$, present ion concentration in growth media + added ion concentration for each ion level

$$TF = [Cd]_{\text{shoot}} / [Cd]_{\text{root}} \quad (4)$$

$$\text{TAR of ion } (\mu\text{g g}^{-1}\text{ DW day}^{-1}) = ([\text{ion}]_{\text{shoot}} \times DW_{\text{shoot}}) + ([\text{ion}]_{\text{root}} \times DW_{\text{root}}) / \text{growth day} \times (DW_{\text{shoot}} + DW_{\text{root}}) \quad (5)$$

$$\text{Net Acc } (\mu\text{g/g DW}) = \text{total amount of ion in whole plant } (\mu\text{g}) / \text{root DW (g)} \quad (6)$$

Physiological and Biochemical Analyses

The physiological and biochemical analysis were determined in fresh leaf samples before harvest. The fresh leaf samples (200 mg) were cut into small pieces and were extracted with 10 mL of acetone (90% v v^{-1}) in a homogenizer. After filtering with Whatman No. 4 filter paper, the absorbance of the extract was measured at 663, 645, and 470 nm using a UV-Vis spectrophotometer (Shimadzu UV-1201; Tokyo). The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a+b* (Chl *a+b*), and carotenoids (Car) were calculated according to the formula of Lichtenthaler (1987). The MP for the shoot disc samples was measured by the electrical conductivity (EC) method as described by Yan et al. (1996). The RWC was determined using a composite sample of leaved discs (1 cm), which were weighed to record fresh weight (FW), floated in distilled water for 4 h to determined turgid weight (TW) then oven-dried at 70 ºC for 48 h to measure dry weight (DW). The RWC were calculated by the Equation 7.

RWC = (FW-DW) / (TW-DW) (7)

Lipid peroxidation of leaves is a good indicator for assessing membrane damage and was estimated by the level of malondialdehyde (MDA), the end product of lipid peroxidation, described by Hodges et al. (1999). In brief, fresh leaf samples (250 mg) was homogenized by a homogenizer (Heidolph, Diax 900) in 5 mL 0.1% trichloroacetic acid (TCA) and the homogenate was centrifuged at 5000 g for 5 min. After that, 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA) were added to 1 mL aliquot of the supernatant. The mixture was heated in boiling water bath (95 ºC) for 15 min and allowed to cool in ice bath quickly. The supernatant was centrifuged at 10000 g for 5 min and resulting supernatant was used for spectrophotometric determination of MDA. The absorbance at 532 nm was recorded and corrected for nonspecific absorbance at 600 nm. The MDA concentration was calculated by means of an extinction coefficient of (E = 155 mM/cm).

The hydrogen peroxide (H_2O_2) content of the leaves was extracted and estimated as described by Mukherjee and Choudhuri (1983). Fresh leaf samples (250 mg) was homogenized by a homogenizer (Heidolph, Diax 900) in 5 mL with cold acetone and filtered. An aliquot (1 mL) of the extracted solution was mixed with 4 mL of titanium dioxide (TiO_2) reaction solution in TiO_2 (0.06%, w/v), potassium sulphate (K_2SO_4) (0.6%, w/v), and sulphuric acid (H_2SO_4) (10 %, v/v) and added 5 mL concentrated ammonia (NH_3) solution. The mixture was centrifuged at 10000 g for 5 min. The intensity of yellow color of the supernatant was measured at 415 nm. The concentration of H_2O_2 was calculated from a standard curve plotted with the range of 100–1000 nmol H_2O_2.

Free proline content was extracted from 250 g fresh leaf samples homogenized with 5 mL of 3% (w/v) sulfosalicylic acid at 4ºC and estimated by ninhydrin reagent Bates et al. (1973).

Statistical analyses

The experimental design was a completely randomized factorial design with three replicates and obtained data were analyzed by ANOVA. The levels of significance are represented by * at $P< 0.05$, ** at $P< 0.01$, *** at $P< 0.001$, and ns: non-significant. The statistical tests were performed by using JMP package program (SAS Institute Inc., Cary, NC). The differences of means among different Cd treatments within a variety were compared by Duncan's multiple-range test ($\alpha: 0.05$).

Results and Discussion

The Cd exposure progressively depressed plant growth, and increasing Cd levels dramatic decreased the shoot and root biomass in both purslane varieties (Table 1). The shoot and root DW of varieties significantly decreased with increasing level of Cd in comparison with the control. For example, the shoot DW decreased by 17.3% and 34.9% for cv. İstanbul and wild variety of purslane while the root DW reduced by 18.5% and 45.9%, respectively, at 50 µM Cd treatment. Furthermore, at the highest Cd treatment, shoot DW decreased by 68.9%, and 88.9% for cv. İstanbul and wild variety of purslane while the root DW diminished by 64.8%, and 91.6%, respectively. On the basis of the percent reductions in the shoot dry biomass, wild variety can be suggested as Cd-sensitive and cv. İstanbul as Cd-tolerant. The suppression of plant growth might be due to Cd-phytotoxic effects on synthesis of cell wall (Barcelo et al. 1990), and enhanced production and accumulation of ROS which damaged photosynthetic activity by decreasing chloroplastic pigments (Panda et al. 2011).

Table 1 The changes in fresh and dry biomass and root length of purslane varieties exposed to Cd

Cd treatments (μM)	Fresh Weight (g/10 plant)		Dry Weight (g/10 plant)		Root Length (cm)	Reduction in biomass as % of control	
	Shoot	Root	Shoot	Root		Shoot	Root
P. oleracea L. cv. İstanbul							
0	$54.6^{\pm 0.90a}$	$3.31^{\pm 0.45a}$	$2.48^{\pm 0.05a}$	$0.28^{\pm 0.01a}$	$13.0^{\pm 1.21ab}$	-	-
50	$51.6^{\pm 1.26a}$	$2.79^{\pm 0.06ab}$	$2.30^{\pm 0.08a}$	$0.26^{\pm 0.01b}$	$13.8^{\pm 0.69a}$	7.3	8.5
100	$48.7^{\pm 1.74a}$	$3.29^{\pm 0.12abc}$	$2.02^{\pm 0.17a}$	$0.26^{\pm 0.00b}$	$15.7^{\pm 1.62a}$	18.5	9.2
200	$47.9^{\pm 5.03ab}$	$3.18^{\pm 0.15bc}$	$1.99^{\pm 0.25a}$	$0.26^{\pm 0.01b}$	$15.8^{\pm 0.93a}$	19.8	8.5
400	$40.6^{\pm 1.26b}$	$2.04^{\pm 0.29c}$	$1.57^{\pm 0.13b}$	$0.17^{\pm 0.01c}$	$15.1^{\pm 1.55a}$	36.7	39.8
800	$18.3^{\pm 2.92c}$	$1.27^{\pm 0.18d}$	$0.77^{\pm 0.14c}$	$0.10^{\pm 0.06d}$	$9.5^{\pm 0.25b}$	68.9	64.8
P. oleracea L. Wild variety							
0	$58.5^{\pm 2.79a}$	$4.75^{\pm 0.60a}$	$2.98^{\pm 0.04a}$	$0.39^{\pm 0.07a}$	$18.7^{\pm 0.44a}$	-	-
50	$40.9^{\pm 4.90b}$	$3.50^{\pm 0.26ab}$	$1.94^{\pm 0.25b}$	$0.21^{\pm 0.05b}$	$18.2^{\pm 0.15a}$	34.9	45.9
100	$32.3^{\pm 4.64bc}$	$2.67^{\pm 0.41b}$	$1.68^{\pm 0.27b}$	$0.17^{\pm 0.03bc}$	$14.6^{\pm 3.36b}$	43.6	56.1
200	$29.4^{\pm 2.99bc}$	$2.66^{\pm 0.59b}$	$1.45^{\pm 0.18b}$	$0.17^{\pm 0.05bc}$	$13.6^{\pm 0.23b}$	51.3	56.1
400	$29.2^{\pm 4.87c}$	$2.59^{\pm 0.43b}$	$1.43^{\pm 0.23b}$	$0.17^{\pm 0.03bc}$	$13.2^{\pm 0.62b}$	52.0	57.4
800	$5.2^{\pm 0.33d}$	$0.72^{\pm 0.02c}$	$0.33^{\pm 0.04c}$	$0.04^{\pm 0.02c}$	$12.8^{\pm 0.17b}$	88.9	91.6
ANOVA: *F* values							
Variety (V)	34.6***	0.7 ns	4.9*	2.3 ns	3.3 ns		
Cd	42.3***	16.4***	35.0***	14.7***	3.6*		
V x Cd	3.0*	2.9*	2.3 ns	2.7*	4.0**		

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

Root length linearly decreased for cv. İstanbul, but the changes for wild variety was different with Cd treatments as compared to control (Table 1). At 800 μM Cd treatment, however, root length in cv. İstanbul and wild variety of purslane was decreased by 26.9%, and 31.6%, respectively. It was determined that both FW and DW of the shoot and root, and root length were notably affected by the interaction of variety and Cd treatment, except for shoot FW. The reduction of plant growth parameters caused by Cd treatment has been demonstrated in different plant species such as perennial ryegrass (Chen et al. 2018; Wang et al. 2013), sunflower (Samet et al. 2017), *Solanaceae* plants (Çikili et al. 2016), safflower (Moradi and Ehsanzadeh 2015; Shi et al. 2010), purslane seedling (Naz et al. 2013), soybean (Shamsi et al. 2010), lettuce seedlings (Xu et al. 2015), and barley (Guo et al. 2007).

Cd exposure resulted in an increment of Cd concentration and uptake in both shoot and root (Table 2). Evidently exposure of plants to Cd stress caused that Cd was accumulated at higher concentrations in roots than in shoots in both purslane varieties, but root Cd uptakes were not. At the highest level of Cd treatment compared to the control, the shoot and root Cd concentrations increased by 198-, and 141-fold for cv. İstanbul, and by 70-, and 157-fold for wild variety, respectively. Also, the shoot and root Cd uptakes increased by 64-, and 48-fold for cv. İstanbul at the highest Cd treatment in comparison with the control, whereas these increments was found 8-, and 18-fold for wild variety, respectively. Besides, being less tolerance to high Cd concentrations according to cv. İstanbul, it is an imperfection for wild variety. Hence, cv. İstanbul might be considered as Cd accumulator than wild variety. In view of the Cd distribution, the roots of purslane varieties accumulated much higher Cd than the shoots with Cd exposure (Table 2). The cell walls of roots play a significant role in HM tolerance in plants due to the fact that the root is in direct contact with Cd as explained by He et al. (2008). Cd accumulation in roots might be resulted from cross-linking of Cd to carboxyl groups of the cell wall and/or to interactions with thiol residues of soluble proteins (Leita et al. 1993). The high Cd concentration was mainly determined in roots and old leaves of sunflower by virtue of Cd toxicity as reported De Maria et al. (2013), who elucidated that sunflower tend to avoid toxicity in the physiologically most active parts of the plants by decreasing Cd translocation to the epigeous part, and by promoting the re-translocation of toxic metals from shoots to the roots. Many researchers have reported that Cd is accumulated more in the roots than in the shoots in plant species and/or their genotypes such as perennial ryegrass (Wang et al. 2013), *Solanaceae* plants (Çikili et al. 2016), safflower (Moradi and Ehsanzadeh 2015; Shi et al. 2010), and soybean (Shamsi et al. 2010). The Cd is more toxic to purslane seedling, compared to Pb and Zn, and also the roots of purslane seedlings are more sensitive to the HMs (Cd, Pb, and Zn) in comparison with shoot as have stated by Naz et al. (2013).

Table 2 The changes in concentration, uptake, and distribution of Cd in plant shoot and root of purslane varieties exposed to Cd

Cd treatments	Cd (μg g^{-1} DW)		Cd distribution (%)		Cd uptake (μg $plant^{-1}$)	
(μM)	Shoot	Root	Shoot	Root	Shoot	Root
P. oleracea L. cv. İstanbul						
0	$0.9^{\pm0.13c}$	$2.7^{\pm0.18f}$	24.3	75.7	$2.2^{\pm.0.36c}$	$0.8^{\pm0.04e}$
50	$17.5^{\pm0.59c}$	$94.5^{\pm0.12e}$	15.7	84.3	$40.2^{\pm2.37c}$	$24.5^{\pm1.83d}$
100	$24.3^{\pm1.05c}$	$120.8^{\pm12.0d}$	17.0	83.0	$49.5^{\pm6.06bc}$	$31.2^{\pm2.81cd}$
200	$27.7^{\pm0.87b}$	$161.0^{\pm1.06c}$	26.4	73.6	$114.2^{\pm13.4a}$	$42.1^{\pm1.78ab}$
400	$60.9^{\pm3.32b}$	$282.3^{\pm2.84b}$	17.7	82.3	$96.0^{\pm11.4ab}$	$48.2^{\pm2.36a}$
800	$177.8^{\pm21.1a}$	$381.1^{\pm11.3a}$	31.6	68.4	$141.2^{\pm35.7a}$	$38.1^{\pm3.40bc}$
P. oleracea L. Wild variety						
0	$1.5^{\pm0.29e}$	$2.9^{\pm0.41e}$	32.2	65.8	$4.4^{\pm0.89b}$	$1.1^{\pm0.19c}$
50	$22.5^{\pm1.87d}$	$57.2^{\pm0.58d}$	28.1	71.9	$42.6^{\pm2.24a}$	$12.1^{\pm2.78b}$
100	$26.9^{\pm0.64c}$	$87.5^{\pm2.05c}$	23.6	76.5	$44.9^{\pm6.16a}$	$15.2^{\pm2.59b}$
200	$34.8^{\pm1.10b}$	$162.9^{\pm8.04b}$	17.7	82.4	$50.1^{\pm4.80a}$	$27.5^{\pm7.57a}$
400	$32.5^{\pm0.44b}$	$88.4^{\pm5.28c}$	27.0	73.0	$46.8^{\pm7.84a}$	$14.5^{\pm1.73b}$
800	$105.5^{\pm2.27a}$	$455.6^{\pm2.94a}$	18.8	81.2	$34.7^{\pm4.86a}$	$20.0^{\pm0.50ab}$
ANOVA: *F* values						
Variety (V)	28.4***	83.4***			27.2***	83.6***
Cd	125.5***	1156.0***			13.5***	34.0***
V x Cd	11.2***	112.4***			6.6***	6.8***

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

The shoot and root BCF remarkably decreased at all Cd levels as compared to the control, and the BCF was greater in the roots than in the shoots (Table 3). Furthermore, both the shoot and root BCF in both purslane varieties were in excess of the critical level for a Cd-hyperaccumulator, currently acknowledged as BCF> 1 (Baker 1981). To give more precise values a Cd hyperaccumulator should have > 100 μg g^{-1} DW (0.01%, w/w) (Baker 1981). Hyperaccumulators, efficient plants root-to-shoot transport system and indicating enhanced capacity for detoxification, have an inherent capacity to absorb metal at levels 50-500 times greater than normal plants (McGrath and Zhao 2003). The BCF levels mostly tend to diminish depending on increasing concentrations of HMs in growth media (Zhao et al. 2003). The BCF is typically greater than one in metal-accumulating species; whereas that of excluding species is often lower than it (Baker 1981).

The partitioning of Cd to different plant organs plays a substantial role in toxicity of Cd to plants, and translocation factor is described as ratio of HMs in plant shoot to that in plant root. The TF of Cd decreased from 0.56 to 0.23 for wild variety; however, increased from 0.33 to 0.47 for cv. İstanbul at the highest Cd treatment (Table 3). The TF of two purslane varieties were substantially lesser than the critical level (TF > 1).

The total accumulation rate (TAR) is an index parameter which has been greatly used in bioaccumulation studies and is a measure of HM uptake by plants (Zhu et al. 1999). The TAR of Cd and Net Acc increased with all Cd levels in comparison with the control (Table 3). Irrespective of Cd treatments, Net Acc in cv. İstanbul (639.3 μg g^{-1} DW) was higher than in wild variety (465.6 μg g^{-1} DW) while the TAR of Cd in cv. İstanbul was more high than by nearly 2-fold in wild variety. Results supporting these findings have been revealed by Ekvall and Greger (2003), who explained that genotypic and/or ecotypic differences exist for Cd uptake and translocation to shoots, and an increment in the TAR of Cd were reported in sunflower (Samet et al. 2017), in *Solanaceae* plants (Çikili et al. 2016), and in carrot (Sharma and Agrawal 2006). Moradi and Ehsanzadeh (2015) stated that net accumulation of Cd via roots significantly increased safflower genotypes subjected to increasing concentrations of Cd. Both the accumulated and translocated Cd/HM amounts of plants/accumulators vary with species and genotypes as an according to Tran and Popova (2013). Some researchers have indicated that much of Cd taken up by plants is retained in roots and only a portion is translocated to shoots (Tran and Popova 2013; Guo et al. 2007; Benavides et al. 2005).

The content of photosynthetic pigments (Chl *a*, *b*, *a+b* and Car) of leaves in both purslane varieties were dramatically decreased by increasing of supplied Cd to the soil, except for 50 μM Cd treatment in wild variety (Table 4). The content of photosynthetic pigments increased with 50 μM Cd treatments for wild variety and then remarkably decreased with increasing Cd levels compared to the control. For example, the reductions of Chl *a*,

Chl *b*, Chl *a+b* and Car content was found by 25.0%, 30.3%, 25.8%, and 21.2% for cv. İstanbul, and by 79.3%, 51.6%, 75.1%, and 52.8% for wild variety, respectively, in plants subjected to high Cd level in comparison with the control. It is well known that Cd restrains photosynthesis and decreases chlorophyll content. The decreases of chlorophyll content in both purslane varieties could be associated with chlorophyll degradation of and/or disorders in its biosynthesis and the reduction of thylakoid membrane integrity (Sandalio et al. 2001). In support of these findings, the reduction of Chl content with an increasing Cd content occurs in some plants, including perennial ryegrass (Chen et al. 2018; Wang et al. 2013), sunflower (Samet et al. 2017), *Solanaceae* plants (Çikili et al. 2016), safflower (Moradi and Ehsanzadeh 2015; Shi et al. 2010), and lettuce seedlings (Xu et al. 2015). Although an interaction of Cd and purslane varieties on the ratio of Chl *a*/*b* and Car/Chl were significant, the ratio of Chl *a*/*b* decreased for wild variety at the highest Cd level, and these changes was non-significant for cv. İstanbul with Cd treatments (Table 4). The ratio of Car/Chl was remarkably accrued at the highest Cd level in both purslane varieties. Shi et al. (2010) stated that the ratio of Car/Chl considerably increased in safflower cultivar which exhibited the high photosynthetic performance at high Cd level. Moreover, excess of Cd decreased the ratio of Chl (*a+b*)/Car in leaf sections at different age of of *Zea mays* seedlings as mentioned by Dresler et al. (2014).

Table 3 The changes in bioconcentration factor (BCF), translocation factor (TF), total accumulation rate (TAR), and net accumulation of Cd via root (Net Acc) of purslane varieties exposed to Cd

Cd treatments (μM)	BCF		TF	TAR of Cd ($\mu g\ g^{-1}$ DW day^{-1})	Net Acc ($\mu g\ g^{-1}$ DW)
	Shoot	Root			
P. oleracea L. cv. İstanbul					
0	$21.7^{\pm3.33a}$	$66.9^{\pm4.59a}$	$0.33^{\pm0.05b}$	$0.35^{\pm0.05c}$	$10.3^{\pm1.42d}$
50	$3.1^{\pm0.11b}$	$16.7^{\pm1.08b}$	$0.17^{\pm0.00c}$	$4.23^{\pm0.68b}$	$249.3^{\pm13.8cd}$
100	$2.2^{\pm0.09b}$	$10.7^{\pm1.06bc}$	$0.21^{\pm0.03c}$	$8.06^{\pm1.07b}$	$312.1^{\pm17.8bcd}$
200	$2.6^{\pm0.04b}$	$7.2^{\pm0.05cd}$	$0.36^{\pm0.01b}$	$15.63^{\pm3.11a}$	$595.3^{\pm37.7bc}$
400	$1.4^{\pm0.07b}$	$6.3^{\pm0.06cd}$	$0.22^{\pm0.01c}$	$11.05^{\pm1.73ab}$	$842.7^{\pm60.1b}$
800	$2.0^{\pm0.23b}$	$4.2^{\pm0.13d}$	$0.47^{\pm0.06a}$	$7.18^{\pm2.17b}$	$1825.6^{\pm417a}$
P. oleracea L. Wild variety					
0	$39.7^{\pm7.26a}$	$71.8^{\pm10.2a}$	$0.56^{\pm0.09}$	$0.81^{\pm0.14b}$	$14.3^{\pm2.02d}$
50	$4.0^{\pm0.33b}$	$10.1^{\pm0.10b}$	$0.39^{\pm0.03}$	$5.21^{\pm1.07a}$	$281.1^{\pm56.0c}$
100	$2.4^{\pm0.06b}$	$7.8^{\pm0.18b}$	$0.31^{\pm0.01}$	$5.00^{\pm1.18a}$	$355.9^{\pm53.5bc}$
200	$1.6^{\pm0.05b}$	$7.2^{\pm0.36b}$	$0.26^{\pm0.01}$	$5.53^{\pm0.88a}$	$530.4^{\pm143.0b}$
400	$0.7^{\pm0.01b}$	$2.0^{\pm0.12b}$	$0.37^{\pm0.03}$	$4.48^{\pm1.27a}$	$369.7^{\pm26.9bc}$
800	$1.2^{\pm0.02b}$	$5.1^{\pm0.03b}$	$0.23^{\pm0.01}$	$0.90^{\pm0.18b}$	$1242.4^{\pm98.0a}$
ANOVA: *F* values					
Variety (V)	2.9 ns	0.5 ns	2.4 ns	31.8***	5.0*
Cd	46.0***	120.6***	2.6*	11.5***	30.9***
V x Cd	3.7*	0.8 ns	5.3***	3.6*	2.8 ns

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

The interaction between varieties and Cd treatments on the concentration of Zn, Ca, and Na significantly variations in both shoot and root, but it was no significant effect on the K concentration in shoot (Table 5). The Cd stress significantly increased the concentrations of Zn and Na in both shoot and root of two varieties, as well the K concentration in shoot. In shoot and root of purslane varieties, the Ca concentration was diminished by Cd treatments, but it was enhanced in shoot of wild variety. As known, Cd competes with macronutrients such as P, K, Ca, and Mg, and micronutrients such as Zn, Fe, Mn, and Cu for the same transmembrane carrier protein (Rivetta et al. 1997; Sanitá di-Toppi and Gabbrielli 1999). The distribution of toxic metal might affected due to the competition between toxic metal and nutrients in the plant for binding sites in different compartments including plasma membrane, cell wall, and the cell (Nazar et al. 2012). Therefore, the Cd may interfere with nutrient uptake due to its effect on the permeability of plasma membranes. The changes in the Zn, K, and Na concentrations might be expounded by increasing nutrient absorption due to the effect of Cd on plasmalemma permeability and an accumulative effect due to the reduction of growth resulting from Cd toxicity. Solti et al. (2011) have explained that Cd can inhibit mineral nutrition by competition between this metal and other metal ion on poplar (*Populus jaquemontiana* var. glauca). These authors also have mentioned two mechanisms as regards inhibiting metal absorption i.e., inhibition the chelating process of metal ion, like Fe, and the loading of ion into the xylem, and the influence of Cd on Ca in competition for Ca-transporters. The Cd entry through the Ca channel in the leaves results in the disturbing the plant-water relationship, which causing stomatal closure in many plants, leading to lower transpiration rate, and inhibition of photosynthesis due to the adverse effects on chlorophyll metabolism

(Nazar et al. 2012). Zhang et al. (2002) determined that there was an increment in the concentrations of K, Fe, Zn, and Cu of wheat genotypes at the seedling stage, and a reduction in the concentrations of Ca and Mg. Furthermore, many researchers reported that excess Cd increased the concentration of Zn in shoot of tomato and goldenberry (Çikili et al. 2016), rape and wheat genotypes (Zembala et al. 2010), lettuce seedling (Xu et al. 2015), and the Ca concentration in perennial ryegrass (Chen at al 2018). In contrast, with increasing of Cd application, it was reported the reduction in K, Ca and Zn contents of shoot of pea (Sandalio et al. 2001), in K, Ca and Zn contents in roots of soybean genotypes (Shamsi et al. 2010) and barley genotypes (Guo et al. 2007), in K and Ca contents in shoot and root of rape and wheat genotypes (Zembala et al. 2010), in the Zn concentration of shoot and root in lettuce seedling (Xu et al. 2015), and in the Ca concentration of shoot and root perennial ryegrass (Chen at al 2018).

Table 4 The changes in photosynthetic pigments in leaves of purslane varieties exposed to Cd

Cd treatments (µM)	Chl *a*	Chl *b*	Chl *a+b*	Car	Chl *a/b*	Car/Chl
	µg g^{-1} FW					
	P. oleracea L. cv. İstanbul					
0	$133.1^{\pm3.7a}$	$26.7^{\pm1.6a}$	$159.8^{\pm5.3a}$	$78.1^{\pm2.1a}$	$5.01^{\pm0.16}$	$0.489^{\pm0.004bc}$
50	$116.8^{\pm4.8bc}$	$20.9^{\pm1.1bcd}$	$137.7^{\pm5.5bc}$	$67.9^{\pm2.1b}$	$5.60^{\pm0.25}$	$0.493^{\pm0.004bc}$
100	$101.5^{\pm6.4d}$	$17.5^{\pm1.4d}$	$119.0^{\pm7.6d}$	$59.7^{\pm3.0c}$	$5.83^{\pm0.23}$	$0.503^{\pm0.007ab}$
200	$105.4^{\pm0.3cd}$	$22.1^{\pm2.1bc}$	$127.5^{\pm2.2cd}$	$62.0^{\pm0.7c}$	$4.86^{\pm0.49}$	$0.486^{\pm0.11bc}$
400	$119.1^{\pm1.6b}$	$23.7^{\pm0.8ab}$	$142.8^{\pm0.8b}$	$68.2^{\pm0.8b}$	$5.03^{\pm0.25}$	$0.477^{\pm0.006c}$
800	$99.8^{\pm2.9d}$	$18.6^{\pm1.1cd}$	$118.5^{\pm2.2d}$	$61.6^{\pm0.4c}$	$5.41^{\pm0.41}$	$0.520^{\pm0.007a}$
	P. oleracea L. Wild variety					
0	$199.2^{\pm7.8b}$	$35.3^{\pm1.0b}$	$234.5^{\pm5.7b}$	$118.8^{\pm2.5b}$	$5.64^{\pm0.06a}$	$0.507^{\pm0.002b}$
50	$234.5^{\pm5.1a}$	$44.8^{\pm0.7a}$	$279.3^{\pm4.9a}$	$136.2^{\pm2.8a}$	$5.24^{\pm0.17a}$	$0.488^{\pm0.002b}$
100	$179.5^{\pm2.4c}$	$32.5^{\pm1.5bc}$	$211.9^{\pm3.9c}$	$106.7^{\pm0.9c}$	$5.54^{\pm0.18a}$	$0.504^{\pm0.005b}$
200	$142.8^{\pm3.3d}$	$27.6^{\pm3.0d}$	$172.4^{\pm6.2d}$	$86.4^{\pm2.6d}$	$5.27^{\pm0.45a}$	$0.507^{\pm0.008b}$
400	$144.1^{\pm1.8d}$	$29.0^{\pm0.5cd}$	$173.1^{\pm2.2d}$	$86.1^{\pm0.2d}$	$4.98^{\pm0.05a}$	$0.498^{\pm0.006b}$
800	$41.3^{\pm0.5e}$	$17.1^{\pm0.6e}$	$58.4^{\pm0.6e}$	$56.1^{\pm0.8e}$	$2.43^{\pm0.10b}$	$0.961^{\pm0.021a}$
ANOVA: *F* values						
Variety (V)	447.7***	127.1***	429.8***	898.4***	7.81*	286.5***
Cd	211.2***	26.7***	178.9***	150.0***	10.15***	281.9***
V x Cd	137.9***	18.7***	115.8***	95.1***	11.35***	216.4***

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

Table 5 The changes in the concentration of Zn, K, Ca, and Na in shoot and root of purslane varieties exposed to Cd

Cd treatments (µM)	Zn (µg g^{-1} DW)		K (mg g^{-1} DW)		Ca (mg g^{-1} DW)		Na (mg g^{-1} DW)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	P. oleracea L. cv. İstanbul							
0	$65.9^{\pm2.0c}$	$36.7^{\pm1.2c}$	$77.4^{\pm2.5b}$	$40.5^{\pm1.5}$	$8.97^{\pm0.06a}$	$3.63^{\pm0.04b}$	$1.09^{\pm0.05b}$	$1.61^{\pm0.01d}$
50	$76.9^{\pm1.3b}$	$50.7^{\pm2.2b}$	$82.6^{\pm0.4b}$	$42.0^{\pm3.2}$	$8.30^{\pm0.42ab}$	$3.72^{\pm0.06ab}$	$1.06^{\pm0.02b}$	$1.37^{\pm0.00e}$
100	$87.1^{\pm1.2a}$	$55.6^{\pm1.7b}$	$85.3^{\pm1.3b}$	$42.9^{\pm0.6}$	$7.15^{\pm0.34bc}$	$3.71^{\pm0.06ab}$	$1.26^{\pm0.02a}$	$2.22^{\pm0.01b}$
200	$83.4^{\pm1.5ab}$	$53.1^{\pm3.0b}$	$87.7^{\pm1.8b}$	$40.3^{\pm0.3}$	$5.92^{\pm0.53cd}$	$3.95^{\pm0.19a}$	$1.29^{\pm0.02a}$	$2.59^{\pm0.01a}$
400	$77.2^{\pm5.0b}$	$59.2^{\pm5.3b}$	$102.6^{\pm1.0a}$	$39.1^{\pm0.1}$	$5.59^{\pm0.85d}$	$3.91^{\pm0.05ab}$	$1.32^{\pm0.05a}$	$2.23^{\pm0.00b}$
800	$82.8^{\pm1.9ab}$	$73.9^{\pm0.7a}$	$104.9^{\pm7.6a}$	$38.6^{\pm0.1}$	$4.66^{\pm0.26d}$	$2.77^{\pm0.05c}$	$1.28^{\pm0.03a}$	$2.03^{\pm0.00c}$
	P. oleracea L. Wild variety							
0	$54.4^{\pm1.5d}$	$39.8^{\pm0.3c}$	$90.9^{\pm0.5bc}$	$37.3^{\pm0.1c}$	$3.70^{\pm0.12c}$	$2.86^{\pm0.14a}$	$1.16^{\pm0.03c}$	$1.60^{\pm0.10d}$
50	$60.6^{\pm2.7c}$	$56.4^{\pm3.6b}$	$85.6^{\pm3.2c}$	$41.2^{\pm0.5b}$	$7.15^{\pm0.21a}$	$2.45^{\pm0.41a}$	$1.13^{\pm0.11c}$	$2.05^{\pm0.04bc}$
100	$68.1^{\pm3.1b}$	$56.9^{\pm1.7b}$	$98.3^{\pm5.4b}$	$40.6^{\pm2.0bc}$	$5.75^{\pm0.26b}$	$1.50^{\pm0.08b}$	$1.52^{\pm0.01b}$	$1.94^{\pm0.14bc}$
200	$61.5^{\pm1.0c}$	$63.0^{\pm3.8b}$	$95.0^{\pm7.2bc}$	$41.0^{\pm1.5b}$	$7.05^{\pm0.20a}$	$1.22^{\pm0.03bc}$	$1.41^{\pm0.04b}$	$2.64^{\pm0.12a}$
400	$70.0^{\pm1.4b}$	$58.6^{\pm0.9b}$	$96.2^{\pm1.1bc}$	$45.9^{\pm0.6a}$	$5.75^{\pm0.14b}$	$0.83^{\pm0.06c}$	$1.43^{\pm0.06b}$	$2.15^{\pm0.12b}$
800	$79.3^{\pm0.7a}$	$101.6^{\pm1.6a}$	$112.7^{\pm1.2a}$	$47.1^{\pm0.1a}$	$6.02^{\pm0.02b}$	$0.76^{\pm0.01c}$	$1.97^{\pm0.03a}$	$1.82^{\pm0.01cd}$
ANOVA: *F* values								
Variety (V)	103.5***	27.5***	9.06**	4.8*	17.1***	494.4***	48.1***	0.3 ns
Cd	21.0***	77.1***	13.3***	2.5 ns	10.5***	22.0***	33.8***	47.3***
V x Cd	4.95**	8.1***	2.0 ns	7.2***	22.9***	29.5***	16.8***	10.7***

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

A significant interaction between purslane varieties and Cd levels on the MP, RWC, lipid peroxidation, H_2O_2 content, and proline accumulation was given in the Table 6. The MP enhanced progressively with increasing of Cd supply in shoot of cv. Istanbul, whereas it diminished in wild variety. The significant variations about the MP in purslane varieties were observed by Cd exposure, and it was supposed that the reductions in the MP of wild variety might be related to an increment in the shoot Ca concentration with Cd exposure. It was conspicuous that the Ca concentration in shoot of wild variety substantially enhanced with increasing Cd exposure, but it diminished in shoot of cv. İstanbul (Table 5). It is well known that Ca is essential nutrient element to strengthen the cell walls and cell division, and it is possible that the Ca efflux in cell may be impaired the cell integrity. The presence of Ca^{2+} declined the Cd- stimulated leakage of solutes in bean (*Phaseolus vulgaris* L.) as previously reported by Fuhrer (1982). Zembala et al. (2010) have stated that Cd stress bring about a decrease in plasmalemma fluidity and an increment in ion leakage of leaves in rape and wheat genotypes. Besides, the Cd is responsible for structural damage to cellular organelles, like increased membrane permeability thus leading to leakage of intercellular contents (Ahmad et al. 2016). It was demonstrated that Cd stress was in charge of electrolyte leakage in mustard Ahmad et al. (2016) and an increment in the MP for sunflower (Samet et al. 2017).

Table 6 The changes in membrane permeability (MP), relative water content (RWC), contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), and proline accumulation in leaves of purslane varieties exposed to Cd

Cd treatments (μM)	MP (EC %)	RWC	MDA (nmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	Proline (nmol g^{-1} FW)
P. oleracea L. cv. İstanbul					
0	$21.6^{\pm0.64c}$	$92.3^{\pm0.11}$	$0.95^{\pm0.05b}$	$1.30^{\pm0.03c}$	$195.6^{\pm9.7a}$
50	$32.0^{\pm3.82b}$	$91.5^{\pm0.07}$	$1.03^{\pm0.06b}$	$5.03^{\pm0.40a}$	$142.5^{\pm4.4b}$
100	$35.2^{\pm2.95b}$	$90.9^{\pm0.49}$	$1.05^{\pm0.05b}$	$4.28^{\pm0.17a}$	$111.9^{\pm5.8.bc}$
200	$48.3^{\pm0.40a}$	$90.3^{\pm1.03}$	$1.08^{\pm0.06b}$	$3.30^{\pm0.43b}$	$99.1^{\pm9.8cd}$
400	$55.6^{\pm0.49a}$	$90.4^{\pm0.73}$	$1.12^{\pm0.08b}$	$2.00^{\pm0.03c}$	$57.2^{\pm8.5e}$
800	$53.3^{\pm4.54a}$	$89.8^{\pm0.43}$	$1.48^{\pm0.02a}$	$1.43^{\pm0.10c}$	$73.1^{\pm8.5de}$
P. oleracea L. Wild variety					
0	$52.5^{\pm0.73a}$	$90.4^{\pm0.44b}$	$0.86^{\pm0.03c}$	$1.33^{\pm0.17c}$	$200.4^{\pm4.1a}$
50	$48.1^{\pm1.99b}$	$93.4^{\pm0.40a}$	$0.82^{\pm0.04c}$	$3.59^{\pm0.26b}$	$208.5^{\pm9.7a}$
100	$42.3^{\pm0.19c}$	$87.8^{\pm0.40c}$	$0.97^{\pm0.04b}$	$4.88^{\pm0.45a}$	$195.6^{\pm3.8a}$
200	$28.3^{\pm0.73d}$	$87.5^{\pm0.68c}$	$1.03^{\pm0.01b}$	$2.03^{\pm0.17c}$	$157.0^{\pm3.0b}$
400	$29.9^{\pm1.31d}$	$86.8^{\pm0.34c}$	$0.99^{\pm0.02b}$	$1.92^{\pm0.34c}$	$163.4^{\pm3.3b}$
800	$49.5^{\pm0.28ab}$	$81.6^{\pm0.23d}$	$1.43^{\pm0.04a}$	$1.32^{\pm0.04c}$	$150.2^{\pm1.8b}$
ANOVA: *F* values					
Variety (V)	0.4 ns	105.4***	14.5***	6.2*	170.2***
Cd	13.0***	44.5***	39.4***	59.8***	33.4***
V x Cd	54.3***	21.0***	1.0 ns	4.6**	7.6***

Values are expressed as means ±SE of three replicates (n = 3). Different letters in the same column for each variety are significantly different according to the LSD ($\alpha < 0.05$).

The RWC as an indicator of phytotoxicity after HM stress was reduced significantly in both purslane varieties, except for 50 μM Cd treatments for wild variety (Table 6). Irrespective of Cd treatments, the RWC in wild variety was more reduction than those of cv. İstanbul. A reduction in RWC might be related to water-absorbing capacity with varying levels as well its ability to prevent water loss through stomata (Bayoumi et al. 2008). Also, the other reason might be that varieties have production capacity even at high level of H_2O_2 and lipid peroxidation. The reduction in RWC in the present study confirms the findings of Ahmad et al. (2016) in mustard. Furthermore, De Maria et al. (2013) reported that leaf osmotic potential was decreased with increasing the levels of Cd in sunflower plants, but no changes significant in RWC.

Lipid peroxidation, the H_2O_2 content, and proline accumulation were notably increased by Cd treatments in both purslane varieties; however, the changes in the H_2O_2 content were significant up to 200 μM Cd level (Table 6). The Cd as a non-redox metal unable to perform single electron transfer reactions does not produce ROS such as the singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and H_2O_2; however, it generates oxidative stress by interfering with the antioxidant defense system (Tran and Popova 2013, Benavides et al. 2005). Excess Cd can lead to oxidative stress by virtue of the increase in ROS, which could potentiate the accumulation of MDA, an indicator of Cd-induced oxidative damage to the membranes (Tran and Popova 2013). Many studies revealed that increasing Cd levels enhanced the contents of MDA and H_2O_2 in plant species, such as lettuce (Xu et al. 2015), perennial ryegrass (Chen et al. 2018; Wang et al. 2013), soybean genotypes (Shamsi

et al. 2010), and safflower genotypes (Moradi and Ehsanzadeh 2015). Singh and Agrawal (2007) reported that exposed to HMs (Cd, Ni, Zn, Pb, Cr, and Cu) due to sewage sludge amendment enhanced lipid peroxidation in *Beta vulgaris* plants.

The proline accumulation was decreased with increasing level of Cd in shoot of both varieties. When compared to control, the reduction in proline accumulation at the highest Cd level was 62.6% in cv. Istanbul and 25.0% in wild variety. Also, regardless of Cd treatment, the proline accumulation of wild variety was high than that of cv. İstanbul. The present study shows the accompanying reduction in the proline content with increasing of Cd exposure in two purslane varieties. Similar results was reported by Nikolić et al. (2008), who stated that proline content was not markedly altered in plants exposed to 10^{-5} M Cd in leaves of hybrid poplar (*Populus nigra* × *maximowitzii* × *P.nigra* var. *Italica*); however, when exposed Cd enhanced to 10^{-4} M, proline accumulation reduced by 21.7% in leaves, and increased by 97.8% in roots. Proline is involved in HM detoxification processes, and free proline chelates Cd ions, resulting in the formation of a non-toxic Cd-proline complex (Sharma et al. 1998). These reductions in proline accumulation in shoot of purslane varieties might be explained that the quantity of free proline in cell will have reduced in consequence of an increment in quantity of non-toxic Cd-proline complex with increasing shoot Cd concentration. In contrast to our findings, Cd-induced increasing proline accumulation is a frequently noticed phenomenon, and previously documented for several plants such as mustard (Ahmad et al. 2016), safflower genotypes (Moradi and Ehsanzadeh 2015), and wheat (Tripathi et al. 2013). Tripathi et al. (2013) explained this fact that an increment in proline accumulation due to Cu and Cd exposure was negatively correlated with the wheat growth, and the activities of pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) increased, but the activity of proline dehydrogenase (PDH) reduced sharply in the early phase (up to12 h) of metal exposure.

Moreover, Singh and Agrawal (2007) reported an increment in proline contents for *Beta vulgaris* with the increasing concentration of HMs in soil due to sewage sludge amendment.

Conclusion

The present study clearly indicated that Cd exposure progressively depressed plant growth in purslane varieties and caused the reductions in contents of photosynthetic pigments, RWC, the BCF of shoot and root, and proline accumulation. The concentrations of Cd, Zn, and Na in shoot and root, net Cd accumulation via root, and TAR of Cd were increased by Cd exposure. The root length, the MP of the leaves, TF of Cd and the concentrations of K and Ca were varied according to varieties. The reductions in biomass production and the contents of photosynthetic pigments in wild variety were higher than in cv. Istanbul. Based on these results, this study suggested that the effect of Cd stress was more obvious in wild variety (Cd-sensitive) than in cv. Istanbul (Cd-tolerant).

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Araştırma Makalesi/*Research Article (Original Paper)*

The Effect of Different Application Times of GA_3 on Physical Characteristics of Black Myrtle Berries (*Myrtus communis* L.)

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Abstract: Myrtle (*Myrtus communis* L.) is a widespread Mediterranean shrub that is one of the most characteristic species in Turkey. Its berries and leaves are employed for food, pharmacology and cosmetic. Nowadays, there is a big demand for black myrtle fruits because of its high antioxidant capacity. But it contains high amount of seeds and this situation reduces marketing and production. Gibberellic acid (GA_3) is one of the most decreased seed number from 9.20 (control) to 2.17 seed/fruit. In addition, with three consecutive application of GA_3, seed rate decreased to 5.38% while 14.54% in control. Additionally important hormones used to improve fruit quality and provide seedlessness. The main objective of this study is to investigate the effects of GA_3 on seedlesness and quality in black myrtle fruits. Studies were carried out in the orchard of black myrtle cultivar located in Antalya. At different flowering stages, GA_3 dose of 100 ppm were applied to the whole of the plants. Some applications significantly reduced seed number in fruits. Three applications of GA_3 at ballon, full bloom and fruit set stages consecutively, these GA_3 applications caused the total seed weight reduced to 29.92 (mg/fruit) from 99.13 (mg/fruit) in control. Some GA_3 applications resulted in fully seedless fruits up to 12.00%. In addition, GA_3 applications did not change at fruit weight and fruit size of fruits.

Key words: *Myrtus communis*, Growth regulators, Fruit, Seedlessness

GA_3'in Farklı Uygulama Zamanlarının Siyah Mersin (*Myrtus communis* L.) Meyvelerinin Fiziksel Özellikleri Üzerine Etkisi

Özet: Mersin, Türkiye'de en karakteristik türlerden biri olan yaygın Akdeniz çalısıdır. Meyveleri ve yaprakları gıda, farmakoloji ve kozmetikte kullanılmaktadır. Günümüzde yüksek antioksidan kapasitesi nedeniyle siyah mersin meyvelerine büyük bir talep bulunmaktadır. Ancak meyveleri yüksek miktarda çekirdek içermekte ve bu durum meyvelerin pazarlamasını ve üretimi azaltmaktadır. Bu çalışmanın temel amacı, siyah mersin meyvelerinde Gibberellic acid (GA3)'in çekirdek ve meyve kalitesi üzerindeki etkilerini araştırmaktır. Çalışmalar Antalya'da bulunan siyah mersin bahçesinde gerçekleştirilmiştir. Çiçeklenmenin farklı aşamalarında, tüm bitkilere 100 ppm GA_3 uygulanmıştır. Bazı uygulamalar meyvelerdeki çekirdek sayısını önemli ölçüde azaltmıştır. Balon, tam çiçeklenme ve meyve tutumunda GA_3'in ardı ardına üç kez uygulanması çekirdek sayısının 9.20'den (kontrol) ile 2.17'ye (adet/meyve) düşmesine neden olmuştur. Ayrıca, üç ardışık GA_3 uygulamasıyla, çekirdek oranı kontrolde %14.54 den %5.38'e azalmıştır. Ek olarak GA_3 uygulamaları, kontrolde 99.13 (mg / meyve) olan toplam çekirdek ağırlığının 29.92'ye (mg /meyve) düşmesine neden olmuştur. Çalışmada bazı GA_3 uygulamalarında %12.00 oranında tamamen çekirdeksiz meyvelerin oluştuğu belirlenmiştir. Ayrca, GA_3 uygulamaları sonucunda meyve ağırlığı ve meyve büyüklüğünde değişim olmamıştır.

Anahtar Kelimeler: *Myrtus communis*, Büyüme düzenleyici madde, Meyve, Çekirdeksizlik

Introduction

Myrtle (*Myrtus communis* L.) is a widespread Mediterranean shrub that is one of the most characteristic species in Turkey. It grows naturally especially in 500-600 m above sea level in the coastal areas of Aegean, Marmara and Mediterranean regions that have the Mediterranean climate (Oğur 1994; Baytop 1999). Furthermore, there is no registered myrtle cultivar in Turkey (Uzun and Bayır 2012).

M. communis L. is belonging to the Myrtaceae family. This family contains stilbene compounds that have some significant biological activities (Keskin and Kunter 2017).

Myrtle is traditionally used in the treatment of stomach, hypoglycemic and oral diseases. It is also used for constipation, building an appetite, reducing hemorrhage and externally for wound healing (Duke 1988; Twaij *et al.* 1989; Ogur 1994; Ozek *et al*. 2000). *M. communis* has been used for medicinal and nutritional purposes since ancient times. The leaves and fruits of myrtle traditionally used as antiseptic, disinfectant, and hypoglycemic agents (Barboni *et al*. 2010).

Myrtle is known as "Mersin" or "Murt" or "Hambeles" in Mediterranean Region of Turkey (Aydın and Özcan 2007). Myrtle fruits have white and black colour. White myrtles which are called Hambeles are mostly cultivated and consumed mainly as fresh fruit in Turkey. Mersin fruits are collected from the naturally grown areas by local people and sold in the regional markets. The black myrtle fruits are mainly consumed as fresh fruit (edible), dried fruit and fruit tea, also it is used in the marmalade and jam production. Due to the fact that the fruit shelf life of black myrtle is quite long (about 3-4 months), it remains in the market for a long time for fresh consumption. Black myrtle has a lot of small seeds inside while Hambeles with greater fruit size contains rudimentary seeds but shelf-life is very short. In Italy, particularly in Sardinia, black berry is used to produce the characteristic myrtle liqueur (Mulas *et al.* 2000).

Nowadays myrtle berries and leaves are mostly employed for the formulation of liquors and the good antioxidant activity of myrtle liquors has also been reported (Tuberoso *et al*. 2010). Black berries, that are astringent but sweetish and edible, contain flavonoids and anthocyanins (mainly myricetin glycosides), having strong antioxidant properties (Barboni *et al*. 2010). There is great interest for black myrtle fruits because of their high antioxidant capacities and phenolic properties. However, the main complained subject of black myrtle which is emphasized by fresh-fruit consumer is the large number of hard seeds inside fruit.

Gibberellic acid (GA3) is one of the most important hormones used to improve fruit quality and provide seedlessness. With GA_3 applications, successful results have been obtained in seedless fruit production in some fruit types such as grapes, loquat, pears, and cherries at various stages of production. 100 ppm GA_3 applications provided seedlessness before the flowering in Çavuş, Balbal ve Hamburg Misketi grape varieties (Fidan 1969). Also 250 ppm GA_3 seedless fruit production has been provided in loquat (Goubran and El-Zeftawi 1986; Lin *et al.* 1999). These ratios varied according to type, dose and time. Its known to increase the parthenocarpic fruit production like auxins and even they are sometimes more efficient (Seçer 1989; Westwood 1993; Erio 1998; Bora and Sarma 2006). Therefore, this study was conducted to determine the effects of gibberellic acid applications in different times around flowering on seedlesness and some physical fruit characteristics in the black myrtle fruits.

Material and Methods

The research was carried out in the orchard of 7 years old of "Yakup" black myrtle cultivar located in Antalya. Trees were planted 3x3 m apart and grown on terra-rosa soil with drip irrigation. GA_3 (G7645-sigma) applications were made at the dates specified in 2017 (Table 1). Before the applications, GA_3 was dissolved in ethyl alcohol at a dose of 100 ppm and 0.1% spreader adhesive (Wax-Wet) was added. At different flowering stages, GA_3 dose of 100 ppm was applied to the whole of the plants. In the control application, only water + emissive adhesive mixture was applied to the trees. The applications were done in 3 replicates as each tree is one replicate. Fruits were harvested when soluble solids reached 21% on 16 November 2017. In the harvested fruits; fruit weight (mg), fruit volume (ml), fruit diameter and length (mm), developed seed number per fruit (number/fruit), undeveloped (rudimentary; seeds have 2 mg or less weight) seeds (seed/fruit), total seed weight (mg/fruit), seed ratio (%) and seedless fruit ratio (%) were determined. The study was set up according to the "Randomized Blocks Experimental Design". In the variance analysis for the applications, the differences between the averages were determined with Tukey multiple comparison test at 5% level.

Table 1. GA_3 application periods and doses

Application	GA_3 Aplication Periods	GA_3 Doses
Control	Ballon (B)	0 ppm
1st App.	Ballon (B)	100 ppm
2nd App.	Ballon (B) + Full Bloom (FB)	100 ppm + 100 ppm
3rd App.	Ballon (B) + Full Bloom (FB) + Fruit Set (FS)	100 ppm + 100 ppm+100 ppm
4th App.	Full Bloom (FB)	100 ppm
5th App.	Full Bloom (FB) + Fruit Set (FS)	100 ppm + 100 ppm
6th App.	Fruit Set (FS)	100 ppm

Results and Discussion

The results of the GA_3 application on the fruit characteristics of black myrtle are given in Table 2. In the study, the statistical difference between applications in terms of fruit weight was not significant. With applications, the highest value of fruit weight was obtained in the control group (680.70 mg) while the lowest value was determined with one application of GA_3 at fruit set stage (544.36 mg) (Table 2). Similarly, Alım and Uzun (2017) indicated that 100 ppm GA_3 application to black myrtle fruits at different times was not effected on fruit weight. In addition, Uzun *et al.* (2016) determined that the fruit weight of black myrtle (Yakup) is compatible with the results obtained with values between 0.76-0.90 g. The effect of GA_3 on the fruit weight and volume vary depending on the dose, time, and fruit variety. Fruit weight and fruit size values (volume, diameter and length) are not significantly affected by GA_3 treatments. Fruit weight ranged from 544.36 mg/fruit in fruit set treatment to 680.70 mg/fruit in control. According to a study made in strawberry fruit weight showed a decrease with application of GA_3, when compared to the control group (13.7 g); 50 and 100 ppm GA_3 treatments statistically decreased fruit weight of strawberry (13.01 g and 12.33 g, respectively) (Eshghi *et al*. 2012).

When assessed for effect on fruit volume of GA_3 applications, it was similar to fruit weight values. The highest fruit volume value (0.83 ml) was found in the control, while the lowest was found in two applications of GA_3 at and Full Bloom stages consecutively and one application of GA_3 at fruit set stage (0.67 ml). According to a study made in strawberry, fruit volume showed a statistical decrease with the application of GA_3 (Gündogdu *et al*. 2017).

The effect of the applications GA_3 on the fruit diameter and length were not statistically significant. The highest value of fruit diameter and length was obtained in the control group (10.57 mm and 12.20 mm, respectively) while the lowest value of fruit diameter was determined with three applications of GA_3 at ballon, full bloom and fruit set stages consecutively (9.28 mm), fruit length was determined with two applications of GA_3 at Ballon and Full Bloom stages consecutively (10.64 mm). In another study, Alım and Uzun (2017) reported that the diameter of the fruit decreased in parallel with the number of seeds.

In the study, the statistical difference between applications in terms of fruit flesh ratio is significant. The highest fruit flesh ratio (94.62%) was found in three applications of GA_3 at ballon, full bloom and fruit set stages consecutively, while the lowest was found in the control (85.46%). Although the developed number of seeds decreased, GA_3 applications increased the rate of fruit flesh ratio. Moreover, these applications did not affect the fruit weight. This is a very important result in terms of fruit quality.

Although the effect of GA_3 applications on seedless fruit rate was not statistically significant in the study, the highest seedless fruit ratio was found in three applications of GA_3 at ballon, full bloom and fruit set stages consecutively (12.00%), while the lowest was found in the control, one applications of GA_3 at ballon stage, two applications of GA_3 at ballon and full bloom stages consecutively and application of GA_3 at full bloom stages (0.00%). Similarly, Alım and Uzun (2017) indicated that (B+FB+FS) 100 ppm GA_3 applications at ballon, full bloom and fruit set stages consecutively (10.67%) increased seedless fruit ratio compared control fruits (0.00%).

Table 2. Effects of GA_3 applications on black myrtle fruit characteristics

Application	Fruit Weight (mg)	Fruit Volume (ml)	Fruit Diameter (mm)	Fruit Length (mm)	Fruit Flesh Ratio (%)	Seedless Fruit Ratio (%)
Control	680.70	0.83	10.57	12.20	85.46c*	0.00
1st App.	656.26	0.80	10.30	11.56	90.06b	0.00
2nd App.	549.26	0.67	9.75	10.64	90.41b	0.00
3rd App.	578.69	0.71	9.28	10.90	94.62a	12.00
4th App.	657.62	0.80	10.40	11.51	91.51ab	0.00
5th App.	644.44	0.79	9.94	11.96	93.64ab	2.67
6th App.	544.36	0.67	9.48	10.90	91.64ab	6.67

*There are significant differences ($p<0.05$) between means indicated same later in the same column

GA_3 applications were statistically significant on the number of developed seeds of black myrtle fruits depending on the application time. The applications caused a significant reduction in the number of seed of fruits compared to the control. The lowest developed seed number was found in three applications of GA_3 at ballon, full bloom and fruit set stages consecutively (2.17 seed/fruit), while the highest was found in the control (9.20 seed/fruit). GA_3 treatments have reduced the number of seeds, while fruit weight and fruit size did not change (Table 3).

The seed number results of the present study are similar to the studies done by Alım and Uzun (2017) and Uzun *et al.* (2016) in black myrtle; Fellman *et al*. (1991) in grapes and Aslmoshtaghi and Shahshavar 2013) in loquat.

Table 3. Effects of GA_3 applications on black myrtle seeds characteristics

Application	Developed Seed (seed/fruit)	Rudimentary Seed (seed/fruit)	Total Seed Weight (mg/fruit)	Seed Ratio (%)
Control	9.20a*	1.71	99.13a	14.54a
1st App.	5.69b	1.83	65.36b	9.94b
2nd App.	4.43bc	1.80	52.64bc	9.59b
3rd App.	2.17c	1.05	29.92c	5.38c
4th App.	4.72bc	1.97	55.99bc	8.49bc
5th App.	3.13bc	1.62	40.29bc	6.35bc
6th App.	3.64bc	1.21	46.14bc	8.36bc

*There are significant differences ($p<0.05$) between means indicated same later in the same column

In the study, difference between the applications in terms of total seed weight and seed ratio was determined. Total seed weight was reduced in parallel with the number of developed seed when compared to control.

In the present study, it was found that total developed seed weight was the lowest in three applications of GA_3 at ballon, full bloom and fruit set stages consecutively. At the same time, this application was found to contain the least number of developed seed (29.92 mg/fruit), while the highest was found in the control (99.13 mg/fruit). Similarly, Alım and Uzun (2017) indicated that two applications of GA_3 at ballon and full bloom stages (10.67%) decreased total seed weight compared control fruits (94.27 mg/fruit). Furthermore, Lu *et al.* (1997) reported that lowered the total seed weight of GA_3 applications in grape.

As a result of the study, it was determined that GA_3 applications influenced the fruit quality of black myrtle. Some applications significantly reduced seed number in fruits. Three applications of GA_3 at ballon, full bloom and fruit set stages consecutively decreased seed number. In general, three applications of GA_3 at ballon, full bloom and fruit set stages consecutively increased the fruit quality more.

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Araştırma Makalesi/*Research Article (Original Paper)*

Determination of the Considerations of the Farmers about Irrigation Organizations by Factor Analysis

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Abstract: In this study, the considerations of the farmers who were affiliated to the organizations undertaking the irrigation administration in Edirne, Kırklareli, Tekirdağ and Çanakkale provinces about irrigation organizations were analyzed. Within the scope of the study, surveys were conducted in 70 irrigation cooperatives, 67 municipality and legal entities, 7 irrigation unions and 1 DSI irrigation organization. Total of 567, 113, 227 and 7 surveys were conducted in the irrigation facilities administrated by irrigation cooperatives, irrigation unions, municipality and legal entities and DSI, respectively. Total of 301 surveys in 74 villages of 9 districts in Edirne, 168 surveys in 43 villages of 5 districts in Tekirdağ, 156 surveys in 36 villages of 4 districts in Kırklareli and 289 surveys in 69 villages of 11 districts in Çanakkale were conducted. The conclusions of the farmers were evaluated by using five point likert scale. It was determined that the irrigation organizations did not notice the training of the farmers, did not include these trainings in their working schedules and similarly, they did not conduct studies aimed at the conscious and balanced use of the agricultural inputs. Factor analysis was used for the evaluation of the considerations of the farmers about irrigation organizations. KMO and Barlett test statistics was used in order to examine the convenience of the considerations to factor analysis and KMO value was found as 0.891. According to factor analysis results, 13 variables were gathered in three factor groups named as "Administration", "Ownership and Service" and "Consciousness Raising".

Keywords: Irrigation organization, factor analysis, farmer

Introduction

Water management is defined as the development, distribution and usage of the water sources. The main purpose in the administration of the irrigation organizations is to increase the farmers' incomes and therefore realize the efficient distribution and usage of the water sources. Irrigation management can be defined as an organization which provides the distribution and usage of the water in order to realize the irrigation purposes in the agriculture. The agricultural irrigation management studies in our country include the studies such as the general irrigation planning before irrigation season, preparation, application and observation of the water distribution programs in the irrigations season and the evaluations at the end of the irrigation season (Eminoğlu 2007).

Development of the soil and water sources and determination of the utilization principles are required in order to provide the rural development and increase the production in the agriculture sector. The studies aimed at the composing of the agricultural foundation, efficient management and usage of the sources are significant for the development of the soil and water sources. On the other hand, farmers' full economic and social participation to the irrigation administration and rational management of the irrigation organizations should be provided in order to maintain the sustainability of the utilization from the soil and water sources. Mental, physical and financial participation of the farmers will allow the efficient usage of the farmers. In this regard, determination of the suitable administration types is required for the determination of the policies aimed at the transfer of the irrigation facilities to the users.

Nowadays, irrigation administration is generally done by irrigation unions, irrigation cooperatives and municipalities or legal entities. The problems increased due to the increases in the irrigation areas and the yield expected from irrigation could not reach to the desired levels. Concordantly, the government left the irrigation administration to the unions and other organizations for more efficient and economic irrigation and irrigation administration (Özkan et al. 2011).

Government Irrigation Administration: The administration, maintenance and repair of the irrigation facilities are done by the government organizations after the construction of the irrigation facilities. Government irrigation administration in our country is observed in the irrigation facilities constructed by DSI in accordance with the law no 6200.

Irrigation Cooperative Administration: Irrigation cooperatives are the organizations which the farmers organize by combining the economic potentials in order to utilize from underground and ground sources due to the law no 1163 (Ertan and Kaya 2006). The management of the irrigation cooperative consists of the general board, board of management and supervisory board. The aim of the irrigation cooperatives is to obtain the water for the agricultural production to the farmers, make equitable distribution and provide the efficient usage of the water.
Local Government Administration (Municipalities and Legal Entities): The local governments are significant for the sustainability of the services in spite of the main competent central organizations in water management area. The administration, maintenance and repair of the irrigation facility are carried out by the decisions of the mayor and the councilors. The structure of the personnel differs according to the importance and size of the facility. Generally, water distribution planning is not done in the local governments and irrigation is done as giving water to the farmers according to the reserve (Akıllı 2011).

Irrigation Union Administration: The administration of the irrigation facilities is done by the irrigation unions. The irrigation union conducts the administration, maintenance and repair activities of the facility according to the principles in the transfer contract. The responsibilities of the irrigation union are to repay the participation price of the facility, collect the share, water service price and the fine, contribute to the realization of the production targets and pay the administration and maintenance costs for the common facilities.

Süheri and Topak (2005) compared three water user organizations, two irrigation cooperatives and two municipality operated organizations in Konya Plain and indicated that water user associated with organizations measured water at source and delivered points regularly. Sayın et al. (2013) compared 29 irrigation organizations in the province of Antalya using a number of performance indicators. Sufficiency, efficiency, sustainability and producer satisfaction were used as criteria for the productivity of irrigation networks. Aydoğdu et al. (2015) determined the views and perceptions of the presidents' to water management and operations, implemented related to Water User Associations' regularly. Ünver (2016) examined water resources management irrigation cooperatives and cooperatives partners. Administrative and practical operation of irrigation cooperatives were evaluated for this purpose. Environmental awareness was revealed concerning the manufacturer of water resources in the region. The opinions of the farmers who were the partner of irrigation cooperatives were evaluated for water management.

The considerations of the farmers about irrigation organizations in Edirne, Kırklareli, Tekirdağ and Çanakkale provinces were examined in this study. It will be utilized from the results for the solution of the irrigation administration problems, determination and application of the policies related with the subject.

Material and Method

Data were collected from the farmers who utilized from the irrigation organizations operated by irrigation cooperatives and municipalities in Edirne, Kırklareli, Tekirdağ and Çanakkale provinces and irrigation unions in Çanakkale province. This study was mainly carried out in irrigation cooperatives, irrigation unions and municipalities. Besides, one irrigation organization which was managed by DSI was included to the study. Surveys were conducted in 70 irrigation cooperatives, 7 irrigation unions, 67 municipality and legal entities and 1 DSI irrigation organization.

The surveys were conducted in all of the irrigation cooperatives, irrigation unions and municipality and legal entities. The sample size of the producers was calculated according to the sampling method in the previous studies (Alder and Roessler 1977; Aksoy et al. 1996). The sampling unit was composed of the producers who were randomly selected from each irrigation facility. The sample size was determined to be as 4 farmers from each irrigation facility and this number was considered to be enough for each irrigation facility.

Total of 567 surveys were conducted in the irrigation organizations administrated by the irrigation cooperatives. Besides, 113 surveys in irrigation unions, 227 surveys in municipality and legal entities and 7 surveys in the irrigation organization administrated by DSI were conducted.

Total of 301 surveys in 74 villages of 9 districts in Edirne, 168 surveys in 43 villages of 5 districts in Tekirdağ, 156 surveys in 36 villages of 4 districts in Kırklareli and 289 surveys in 69 villages of 11 districts in Çanakkale province were conducted.

Descriptive statistics were applied to the data. For this purpose, it was utilized from averages, frequency distributions, minimum and maximum values. T test was used for the analysis of normally distributed continuous data.

The considerations of the farmers about the irrigation organizations were measured by 5 point likert scale and evaluated by factor analysis. A Likert Scale is a type of rating scale used to measure attitudes or opinions. With this scale, respondents are asked to rate items on a level of agreement.

- Strongly agree
- Agree
- Neutral
- Disagree
- Strongly disagree

The aim of factor analysis is to summarize the relationship between the variables. This relationship can be explained with some variables of factors derived from original variables. The aim is to present a comprehensible solution (Gorsuch 1983). Generally, the first step of factor analysis is to explain the interaction between the variables. Correlation coefficient is used for the scale of this relationship. The correlation matrix indicates that the relationship between the variables is positive. Besides, it indicates that whether the correlations can be explained by smaller hypothetic variables or not (Kim and Mueller 1978). Mathematically, factor analysis is similar to multiple regression analysis. The specific variables group by undertaking a factor and the data are grouped by considering the total variance. The conformity of the data to factor analysis is determined by Bartlett Test of Sphericity and Kaiser-Meyer-Olkin test. Bartlett Test of Sphericity tests the probability that there are high ratio correlations between some variables. According to Bartlett Test of Sphericity, factor analysis cannot be done if "Correlation matrix is unit matrix" hypothesis is not rejected (Tucker and LaFleur 1991).

Coefficient of partial correlation is another indicator of the relationship between the variables. Kaiser-Meyer-Olkin (KMO) test is an index which compares the size of the observed correlation coefficients. KMO value limits are as follows;

- > 0.90 perfect,
- 0.80-0.90, excellent
- 0.70-0.80, good,
- 0.60-0.70, normal,
- < 0.60, inacceptable (Pett et al. 2003).

Alpha (α) model was used in order to analyze the reliability of the scales. This coefficient is between 0 and 1 and is named as Cronbach Alpha Coefficient. Alpha coefficient limits are as follows;

- $0.00 \leq \alpha < 0.40$ the scale is not reliable,
- $0.40 \leq \alpha < 0.60$, the reliability of the scale is low,
- $0.60 \leq \alpha < 0.80$, the scale is rather reliable,
- $0.80 \leq \alpha < 1.00$, the scale is very reliable (Kalaycı et al. 2005).

Results

When the age distributions of the farmers were examined, it was determined that 31.2% of the farmers were in 41-50 age interval and 30.9% of the farmers were in 51-60 age interval. Besides, 19.3%, 4.5% and 14.1% of the farmers were in 31-40 age interval, 20-30 age interval and over the age of 60 years, respectively. It was determined that 70.6% of the farmers were primary school graduate and 14.5% and 13.2% of the farmers were high-school and secondary school graduate, respectively. Approximately, 2/3 of the farmers (57.4%) of the farmers had 4-6 persons and 34.5% of the farmers had 1-3 persons in their families.

It was concluded that 25.7% of the farmers participated in the administration of the irrigation organization recently whereas 42.5% of the farmers participated in the administration of the irrigation organization in the past. Besides, 52.5%, 19.1% and 15.6% of the farmers stated that the irrigation administration should be done by irrigation cooperatives, DSI and municipalities, respectively.

Generally, it was concluded that the evaluations of the farmers in terms of some considerations of the irrigation

organizations were not different. The certain two results were determined as that the irrigation organizations did not care the trainings of the farmers, did not include these trainings to the working programs and did not perform studies aimed at the conscious use of the agricultural inputs (Table 1).

Table 1. Considerations of the farmers about irrigation organizations

	Irrigation Organization			
	Irrigation Cooperative	Municipality/Legal Entity	Irrigation Union	DSI
Directors of the irrigation organization are reliable	4.01	4.32	3.64	4.86
Directors of the irrigation organization fulfil the responsibilities	3.85	4.15	3.38	4.29
I feel myself as a piece of the irrigation organization	4.26	4.27	4.00	3.57
I regularly attend to the general assembly of the irrigation organization	4.20	-	2.99	2.00
I find the irrigation organization successful on the decisions.	3.66	3.92	3.18	4.29
Irrigation organization performs training studies adequately.	1.64	1.77	1.58	1.71
My agricultural production increased after participating to the irrigation organization	3.63	3.90	3.73	4.43
My technical knowledge increased after participating to the irrigation organization	2.82	3.10	2.57	3.43
Irrigation organization provides efficient service (irrigation channel construction, repair and maintenance)	3.53	3.28	3.73	5.00
I contribute to the development of the irrigation organization	4.12	3.77	3.54	3.57
I give opinion for the development of the irrigation organization	3.68	3.62	3.11	3.33
Irrigation organization encourages the irrigation organization for conscious agricultural input usage (seed-fertilizer-pesticide)	1.69	1.93	1.60	1.71
I think that irrigation organization is administrated well	3.72	3.89	3.34	4.14
Irrigation organization is in relationship with the other agricultural organizations	3.92	4.27	3.68	4.50

It was utilized from factor analysis on the evaluation of the considerations of the farmers about irrigation organizations. The considerations of the farmers, who were the members of irrigation cooperatives, municipalities and irrigation unions, were evaluated. DSI was excluded from the analysis as it was not sufficient to be considered. The considerations of the farmers were measured by 5 point likert scale. The reliability of the scale was measured by Cronbach's Alpha test and this value was found as 0.848. The scale was accepted as reliable as this value was close to 1.

KMO and Barlett test were used in order to analyze the convenience of the considerations to factor analysis. KMO value was found as 0.891. The values between 0.80 and 0.90 were specified as "good". As seen in Table 2, Bartlett Test of Sphericity significance level value was 0.00. H0 hypothesis was rejected as this value was less than 5% error margin. In other words, Bartlett Test of Sphericity was found significant ($\chi^2 = 455954$, p=0.000).

Table 2. KMO and Bartlett's test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy		0.891
Bartlett's Test of Sphericity	Chi-Square	4559.544
	df	78
	Sig,	0.000

The factor analysis results are given in Table 3. Factor rotation was done for the interpretation of the factors. Varimax method was preferred for factor rotation (Albayrak 2006). Rotated factor loadings matrix which was obtained from 13 variables and 3 factors were given in Table 3.

The first factor group was named as "Administration". This group indicated how the irrigation organizations were managed and whether the directors were reliable or not. Besides, the relationship of the irrigation organization with the other agricultural organizations" was an important factor for the farmers.

Table 3. Rotated factor loadings matrix

		Component 1	2	3
Administration	Directors of the irrigation organization fulfil the responsibilities	**0.873**	0.124	0.12
	I think that irrigation organization is administrated well	**0.868**	0.203	0.127
	Directors of the irrigation organization are reliable	**0.865**	0.123	0.119
	I find the irrigation organization successful on the decisions.	**0.816**	0.216	0.12
	Irrigation organization is in relationship with the other agricultural organizations	**0.688**	0.26	0.053
	Irrigation organization provide efficient service (irrigation channel construction, repair and maintenance)	**0.621**	0.091	0.06
Ownership and Service	I contribute to the development of the irrigation organization	0.067	**0.672**	-0.186
	I give opinion for the development of the irrigation organization	0.102	**0.629**	0.147
	I feel myself as a piece of the irrigation organization	0.441	**0.578**	-0.165
	My technical knowledge increased after participating to the irrigation organization	0.183	**0.554**	0.468
	My agricultural production increased after participating to the irrigation organization	0.261	**0.547**	0.22
Consciousness Raising	Irrigation organization encourages the irrigation organization for conscious agricultural input usage (seed-fertilizer-pesticide)	0.028	-0.048	**0.8**
	Irrigation organization performs training studies adequately.	0.19	0.098	**0.717**

According to the results, the second factor group was named as "Ownership and Service". The considerations of the farmers for the development of the irrigation organizations and arrangement of the general meetings by the participation of the farmers were significant subjects. The farmers stated that they agreed with the consideration such as "My agricultural production increased after participating to the irrigation organization". It was concluded that the irrigation organizations had a significant part in the rural development (Table 3).

The third group was named as "Consciousness Raising". The considerations such as "Irrigation organization encourages the irrigation organization for conscious agricultural input usage (seed-fertilizer-pesticide)" and "Irrigation organization performs training studies adequately" were gathered under this factor group. According to the results, it was determined that the irrigation organizations were inefficient in terms of the training of the farmers (Table 3).

According to the t test results, the differences between the irrigation organizations managed by irrigation cooperatives and municipality and legal entities in terms of the scores obtained from the factor groups named as "Administration" and "Consciousness Raising" was determined to be statistically significant in 1% confidence level.

The differences between the irrigation organizations managed by irrigation unions and municipality and legal entities in terms of the scores obtained from the factor groups named as "Administration", "and "Consciousness Raising" were determined to be statistically significant in 1% confidence level and the difference was determined to be statistically significant in 5% confidence level in terms of the factor group named as Ownership and Service"

The differences between the irrigation organizations managed by irrigation cooperatives and irrigation unions in terms of the scores obtained from the factor groups named as "Administration" was determined to be statistically significant in 5% confidence level and the difference was determined to be statistically significant in 1% confidence level in terms of the factor group named as Ownership and Service" (Table 4).

Table 4. t test results

	Administration		Ownership and Service		Consciousness Raising	
	ave.	p	ave.	p	ave.	p
Irrigation Cooperative Municipality/Legal Entity	-0.021 0.186	0.007*	0.071 -0.017	0.284	-0.076 0.212	0.001*
Municipality/Legal Entity Irrigation Union	0.186 -0.257	0.000*	-0.017 -0.304	0.020*	0.212 -0.047	0.025*
Irrigation Cooperative Irrigation Union	-0.021 -0.257	0.027*	0.071 -0.304	0.001*	-0.076 -0.047	0.772

Conclusions

It was concluded that there were not training and extension activities in the irrigation organizations. The trainings were not performed sufficiently for the farmers in the irrigation organizations under the administration of irrigation cooperatives, irrigation unions and municipality/legal entities. As seen from the results, the confidence of the farmers to the directors and ownership levels of the irrigation organizations were above average and good. However, it was concluded that the farmers could not get enough support from the irrigation organizations on increasing their technical information and use of conscious agricultural inputs. The perceptions and evaluation levels of the farmers on consciousness raising or training from the irrigation organizations were below the average. Then, the irrigation organizations, especially irrigation cooperatives, should train the members with the training and extension activities on the agricultural subjects. Likewise, training subject takes part among the principles of cooperative especially in cooperative organizations.

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Araştırma Makalesi/*Research Article (Original Paper)*

University Students' Environmental Policy Preferences A Case Study in Ege University

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Abstract: Environmental awareness has increased significantly in recent years in parallel to rising environmental problems. The environmental consciousness and awareness of University students which form a great part of young are extremely important in terms of determining applicable environmental policies for increasing environmental problems. Undoubtedly, future policy makers will come out of today's university youth. In this context, the aim of this study was to determine the views of university students regarding the effects of agriculture and industry on the environment and to suggest alternative environmental measures and policies. The main material of the study was obtained from face to face interviews with 379 undergraduate students of 12 faculties in Ege University. The students' environmental awareness was determined by five-point Likert Scale. On the other hand, Best-Worst analysis was used in designating the trends about measures to take for protection of environment. Research result indicated that even as "human health" was the most affected factor in uncontrolled release of harmful waste conditions; the most effective incentive for waste control would be the "provision of tax exemptions for enterprises with waste control facilities". The students also stated that the most effective punishment for those who left behind the waste would be "decertification of enterprise".

Keywords: Environmental Problems, Best-Worst Analysis, University Students.

Üniversite Öğrencilerinin Çevre Politikası Tercihleri: Ege Üniversitesi Örneği

Özet: Çevresel farkındalık, çevre sorunlarının arttığı son yıllarda giderek daha da önemli hale gelmiştir. Özellikle gençlerin çevreye karşı duyarlılığı ve çevre sorunları konusundaki farkındalıkları gelecek açısından çok şey ifade etmektedir. Hiç şüphesiz, geleceğin politika yapıcıları bugünün üniversiteli gençlerinin arasından çıkacaktır. Bu bağlamda bu çalışmanın amacı; üniversite öğrencilerinin tarım ve sanayinin çevre üzerindeki etkilerine yönelik görüşlerini belirleyerek, bu doğrultuda alternatif çevresel önlemler ve politikalar önermektir. Çalışmanın ana materyalini Ege üniversitesinin 12 fakültesindeki 379 lisans öğrencisiyle yüz yüze yapılan anketler aracılığıyla toplanan veriler oluşturmaktadır. Üniversite öğrencilerinin çevre bilinci beş noktalı likert ölçeği ile belirlenirken; çevreyi korumak için alınması gereken önlemler hakkındaki eğilimleri belirlenirken Best-Worst analizinden yararlanılmıştır. Araştırma sonucunda, zararlı atıkların kontrolsüzce çevreye bırakılmasından en çok etkilenen unsurun "insan sağlığı" olduğu; atık kontrolü için en etkili teşvikin ise "atık kontrol tesisi olan işletmeler için vergi muafiyeti sağlanması" olacağı sonucuna ulaşılmıştır. Öğrenciler ayrıca, çevreye atık bırakanlara verilecek en etkili cezanın "işletme ruhsatının iptal edilmesi" olacağını belirtmişlerdir.

Anahtar kelimeler: Çevre Sorunları, Best-Worst Analizi, Üniversite öğrencileri.

Introduction

Environmental problems has been one of the most important problems of our recent past and present time. Taking into consideration the continuing of the problem, it is clear that the measures already taken and the activities made have not been suffient (Atış et al. 2017). Environmental awareness has increased significantly in recent years in parallel to rising environmental problems. The environmental consciousness and awareness of the young students is extremely important in terms of determining applicable environmental policies for increasing environmental problems (Atış et al. 2016). Student's environmental attitudes is particularly important because they ultimately will be affected by and will need to provide solutions to environmental problems arising from present-day actions. As future scientists, policymakers, consumers, and voters, today's youth will be responsible for "fixing" the environment, and they will be the ones who must be persuaded to adopt and pay the costs of future environmental policies (Bradley et al. 1999).

The aim of this study is to determine the views of university students regarding the effects of agriculture and industry on the environment and to suggest alternative environmental measures and policies. In this framework, this research is also expected to lead to the creation of the right environmental policies for the future of today's university students, who would be the decision makers of the future, and their expectations about the future of the environment.

In the international literature, there are studies that address the issue of environmental sensitivity and awareness in the dimension of youth or university students (Paraskevopoulas et al. 1998; Bradley et al. 1999; Wong 2003; Duan and Fortner 2005; Maffia et al. 2011; Abbas and Singh 2014; Aggelis et al. 2016; Keinonen et al. 2016). The previous studies on the subject are as follows (Özdemir et al. 2004; Gülüm 2011; Müderrisoğlu and Altanlar 2011; Sayan and Kaya 2016; Çelik et al. 2016; Atış et al. 2017; Beser et al. 2017). It seems studies focusing on university students' perceptions, attitudes and behaviors towards the impacts of both agriculture and industry on the environment is scarce, although there exit a number of studies that examine university students' perceptions, attitudes and behaviors towards the environment.

The literatüre showed that in various countries, there were many researches that used the Best-Worst scaling in different areas (Flynn et al. 2007; Jaeger et al. 2008; Louviere and Towhidul 2008; Cohen et al. 2009; Lagerkvist et al. 2012; Erdem et al. 2012; Lagerkvist 2013; Lancsar et al. 2013; Loose and Lockshin 2013), but the number of studies that were analyzed by this method is quite limited in Turkey (Atış et al. 2016; Azak et al. 2016; Çiftçi 2016). On the other hand, by the reason of being the first study using the Best-Worst method in subject of environmental awareness and preferences in Turkey, it is thought to be very important because of the contributions to the literature.

Materials and Methods

The main material of this study is consist of the data obtained from the students registered at the faculties of Ege University in the 2013-2014 academic year. The data is obtained by face to face interviews with students using a questionnaire designed for the purpose of the study. Secondary data of the study is provided from the Department of Students Affairs of the Ege University and Student Affairs Services of the Faculties. The number of the students interviewed with in this study is determined with the Proportional Sampling Method (Miran 2002; Newbold et al. 2012).

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^2 + p(1-p)}$$

n: sample volume
N: The number of students in the population
p: rate of the number of students in the population (to access a maximum volume of sample, 0.50 was taken)
q^2px: Variance of proportion

According to the formula, sample size was determined as 379 with 95% confidence interval and 5% margin of error. The number of students interviewed at the faculties is determined according to the share of each faculty in the main population (Table 1). It has been paid attention to the fact that the distribution of surveys in faculties according to their classes is proportionally close to each other.

Table 1. Distribution of Students Participating in the Survey by Faculties

Faculty	**Frequency**	**%**
Engineering	70	18,5
Literature	70	18,5
Science	50	13,2
Medical School	32	8,4
Agriculture	31	8,2
Education	28	7,4
Economics and Administrative Sciences	27	7,1
Communication	26	6,9
Dentistry	13	3,4
Nursing	12	3,2
Pharmacy	11	2,9
Fisheries Faculty	9	2,4
TOTAL	**379**	100,0

Best-Worst analysis was used to designate the trends about measures that have to be taken to protect environment.

Best-Worst Methods

Maximum difference scaling (MaxDiff) or Best-Worst is a discrete choice model first described by Jordan Louviere in 1987 while on the faculty at the University of Alberta. The first working papers and publications occurred in the early 1990s. With MaxDiff, survey respondents are shown a set of the possible items and are asked to indicate the best and worst items (or most and least important, or most and least appealing, etc.). According to Louviere, MaxDiff assumes that respondents evaluate all possible pairs of items within the displayed set and choose the pair that reflects the maximum difference in preference or importance (Azak et al. 2016; Çiftçi 2016). MaxDiff may be thought of as a variation of the method of Paired Comparisons. Consider a set in which a respondent evaluates four items: A, B, C and D. If the respondent says that A is best and D is worst, these two responses inform us on five of six possible implied paired comparisons: A > B, A > C, A > D, B > D, C > D. The only paired comparison that cannot be inferred is B vs. C. In a choice among five items, MaxDiff questioning informs on seven of ten implied paired comparisons (Lagerkvist 2013). MaxDiff questionnaires are relatively easy for most respondents to understand. Furthermore, humans are much better at judging items at extremes than in discriminating among items of middling importance or preference. And since the responses involve choices of items rather than expressing strength of preference, there is no opportunity for scale use bias (Flynn et al. 2007; Louviere et al. 2012).

On a more technical level, if there are k attributes to be scaled, and they are placed in C subsets, there are k(k-1)/2 "BW" pairs associated with each subset. That means that each choice set contains k(k-1) possible choice options (namely, all the BW and WB pairs). For any given subset presented to an interviewee, he/she implicitly chooses from k(k-1) pairs (Goodman et al. 2005).

Results and Discussion

General Characteristics of Students

The average age of the interviewed students is 22 and between 17 and 37 years of age. In their study, Celik et al. (2016), Sayan and Kaya (2016) and Baser et al. (2017) found the average age of the students to be 21.8, 21.21 and 21.16, respectively. More than half (56,7%) of the respondents are female students. Kaya et al. (2016) examined 136 high school students and 35.5% of the students were female. While 34% of the students living with their own family, 33% stay in the dormitory and 30% stay in the student's residency. Celik et al. (2016) reported that 49.9% of the students were staying in dormitories, 32.5% stay in the student's residency, 13.9% of the students living with their own family and 3.7% with their relatives. The income average of the families of the students is 2768 TL and the average of the pocket money is 505 TL (Table 2). Baser et al. (2017), in their study conducted with nursing students of Dokuz Eylül University in Izmir, determined that 63.9 % of the students had low family income, while 23.3% had medium and 12.8% had high family income. Wong (2003) reported that about 16 % of students were from wealthy or better-off families while the majority came from ordinary or poor family backgrounds.

Table 2: Some Socio-Demographic Characteristics of Students

Gender	**Frequency**	**%**	
Male	164	43,3	
Female	215	56,7	
Residence Place	**Frequency**	**%**	
Own family	130	34,3	
Dormitory	124	32,7	
Student House	114	30,1	
Others	11	2,9	
TOTAL	379	100,0	
Family Income Status and Monthly Allowance From Family of Students (TL)			
	Mean	**Min.**	**Max.**	**Std.Deviation**
Monthly Income Family	2768,21	300	10000	1519,50
Monthly Allowance from Family	505,22	0	3000	373,73

Environmental impact of agriculture and industry sectors

The students were given statements about the effects of the agricultural sector on the environment and their participation levels were determined. According to this, the students agree in the statement "chemical fertilizers pollute the environment considerably", with an average of 4.21. In addition, the agreement of the students in all other expressions on the environmental impact of various agricultural activities indicates that agricultural production pollutes the environment. (Table 3).

Table 3: Level of Participation of Students to the Impact of Agricultural Sector on the Environment

Impact of Agricultural Sector on the Environment	**1**	**2**	**3**	**4**	**5**	**Scale Average**
Farmer behavior and decisions have a significant impact on the environment	22	27	74	122	134	3.84
Excessive irrigation is harmful to plant and soil	12	23	74	128	142	3.96
Chemical fertilizers pollute the environment considerably	8	21	48	108	194	4.21
Agricultural pesticides pollute the environment considerably	15	30	62	121	151	3.96
Farmers have to reduce the use of chemical fertilizers and pesticides to protect the environment	20	23	57	95	184	4.05

1: Completely disagree, 5: Completely agree

The students stated that they completely agreed in the statement "Industry-based air pollution is an important environmental problem", with an average of 4.55 in first rank. Besides, students stated that they agreed almost all of the environmental problems from industry-borne (Table 4).

Table 4. Level of Participation of Students to the Impact of Industrial Sector on the Environment

Impact of Industrial Sector on the Environment	**1**	**2**	**3**	**4**	**5**	**Scale Average**
Industrialists' behavior and decisions have a significant impact on the environment	13	14	42	90	220	4.29
Chemicals used in the industry harm the environment	8	12	30	65	264	4.49
Industrial air pollution is an important environmental problem	8	9	26	60	276	4.55
Industrial water pollution is an important environmental problem	12	13	19	59	276	4.51
Industrial soil pollution is an important environmental problem	11	6	25	71	266	4.52
Industrialists have to head environmentally friendly clean technology to protect the environment	15	17	21	53	273	4.46

1: Completely disagree……5: Completely agree

Analysis of students' environmental policy preferences with the best-worst method

In this section, which is prepared based upon the fact that some sub-sectors of the industry threats the environment more than the others, the students pointed out that at the most "manufacturing industry" and at the least "transportation and storage" sub-sectors as threats (Table 5 and Figure 1). Maffia et al. (2011) reported that 0.8% of the university students considered the mining sector as a threat to the environment.

Table5. The sub-sectors of the industry that the environment most.

Attiributes	**Best**	**Worst**	**B-W**	**Mean (B-W)**
Mining and quarrying	106	76	30	0,0789
Manufacturing industry (food, textile, skin, paper, paint, chemical etc.)	**215**	22	193	0,5079
Cconstruction industry	31	118	-87	-0,2289
Transportation and storage	27	**163**	-136	-0,3579

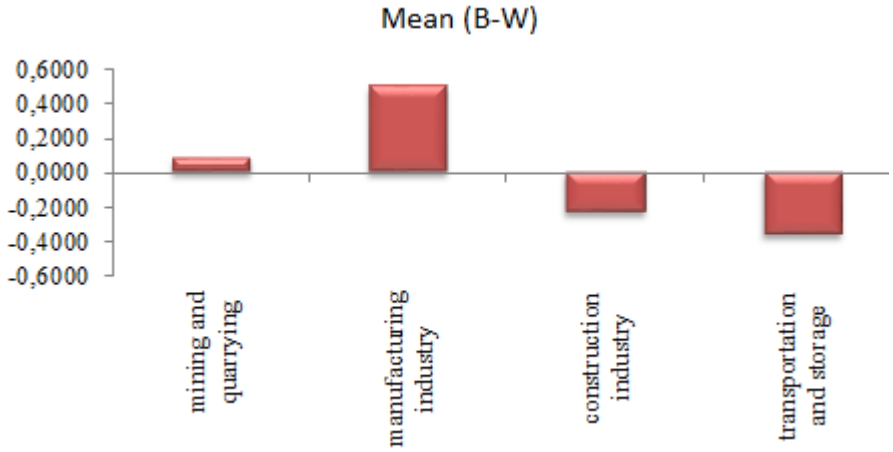

Figure 1. The sub-sectors of the industry that threats the environment most.

Likewise, it is understood that the students see the "agriculture input industry (machine, fertilizer, seed etc.) as the most threating and the "plant production as the least threating sub-sectors sub-sectors of agriculture to the environment (Table 6, Figure 2).

Table6. Comparative threating levels of sub-sectors of agriculture to the environment

Attiributes	**Best**	**Worst**	**B-W**	**Mean (B-W)**
Plant production	28	**230**	-202	-0,5316
Animal production	24	82	-58	-0,1526
Industry providings input to agriculture (machine, fertilizer, seed etc.)	**179**	30	149	0,3921
Agricultural product processing industry	148	37	111	0,2921

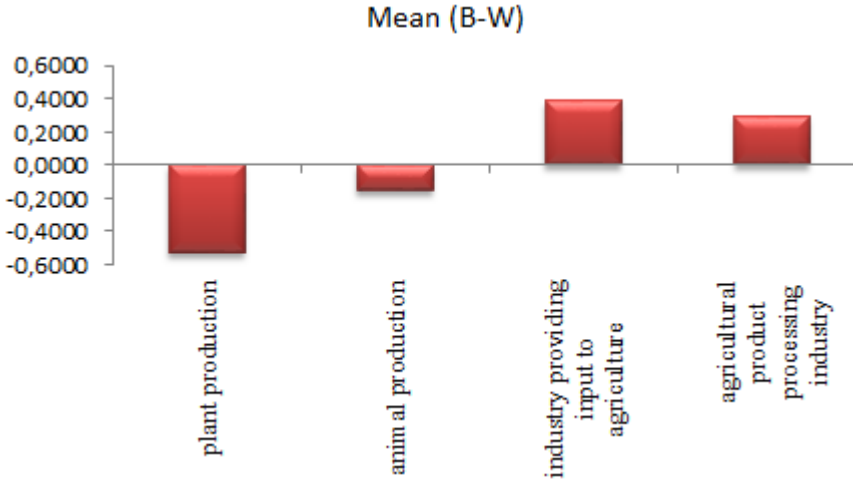

Figure 2. Comparative threating levels of sub-sectors of agriculture to the environment.

In terms of elements affected by uncontrolled release of harmful wastes to the environment, the students ranked the "human health" and the "animal product quality" as the most and the least affected elements, respectively. (Table7, Figure 3).

Table7. Elements affected by uncontrolled release of harmful wastes to the environment

Attiributes	Best	Worst	B-W	Mean (B-W)
Natural vegetation	72	29	43	0,1132
Animals in the nature	25	18	7	0,0184
Atmosphere-air	63	49	14	0,0368
Human health	**113**	22	91	0,2395
Microorganisms in soil and soil	60	78	-18	-0,0474
Plant product quality	9	22	-13	-0,0342
Animal product quality	9	**85**	-76	-0,2000
Underground and surface waters in the nature	28	76	-48	-0,1263

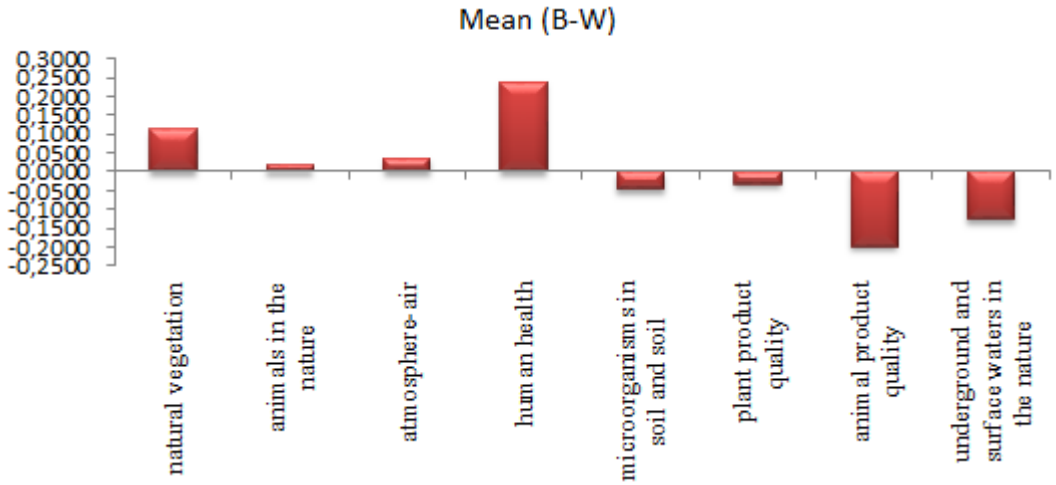

Figure 3. Elements affected by uncontrolled release of harmful wastes to the environment.

In terms of incentives effective in waste control, the students considered "tax exemptions must be provided to waste control facilities" incentives as the most effective incentives option and the "appropriate credit facilities should be provided to those with waste control facilities" as the least effective option (Table 8, Figure 4).

Table 8. Incentives effective in waste control

Attiributes	Best	Worst	B-W	Mean (B-W)
Payment for waste control facility by amount of waste	**75**	51	24	0,0632
Annual fixed payment for enterprises with waste control facility	24	57	-33	-0,0868
Cheap raw materials should be supplied to enterprises with waste control facilities	45	31	14	0,0368
Businesses with waste control facility must be certified	72	48	24	0,0632
The most effective method among businesses should be rewarded and announced.	66	60	6	0,0158
Tax exemptions must be provided to waste control facilities	60	25	35	0,0921
Waste control plants should be given a price premium for their products.	9	42	-33	-0,0868
Appropriate credit facilities should be provided to those with waste control facilities.	28	**65**	-37	-0,0974

The student ranked the most and the least effective punishments to those who left behind waste to the environment as "revocation of business license" and "monetary penalty given to the owner of the business and taken to the training program", respectively. (Table 9, Figure 5). Wong (2003) stated that as a preference of environmental policy, most students (57%) regarded ‘strengthening policy implementation’ as the most important approach and 17% of the students stated that they wanted heavy penalties for those who pollute the environment.

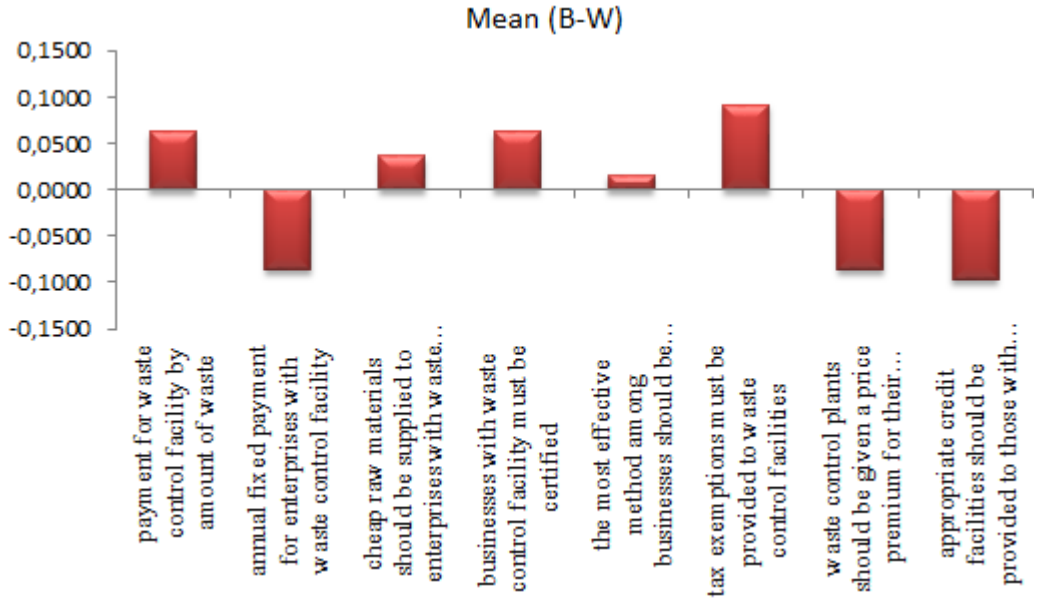

Figure 4. Incentives that can be effective in waste control.

Table 9. The punishment that can be given to those who leave behind waste to the environment.

Attiributes	**Best**	**Worst**	**B-W**	**Mean (B-W)**
Monetary penalty	53	81	-28	-0,0737
Temporarily shut down the business	43	36	7	0,0184
Announcement of the business in the list of harmful persons	22	61	-39	-0,1026
the company's product labels have "harmed the environment"	51	38	13	0,0342
Revocation of business license.	**153**	15	138	0,3632
Alerting the owner to the training program.	14	**120**	-106	-0,2789
Monetary penalty given to the owner of the business and taken to the training program.	43	28	15	0,0395

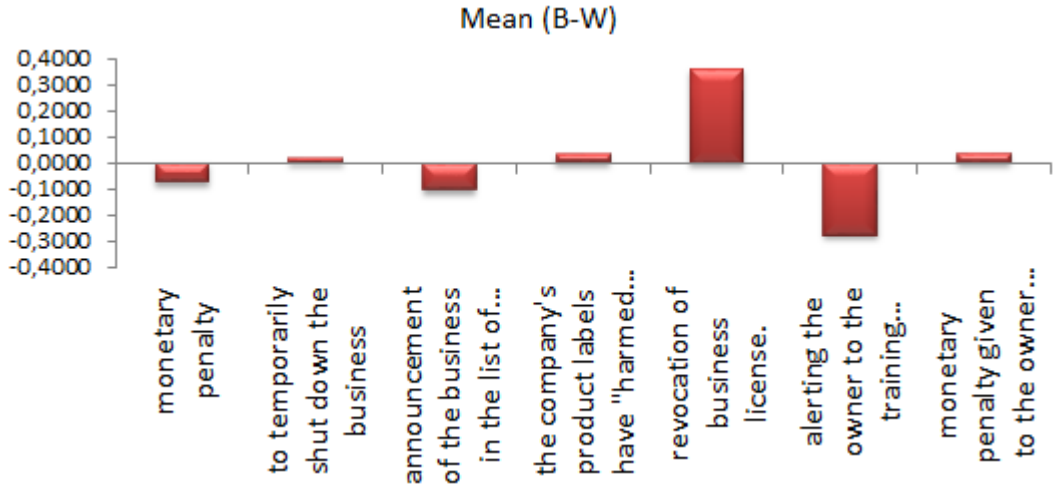

Figure 5. The punishment that can be given to those who leave behind waste to the environment.

Conclusions

The aim of this study is to determine the views of university students regarding the effects of agriculture and industry on the environment and to suggest alternative environmental measures and policies. When the results of the survey are evaluated, it is understood that university students are aware of the high environmental sensitivities, the agricultural and industrial sectors and the pressures that these sectors place on the environment. University students think agriculture is also effective in environmental pollution, but industrial sector is much

more effective. In this sense, it seems possible to achieve the participation of today's youth in policies to be implemented in order to reduce the impact of agriculture and especially the industrial sector on environmental pollution. Together with the most significant incentive to provide tax exemptions to businesses with waste control facilities, the students have different ideas about effective incentives for waste control. However, there is a greater consensus on penalties for those who leave the area, and they approve more radical punishments, such as the cancellation of business license. This suggests that success in today's youth can be achieved with respect to future measures and the implementation of these measures.

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Araştırma Makalesi/*Research Article (Original Paper)*

Aromatic Composition of Pink Skinned Grape Cultivar: Gülüzümü (*V.vinifera* L.)

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Abstract: Gülüzümü is a grape cultivar used for fresh consumption. In addition to table grape characteristics, it is also used for traditional must products thanks to its berry skin color and flavor properties. This variety, which is one of the important genetic resources of Ankara province, come into prominence with its attractive "pink" skin color and unique "rosy" flavor. In this study, the aroma composition of Gülüzümü was investigated on the grape material obtained from Ankara-Beypazarı district, which is the original ecology of the variety. Analysis was carried out using solid-phase microextraction technique with gas chromatography and mass spectrometry. Results were expressed in peak area (%) as a mean value. A total of 28 volatile compounds were identified. These compounds included 5 acids, 5 alcohols, 2 C_6 compounds, 3 terpenes, 1 C_{13} norisoprenoid and 12 aldehydes and ketones. The quantities of other compounds were detected very low. The relative distribution of the aroma compounds was; C_6 compounds 67.9%, aldehydes and ketones 10%, acids and alcohols 4.9%, terpenes 2.5%, C_{13} norisoprenoids 0.3% and other compounds 9.6%. The C_6-compounds (hexanal and 2-hexenal) that have vegetal flavor had the highest values in total aroma composition of Gülüzümü. Although terpene compounds are proportionally low in the aromatic composition, nerol, the main compound that contributes rosy flavor, was the compound that detected high amount. Benzene acetaldehyde (hyacinth), 2-ethyl hexanol (floral), benzene methanol (fruity), maltol (caramel), heptanoic acid (sour) and acetic acid (vinegar) were determined as proportionally important compounds in total aroma composition of Gülüzümü.

Keywords: Volatiles, flavor, table grape, GC-MS analysis, *V.vinifera* L.

Introduction

In grapes, flavor composition is defined as a combination of sugars, acids and volatiles. Many factors such as grape cultivar, climate, ripening degree, cultural practices and vegetation year are influential on chemical composition of grapes (Duan et al. 2014, Keskin et al. 2013). Among these metabolites, aroma composition is an important quality parameter for not only wine grapes but also table grapes due to determine consumer acceptance. The sensory effect of aroma compounds is dependent on their concentration and olfactory perception threshold. Their concentration in grapes and wines generally ranging from several mgL^{-1} to a few ngL^{-1}, or less (Ribereau-Gayon et al. 2006).mStudies on aroma composition in literature have focused on wines and wine grapes and it is obvious that there is a lack of knowledge in table grapes. However, aromatic and organoleptic properties are important quality parameters for table grapes as it determines the quality of fresh consumption (Kunter et al. 2013).

Gülüzümü is an indigenous germplasm for Ankara province. It is well known but locally grown cultivar. Berries are characterized as thin-skinned, pink-rosy colored and used for fresh consumption (Cantürk et al. 2015). The cultivar has a distinct flavor that has not yet been investigated in terms of aroma composition. Therefore, in the study, it is aimed to determine the aromatic composition of Gülüzümü cultivar.

Material and Methods

Plant material

The study was performed during the growing season of 2016 on cv. Gülüzümü (*V.vinifera* L.). Grapes were cultivated in the vineyards of Research Station for Viticulture in Kalecik, Ankara (670 m above sea level). The vineyard was planted in 2005 with 1.5 x 3 m row spacing. Grapevines were grafted on 1103P rootstock and

trained bilateral cordon. Characteristics of vineyard soil were clay-loam. The research region has characteristics of continental climate, which is quite warm and arid, with limited precipitation. Grapes of Gülüzümü were harvested on September 9, 2016 and grape samples were stored at -25ºC until analysis.

Extraction of aroma compounds

Solid phase microextraction technique was used for the extraction of aroma compounds (Shalit et al. 2001). Berry samples were homogenized in a blender and 8 g of homogenate was transferred to a vial. 2 ml of saturated NaCl solution were added on and vortexed for 30 sec. The sample was equilibrated at 65ºC for 30 min with 65 µm polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB) (Supelco, Bellefonte, PA, USA) under stirring and then inserted into the injection port of GC for 5 min. The fiber was conditioned in GC injection port at 200 ºC for 20 min before analysis.

GC-MS analysis

GC-MS analysis of aroma compounds was performed with Shimadzu GCMS-QP-2010 Chromatograph equipped with a capillary column (Restek RTX-5) (30m x 0.25mm x i.d., 0.25µm film thickness). The carrier gas was used helium with 1 mL/min flow rate. Injection temperature was 250 ºC and the oven temperature was set to increase from 40 ºC to 240 ºC at a rate of 4 ºC/min. Identification of aroma compounds was done by comparing their mass spectra and retention time from the WILEY and NIST library. The results were expressed in peak area (%) as a mean value.

Results and Discussion

In the study, a total of 28 volatile compounds were identified in Gülüzümü (Table 1). Distribution and percentage of the detected compounds have shown in Figure 1.

Table 1. Volatile composition of Gülüzümü. RT: Retention time (min), RI: Retention index

Aroma compounds	RI	RT	Mean peak area (%)
Acetic acid	685	1.944	1.38
Hexanal	811	6.233	36.94
2-Hexanal	872	8.435	30.99
Methyl ester hexanoic acid	958	11.663	0.90
2-Heptanal	993	13.005	0.62
Benzaldehyde	995	13.103	0.73
1-Octen-3-ol	1019	14.028	0.70
Heptanoic acid	1024	14.212	1.24
6-Methyl-5-hepten-2-one	1028	14.356	0.35
2-Pentyl furan	1032	14.504	0.76
2,4 Heptadienal	1038	14.751	0.67
2-Ethylhexanol	1073	16.112	2.07
Benzene methanol	1078	16.300	1.24
Benzene acetaldehyde	1087	16.656	2.70
2-Octenal	1103	17.268	1.21
1-Phenyl ethanone	1112	17.579	0.30
2-Octen-1-ol	1115	17.710	0.37
Cyclohexane methanol	1119	17.837	0.90
Nonanal	1153	19.132	0.68
Benzene ethanol	1163	19.488	0.51
Maltol	1194	20.629	1.31
2,6-Nonadienal	1205	21.026	0.38
Benzoic acid	1216	21.415	0.53
Methyl salicylate	1249	22.593	0.83
Dodecanal	1260	22.981	0.31
Nerol	1312	24.793	1.31
β-Ionone	1557	32.449	0.25
α-Farnesene	1576	33.039	0.32

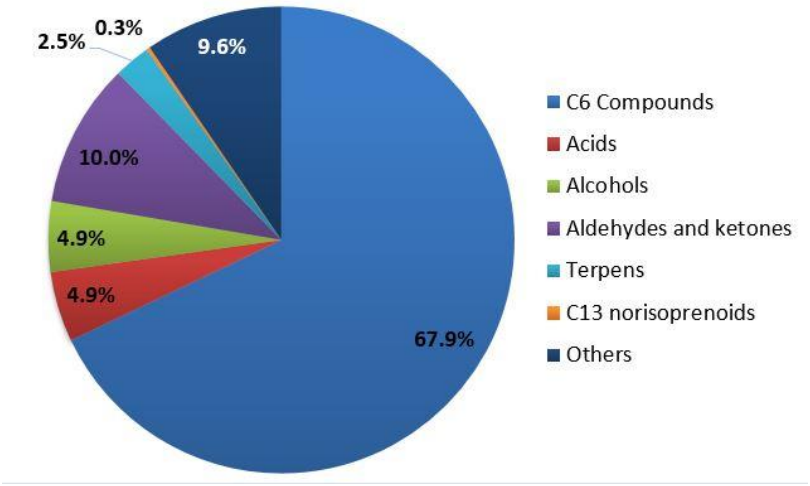

Figure. 1. Percentage of the aroma compounds of Gülüzümü.

The C_6-compounds were the most abundant compounds found in Gülüzümü. In this group, hexanal (37%) and 2-hexanal (31%) were detected and they accounted for more than half of the total aroma composition with 67.9%. Linoleic and linolenic acids in the plasma membrane produce saturated and unsaturated C_6 alcohols and aldehydes via lipoxygenase activity. Content of C_6 compounds depends on several factors like grape cultivar, ripeness degree, temperature regime and duration of fermentation (Moreno and Peinado 2012). They produce green or vegetal aroma. Some authors also indicated that C_6 compounds are the most abundant volatiles present grapes (Song et al. 2012; Wu et al. 2016).

The other important aromatic group of Gülüzümü was aldehydes and ketones. 12 compounds were detected in this group and they accounted for 10% of total aroma composition. They contribute the unique aromatic character of grape cultivars with their floral, vegetal, fatty and sweet flavor. Benzene acetaldehyde with hyacinth (2.7%), maltol with caramel (1.3%) and 2-octenal with green, leafy aromas (1.2%) were the most abundant compounds in this group.

Acids and alcohols were detected approximately the same quantity (4.9%). Acetic acid was the dominant acid in the cultivar with its characteristic vinegar odor (1.4%). The other acids identified were methyl salicylate, methyl ester hexanoic, benzoic and heptanoic acids. 1-Octen-3-ol, 2-octen-1-ol, 2-ethylhexanol, benzene methanol and benzene ethanol were determined as alcohols. Among these, 2-ethylhexanol with floral (2.1%) and benzene methanol with fruity odor (1.2%) were determined in high quantities.

Terpene compounds had a minor contribution of 2.5% to the total flavor of the cultivar. A total of 3 terpene compounds, nerol, cyclohexane methanol and α-farnesene were identified in Gülüzümü. Terpene compounds are responsible for the characteristic Muscat flavor of grapes and wines. They begin to synthesize at berry set and their quantity gradually increases until maturity (Günata et al. 1985; Vilanova et al. 2012). Although total terpene concentration was low, nerol was one of the most abundant compounds of Gülüzümü (1.3%) with its strong rosy characteristic. Cyclohexane methanol was also considered as an important compound (0.9%) contributes to floral odor. It can be concluded that these two compounds play a major role in the formation of a unique rosy and floral aroma of Gülüzümü. α-Farnesene was identified in very low quantity.

β-ionone was the only compound identified from the C_{13} norisoprenoids. It generates characteristic violet flavor, although its amount was very low (0.25%). Although the concentration of norisoprenoids is generally low, they contribute to the formation of the characteristic flavor of grapes and wines due to their strong aromatic properties (Razungles et al. 1998; Moreno and Peinado 2012).

Other compounds detected at very low quantities constituted 9.6% of the total aroma composition in Gülüzümü. Sensory characteristics of detected compounds were presented in Table 2.

In this study, the first findings on the flavor characteristics of Gülüzümü an important native cultivar of Ankara province were obtained. In addition to its attractive pink skin color, identification of the compounds that generate its unique flavor is important in terms of protection and evaluation of our native genetic resources. Moreover, the present study has contributed to the sources which are very limited in table grape cultivars.

Table 2. Aroma description of volatile compounds in Gülüzümü

Compounds	Aroma description
C_6 Compounds	
Hexanal	Vegetal, grassy, green
2-Hexanal	Green, sweet, fruity
Aldehydes and ketones	
2-Heptanal	Pungent, green, mild fatty
Benzaldehyde	Sweet, strong almond
6-Methyl-5-hepten-2-one	Fatty, green, citrus
2,4 Heptadienal	Fatty, green
Benzene acetaldehyde	Green, floral, sweet hyacinth
2-Octenal	Green, leafy, mild fatty
Nonanal	Citrus, rosy, fatty
2,6-Nonadienal	Fresh, cucumber
Maltol	Caramel, candy, jam like
Dodecanal	Fatty, violet, woody
1-Phenyl ethanone	Sweet, pungent, strong medical
2-Pentyl furan	Fruity, green bean, vegetable
Terpenes	
Nerol	Fresh, sweet, rose
Cyclohexane methanol	Fresh, floral, magnolia, grass
α-Farnesene	Fruity, vegetal
Acids	
Acetic acid	Strong, pungent vinegar
Methyl ester hexanoic acid	Ether-like, pineapple
Methyl salicylate	Minty, spicy, sweet
Benzoic acid	Almond
Heptanoic acid	Sour, fatty
Alcohols	
1-Octen-3-ol	Sweet, earthy, lavender, rose, hay
2-Octen-1-ol	Fatty, mild hazelnut, waxy, cream
2-Ethyl hexanol	Mild sweet, floral
Benzene methanol	Fruity, sweet
Benzene ethanol	Fresh, rose, honey
C_{13} norisoprenoids	
β-Ionone	Violet, fruity, floral

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Chemical Changes During Storage in Hazelnuts Separated from Husks by Patoz and Hand

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Abstract: This study was carried out to determine the chemical changes during storage in 'Tombul', 'Palaz' and 'Kalınkara' hazelnuts that separated from husks by patoz and hand in province of Giresun (Turkey) in 2014. In the study, it was researched kernel moisture (%), total fat (%), crude protein (%), ash (%), free fatty acids (%), peroxide (meqO2/kg) and colour values with 3 months intervals during 9 months in storage room at ambient temperature. As results of the study, in the samples separated by patoz, the fat content was more stable during storage; the free fatty acids and the a* value was more changed, and the peroxide value was higher at the end of the storage. In addition, it was determined that there was no significant effect of the separation methods on moisture, protein and ash ratio, and moisture decreased, protein increased during storage; peroxide value was determined only at the end of the storage.

Keywords: *Corylus avellana*, hazelnut, husking, storage, chemical properties

Elle ve Patozla Ayıklanan Fındıklarda Depolama Süresince Meydana Gelen Kimyasal Değişimler

Özet: Bu çalışma Tombul', 'Palaz' ve 'Kalınkara' fındık çeşitlerinde elle ve patozla ayıklanan ürünlerde depolama süresince meydana gelen kimyasal değişimleri belirlemek amacıyla 2014 yılında Giresun'da yürütülmüştür. Çalışmada iç fındıkta nem (%), toplam yağ (%), ham protein (%), kül (%), serbest yağ asitleri (%), peroksit (meq02/kg) ve fındık ununda renk değerleri 9 ay boyunca oda koşullarında üçer ay aralıklarla araştırılmıştır. Çalışma sonucunda; patozla ayıklanan fındık meyvelerinin yağ içeriklerinin depolama süresince daha stabil olduğu, serbest yağ asitleri ile renk a* değerlerinde daha fazla değişim olduğu ve depolama sonundaki peroksit değerinin daha yüksek olduğu belirlenmiştir. Bunun yanında, ayıklama yöntemlerinin nem, protein ve kül içeriğine etkisinin önemsiz olduğu, depolama süresince nemin azaldığı, proteinin arttığı, peroksit değerinin ise sadece depolama sonunda ortaya çıktığı görülmüştür.

Anahtar kelimeler: *Corylus avellana*, fındık,ayıklama, depolama, kimyasal özellikler

Introduction

Historical documents record that hazelnut was growing in northern Turkey's Black Sea coastal area approximately 2500 years ago, and it has been transported to many other countries during the last six centuries. Turkey is the world leader and dominates world markets in hazelnut production and export. Today, Turkey is the top exporter of hazelnuts and hazelnut products, with 240,000-270,000 t kernels exported (85% of the world export) (Anıl et al. 2016).

Husking is one of the last processes that can affect the parameters such as nut output, husking efficiency, blank-nut separation efficiency, kernel loss, damaged-nut ratio and husk-separation efficiency. Therefore, taking values of optimum performance characteristics of the husker into consideration, a new design should be developed in order to remove the adjustment procedure for the upper and lower separation systems (Beyhan et al. 2009). In the design of machineries used for harvesting, husking and processing of agricultural products, the physico-mechanical properties of the products are the most important parameters that should be known. The degree of mechanization in the husking and processing of hazelnuts in Turkey is increasing (Beyhan et al. 1994).

This study was carried out to determine the chemical changes during storage in hazelnuts that separated from husks by patoz and hand.

Materials and Methods

This research was carried out in 2014 on a hazelnut orchard of Hazelnut Research Institute in the province of Giresun (Turkey). 'Tombul', 'Palaz' and 'Kalınkara' hazelnut cultivars have been used as plant material. In the study, the husking machine with local name 'patoz' was used to separate the nuts from husk.

The hazelnuts analyzed in this experiment were harvested in August 11-15, 2014. The nuts with husks were spread separately onto the concrete ground. They were exposed to natural sunlight for three days before separation operation. At the end of the drying process, half of the samples of each cultivar was separated by husker and the other half by hand. After the husking process the hazelnuts were dried again for 2 days in natural sunlight. And then the in-shell hazelnuts were stored for 9 months in jute bags in storage room at ambient temperature.

The analysis of kernel moisture, total fat, crude protein, ash, free fatty acids, peroxide and colour values (in kernel powder) in the cultivars were performed on the product at beginning and after three, six and nine months of storage. Moisture of kernel grinded with blender was determined using moisture determination device (Precisa XM 50); total fat by soxhlet method and crude protein by kjeldahl method were determined (James 1995); free fatty acid content and peroxide value were determined in accordance with AOAC methods (AOAC 1990 a and b, respectively). The ash content of the samples was determined by incineration in an ash furnace until white ash was obtained at 5250 °C (Anonymous, 1962). Color values (L*, a*, b*) on kernel powder were determined using Konica Minolta CR-400 Chroma Meter instrument.

The experiment was designed according to randomized plots with two replications. 6 kg nuts for each replication in jute bags were used, in other words 96 kg nuts for each cultivar and 288 kg nuts for a total of three cultivars.
Statistical analyzes were performed using the SAS-JMP program. The LSD test has been applied to compare the differences between means.

Results and Discussion

The analysis of variance detected significant differences for kernel moisture during storage periods in 'Palaz' hazelnut separated by patoz, and in 'Kalınkara' hazelnut separated by hand. On the other hand, moisture were not significant according to separate methods and storage periods in 'Tombul', and in the hand samples of 'Palaz', patoz samples of 'Kalınkara' (Table 1). The highest level of kernel moisture content of the cultivars at the beginning of storage, and the lowest level at the end of the storage except of samples separated by hand of 'Tombul'. Turan (2017) also stated that the moisture content of hazelnuts stored at room temperature for 18 months decreased during storage.

As expected, crude protein increased with storage in all samples, and this value was not differed according to cultivars and separation methods. In the other studies where in-shell hazelnuts were stored under the same conditions, it was determined that during the storage period, the ratio of the crude protein to the 9th month was increased (Bostan and Koç-Güler 2016); the fluctuations in protein value parallel to the changes in moisture content during storage, especially when this value is higher at the beginning of storage and this is due to the decreasing moisture content during storage (Turan 2017); in another study this value fluctuated during storage (Koç Güler 2015), and protein content did not show a one-sided change during storage (Çakırmelikokğlu et al. 1993). In our study, it is thought that the ratio of protein increases during storage depending on the decreasing moisture content. On the other hand, Özçağıran et al (2014) stated that protein content contents of hazelnut kernels varies between 10% and 24%, and 100 g of hazelnut kernels fulfils 22% of daily protein need of a human. In this study the highest protein ratios were in 'Tombul' (24,78%), 'Palaz' (22,96%) and 'Kalınkara' (19,88%) cultivars, respectively.

Table 1. Changes of parameters in the kernels during the storage period

Cultivars	**'Tombul'**		**'Palaz'**		**'Kalınkara'**	
Separation	**Hand**	**Patoz**	**Hand**	**Patoz**	**Hand**	**Patoz**
Storage			**Moisture (%)**			
Beginning	4,17	3.70	4,55	4.24 a**	4,16 b*	4,04
3rd month	4,35	3.69	4,53	4.30 a	4,71 a	3,76
6th month	4,20	3.54	4,65	3.88 b	4,23 b	3,86
9th month	4,25	3.67	4,34	3.97 b	3,91 b	3,90
			Crude protein (%)			
Beginning	17.97 b**	17.91 b**	16.28 b**	17,29 b**	13,55 b**	14,89 b**
3rd month	18.38 b	19.08 b	17.16 b	17,92 b	14,58 b	15,08 b
6th month	18.75 b	19.16 b	17.37 b	17,96 b	14,69 b	14,91 b
9th month	24.70 a	24.78 a	22.96 a	21,50 a	18,94 a	19,88 a
			Total fat (%)			
Beginning	48,60 b**	57.69	53,15	57,59	58,22 b*	63,08
3rd month	61.04 a	57.97	59,50	59,20	62,12 a	64,25
6th month	58.62 a	59.73	57,62	59,21	62,22 a	64,15
9th month	58.91 a	57.22	54,91	60,12	60,99 a	64,55
			Free fatty acid (%)			
Beginning	0.25	0.22 b*	0,36	0,30	0,47	0,28 b*
3rd month	0.30	0.28 b	0,35	0,29	0,43	0,43 ab
6th month	0.35	0.37 a	0,38	0,34	0,51	0,55 a
9th month	0.27	0.26 b	0,29	0,31	0,44	0,34 b
			Peroxide value (meqO_2/kg)			
Beginning	0.00 b**	0,00 b**	0,00 b**	0,00 b**	0,00 b**	0,00 b*
3rd month	0.00 b	0,00 b	0.00 b	0,00 b	0,00 b	0,00 b
6th month	0.00 b	0,00 b	0.00 b	0,00 b	0,00 b	0,00 b
9th month	0.71 a	2,05 a	1.03 a	1,12 a	0,06 a	0,79 a
			Ash (%)			
Beginning	2,63	2.52	2,63	2,78	2,27	2,22
3rd month	2.92	3,01	3,21	3,04	2,54	2,59
6th month	2.96	2,31	3,00	2,39	2,54	2,55
9th month	2.78	2,80	2,78	2,78	2,46	2,79
			L*			
Beginning	67.74 c**	69.53 b**	70.34 a**	64,65 b**	66,84 a*	64,23 b**
3rd month	73.35 a	72.26 a	72.83 a	69,66 a	67,09 a	69,11 a
6th month	70.00 b	70.58 ab	71.34 a	68,83 a	69,35 a	68,35 a
9th month	64.09 d	65.68 c	62.49 b	61,89 c	59,30 b	62,11 b
			a*			
Beginning	2.78	2,48	2,75	3,84 a**	3,27	4,22 a**
3rd month	2.33	2,85	2,56	2,93 b	3,99	3,06 b
6th month	2.44	2,41	2,60	2,94 b	3,46	2,90 b
9th month	2.77	2,47	2,86	2,85 b	3,46	2,90 b
			b*			
Beginning	20.25 a**	20,29 a**	18,95 a**	16,25 b**	19,64 a**	16,44 b**
3rd month	20.11 a	19,77 a	18,65 ab	18,46 a	19,80 a	19,61 a
6th month	20.28 a	19,40 a	17,96 b	17,28 ab	19,49 a	19,30 a
9th month	14.17 b	15,77 b	14,54 c	14,52 c	14,92 b	14,97 c

Means in the same column with different letters are statistically significant

*,** Significant at P= 0.05 and P= 0.01, respectively

Fat rate significant changed during storage period only in the samples separated by hand in 'Tombul' and 'Kalınkara' cultivars. The highest values were generally observed at the end of storage, but no significant change was observed between third and ninth months. In the previous studies, it was found that there was no change in the oil content

during storage (Çakırmelikokğlu et al. 1993); the fat values at the end of storage were slightly higher in 'Tombul', 'Palaz' and 'Kalınkara' cultivars which were stored in the cold storage for 12 months, and there were significant differences between the cultivars (Ağar et al. 1995); in the same cultivars stored on different conditions, the fat rate increased during storage and that there are no significant differences between these cultivars (Koyuncu 2004); the fat rate was more stable during storage in 'Palaz' and 'Kalınkara' than 'Tombul' stored in room at ambient temperature (Bostan and Koç Güler 2016); irregular variation of total fat value during storage period and decreased total fat value at the end of the storage may have resulted from the heterogeneity of the samples (Koç Güler et al. 2017); the fat rate was changed during storage (Turan 2017). It is seen that different results are obtained regarding the change of oil content during storage. In our research, it is thought that the high oil content at the end of storage is related to the decrease of moisture content.

Free fatty acids significant changed during storage period only in the samples separated by patoz in 'Tombul' and 'Kalınkara' cultivars. In general, it was determined that this value increased up to 9th month, decreased at 9th month but it was higher than the beginning value and it was found that it did not reach 1% in any application. Dermirci Ercoşkun (2009) stated that the hazelnut, which has a high oil content, is chemically or enzymatically hydrolyzed in the storage stage and is exposed to free fatty acid generation. On the other hand Bostan and Koç Güler (2016) stated that this value was 0.20% at the beginning of the storage, 0.34% at the 9th month and decreased to 0.31% at the 12th month after 9th month but it was higher than the beginning value. Turan (2017) also determined that during the storage period, the value of fatty acids generally decreased. Both studies support our results. 1% for free fatty acid is an important upper limit and the quality of the food falls above this value (Koç Güler et al. 2017). In our study, this value was well below 1% in all applications.

Peroxide, which is produced as a result of oxidation of unsaturated fatty acids, is an important factor for the storage period in hazelnut and causes quality deterioration (Dermirci Ercoşkun 2009). In the study peroxide value was determined in all cultivars and applications only at the end of storage (9th month). The highest values were observed for the samples separated by patoz, especially in 'Tombul' (2.05%). In a study, peroxide values significantly increased during the storage period but such an increase was not constant (Koç Güler et al. 2017). Bostan and Koç Güler (2016), Turan (2017) and Akçin (2018) also stated that the peroxide value reached the highest level at the end of storage. In case of hazelnut sales, if the buyer does not have a special request, it is stated that the peroxide value is desirable below 1 meqO_2/kg (Koç Güler 2015). In our study, only at the end of storage this value was determined to be over 1% in Tombul (patoz application) and Palaz (hand and patoz applications) cultivars. In addition, this value was generally higher in patoz applications.

Ash value did not change significantly during the storage period compared to the separation method.

Change in color are quite different regarding the variety, and these changes can cause loss of taste, vitamins and protein and decrease in nutritional value in hazelnut kernels (Lopez et al. 1997). In the study storage time had a significant effect on L* and b* color values on kernel powder. The lowest values were determined at the end of storage. The effect of separation by patoz on changing of a* value was significant, and the lowest values also observed at the end of storage. Koç Güler et al. (2017) reported that storage period significantly affected the color of hazelnut kernel, and L*, a* and b* values decreased at the end of storage.

Conclusions

At the result of storage in room at ambient temperature for 9 months of in-shell hazelnuts separated by patoz and hand, the following results were obtained:

- The moisture content generally decreased during storage and there was no significant effect of the separation methods,
- The protein ratio increased with storage, depending on the moisture content, and was not vary with the separation methods,
- The fat rate of samples separated by patoz was more stable than that of hand-separated samples during storage, and generally the highest values were observed at the end of storage, depending on the moisture content,
- The free fatty acids in the samples separated by patoz showed more changes during storage compared to the hand-separated samples,

- The highest peroxide values in the samples at the end of storage were found in the samples separated by patoz,
- There were no significant changes in the ash values of the samples separated both methods, and
- The changes in the a* value of the samples separated by patoz were generally significant, while the changes in the values of L* and b* were insignificant, and the lowest values of these traits were observed at the end of storage.

As a result, it can be said that especially free fatty acid and peroxide values of hazelnut kernels which were separated by patoz machine were changed more during storage. It is therefore advisable to pay attention to this issue when designing patoz machines.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Effects of Rootstock Cutting Thickness on Final Take, Quality of Potted Grapevine Saplings

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Abstract: In grapevine propagation, dormant cutting quality of rootstock and scion is as equally important as the quantity. The best length and thickness of cuttings for propagation were determined by researches. Cuttings should be at least 7-12 mm in diameter at the top. The length can vary from 30 to 40 cm, depending on location and soil type. In this study, the effects of rootstock cutting thickness (diameter) on rafting success, final take and quality of potted grapevine plants were investigated. The dormant cuttings of 5BB, 1103 P and 110 R dormant cuttings were grouped by their diameters (6-9 mm,10-13 mm, 14 mm≤), then scions of Narince cultivars were grafted by omega grafting machine on cuttings. In the study, the callusing and rooting perfomance of grafted cuttings and final take were evaluated. The ratios of round callusing on graft union in 5BB and 1103P rootstocks decreased with increasing of cutting thickness. The callusing formation on base part in three rootstocks increased with increasing of cutting thickness, but root formation decreased. Total final take ratios ranged from 94 % to 98%. The ratios of total final take decreased with increasing in cutting thickness. As a result, it has been successufully exhibited that cutting with a thickness of 7-18 mm can be used in grapevine saplings production.

Keywords: 5BB,110 R, 1103 P, Shoot lenght, Root fresh weight, Potted-grapevine

Özet: Asmanın çoğaltılmasında, çoğaltma materyali olarak kullanılan çelik ve kalemlerinin miktarı kadar kalitesi de aynı derecede önemlidir. Çoğaltma için en iyi çelik uzunluk ve kalınlığı araştırmalarla belirlenmektedir. Çelik uzunluğu bölge ve toprak tipine göre 30-40 cm arasında değişmektedir. Bu araştırmada, aşılı tüplü asma fidanı üretiminde anaç çapının aşı başarısı, fidan randımanı ve kalitesine etkileri araştırılmıştır. 5BB, 1103 P ve 110 R anaçlarına ait odun çelikleri çaplarına göre üç boya (6-9 mm,10-13 mm, 14 mm≤) tasnif edildi, daha sonra Narince çeşidi ile omega aşı makinesi ile aşılandı. Araştırmada aşılı çeliklerin kallus, köklenme performansı ve fidan randıman verileri değerlendirildi. 5BB ve 1103 P anaçlarında çap arttıkça aşı bölgesinde çepeçevre kallus oluşumu azalmıştır. Üç anaçta da genellikle çelik çapı arttıkça aşılı çeliklerin bazal kısmında kallus oluşumu artarken kök oluşumu ise azalmıştır. Toplam fidan randımanları % 94-98 arasında değişmiştir. Çelik çapı arttıkça toplam fidan randımanı azalmıştır. Sonuç olarak, 7-18 mm çapa sahip çeliklerin aşılı asma fidanı üretiminde kullanılabileceği ortaya koyulmuştur.

Anahtar kelimeler:5BB,110 R, 1103 P, Sürgün uzunluğu, Kök yaş ağırlığı, Tüplü asma fidanı

Introduction

Grafted grapevine saplings on different rootstocks are commonly used in modern viticulture practices to prevent the spread of Phylloxera pest or to benefit from various advantages of the rootstocks. Today, it is estimated that80–85% of the vineyards worldwide are using rootstocks.

Indoor grafted grapevine sapling production over the benches has been used worldwide since 1930s (Alley, 1980). Grafted cuttings are transplanted into fields or greenhouse after grafting, callusing, adaptation and second waxing. Potted grapevine saplings are produced through rooting grafted scions under greenhouse conditions (Çelik et al., 1998).

The cuttings used in grafted grapevine sapling production should be supplied from pure or hybrid grapevine rootstocks. Scion characteristics of grapevine rootstocks were classified according to TSE 4027: The 1st class scions have 3-5 buds, 7-10 mm in diameter and 35-45 cm long; the 2nd class scions have 3-5 buds, 5-7 mm in diameter 35-45 cm long (Çelik et al., 1998).

Characteristics of the plant material are the primary factors influencing success in sapling production. In grapevine sapling production, rooting capacity of the rootstocks, scion taking times, health and development status of the main plant, preservation conditions and etc. Issues have significant effects on success in production. Especially the nutrition and lignification status of the rootstock are quite effective in callusing and rooting of the grafted scions (Dardeniz et al.,2007; 2008; Rodoplu and Dardeniz, 2015).

For a well lignification in scions, core/cane diameter ratio should be 1/2 (Çelik et al., 1998). Before grafting, rootstocks and scions should be subjected to diameter classification (6-8, 8-10, 10-12 mm and etc.) to improve productivity in grafting and to improve compatibility of the scions in diameter (Çelik et al.,1995).

Many external factors such as cutting length and diameter, indole-3-butyric acid (IBA) concentration, date of cutting collection, and preheating can affect and increase rooting of hardwood cuttings of many species (Tofanelli et al., 2003)

Beyl et al. (1995) investigated rooting characteristics of ligneous scions of 13 *Actinidia arguta* kiwi genotypes. Results indicated that scion lengths and diameters were effective in root development and reported the best outcomes for scions 2-8 mm in diameter and 8 cm long. They also pointed out the results varied based on the genotypes.

Çelik and Gargın (2009) investigated the effects of different IBA concentrations and scion thickness on rooting of four different grapevine rootstocks. Results showed that the best rooting ratio for 420A rootstock and lower number of roots, root fresh and dry weights for 110R rootstock than the others. They also indicated that scion thickness did not have significant effects on rooting.

Doğan et al. (2016) investigated the effects of scion diameter (4-7mm, 8-10 mm, 10-12 mm), rooting medium and IBA concentrations on rooting and root quality of 3 grapevine rootstocks (5BB, 420 A and 41B). Researchers reported for rooting and root quality parameters that the best outcomes were achieved from 10-12 mm scions for rooting ratio, from 8-10 mm scions for number of roots, from 10-12 mm scions for root length and from 10-12 mm scions for root fresh weight. In general, medium or thick scions exhibited better performance values.

This study was carried out to determine the effects of scion diameter on the success of callusing, final take and quality of shoot and root in grafted potted grapevine sapling production.

Material and Methods

This study was conducted at Grapevine Sapling Production Unit of Tokat Gaziosmanpaşa University in the year 2014. The scions of 5BB, 110 R and 1103 P rootstocks and cuttings of Narince grape cultivar were used as the material of the study. Omega-cut grafting tool was used in grafting. For potted sapling production, 1 l plastic bags filled with 1:1 perlite:peat mixture were used for all treatments. Pine chips and plastic crates were used for callusing of grafted scions.

The scions and cuttings used in this study were divided into 3 different thickness (diameter) groups (Diameter 1: 6-9 mm, Diameter 2: 10-13 mm, Diameter 3: 14-18 mm). Standard procedures were practiced throughout the stages of grafted grapevine production as of preparation of scions for grafting, water immersion, sanitation (thermotherapy, fungicide treatments), bench grafting, the first waxing, callusing, adaptation, the second waxing, 2000 ppm IBA treatments, planting into tubes and arboriculture (Akman and Ilgın, 1991; Ilgın et al., 1990; Çelik, 1983).

Data were collected at two periods.

1-Following the removal of grafted scions from the callusing unit: Grafting success ratio (%), callus development ratio on granting section (%), root and callus development ratio on basal sections of the scions.

2-Following the development of grapevine saplings: Total final take, shoot length (cm), shoot and root fresh and dry weight (g).

Figure 1. Pre-grafted cuttings were classified according to their diameter.

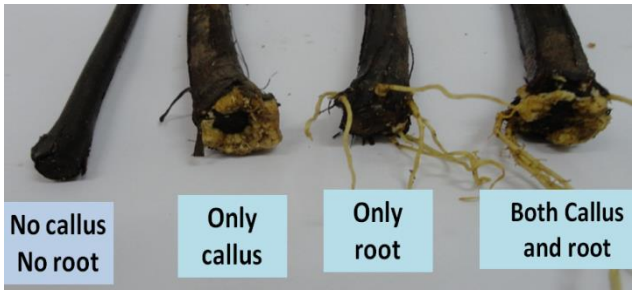

Figure 2. The root and callus development on the basal of cutting.

Experiments were conducted in randomized plots experimental design with two factors (rootstock and cutting diameter) and 4 replications with 25 grafting for each replication. Experimental results were subjected to variance analysis and means were compared with Duncan's multiple range test.

Results and Discussion

Scion thickness had significant effects on grafting success ratios. Success ratios varied between 94,0-100,0% and success ratios were generally identified as 100% in scions with a diameter greater than 10 mm (Table 1). Scion thickness also had significant effects on callus development ratio around the grafting section and overall callus development ratio of 5BB and 1103P rootstocks at %5 level. Callus development ratios around the grafting sections varied between 61-89% and overall callus development ratios varied between 82.3-96.2%. The greatest surrounding and overall callus development ratio was observed in 1103P rootstock and the lowest values were seen in 110R rootstock (Table 1).

Table 1. Effect of cutting thickness on callus development and grafting success ratios

Rootstock	Thickness	Grafting success ratio(%)	Callus development ratio (%)				
			25	50	75	100	Average Callus Ratio
5BB	6-9 mm	100.0a	3	2	13	82a	93.5 a
	10-13 mm	100.0a	1	2	11	86a	95.5 a
	14 mm≤	100.0a	14	20	32	34b	71.5 b
1103 P	6-9 mm	99.0a	2	1	7	89a	96.21 a
	10-13 mm	100.0a	1	7	14	78b	92.25 b
	14 mm≤	100.0a	1	14	24	61c	86.25 c
110 R	6-9 mm	94.0a	7	8	13	66a	86.70 a
	10-13 mm	100.0a	13	7	18	62a	82.25 a
	14 mm≤	100.0a	2	8	27	63a	87.75 a

*Each rootstock has been evaluated in its own right
** The means indicated with the same small letter in the same column are not significantly different ($p<0.05$)

Previous researchers indicated that rootstock-scion compatibility (Kester,1965; Coombe and Dry,1992), rootstock/scion combination (Türkben and Sivritepe,2000; Dardeniz and Şahin, 2005; Cakir at al., 2015; İşçi et al., 2015; Köse et al., 2015) and lignification levels of the scions (Exadaktylou et al., 2009) had significant effects on callus development around the grafting section.

Scion thickness had significant effects on callus, root and callus+root development on basal sections of the scions at 5% level. Callus development ratios on basal sections of the scions varied between 5-97%; callus+root development ratios varied between 3-88%. With regard to callus+root development ratios on basal sections of the scions, the best performance was observed in 1103P and the worst performance was observed in 110R rootstock (Table 2).

Table 2. Effect of cutting thickness on callus and root development in the basal part of the cuttings

Rootstock	Thickness	Callus and root development rate on the basal part of cuttings (%)			
		No callus – noroot	Only Callus	Only Root	Both callus and root
5BB	6-9 mm	7	46c	3b	44a
	10-13 mm	3	69a	0c	28b
	14 mm≤	10	62b	10a	18c
1103 P	6-9 mm	0	5c	7a	88a
	10-13 mm	0	19b	6a	75b
	14 mm≤	0	43a	2b	55c
110 R	6-9 mm	14	77c	0a	9a
	10-13 mm	8	86b	0a	6ab
	14 mm≤	0	97a	0a	3b

*Each rootstock has been evaluated in its own right

** The means indicated with the same small letter in the same column are not significantly different ($p<0.05$)

Lower performance of the grafted scions of 110R rootstock in callus and root development than the other rootstocks probable resulted from the genetic differences between the rootstocks. Thusly, Sucu and Yağcı (2015) investigated the effects of pre-grafting storage durations in callusing rooms on final take of the saplings and indicated that 100R and 140 Ru rootstocks started physiological activity later than the other rootstocks.

It was reported in another study carried out about the scion diameter and callus-root development of Gisela 5 rootstocks that callusing and rooting ratios decreased with increasing scion diameters and the greatest values were obtained from the thinnest (6-8 mm) scions (Exadaktylou et al., 2009).

In previous studies, significant effects of rootstock development and vigor (Williams and Smith, 1991; Tandonnet et al., 2010), root structure (Jogaiah et al., 2013), lignification status of the scions and genotypes (Beyl et al., 1995), scion taking time, scion length, scion diameter, scion position, pre-heating and hormone treatments (Hartmann et al., 2002; Tofanelli et al., 2003) on rooting and root parameters of ligneous scions of several cultivars were reported.

Scion thickness had significant effects on final take of the saplings. Final takes varied between 94-98%, which were quite a high ratio. While final takes decreased with increasing scion diameters in 5BB and 1103 P rootstocks, a relative increase was observed in 110R rootstock (Table 3).

Table 3. Effect of cutting thickness on total final take

	5BB Cutting thickness (mm)			1103 P Cutting thickness (mm)			110 R Cutting thickness (mm)		
	6-9	**10-13**	**14+**	**6-9**	**10-13**	**14+**	**6-9**	**10-13**	**14+**
Final take ratio (%)	96a	94a	88b	98a	94b	86c	90b	98a	96a

*Each rootstock has been evaluated in its own right

** The means indicated with the same small letter in the same column are not significantly different ($p<0.05$)

Exadaktylou et al. (2009) reported decreasing rooting ratios with increasing scion diameters in Gisela 5 scions. On the other hand, Dogan et al. (2016) reported increasing rooting ratios with increasing scion diameters in 3 grapevine rootstocks and also indicated varying rooting ratios with the rooting mediums and scions diameters. Çelik and Gargın(2009) indicated that scion diameters did not have significant effects on rooting ratios of hard-rooting 41 B, 110 R and 420 A grapevine rootstocks.

Several factors effects total final take in grafted grapevine sapling production. Such factors include rootstock/cultivar combinations, rooting capacity of the rootstocks, frost damage on winter buds, practices in grafting phase, grafted scion planting time and care conditions (Çelik and Ağaoğlu, 1979; Cangi, 1998; Baydar and Ece, 2005; Dardeniz and Şahin, 2005; Cangi et al., 2015). Quite high final takes of the present study indicated that the scions were quite healthy, well-lignified and optimum experimental conditions were provided.

Shoot and root parameters were determined when the potted grapevine saplings reached to a stage of planting. While scion thickness significantly influenced shoot fresh and dry weights of 5BB rootstock, the differences in

shoot lengths were not found to be significant. Shoot lengths of potted saplings varied between 13,95 - 38,50 cm. Shoot fresh weights varied between 6.57 - 13.20 g/vine, shoot dry weights varied between 1,05 - 1,91 g/vine. The heaviest and the longest shoots were observed in 1103P rootstock and the shortest and the lightest shoots were observed in 110R rootstocks. Scion thickness had relatively positive effects on shoot weights of 5BB and 1103P rootstocks (Table 4).

Table 4. Effect of root diameter on shoot parameters

Parameters	5BB Cutting thickness (mm)			1103 P Cutting thickness (mm)			110 R Cutting thickness (mm)		
	6-9	**10-13**	**14+**	**6-9**	**10-13**	**14+**	**6-9**	**10-13**	**14+**
Shoot length (cm)	32.45a	31.21 a	33.30a	36.61a	36.91 a	38.50a	19.10a	13.95a	22.00a
Shoot fresh weight (g/sapling)	9.06 b	10.83 ab	15.04a	11.68a	12.63a	13.20a	8.07 a	6.57 a	7.75 a
Shoot dry weight (g/sapling)	1.29 b	1.72ab	2.31a	1.81 a	1.84 a	191 a	1.24 a	1.05 a	1.20 a

*Each rootstock has been evaluated in its own right
** The means indicated with the same small letter in the same column are not significantly different ($p<0.05$)

It was reported in previous studies about the potted grapevine sapling production that shoot lengths varied with grafting combinations (Kılıç, 2014) and scion thickness did not have any significant effects on shoot lengths of the American grapevine saplings (Çelik and Gargın, 2009).

While the effects of scion thickness on root fresh weights were not found to be significant, scion thickness had significant effects on root dry weights of 5BB rootstock. Root fresh weights of the saplings varied between 1.60 - 3.26 g/vine and root dry weights varied between 0.08 - 0.6 g/vine. In general, weaker root developments were observed in 110R and 1103P rootstock with increasing scion thicknesses (Table 5).

It was reported in previous studies about grapevine sapling production that number of roots, root lengths, root development levels or weights varied with the rootstocks and similar with the present findings, the 110R rootstock had lower performance values with regard to these parameters (Çelik and Gargın, 2009; Sucu and Yağcı, 2015; Köse et al., 2015).

In scion-propagation, quality and successful productions, shoot and root development are influenced by various factors including scion diameter, scion length, plant genotype, scion taking time, lignification level and etc. (Mannini and Schneider, 1990; Beyl et al., 1995; Hartmann et al., 2002; Tofanelli et al., 2003; Çelik and Gargın, 2009).

Table 5.Effect of cutting thickness on root fresh and dry weight

Parameters	5BB Cutting thickness (mm)			1103 P Cutting thickness (mm)			110 R Cutting thickness (mm)		
	6-9	**10-13**	**14+**	**6-9**	**10-13**	**14+**	**6-9**	**10-13**	**14+**
Root fresh weigt (g)	2.49 a	2.24a	3.26 a	3.13 a	3.00a	1.60 a	3.08 a	2.22 a	2.00a
Root dry weight (g)	0.6 a	0.14b	0.20b	0.18 a	0.20a	0.08 a	0.18 a	0.11 a	0.11a

*Each rootstock has been evaluated in its own right
** The means indicated with the same small letter in the same column are not significantly different ($p<0.05$)

Conclusion

The following conclusions were drawn from the present study carried out to determine the effects of 3 different scion diameters of 5BB, 1103 P and 110 R rootstocks on potted grafted grapevine sapling production in greenhouse and sapling development in vineyard;

* In grafted scions, basal root and callus development varied with the rootstock genotype and scion diameter. Callus+root development generally decreased with increasing scion diameters.
* Quite high final takes (94-98%) were achieved in all rootstocks.
* Sapling root and shoot development were influenced by rootstock genotypes.
* Shoot biomass increased, but root biomass decreased with increasing scion diameters.
* With regard to sapling final take and quality, 5BB and 1103P rootstocks had better performance with thin and medium (6-13 mm) diameter scions and 110R had better performance with thick (14-18 mm) scions.

It was finally concluded that 7-18 mm thick scions could reliably be used in potted grapevine sapling production.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effect of Preharvest Applications on Storage and Quality Properties of Miho Wase Mandarin

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Abstract: In this study, liquid Nutri-Cal, powdered Sulfon and water (control) from leaves were applied to the Miho-Wase mandarin before harvest. The fruits were stored for 3 months under conditions containing 1°C temperature and 90% relative humidity. The fruit was kept in the shelf life for 7 days. The weight loss, color change, fruit juice amount, titratable acidity (TA) amount, total soluble solids (TSS) amount, pH and decay values of the fruits kept store and shelf life were evaluated periodically. When all the data were evaluated, Nutri-Cal liquid fertilizer application, which was applied to fruits for 1 month before 15 days, was found to have a positive effect on quality criteria during storage and shelf life.

Keywords: Mandarin, Storage, Nutri-Cal, Sulfon, Quality

Introduction

Citrus production in the world is increasing. According to FAO in the world there are about 140 000 000 tons of citrus production. Citrus production in our country as well as in the world is increasing rapidly. According to TUİK in 2016, there are approximately 4.300.000 tons of citrus fruit production in our country. This is 1.800.000 tons of orange, mandarin 1.400.000 tons, 850.000 tons of lemon, grapefruit 300.000 tons, while the vicinity of 2.000 tons of citrus (Anonymous, 2017). In our country the production of the total citrus is 88.38% in Mediterranean region and 10,93% in the Aegean region. Citrus flavors, is the leading product in terms of smell and colors are quite attractive garden. Storage factors affecting the preservation of garden products without significant losses; the temperature, the composition of the storage atmosphere, the relative humidity of the store air, and the air movement in the storage. These storage factors, especially the temperature and the composition of the gases in the tank atmosphere, slow down respiration and delay the maturation of two important influences (Canan et al. 2015).

Many studies on the preservation of citrus fruits have reported that the citrus fruits have a significant effect on the degree of preservation of the products after storage and on the preservation conditions of the species, variety, ecology of the cultivated region, rootstock used (Özdemir and Dündar 2006; Özdemir and Kahraman 2004).

10 min UV-C light application alone, calcium and hot water applications are applied individually or in combination. The 'Satsuma' fruit was stored at 5-10ºC for 90 days at a humidity of 90-95% for 2 months. Hot water and calcium chloride ($CaCl_2$) the amount of calcium in the treated fruit peel was found to slightly increase. In general, all applications except for applications where calcium has provided effective disease control. 53ºC 3 minutes in hot water applications besides having good control of the disease, the application of UV-C light show as negative and because of the many quality parameters were found to positively affect the most appropriate application (Şen and Karaçalı 2003).

Karamustafaoğlu (2008), after garden, harvest and harvest against *Phytophthora citrophthora* brown rot, *Penicillium digitatum* and *P. italicum* green and blue rot, *Lasiodiplodia theobromae* sap rot, *Alternaria citri* black rot and *Geotricum candidum* common rot in Mediterranean region. It was aimed to protect the orange product during the period. For this purpose, "sodium carbonate", which is used especially in organic agriculture and citrus diseases after harvest in California, is included. In addition, some fungicides known to be effective in the packaging house phase have also been used. This study was conducted at the Washington Navel orange. The best results were obtained from Fosetyl-Al and Thiophanate-methyl applications in the growing period before

the harvest and after 14 days at the temperature of 7-10°C in the cold air storage and after 7 days at the ambient temperature, the deterioration did not occur at the end of storage.

Two citrus species 'Swingle' citrumelo [*C. Macfad.* x *Poncirus trifoliate* (L.) Raf.] grafted 'Valencia' sweet orange [*Citrus sinensis* (L.) Osbeck] and 'Lisbon' lemon (*C. limon* L.), nitrogen-15 administration and made on the dispensing operation of orange trees in N content was found lower than lemon trees. For the use of N, the orange trees were found to be less effective than the lemon trees (Boaretto et al 2010).

Some growth Aegean regulators 'Satsuma' mandarin (*Citrus unshiu* Mar.) before harvesting on a commercial orchard in Selcuk district to determine the effect on storability on the tree giberellic acid (GA_3 10 ppm) of one and two times, 2,4-diclorofenoksi acetic acid (2,4-D, 16 ppm) was applied once. The first harvest was made at the time of the normal harvest time and thereafter 3 harvests were made at intervals of one month. The fruit from untreated trees in the first harvest were stored at 5±0.5ºC, 90-95% relative humidity. Each term in the fruit harvested and removed from storage; The quality parameters were investigated. pouring rates were determined again from the second fruit harvest. Some quality losses, including puffiness, were significantly inhibited in GA_3-applied trees 2 times. The 2,4-D and 2-times GA_3 treatments have significantly reduced spillage (Şen et al 2009).

Güneri et al (2012), in their research, Valencia orange variety was grown in alkaline soil, high pH due to soil sulfur, gypsum and citric acid ammonium sulfate foliar applications were made. According to the results of two-year nutrient element analysis; the amount of P that can be taken into the soil in the first year and the amount of Na in the second year were found to be low in sulfur and gypsum applications ($p < 0.01$). The amount of Zn in the fruit pulp increased in the second year of application ($p < 0.05$) and Mn increased in the first year ($p < 0.01$).

The aim of this study is to observe the effects of pre-harvest liquid Nutri-Cal and powder Sulphon fertilizer application on shelf life and quality of Miho Wase mandarin variety during storage.

Materials and Methods

This study was done in two stages. In the first step, liquid Nutri-Cal and powdered Sulfon were applied from the pre-ground leaf in the special producer's garden. In the years 2015-2016 as the second stage of physical and chemical analysis postharvest Cukurova University Faculty of Agriculture, Department of Horticulture, Postharvest Physiology Laboratory and was carried out in cold storage.

Fertilization two different applications were made at two different times, one month before and 15 days after. Tone 3 L Nutri-Cal first embodiment and second embodiment of liquid toned 3 L Nutri-Cal 3 kg was sprayed into liquid-sulfone powder fertilizer leaves from the tree. Only water was applied to the control trees.

The fruits were stored for 3 months under conditions containing 1°C temperature and 90% relative humidity. The fruit was kept in the shelf life for 7 days. The weight loss, color change, fruit juice, TA, TSS, pH and decay values of the fruits kept storage and shelf life were evaluated periodically (Özdemir and Dündar 2006).

The study randomized plots were established in three replications and 10 fruits on each replication according to experimental design. The data obtained from the experiment were subjected to analysis of variance using JMP statistical package program, differences between means LSD ($p \leq 0.05$) was determined.

Results and Discussion

The weight loss during storage of the Miho Wase mandarin fruits that were taken to the guard increased in all applications. The most weight loss was found in the fruits of Nutri-Cal + Sulfon group. Statistically, treatment, storage time and treatment*storage time were found to be important in weight loss. Weight loss in Miho Wase mandarin fruits in shelf life has increased in all treatments. The most weight loss was found in the fruits of the Nutri-Cal + Sulfon group in the shelf life. Statistically, treatment and shelf life were found to be important in weight loss (Table 1). Türk et al. (2009), there was also an increase in weight loss in their study of Valencia Late Orange and lemon (Canan et al. 2015).

Şen and Karaçalı (2003) observed that after harvesting of UV-C light and some other protective treatments, weight loss was also increased in the study of the effects of quality and stamina power of mandarin.

Table 1. Weight losses (%) during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)			Mean
	1	2	3	
Control	2,78 f	8,12 d	12,47 b	7,79 b
Nutri-Cal	2,68 f	7,41 e	11,82 c	7,30 c
Nutri-Cal+Sulfon	2,81 f	8,30 d	12,90 a	8,00 a
Mean	2,76 c	7,95 b	12,40 a	

$LSD_{0,05}$ Treatment:0,18, $LSD_{0,05}$ST:0,18, $LSD_{0,05}$: Treatment*ST: 0,32; ST:Storage Time

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	6,02	9,57	13,91	18,70	12,05 b
Nutri-Cal	6,31	9,78	13,06	18,56	11,93 b
Nutri-Cal+Sulfon	6,11	10,65	14,73	20,13	12,90 a
Mean	6,15 d	10,00 c	13,90 b	19,13 a	

$LSD_{0,05}$ Treatment:0,53, $LSD_{0,05}$SF: 0,61, $LSD_{0.05}$: Treatment*SF: NS; SF: Shelf Life

In Miho Wase mandarin fruit, the color change in the fruit peel was found to decrease in all three groups of fruit (Table 2). In the control group, h° angle value is high. Statistically, treatment and storage time were found to be important in fruit color. In shelf life Miho Wase Mandarin fruit changes were found to be reduced in all three groups of fruits. It has been determined that h° angle value is higher with applied Nutri-Cal+Sulfones fruit. Statistically, treatment and shelf life were found to be important in h° angle value at shelf life. Şen et al (2009), it was observed that the color values of "Satsuma" mandarin decreased during storage.

Table 2. Color (h°) during storage and shelf life of Miho Wase mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	101,61	97,96	93,81	91,58	96,24 a
Nutri-Cal	97,43	93,35	90,43	88,93	92,54 b
Nutri-Cal+Sulfon	100,76	96,25	94,02	90,43	95,37 a
Mean	99,94 a	95,86 b	92,75 c	90,31 d	

$LSD_{0.05}$ Treatment:1,29, $LSD_{0,05}$ ST: 1,49, $LSD_{0.05}$:Treatment*ST: NS

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	86,84	82,82	82,43	82,08	83,54 c
Nutri-Cal	88,46	84,89	83,71	81,58	84,66 b
Nutri-Cal+Sulfon	92,09	84,27	85,58	83,21	86,29 a
Mean	89,13 a	83,99 b	83,91 b	82,29 c	

$LSD_{0.05}$ Treatment:1,07, $LSD_{0,05}$ SF: 1,24, $LSD_{0.05}$:Treatment*SF: NS

The amount of fruit juice in the Nutri-Cal+Sulfon and Nutri-Cal groups decreased while the amount of fruit juice increased in the fruits of the control group during the storage period of Miho Wase mandarin fruit (Table 3). Statistically, treatment, storage time and treatment*storage time were found to be important in fruit juice at storage. The amount of fruit juice in the Miho Wase mandarin fruit in the shelf life increased. while the amount of fruit juice in the Nutri-Cal+Sulfon and Nutri-Cal groups increased, the fruit juice in the control group decreased (Table 3). Statistically, treatment, shelf and treatment*shelf life were found to be important in fruit juice at shelf life. Şen et al (2009) observed that the Satsuma mandarins decrease in the amount of fruit juice in their work.

During storage, the amount of titratable acid in the Miho Wase mandarin fruit showed increasing value in the fruits of the control group, while the value of the fruits in the Nutri-Cal+Sulfon and Nutri-Cal groups showed a declining value (Table 4). In shelf life Miho Wase mandarin fruits have a titratable acid content, whereas in control and Nutri-Cal groups the values are decreased (Table 4). The treatments are statistically significant. Şen et al (2009) and Türk et al (2009) found that Satsuma Mandarin decreases as in the Nutri-Cal+Sulphone and Nutri-Cal groups.

Table 3. Fruit Juice (%) during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	55,40 c	56,10 bc	56,41 bc	56,77 bc	56,17 b
Nutri-Cal	61,07 a	61,90 a	59,05 a	55,31 c	59,33 a
Nutri-Cal+Sulfon	53,89 c	55,82 c	55,43 c	48,15 d	53,32 c
Mean	56,79 a	57,94 a	56,96 a	53,41 b	

$LSD_{0,05}$Treatment:1,53 $LSD_{0,05}$ST: 1,77 $LSD_{0,05}$Treatment*ST: 3,07.

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	65,41 a	50,27 fg	60,27 abc	58,94 abcd	58,72 a
Nutri-Cal	51,74 efg	53,26 cdefg	62,54 ab	58,63 abcde	56,54 a
Nutri-Cal+Sulfon	48,95 fg	47,68 g	55,48 bcdef	52,89 defg	51,25 b
Mean	55,37 a	50,40 b	59,43 a	56,82 a	

$LSD_{0,05}$Treatment:3,59 $LSD_{0,05}$SF: 4,15 $LSD_{0,05}$Treatment*SF: 7,19.

Table 4. TA (%) during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	0,92	0,83	0,83	0,84	0,85 b
Nutri-Cal	0,92	0,83	1,02	0,91	0,92 a
Nutri-Cal+Sulfon	0,92	0,85	0,89	0,98	0,91 a
Mean	0,92	0,84	0,91	0,91	

$LSD_{0,05}$Treatment: 0,07 $LSD_{0,05}$ ST: NS $LSD_{0,05}$Treatment*ST: NS

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	0,84	0,82	0,81	0,79	0,82 a
Nutri-Cal	0,82	0,86	0,70	0,81	0,79 a
Nutri-Cal+Sulfon	0,67	0,74	0,67	0,60	0,67 b
Mean	0,78	0,81	0,73	0,73	

$LSD_{0,05}$Treatment: 0,07, $LSD_{0,05}$ ST: NS, $LSD_{0,05}$Treatment*ST: NS

Miho Wase has been found to have increased value of TSS in the control of fruits and in the fruits of the Nutri-Cal group. Statistically, treatment, storage time and treatment*storage time were found to be important in TSS at storage. In the shelf life, increasing value of TSS was observed in the fruits in all three applications. Statistically, treatment, shelf life and treatment*shelf life were found to be important in TSS at shelf life (Table 5). Şen et al (2009) in Satsuma mandarin and Türk et al (2009) in Valencia Late oranges found an increase in the TSS in their studies. Statistically, treatment, shelf life and treatment*shelf life were found to be important in TSS at shelf life.

Table 5. TSS (%) during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	9,73	10,13	10,27	10,33	10,12 a
Nutri-Cal	9,87	10,00	10,07	9,80	9,93 a
Nutri-Cal+Sulfon	9,73	9,60	9,87	9,33	9,63 b
Mean	9,78	9,91	10,07	9,82	

$LSD_{0,05}$ Treatment:0,23.$LSD_{0,05}$ST: Ö.D $LSD_{0.05}$Treatment*ST: NS

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	10,13 bcd	10,60 a	10,07 bcd	10,40 abc	10,30 a
Nutri-Cal	9,93 d	10,27 abcd	10,47 ab	9,93 d	10,15 a
Nutri-Cal+Sulfon	8,73 e	10,00 cd	9,93 d	9,87 d	9,63 b
Mean	9,60 b	10,29 a	10,16 a	10,07 a	

$LSD_{0,05}$ Treatment:0,22, $LSD_{0,05}$ST: 0,26, $LSD_{0.05}$Treatment*ST: 0,45.

During storage the pH value of Miho Wase mandarin fruit increased in all three applications. The highest pH value of Nutri-Cal+Sulfon group was found in the fruits. Statistically, treatment and storage time were found to be important in pH at storage. The pH values in shelf life have also been found to be increasing in all three application fruits. The highest pH value of Nutri-Cal+Sulfon group was found in the fruits. Statistically, treatment and shelf life were found to be important in pH at shelf life (Table 6). Türk et al. (2009) also found an increase in the pH values of Valencia Late oranges in their study.

Table 6. pH during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	3,79	3,71	3,88	3,97	3,84 b
Nutri-Cal	3,74	3,63	3,83	3,99	3,80 b
Nutri-Cal+Sulfon	3,79	3,87	3,90	4,02	3,89 a
Mean	3,78 c	3,74 c	3,87 b	3,99 a	

$LSD_{0,05}$ Treatment:0,05 $LSD_{0,05}$ ST: 0,06 $LSD_{0,05}$:Treatment*ST: NS

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	3,82	3,82	3,90	4,05	3,90 b
Nutri-Cal	3,87	3,76	3,99	4,07	3,92 b
Nutri-Cal+Sulfon	4,02	3,92	4,05	4,25	4,06 a
Mean	3,90 c	3,83 d	3,98 b	4,12 a	

$LSD_{0,05}$ Treatment:0,05 $LSD_{0,05}$ SF: 0,06 $LSD_{0,05}$:Treatment*SF: NS

During the storage period, the number of decay fruits increased in all three applications of Miho Wase mandarin fruit. The highest percentage of decay was found in the control group of fruits, followed by the Nutri-Cal+Sulfon and the Nutri-Cal groups. Statistically, treatment, storage time and treatment*storage time were found to be important in decay fruits at storage. In the shelf life, the number of decay fruits was highest in the Nutri-Cal+Sulfon group and the control and Nutri-Cal group fruits were observed. Statistically, treatment, shelf life and treatment*shelf life were found to be important in decay fruits at shelf life (Table 7).

Table 7. Decay (%) during storage and shelf life of Miho Wase Mandarin

Treatment	Storage Time (month)				Mean
	0	1	2	3	
Control	0,00 d	0,00 d	0,00 d	46,67 a	11,67 a
Nutri-Cal	0,00 d	0,00 d	3,33 d	16,67 c	5,00 c
Nutri-Cal+Sulfon	0,00 d	0,00 d	0,00 d	33,33 b	8,33 b
Mean	0,00 b	0,00 b	1,11 b	32,22 a	

$LSD_{0,05}$ Treatment:2,81 $LSD_{0,05}$ST: 3,24 $LSD_{0,05}$:Treatment*ST: 5,62.

Treatment	Shelf Life (month+day)				Mean
	0+7	1+7	2+7	3+7	
Control	0,00 e	3,33 e	20,00 d	46,67 b	17,50 b
Nutri-Cal	0,00 e	3,33 e	6,67 e	30,00 cd	10,00 c
Nutri-Cal+Sulfon	0,00 e	6,67 e	33,33 c	63,33 a	25,83 a
Mean	0,00 c	4,44 c	20,00 b	46,67 a	

$LSD_{0,05}$ Treatment:5,06 $LSD_{0,05}$SF: 5,85 $LSD_{0,05}$:Treatment*SF:10,13.

Conclusion

When all the data were evaluated, Nutri-Cal liquid fertilizer application, which was applied to fruit for 15 days 1 month ago, was found to have a positive effect on quality criteria during storage and shelf life. Different doses may be considered to be effective in the administration of Nutri-Cal + Sulfones.

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Araştırma Makalesi/*Research Article (Original Paper)*

Van Gölü Havzası Endemik Balık Türleri

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Özet: Kapalı bir havza özelliğinde olan Van Gölü Havzası'nda birçok su kaynağı bulunmaktadır. Bu su kaynaklarındaki farklılık kendisini *Alburnus tarichi, Alburnus timarensis, Barbus ercisianus, Capoeta kosswigi ve Oxynoemacheilus ercisianus* gibi endemik balık türlerinde göstermiştir. Bu balık türlerinin ekolojik özelliklerinin bilinmesi, türün havzadaki su kaynaklarında devamlılığının sağlanması açısından önemlidir. Evsel ve sanayi atıkları, akarsu üzerindeki kum ocakları ve kaçak kum alım faaliyetleri, akarsu üzerinde inşa edilen regülatör, menfez ve köprü gibi yapılar, akarsu ıslah çalışmaları, üreme döneminde gerçekleştirilen kaçak avcılık bu türleri tehdit eden başlıca unsurlardır. Bu çalışmada havzada doğal yayılış gösteren endemik balık türlerinin, ekolojik ve biyolojik özellikleri, diğer balık cinslerden ayrılan farklılıkları ve yaşadığı su kaynakları hakkında bazı bilgiler verilerek, sürdürülebilirliklerinin sağlanması amacıyla dikkatlerin bu türler üzerine çekilmesi amaçlanmıştır.

Anahtar kelimeler: Van Gölü Havzası, endemik balık türleri, balık sistematiği, su kaynakları.

Endemic Fish Species of Van Lake Basin

Abstract: The Van Lake Basin is a closed basin that has many water sources. This differentiation in the water resources shows on endemic fish species. All species of fish showing natural distribution in basin are consist of *Albanus tarichi, Alburnus timarensis, Barbus ercisianus, Capoeta kosswigi*, and *Oxynoemacheilus ercisianus*. It is important to know the ecological characteristics of the endemic fish species in order to ensure their continuity in Van Lake Basin. Domestic and industrial wastes, sand quarries and illegal sand extraction activities, regulators, culverts and bridges on the river, river improvement studies and poaching in breeding period are the main threats to these endemic species. The present study, it is aimed to give some information about ecological and biological characteristics of endemic fish species and water resources in Van Lake Basin, differences between the fish species that are separated from other fish species and aiming to draw attention to these species in order to ensure the sustainability of these endemic species.

Keywords: Van Lake Basin, endemic fishes, fish systematics, water resources.

Giriş

Endemik sözcüğü yeryüzü üzerinde belli bir bölgede yayılım gösteren bitkisel ve hayvansal canlılar için iklim ve toprak özelliklerine bağlı kalarak tanımlanmakta iken, endemizm ise yine aynı canlılar için yeryüzündeki belli bir alanda tutulma veya bulunma durumunu ifade etmektedir. Diğer bir ifade ile sadece belli bir bölgede yaşayan ve o bölge dışında yeryüzünün başka alanlarında bulunmayan tüm canlılar o bölgenin endemik canlılarını oluşturmaktadır (Ekim ve Çağlar 2003).

Dünya üzerinde günümüze kadar bildirilmiş 80.000 kadar omurgalı tür bildirilmiştir. Bunlardan geçerli tür sayısının 50.000 civarında olduğu tahmin edilmektedir. Bu geçerli omurgalı türlerinden 34.300 den fazlasını balıklar oluşturmaktadır. Balık tür çeşitliliğinde mercan resifleri gibi bazı sıcak noktalar vardır. Tür çeşitliliğinin zengin olduğu bölgelerden biri de zoocoğrafik açıdan önemli bir geçiş noktası niteliğindeki Anadolu coğrafyasıdır. Komşu coğrafyaların etkisinin yanında Anadolu'nun kendine has coğrafik özellik ve iklim yapısı da tür çeşitliliğimiz artırmıştır. Nitekim ülkemiz iç sularında 370 in üzerinde tür bulunmaktadır ve bunlardan yaklaşık 115 tür ülkemiz için endemiktir (Çiçek ve ark. 2015).

Ülkemizde yaşayan bu endemik balık türleri birçok tehdit ile karşı karşıyadır. Evsel ve sanayi atıkları, akarsu üzerindeki kum ocakları ve kaçak kum alım faaliyetleri, akarsu üzerinde inşa edilen regülatör, menfez ve köprü gibi yapılar, akarsu ıslah çalışmaları, üreme döneminde gerçekleştirilen kaçak avcılık başlıca sorunlar arasında yer almaktadır (Elp 1996; Elp ve ark. 2006a; Şen ve ark. 2015; Atıcı 2017). Bu tür olumsuzluklara örnek olarak yakın zamanda Beyşehir Gölü'ne endemik olan *Alburnus akili* türünün nesli sona ermiş olması verilebilir (İlhan ve ark. 2014).

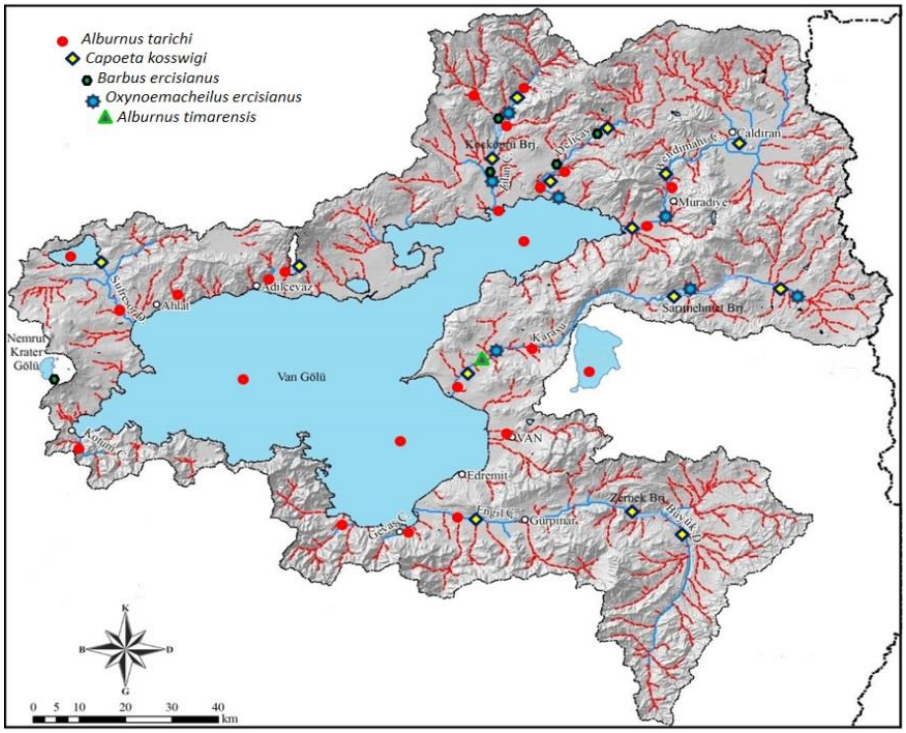
Bu çalışmada, Van Gölü Havzası'na endemik olan inci kefali (*Alburnus tarichi*), timar incisi (*Alburnus timarensis*), siraz (*Capoeta cosswigi*), Erciş bıyıklısı (*Barbus ercisianus*) ve çöpçü balığı (*Oxynoemacheilus ercisianus*)'nın ekolojik ve biyolojik özellikleri, diğer balık türlerinden ayrılan farklılıkları ile yaşadığı su kaynaklarında karşılaştığı çevre sorunları hakkında bazı bilgiler verilerek, bu endemik türlerin sürdürülebilirliğinin sağlanması amacıyla dikkatlerin bu türler üzerine çekilmesi amaçlanmıştır.

Materyal ve Metot

Balık örnekleri toplanırken av aracı olarak serpme ağ, fanyalı ağ ve SAMUS 725 MP marka elektroanestezi cihazı kullanılmıştır. Örnekler havzada yer alan akarsularda aynı gün içerisinde farklı bölgelerine gidilerek yapıldığı gibi, farklı mevsimlerde de örnek toplanarak habitatlarda yayılış gösteren tüm türlere ulaşılmaya çalışılmıştır. Aynı örnekleme yaklaşımı göl ve baraj göllerinde de uygulanmıştır. Örneklemeler sırasında su kaynağında karşılaşılan çevresel sorunlar da not edilmiştir. Elde edilen örnekler bulunduğu bölgeyi tanımlayacak şekilde etiketlenmiş ve %4'lük formaldehitli kaplara konarak laboratuvara getirilmişlerdir. Örnekler bir süre %4'lük formaldehitte bekletildikten sonra alkol serisinden geçirilmiş ve % 70'lik etil alkole alınmıştır (Kottelat ve Freyhof 2007). Fiksasyon işlemi tamamlanan örneklerin taksonomik karakterleri ile ilişkili ölçüm ve sayımları yapılmıştır. Daha sonra çeşitli literatürlerden faydalanılarak sistematik olarak türler belirlenmiştir (Geldiay ve Balık 2009).

Bulgular

Laboratuvar getirilen örneklerden tür tayini yapılıp, alındığı su kaynağına göre havzadaki yayılım bölgeleri Şekil 1'de görüldüğü gibi tespit edilmiştir.



Şekil 1. Van Gölü Havzası'nda yayılım gösteren endemik balıkların bulunduğu noktalar

1. İnci Kefali, Van Balığı *(Alburnus tarichi,* Güldenstadt 1814*)*

Van Gölü havzasına endemik olan inci kefali ile ilgili ilk kayıt Pallas (1811) tarafından bildirilmiştir. Türün 1966 yılında Burdur Gölü'ne ve 1982-1984 yıllarında Erçek Gölü'ne aşılama çalışmaları olmuştur. Burdur Gölü'nde türün populasyon oluşturduğu, ancak sonrasında bir anda ortamdan kaybolduğu ile ilgili kayıt bulunmaktadır (Akşiray 1982). *Alburnus tarichi,* taksonomik olarak Cyprinidae (sazangiller) familyası üyelerindendir. Erçek Gölü'ne aşılanan inci kefali populasyon oluşturmuş olup ticari avcılığı yapılmaktadır (Elp ve Şen 2006).

IUCN Kırmızı liste kategorisi: İnci kefali kırmızı listede Near Threatened (NT) yani "Tehlikeye Yakın" sınıfta yer almaktadır (IUCN 2018a).

Tespit edildiği yerler: İnci kefalinin Van Gölü, Nazik Gölü, Erçek Gölü, Aygır Gölü ve Koçköprü Baraj Gölü ve Van Sazlıkları gibi durgun su ortamları ile Engil, Karasu, Akköprü, Kurubaş, Memedik, Bendimahi Çayı, Deliçay, Zilan, Uludere, Karmuç, Sapur, Güzelkonak, Gevaş gibi akarsulardan yayılış gösterdiği belirlenmiştir (Elp ve ark. 2016).

Ayırt edici özellikler: İnce uzun ve yanlardan hafif basık olan vücut üzerinde siyah renkli sikloid pullar yer almaktadır (Şekil 2). Terminal konumlu ağızda alt çene yukarıya hafif dönmüştür. Yüzgeç ışın sayısı D III/8-9. A III/10-13½. V I/7-8 ve P 14-17 şeklindedir. Yanal çizgi üzerinde 70-90 adet pul yer almaktadır. Farinks dişleri iki sıralı ve 2:5-5:2 formunda iken, omur sayısı 42-45 arasında değişmektedir. Solungaçlarda 21-29 arasında solungaç dikeni bulunmaktadır. Anal yüzgeç dorsal yüzgecin 3-4 pul gerisinden başlamaktadır (Çetinkaya ve Elp 1995; Elp ve ark. 2013).

Şekil 2. Olgun bir inci kefali (*Alburnus tarichi*, Güldenstadt 1814).

Yaşam özellikleri: Tuzlu, tatlı ve acı su ortamlarında yaşayabilen inci kefali akarsu ve göllerde gruplar halinde gezer. Genellikle su kolonunda pelajik bölgede yaşar. Omnivor bir beslenme özelliğine sahip olan inci kefalleri fitoplankton ve zooplankton yanında yumuşakçalar, kurtlar, küçük kabuklular ve böcek larvaları ile beslenmektedir (Danulat ve Selçuk 1992; Geldiay ve Balık 2009). Akarsulara üremek için giren dişi inci kefalleri yumurtalarını akarsularda fazla derin olmayan yerlerdeki otlara, kumlu-çakıllı taban üzerine bırakır. Üreme döneminde (Mayıs-Haziran ayları) erkek inci kefallerinin baş bölgelerinde üreme tüberkülleri oluşmaktadır (Elp 1996; Atıcı ve ark. 2018).

Tehditler: Evsel ve sanayi atıkları, akarsu üzerindeki kum ocakları ve kaçak kum alım faaliyetleri, akarsu üzerinde inşa edilen regülatör, menfez ve köprü gibi yapılar, akarsu ıslah çalışmaları, üreme döneminde gerçekleştirilen kaçak avcılık başlıca sorunlar arasında yer almaktadır (Elp ve ark. 2006a; Şen ve ark. 2015; Atıcı 2017). Üreme döneminde akarsulardan tarımsal faaliyetler için alınan suların Deliçay ve Bendimahi çaylarında hem ölümlere hem de üreme başarısının düşmesine neden olduğu gözlenmiştir.

2. Timar İncisi (*Alburnus timarensis*, Kuru 1980)

Havzaya endemik diğer bir tür olan *Alburnus timarensis* ile ilgili çok fazla çalışma olmamakla birlikte, Kuru (1980) ve Elp ve ark (2013)'nın bu türle ilgili taksonomik özelliklerini ortaya koyup sistematik tanımlamasını yaptıkları çalışmaları bulunmaktadır. *Alburnus timarensis,* taksonomik olarak Cyprinidae (sazangiller) familyasında yer almaktadır.

IUCN Kırmızı liste kategorisi: *Alburnus timarensis* kırmızı listede Critically Endangered (CE) yani "Kritik Olarak Nesli Tehlike" sınıfında yer almaktadır (IUCN 2018b).

Tespit edildiği yerler: *Alburnus timarensis*'in Van Gölü havzası içerisinde Karasu çayında Van gölüne döküldüğü Zeve Şehitliği ile yaklaşık 10 km'lik bir mesafede olan Ablanges köprüsü arasında yayılış gösterdiği, Van Gölü'ne girmediği belirlenmiştir (Elp ve ark. 2013).

Ayırt edici özellikler: *Alburnus timarensis*'te vücut simli, canlı bireylerde renk gümüşimsi, yanal çizgi boyunca soluk pembe-yeşil bir bant bulunmaktadır, bazı bireylerde bant belirsiz olup, ancak belli bir açıdan görülebilmektedir (Şekil 3). Diğer Alburnus türlerinden ayırt edici özellik olarak yanal çizgide 54-68 pul bulunurken, 13-17 solungaç dikenine sahip olması da başka bir özelliğidir. Bazı türlerde vücutta yanal çizginin üstünde siyah noktalar şeklinde lekeler de bulunmaktadır (Elp ve ark. 2013).

Şekil 3. Timar İncisi (*Alburnus timarensis,* Kuru 1980) (Elp ve ark. 2013).

Yaşam özellikleri: *Alburnus timarensis* yayılım gösterdiği Karasu Çayı hızlı akan bir akıntıya sahiptir. Su sıcaklığı yıl boyunca ortalama 12.6 ºC olurken, kışın su sıcaklığı 0 ºC'ye yaklaşmakta, yazın da 25.5 ºC olmaktadır. Akarsuda bulunan yumuşakçalar, kurtlar, küçük kabuklular ve böcek larvaları timar incisinin başlıca besin kaynağını oluşturmaktadır. İnci kefali gibi göle girme özelliği taşımayan timar incisi yaşamının tüm safhasını Karasu Çayı'nda geçirmektedir (Elp ve ark. 2013).

Tehditler: Evsel atıklar, akarsu üzerindeki kum ocakları ve kaçak kum alım faaliyetleri, akarsu üzerinde inşa edilen baraj, gölet, regülatör, menfez ve köprü gibi yapılar, akarsu ıslah çalışmaları, tarımda sulama amacıyla regülatörlerden suyun tarlara aktarılması sonucu akarsuda suyun debisinin azalması başlıca sorunlar arasında yer almaktadır (Elp ve ark. 2006a; Şen ve ark. 2015; Atıcı 2017).

3. Siraz (*Capoeta kosswigi*, Karaman 1969)

Capoeta kosswigi taksonomik olarak Cyprinidae (sazangiller) familyasında yer almaktadır (Karaman 1969).

IUCN Kırmızı liste kategorisi: *Capoeta kosswigi*, kırmızı listede Data Deficient (DD) yani "Eksik Veri" olarak bildirilmiştir (IUCN 2018c).

Ayırt edici özellikler: Yüzgeç ışın sayısı D III- IV 8-9, A III 5 şeklinde olup, yanal çizgide 70-88 adet pul bulunmaktadır. Solungaç diken sayısı 19-24 adettir. Yuvarlakça ve uzun yapılı olan vücut, çok sayıda küçük pullarla örtülüdür. Baş boyu daima vücut yüksekliğinden daha küçüktür. Küçük ve at nalı şeklinde olan ağızda bir çift kısa bıyık bulunur. Burun kısmı sivri yapılı ve uzuncadır (Şekil 4) (Karaman 1969; Geldiay ve Balık 2009).

Tespit edildiği yerler: *Capoeta kosswigi* havzada Nazik ve Aygır Gölü ile Bendimahi, Deliçay, Zilan, Karmuç, Engil ve Karasu derelerinde yayılım göstermektedir (Elp ve ark. 2016; Elp 2017).

Şekil 4. Siraz (*Capoeta kosswigi*, Karaman 1969).

Yaşam özellikleri: Akarsularda hızlı akan, zemini taşlı ve çakıllı bölgelerde yaşamaktadır. Barbus ve alabalık türleri ile yayılım gösterdiği yerlerde de rastlanabilir (Geldiay ve Balık 2009). Bu çalışmada büyük bireylerin daha çok akıntılı bölgelerde yayılış gösterirken, küçük bireylerin durgun alanlarda da bulunduğu tespit edilmiştir. Üreme döneminde erkek bireylerde üreme tüberkülleri denilen beyaz noktalar oluşmaktadır (Elp 2002; Atıcı ve ark. 2018).

4. Erciş Bıyıklısı *(Barbus ercisianus,* Karaman 1971)

Barbus ercisianus, taksonomik olarak Cyprinidae (sazangiller) familyasında yer almaktadır (Geldiay ve Balık 2009).

IUCN Kırmızı liste kategorisi: *Barbus ercisianus,* kırmızı listede Data Deficient (DD) yani "Eksik Veri" olarak bildirilmiştir (IUCN 2018d).

Tespit edildiği yerler: Erciş bıyıklısının havza içerisinde Zilan ve Deliçay ile Nemrut krater gölünde yayılım gösterdiği belirlenmiştir (Elp ve ark. 2016).

Ayırt edici özellikler: Vücut genellikle uzunca ve sikloid pullarla örtülü olup, ventral konumlu ağızda iki çift bıyık yer almaktadır. Burun küt yapıda ve baş büyüktür (Şekil 5). Farinks dişleri üç sıralı 2.3.5-5.3.2, nadiren de 2.3.4-4.3.2 şeklinde sıralıdır. Yüzgeç ışın sayıları D III 8, A III 5 şeklinde, yanal çizgi pul sayısı ise 65-67 adettir. Sırt kısmında belirgin bir karina olmayıp, yüzgeçler ve vücut genelinde koyu renkli benekler bulunmaktadır (Geldiay ve Balık 2009; Elp ve ark. 2006b).

Şekil 5. Erciş Bıyıklısı (*Barbus ercisianus,* Karaman 1971).

Yaşam özellikleri: Genellikle hızlı akışlı zemini çakıllı ve kumlu akarsuları tercih eder. Başlıca besinleri küçük böcek larvaları, *Gammarus, Diaptamus* ve *Daphnia* gibi kabuklular, sivrisinek larvaları ve yumuşakçalar oluşturmaktadır (Geldiay ve Balık 2009). Üreme döneminde erkek bireylerde üreme tüberkülleri denilen beyaz noktalar oluşmaktadır (Atıcı ve ark. 2018).

5. Çöpçü balığı (*Oxynoemacheilus ercisianus*, Erk'akan ve Kuru 1986)

Oxynoemacheilus ercisianus taksonomik olarak Nemacheilidae familyasında yer almaktadır (Erk'akan ve Kuru 1969).

IUCN Kırmızı liste kategorisi: *Oxynoemacheilus ercisianus*, kırmızı listede Endangered (EN) yani "Nesli Tehlikede" olarak bildirilmiştir (IUCN 2018e).

Ayırt edici özellikler: İnce uzun şeklindeki vücutta küçük pullarla kaplıdır. Ventral konumlu ağzın etrafında nispeten iyi gelişmiş dudaklar yer almaktadır. Üç çift kısa bıyıktan iki çifti burun üzerinde, bir çifti de ağız köşelerinde bulunmaktadır (Şekil 6) (Erk'akan ve Kuru 1969).

Tespit edildiği yerler: *Oxynoemacheilus ercisianus* havzada Bendimahi, Deliçay, Zilan, Karmuç, Engil ve Karasu çayında ayrıca havzadaki baraj göllerinden Koçköprü, Zernek ve Sarımehmet Barajları'nda yayılış göstermektedir (Elp ve ark. 2016).

Şekil 6. Çöpçü balığı (*Oxynoemacheilus ercisianus,* Erk'akan ve Kuru 1986).

Yaşam özellikleri: Temiz ve serin olan akarsularda yayılım gösterirken, akarsuların yavaş akan çakıllı kumlu zeminlerini tercih etmektedir. Besininin büyük bir kısmını kurtlar ve böcek larvaları oluşturmaktadır (Geldiay ve Balık 2009).

Tartışma ve Sonuç

Havzaya endemik olan bu beş tür arasından *A. tarichi* en büyük yayılımını Van Gölü'nde gösterirken, Van Gölü'ne dökülen akarsularda da bulunmaktadır. İnci kefali Aygır, Nazik, Erçek, Koçköprü Baraj Gölü ve Van Gölü olmak üzere beş farklı populasyon oluşturmuştur. Yukarıda bildirilen tüm kaynaklarda inci kefalinin yayılış gösterdiği, ancak akarsularda sadece üreme dönemi olan Mayıs-Haziran aylarında görülürken, göl ve baraj göllerinde yılın her ayında yayılış gösterdiği bildirilmiştir. Yapmış olduğumu çalışma ile önceden yapılan örnekleme çalışmaları uyumludur (Kuru 1975; Çetinkaya 1996; Çetinkaya 1999; Çetinkaya 2000; Geldiay ve Balık 2009; Elp ve ark. 2006a; Kocabaş 1999; Elp 2002; Elp ve ark. 2014; Elp ve ark. 2016).
Havzada yayılış gösteren bir diğer endemik Alburnus türü olan *A. timarensis*'in Kuru (1980) tarafından Karasu Çayı ve Zilan Çayı'nda da yayılış gösterdiğini bildirmiştir. Yapılan bu çalışmada da *A. timarensis* sadece Karasu Çayı'nda örneklenmiştir. Bu bulgu Elp ve ark. (2013) tarafından verilen bilgiler ile de uyumludur. Bu tür ile ilgili mevcut veriler populasyon dinamiği ile ilişkili yetersiz olsa da yayılım alanının darlığından dolayı ve Karasu Çayı'na yapılan müdahalelerden dolayı CE kategorisinde yer alması doğru bir yaklaşımdır. Ancak en kısa sürede populasyon yapısı ile ilişkili çalışmalar yapılmalıdır.

Capoeta kosswigi havzada Nazik ve Aygır Gölü ile Bendimahi, Deliçay, Zilan, Karmuç, Engil ve Karasu derelerinde ayrıca Koçköprü, Zernek ve Sarımehmet Baraj Gölleri'nde de yayılış göstermektedir. Bu bulgular önceki çalışmalar ile de uyumludur (Kuru 1975; Evci 1997; Elp 2002). Bu türün büyüme ve üremesi ile ilgili çeşitli yayınlar bulunmakla birlikte populasyonların sürdürülebilirliği ile ilgili çalışmalara ihtiyaç vardır. Bu nedenle kırmızı listede DD statüsünde tanımlanması doğru bir yaklaşımdır.

Erciş bıyıklısı olarak da adlandırılan *Barbus ercisianus* havzada Deliçay ve Zilan akarsuları ile Nemrut Krater Gölü'nde yayılış gösterirken, Kuru (1975) bu türü Erciş bölgesinde Van Gölü'nden bildirmiştir. Yapılan örneklemelerde türün sadece tatlısu ortamında yaşadığı görülmüştür. Ayrıca kırmızı listede Data Deficient (DD) yani "Eksik Veri" olarak bildirilen bu türle ilgili populasyon dinamiği çalışmalarına ihtiyaç bulunmaktadır.

Çöpçü balığı olarak da tanınan *Oxynoemacheilus ercisianus* havzanın doğu, kuzey ve güney bölgelerinde yayılış gösterirken, bu türün birden fazla su kaynağında bulunması ve kendisini avlayan ciddi bir düşmanının olmaması

sebebiyle türün kırmızı listede Endangered (EN) yani "Nesli Tehlikede" yerine Near Threatened (NT) yani "Tehlikeye Yakın" olarak tanımlanması daha doğru bir yaklaşım olacaktır.

Havza için endemik olan bu türlerin özellikle üreme dönemlerinde koruma altına alınması türün devamlılığı açısından oldukça önemlidir. Ürüme döneminde gerçekleştirilen kaçak kum alım faaliyetleri suyun bulandırmasının yanında sedimentte silt yapıdaki küçük parçacıkları tekrar harekete geçirmektedir. Su kolonunda askı haline geçen bu partiküller yumurta üzerine düşerek yumurtalara yapışmakta ve yumurtaların açılmadan ölmesine neden olmaktadır. Ayrıca hareket haline geçen bu partiküller balıkların solungaç yapılarını kapamakta ve ölümlerine neden olmaktadır. Elp ve ark. (2006a), Karasu Çayında 2003 ve 2004 kaçak kum alımları nedeniyle Mayıs-Haziran aylarında toplu balık ölümleri gerçekleştiğini bildirirken, Atıcı (2017) tarafından gerçekleştirilen askıda katı madde (AKM) ve bulanıklık denemelerinde 100 mg/L ve üzeri AKM içeren su ortamlarında inci kefali yumurta ve larvalarında yüksek düzeyde ölümlerin meydana gelmiştir.

Havzada diğer bir sorun ise akarsuda balıkların göçmesini engelleyen yapılardan olan regülatörlerden özellik tarımsal faaliyetlerin gerçekleştirildiği dönemlerde tarlalara su verilmesi sonucu akarsuyun debisi oldukça düşmektedir. Bu dönemlerde tarımsal faaliyet için kullanılan su miktarı doğru belirlenmeli, gereğinden fazla su aktarımından kaçınılmalıdır.

Ülkemiz sularında kirlilik, habitat bozulması, yabancı türlerin aşılanması gibi insan etkenli olumsuz birçok durumla karşı karışayız. Bu olumsuzluklar su kaynakları üzerinde ciddi bir çevresel baskı oluşturmakta, önemli ekolojik ve ekonomik sorunlara yol açabilmektedir (Sülün 2014).

Sonuç olarak, havzadaki bu endemik türlerle ilgili gerekli çalışmalar yapılmalıdır. *Alburnus tarichi* dışında havzaya endemik olan diğer türlerin de neslini devam ettirmesi konusunda sorunlar yaşadığı bilinmeli, inci kefali ile birlikte havzaya endemik diğer balık türleri de içeren koruyucu önlemler alınmalıdır.

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Araştırma Makalesi/*Research Article (Original Paper)*

Serological and Molecular Detection of PVY^{N}, PVY^{C}, PVY^{O} and PVY^{NTN} on Tomato and Pepper in Urmia

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Abstract: Viral diseases are very important on tomato and pepper because of their impact on the quality and quantity of these crops. *Potato virus Y* (PVY) belongs to the genus *Potyvirus* and family *Potyviridae* is one of the most important viruses and has a worldwide distribution on Solanaceae. In order to detection of the virus and determine taxonomic status of strains on tomato and pepper, a total of 88 samples were collected during growing seasons of 2015 and 2016 from different fields of Ziveh, Nazloo, Par, Chongharalouye Pol, Balanej and AliAbad around Urmia and subjected to Indirect-ELISA. The virus was detected in 23 samples. Total RNA was extracted from positive samples in ELISA, and synthesized cDNA was used to amplify a part of the coat protein (CP) gene in RT-PCR using specific primers. Fragments of 609 bp and 549 bp were amplified and sequenced directly. Phylogenetic analysis results showed the existence of PVY strains including N, C and O on tomato samples and NTN on pepper. This is the first report of PVY $^{O, C, N}$ on tomato and PVY^{NTN} on pepper occurrence in Urmia and West-Azarbaijan province of Iran.

Keyword: Solanaceae, PVY, Indirect-ELISA, RT-PCR, Phylogenetic analysis**.**

Introduction

Potato virus Y (PVY); the type species of the genus *Potyvirus* in the family *Potyviridae* is one of the most economically important viruses affecting solanaceous crops including potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), tobacco (*Nicotiana* spp.), eggplant (*Solanum melongena* L.) and pepper (*Capsicum annuum* L.) worldwide (Quenouille et al. 2013). PVY has been reported in almost all parts of the world where its natural hosts occur. This virus can be divided into several strain groups according to host response, resistance gene interactions and serological properties (Singh et al. 2008). The strains include the common strain (PVY^{O}), the tobacco necrosis strain (PVY^{N}), the potato tuber necrosis strain (PVY^{NTN}), the recombinant N:O strain (PVYN:O =PVYN-Wi) as well as several minor strains such as PVY^{C}, PVY^{Z}, PVY^{E} (Hu et al. 2009a;b; Dullemans et al. 2011; Kerlan et al. 2011;Galvino-Costa et al. 2012) and the non-potato strain group (Blanco-Urgoiti et al. 1998). PVY^{O} and PVY^{N} are the basic strains, and from them various isolates/strains have emerged through nucleotide mutations and genome recombination (Nie et al. 2004; 2013). PVY has a single stranded positive RNA genome of ~9700 nucleotides and the particles are flexuous filaments 680–900 nm long and 11–20 nm wide are transmitted by aphid vectors in a non-persistent, non-circulative manner (Wylie et al. 2017). The most widespread recombinant strains are PVY $^{N:O}$ and PVY^{NTN}, which possess one and three recombinant events, respectively (Nie et al. 2013). PVY is wide spread in Iran and some strains of the virus is reported from tobacco, potato, tomato, turnip and pepper from different regions of Iran and the most prevalent strain in Iran is PVY^{O} (Pourrahim et al. 1998; Tousi and Pourrahim 2004; Mostafaie et al. 2006; Massumi et al. 2009; Majdabadi et al. 2011; Hosseini et al. 2011; Seiedy Gilani et al. 2017). Tomato and pepper are grown in most regions of Urmia, but there is no data on PVY strains on these two crops. So the aim of this study was to verify the presence of PVY strains in symptomatic tomato and pepper plants in this region.

Material and Methods

Sample Collection

A set of 88 symptomatic samples of tomato and pepper showing severe or mild mosaic, vein banding, leaf curling, blistering and necrosis on leaves was collected from bean growing areas of Ziveh, Nazloo, Par, Chongharalouye Pol,

Balanej and Ali Abad regions around the city of Urmia during the 2015 and 2016 growing seasons. Each sample was put in a plastic bag, labelled and stored at 4 °C.

Virus detection by Indirect-ELISA

Indirect- enzyme linked immunosorbent assay (Indirect-ELISA) was used for detection of PVY using commercial polyclonal antiserum and anti-rabbit obtained from DSMZ (Braunschweig, Germany). Each sample was diluted 1:5 with antigen coating buffer (100mM $NaHCo_3$, pH=9.6). The diluted plant sap extracts were added to the wells (50 µl). Two replicates were used for each sample. Plates were kept at 4 °C overnight, then rinsed three times with washing buffer (PBST; 10mM Na_2HPo_4, 1.8mM NaH_2Po_4, 140mM NaCl, 0.2 % Tween-20, pH=7.4). Blocking buffer (5% non-fat dry milk+ PBST) were put in the wells and plates were kept at 37 °C for 2 hours, and rinsed three times with washing buffer. Polyclonal PVY antiserum was diluted (1:1000) in blocking buffer and 50 µl was added to the wells and incubated for 3 hours at 37 °C and rinsed three times with washing buffer. Conjugated-anti-rabbit (1:10000) was diluted in blocking buffer and was loaded into each well (50 µl) for 1hour at 37 °C and rinsed again with washing buffer three times. Finally, 10 mg of p-nitrophenyl phosphate (Sigma) in 10 ml of substrate buffer was added to the wells and incubated at room temperature for 30–60 min. Absorbance values were read at 405 nm (A405) using a microplate reader (BioTek ELX-808, USA). Healthy plants were used as negative controls and samples were considered to be positive when the absorbance values at 405 nm values exceeded at least tree time the mean of the negative controls.

Virus detection by RT-PCR

RNA extraction

Total RNA was extracted from 200mg of leaves of randomly selected PVY-positive tomato and pepper samples according to the RNX-plus (Cinnagen, Iran) kit. Extracted RNA stored at -20 or -80ºC for further use.

Reverse transcription-polymerase chain reaction (RT-PCR)

The primer pairs designed by Boonham et al. (2002), were used to amplify fragments of the PVY coat protein (CP) gene (Table1). RT-PCR was performed in two steps. Three µl of extracted RNA was submitted to reverse transcription in a final volume of 20 µl, using 2µl RT buffer 10x (0.5 M Tris-HCl, 0.7 M KCl, 0.1 M $MgCl_2$, pH 8), 1µl DTT (100 mol/µl), 1µl dNTPs (10mMmol/ µl), 0.5 µl RNase inhibitor (10 mmol/µl) and 2 µl Reverse primer (100 pmol /µl) for one hour at 42ºC with 0.5 µl MMuLV reverse transcriptase (200 U/µl). Five µl of the RT reactions were used for PCR. The PCR reaction was carried out in a final volume of 20µl contained 20ng cDNA, 0.2mM of each dNTPs (Cinnagen, Iran), 1.6mM $MgCl_2$, 1U of *Taq* DNA polymerase (Cinnagen, Iran), 0.5µl of each primer pair (20pmol/µl) and 1X PCR buffer. The reaction mixtures were subjected to 35 cycles with the following conditions: 5 min (1 min for the first cycle) denaturation step at 94 ºC, 1 min for annealing at 58 ºC and 1 min (5 min for the last cycle) for primer extension at 72 ºC. PCR tests were performed in a thermal cycler (Mastercycler gradient, Eppendorf, Hamburg, Germany). After a final extension step at 72°C for 5 min, the PCR tubes were held at 4°C or stored at -20°C. The PCR products were analyzed by electrophoresis in 1% agarose gel and stained with DNA safe stain. An ultraviolet (UV) transilluminator was used to visualize DNA bands. The molecular weight of the PCR products were estimated using 1 Kb GenRuler™ ladder (Fermentas).

Table 1. primers used for RT-PCR of PVY strains

Primer name	Sequence 5´-3 ´	polarity	$T_{m\,(C^{\circ})}$	Expected fragment size (bp)
O-9295R	TGTACTGATGCCACCGTCGAAC	reverse		
O-8687F	TCTGGRACACATACWGTRCCR	forward	58	609
N-9236R	CCTTCATTTGAATGTGTGCCTCT	reverse		
N-8687F	TCTGGAACTCAYACTGTGCCAC	forward	58	549
O-9295R	TGTACTGATGCCACCGTCGAAC	reverse		
C-8687F	TCTGGAACWCATACTGTACCAA	forward	58	609
O-9295R	TGTACTGATGCCACCGTCGAAC	reverse		
N-8687F	TCTGGAACTCAYACTGTGCCAC	forward	58	609

R: A/G, W: A/T, Y:C/T

Sequencing and Phylogenetic Analysis

PCR products of 5 ELISA-positive samples were purified and directly sequenced. Sequencing was performed by Macrogen (Seoul, South Korea) on both strands. Sequences were edited and compared with the other PVY sequences deposited in GenBank database (www.ncbi.com) using BLASTn (http://www.ncbi.nlm.nih.gov). Nucleotide sequence similarity and multiple alignment and phylogenetic tree construction of CP gene sequences were carried out using the neighbor-joining (NJ) method and bootstrap analysis replicated 1000 times employing MEGA7 software (Kumar et al. 2016).

Results

Sample collection and ELISA tests

During summer of 2015 and 2016, 88 tomato and pepper samples with virus-like symptoms such as mosaic on leaves, leaf distortion, downward curling, mottling (Figure 1) were collected from tomato and pepper farms in different areas and villages around Urmia and were subjected to Indirect-ELISA that revealed the presence of PVY as shown in Table 2. PVY was detected in all tomato-growing areas including Chongoralloye pol, Aliabad, Nazloo, Lak, Balanej and Ziveh. As pepper is cultivated just in some areas around Urmia, so the number of pepper samples were lower than tomato, but PVY was detected in all pepper sampling areas. Indirect-ELISA test showed different infection rates in various places and the highest infection rate of PVY was found in Nazloo region probably due to virulence of this virus, high population of the vectors in these areas and suitable weather condition for infection.

RT-PCR tests

In addition, RT-PCR test proved the presence of virus PVY in ELISA-positive samples in accordance with ELISA test. At the meantime, specific primers for PVY strains amplified fragments of expected sizes and the expected fragment was amplified in all ELISA-positive plants. PCR products of the expected sizes, 549 bp obtained using the primer pair N-9236R/ N-8687F (Figure 3.1A) and 609 bp obtained using the primer pairs O-9295R/ O-8687F, O-9295R/ C-8687F and O-9295R/ N-8687F (Figure 2.B), indicated a successful amplification of the targeted region.

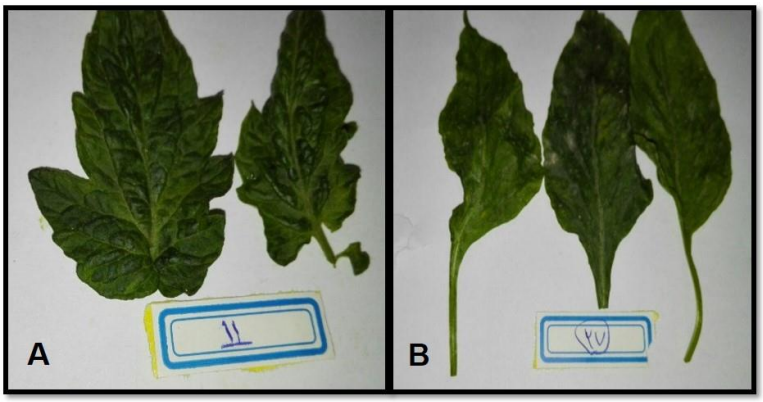

Figure 1. A. tomato, B. pepper with mosaic symptom

Table 2-The number of infected samples and infection ratio in sampling areas

Sampling areas	Total samples		Infected samples		Infection ration	
	tomato	pepper	tomato	pepper	tomato	pepper
Chongoralloye pol	9	0	2	0	22.2	0
Nazloo	8	0	3	0	37.5	0
Par	7	9	2	3	28.57	33.33
Lak	11	8	4	4	36.36	50
Balanej	10	0	2	0	20	0
AliAbad	6	0	1	0	16.6	0
Ziveh	11	8	1	1	9.09	12.5

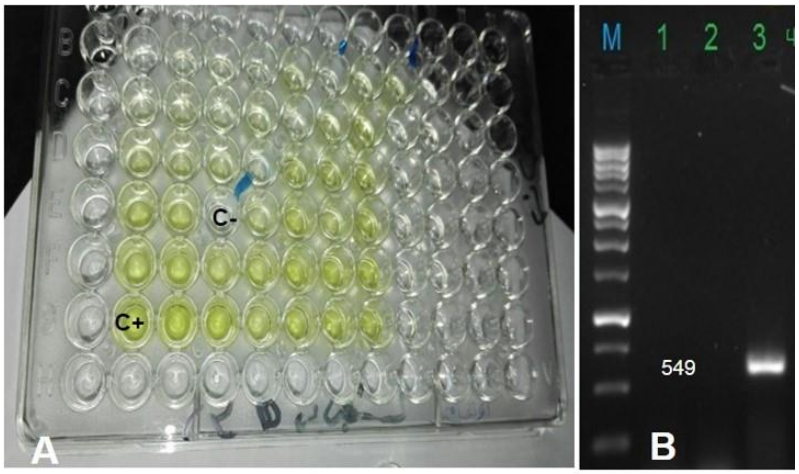

Figure-2. A. Indirect-ELISA results of PVY; ELISA plate showing typical positive and negative reactions. Samples showing positive reaction were similar to the positive control (C^+) as shown by the yellow color and negative control shown as C^-. B. electrophoresis of RT-PCR product related to PVY^{NTN}; 1. Healthy plant 2, 3 PCR product of PVY^{NTN}, M, 1 kb DNA ladder.

Sequencing and Phylogenetic Analysis

The obtained amplicons with expected sizes of 549 bp and 609 bp for 4 PVY strains were sequenced. BLAST searches in NCBI database using these fragments (CP gene) of PVY confirmed the presence of PVY in all of Urmia isolates. Phylogenetic trees on the basis of CP gene of PVY N, NTN, O, C strains were constructed using the neighbor-joining (NJ) method with MEGA7 software (Kumar et al. 2016). The reliability of the tree was assessed by bootstrap analysis with 1000 replication (Figures. 3, 4, 5 and 6). PVY^C isolate of Urmia from tomato was grouped with German isolate of PVY from potato in phylogenetic tree constructed using 16 PVY sequences obtained from GenBank (Figure 3). PVY^N isolate of tomato from Urmia was clustered with PVY^N isolates of Israel, Russia and Serbia that isolated from potato in constructed phylogenetic tree (Figure 4). PVY^O isolate of tomato from Urmia was made a separate cluster of other isolates of PVY^O obtained from the GenBank (Figure 5). PVY^{NTN} isolate of pepper from Urmia was clustered with PVY^{NTN} isolates of South Africa and United Kingdom that isolated from potato in constructed phylogenetic tree (Figure 6). Potato virus X (PVX KPA, accession No. M31541.1) was used as an outgroup in all constructed phylogenetic trees.

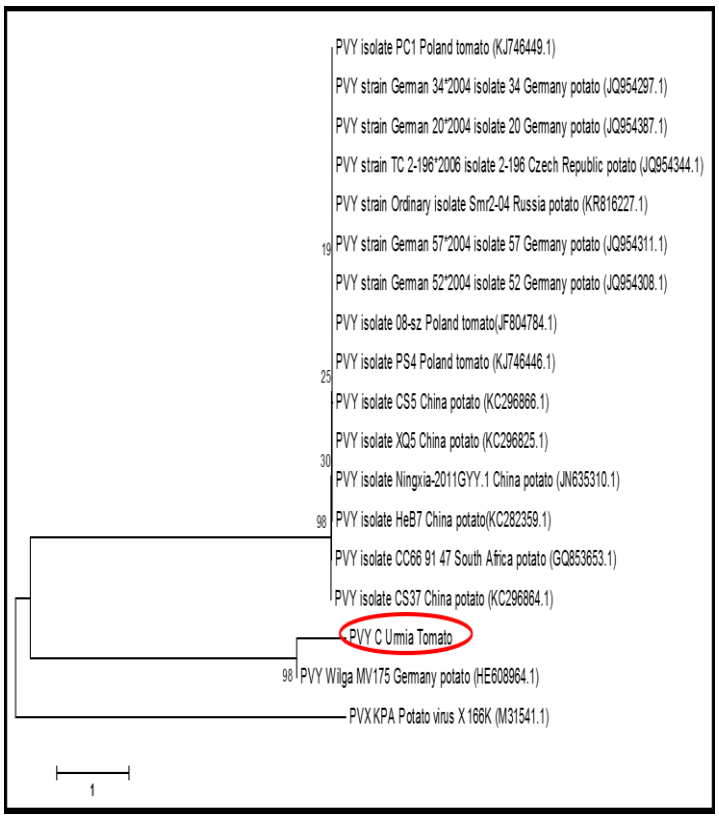

Figure 3. Phylogenetic tree constructed by the neighbor-joining (NJ) of CP gene sequences from 16 PVY sequences and PVY^{C} identified from tomato and *Potato virus X* (PVXKPA) as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

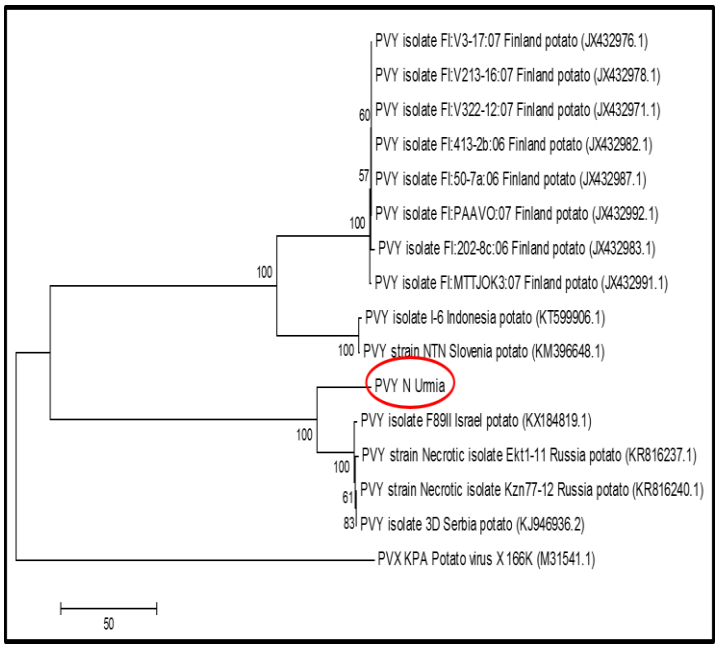

Figure 4. Phylogenetic tree constructed by the neighbor-joining (NJ) of CP gene sequences from 14 PVY sequences and PVY^{N} identified from tomato and *Potato virus X* (PVXKPA) as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

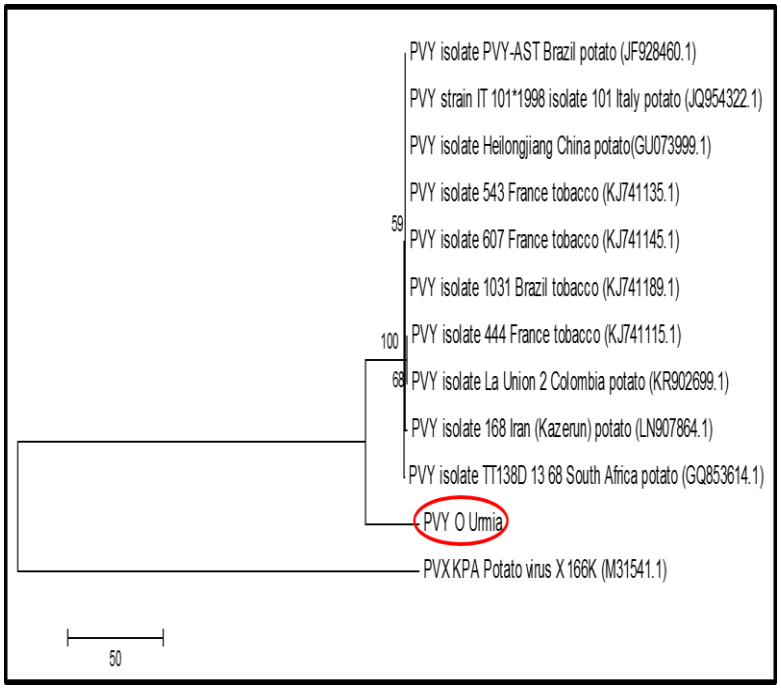

Figure 5. Phylogenetic tree constructed by the neighbor-joining (NJ) of CP gene sequences from 16 PVY sequences and PVY^{O} identified from tomato and *Potato virus X* (PVXKPA) as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

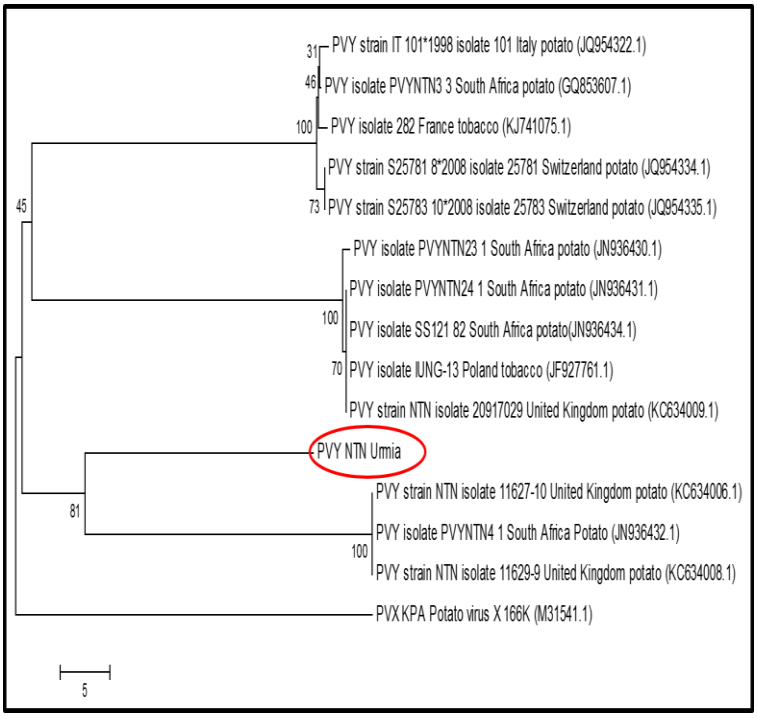

Figure 6. Phylogenetic tree constructed by the neighbor-joining (NJ) of CP gene sequences from 13 PVY sequences and PVY^{NTN} identified from tomato and *Potato virus X* (PVXKPA) as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

Disscussion

PVY was successfully detected in pepper and tomato grown in Urmia by ELISA and RT-PCR using specific primers to 4 strains of PVY. The incidence and distribution of PVY in tomato and pepper growing areas of Urmia were not similar to each other and the rate of infection in some places was higher than others for example in Nazloo and Lak. Investigation on occurrence, spread and determination of PVY strains of most important crops around Urmia are very important especially for implementation of proper diagnostic methods and management techniques, especially breeding for resistance. According to the local observations, symptoms of viral diseases on tomato and also on pepper of this region are increasing considerably in recent years mostly because of earth warming. Our results also showed high rate of PVY infection in the area and confirmed these observations. PVY has been considered as one of the five most important viruses of vegetable crops in the world (Mijatovid et al. 2002) and can be transmitted by several aphid species found in this region in a non-persistent manner. Most of the publications on PVY strains are from potato. PVY strains including PVY^{NTN}, PVY^{O}, PVY^{C} and PVY^{N} strains on potato were reported from different potato growing areas of Iran including Tehran, Khorasan e Razavi, Guilan, Mazandaran, Isfahan and Hamedan provinces (Pourrahim et al. 1998; Tousi and Pourrahim 2004; Maghsoudi et al. 2004; Mostafaie et al. 2006; Massumi et al. 2009; Majdabadi et al. 2011; Hosseini et al. 2011; Maghsoudi et al. 2015). There are a few data on PVY strains isolated from tomato and pepper in Iran. Mostafae et al. (2006) and Massumi et al. (2009) reported the incidence of PVY on pepper and tomato in Tehran and central provinces of Iran for the first time, respectively. They didn't identify the causal strain in their investigations. PVY^{NTN} and PVY^{C} were reported from tomato in Yazd and Golestan provinces by Mirrahimi et al. (2016) and Seiedy Gilani et al. (2017), respectively. This study is the first to confirm the presence of PVY^{O}, PVY^{C} and PVY^{N} strains on tomato and PVY^{NTN} on pepper in Urmia, West Azarbaijan province of Iran and the first of PVY^{NTN} on pepper for Iran. Further work in needed to identify identified strains biologically. The presence of PVY infection can be a potential danger for solanaceous crops including tomato, pepper, potato, eggplant and tobacco growing in Urmia region. Results of this study are the base for further work on ecology, epidemiology, diversity and breeding for resistance to PVY strains prevalent on solanaceous crops in Urmia and also in Iran.

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Araştırma Makalesi/*Research Article (Original Paper)*

Pepper Harvest Mechanism

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Abstract: Due to increased food need, plant cultivation issue has started to come up more often. As in all stages of plant cultivation, the importance of harvesting is also increasing. Due to the consequences of shifting the workforce to different areas in the developing world, tools and machines manufactured for use in agriculture need to be mechanized with greater efficiency. Studies on pepper harvesting mechanisms have been going on for many years. Although mechanisms are operated at certain harvest effectiveness values and mechanisms available in the market produced by some companies have been offered for sale, they have not been fully mechanized. It is estimated that the pepper harvest mechanisms can still be improved. The increasing importance of harvesting mechanisms has created the need to continue such studies in our country.

Key words: pepper, harvest mechanism, agricultural machinery

Introduction

Pepper is a type of vegetable that grows widely in the world and it grows in a hot climate with a high level of consumption. It is a perennial plant in tropical climates. Like tomatoes and eggplant, it is in Solanaceae-eggplant family and within genus capsicum. The most consumed species is *Capsicum annuum* L.

Pepper (*Capsicum annuum* and *Capsicum frutescens*) is originated in central and southern America. After the discovery of the American continent in the 15th century, it began to spread to the world. Today it is widely produced in many parts of the world. It is known that in the 1700s, Jamaican farmers raised four types (cherry, scotch bonnet, bell, and finger) of hot peppers (Anoynomus 1).

The global production of pepper and the amount of consumption per capita (capsium spp.) are regularly increasing. The world production of pepper in 2016 reached approximately 5 462 698 tons. According to the data of 2016 Turkey, it ranks 3rd in the world by 2457 tonnes (Anoynomus 2, Anoynomus 3).

Manual harvesting of pepper increases the production cost. Manual harvest accounts for about 50% of the total production cost. The cost of harvesting with machine can reduce the total cost of production by 10% (Funk and Marshall 2012). In addition, the need for manual hand harvesting is 32.2% of total work force requirement. This requirement may fall below 16.4% with machine harvest (Kang et al. 2016). Because of these advantages, a harvesting machine should be developed which will separate completely from the pepper plant and to minimize damage (Fink and Marshall 2012).

Some parameters must be considered when designing a pepper harvester. These are;

- ✓ Pepper varieties, root attachment strength, body height and thickness, body count, flesh thickness, physical dimensions, maturity grade, branch breaking strength, planting area,
- ✓ Dimensions and the speed of movement of harvest elements,
- ✓ Damages that occur during the harvesting of the plant and its crops and foreign material ratio

Pepper is not matured at the same time and is harvested once or several times for different uses (Gentry 1978).

Experimental first pepper harvesting machines began to be developed in the 1970s. To date, more than 230 machines and 30 different harvesting mechanisms have been tested for 20 different pepper varieties. Pepper harvesting machines are pulled and self-running types are encountered. Many of the harvesting mechanisms used gave acceptable results depending on plant and machine settings. With these mechanisms, about 70-90% of the pepper can be harvested (Akay 2009).

Pepper Harvest Mechanism

Mechanisms are generally mechanisms that separate the peppers from the plant by stripping the peppers on the stalks. Many harvesters used in these mechanisms have been manufactured and used.
These mechanisms are fingers, brushes and spiral types. Fingers, brushes and spirals which are produced from metal or plastic material are mounted on the elements, such as disk, cylinder and chain.

The mechanism developed by Shaw (1975) is shown in Figure 1. This mechanism consists of stripper steel rods placed on a reciprocating double crank mechanism around a center. In experiments conducted on bell peppers, 61% of the product was collected and dropped to 10% of the land.

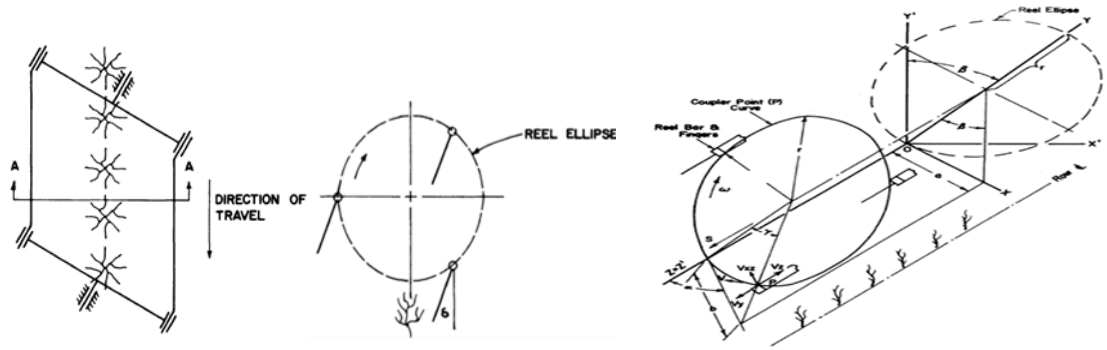

Figure 1. Double crank mechanism

The six-arm two-crank, shocking mechanism designed by Fowler and Shaw (1975) is given in Figure 2. This mechanism enables the separation of the pepper plant by the force of inertia gained by shaking at different frequencies and amplitudes. The best results were obtained at 4.41 Hz frequency and 20.3 cm amplitude with 4 G acceleration value and shaking for 25 s. All of the plants were detached, but 20% of the peppers were damaged.

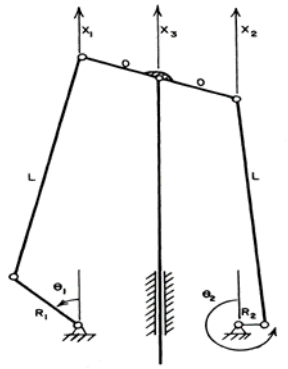

Figure 2. Double crankshaft shocking mechanism

Miles et al. (1978) made a harvesting machine by connecting different spaced comb-shaped stripper on a rotating chain that moves from the tractor (Figure.3). In the experiments, 50-60% of the green chili pepper was harvested in the first pass with a movement of 250 mm s^{-1} in the vertical direction of the fingertip with a spacing of 51 mm. With the second pass, they harvested 80% of the pepper. 38 mm spaced finger arrangement with red chili peppers was harvested 80% in two passes. However, in this interval they encountered a cleaning problem. With improvements in containment and cleaning stated embodiment this problem can be solved.

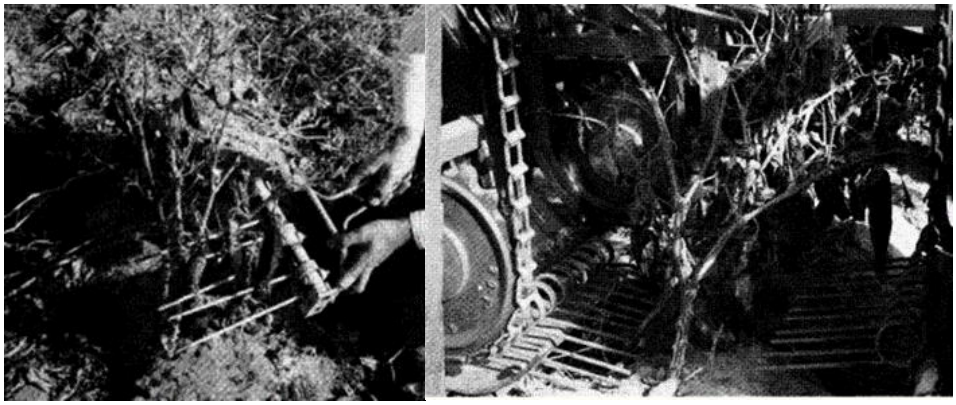
Figure 3. Stripper finger mechanism

Gentry et al. (1978), formed a mechanism with the fingers mounted on the rotary chain (Figure 4). The comb-like fingers separate the pepper plant from the plant by scanning upward. In their study with chili peppers, a decrease in the amount of foreign material collected by increasing the finger spacing from 51 mm to 76 mm was observed. When the speed of the fingers is less than 250 mm s^{-1}, the pepper damage is reduced.

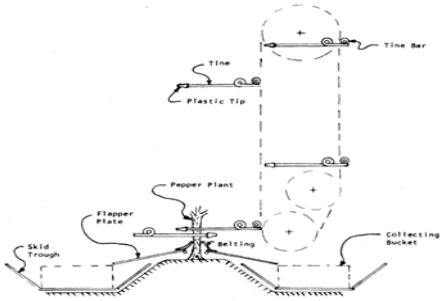

Figure 4. Rotary chain mechanism

Lenker and Nascimento (1982) designed a mechanism for harvesting dry chili peppers (Figure 5).

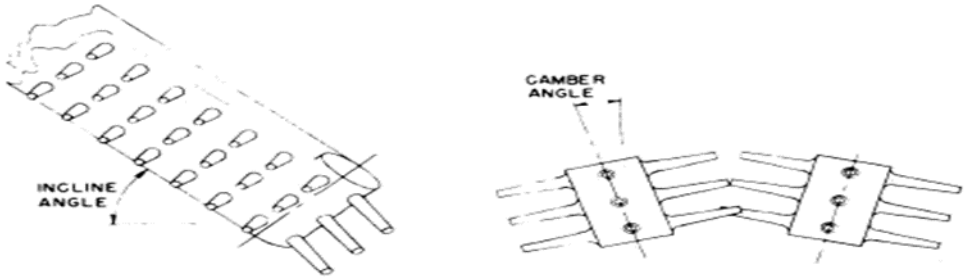

Figure 5. Rotary belt mechanism

The rubber fingers, 32 mm in length, were placed on a rotating belt at 9 mm spacing. However, these fingers have designed a new belt in high yield areas due to the jamming of the peppers between the belts during harvesting. 76 mm long polyurethane fingers were placed at 44 mm spacing. With these fingers, 95% of the product can be seperated from the plant, but only 70-80% can reach the store. To get the pepper dropping from the field, they added the pickup unit that was made from aluminum to the machine. In addition, because of the high amount of foreign material in the store, they added a seperating device in large sizes.

Wilhoit et. al. (1990) devised a mechanism that separates bell peppers from the plant. In this mechanism, the horizontal fingers, which are intermittently placed on the bars connecting between the two disciples rotating in a parallel and inclined position, move upwards elliptically (Figure 6).

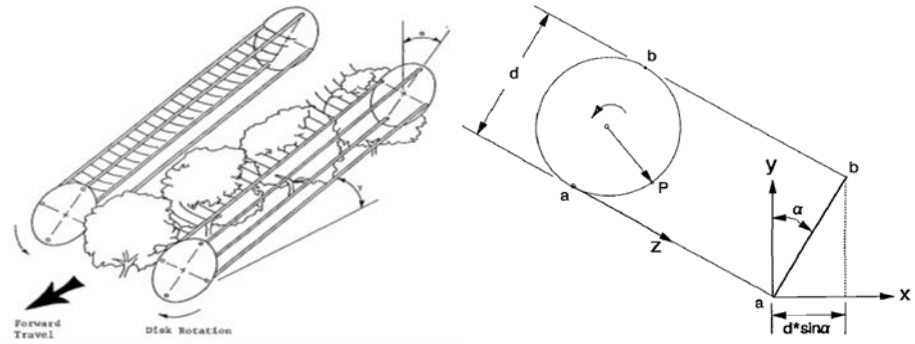

Figure 6. The mechanism by which was developed by Wilhoite et al.

They performed experiments to determine the effect of disk position angle (α) and vertical speed of fingers on harvest performance. As the disk position angle increases from 50° to 58°, it is observed that the fruit damage increases. By changing the angle of the disc, it was seen that the fruit damage was 3-12% and the plant harm was 1-2%. The harvesting efficiency increased from 76% to 84% as the vertical speed of the fingers increased from 0.23 m s^{-1} to 0.53 m s^{-1}. However, they stated that the rates of plant and fruit damage were 1-2% and 4-8%, respectively. They found an average harvesting efficiency of 81% and a fruit loss of 5.9%.During the harvest tests, they found that the plants were not removed from their roots.

By adapting the cotton harvesting mechanism, the cylinder brush arranged as three rows at equal angles on the two cylinders, Palau and Torregrosa (1997) developed a harvesting mechanism (Figure 7).

Figure 7. Brushed mechanism

The brush rotates to scan the plant upwards. The distance between the cylinders can be adjusted between 17-20 cm. However, in the experiments, the gap was fixed to 17 cm. They carried out experiments at feed speeds of 0.27-0.45 m s^{-1}, cylinder rotation speed of 300-500 rpm. In this study, harvest effectiveness for different paprika pepper varieties was determined in the range of 88-93%. During the harvest, it was determined that 4-8% of foreign material came to the collecting chamber and there was 7-12% loss of the product.

In our country, Akay and Özcan (2008) have worked to develop pepper harvesting mechanism. For this purpose, they first determined the breaking strength of the peppers. With a maximum force of 42 N, they detected that peppers could break away from branches. Next, they made a stripper mechanism by positioning spring steel fingers acting in the vertical direction at 15, 20 and 25 mm spacing. The best results were obtained at 80% harvesting efficiency with 20 mm spaced fingers and no significant fruit loss was observed. Then they formed the four bar linkage mechanism in which they placed these stripper fingers opposite to each other. By simulation, they obtained stripper finger orbits. They produced two prototype mechanisms (Figure 8) (Akay and Özcan 2009).

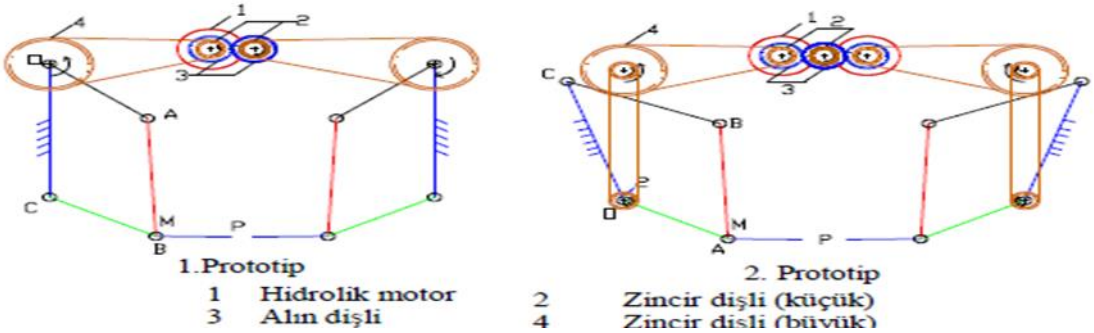

Figure 8. Four bar linkage

While the two mechanisms work with similar principles, the main difference is that the first driven bar is the upper bar, while the second driven bar is the lower bar. In the four-bar mechanism, the driven bar that is driven is a full turn and the other driven bar is the arm pendulum movement. They carried out their work in three different fingers spacing. As a result of the experiments, the first harvesting efficiency of the first mechanism was found to be 72% and the harvesting efficiency of the second mechanism was the highest 65%. We also observed that the product loss ratio was 8% for the first mechanism and 18% for the second mechanism.
Jin et al. (2015) have developed a moving pepper harvester. The harvesting mechanism was formed from steel springs which were mounted on a cylinder (Figure 9).

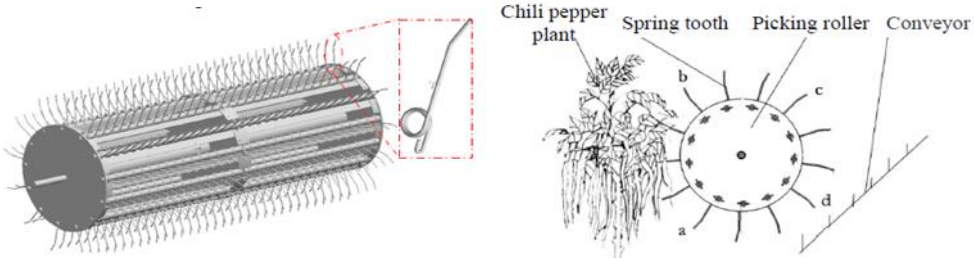

Figure 9. Mechanism developed by Jin et. al.

The working principle is that first passive wheels slowly push the pepper plants forward and form a certain positional angle so that they can slowly and steadily enter the collection mechanism (Figure 10). Depending on the plant height, the picking cylinder can be adjusted and returned freely. Peppers are cut off by a scan and fall on the conveyor belt by the upward rotation of the cylinder.

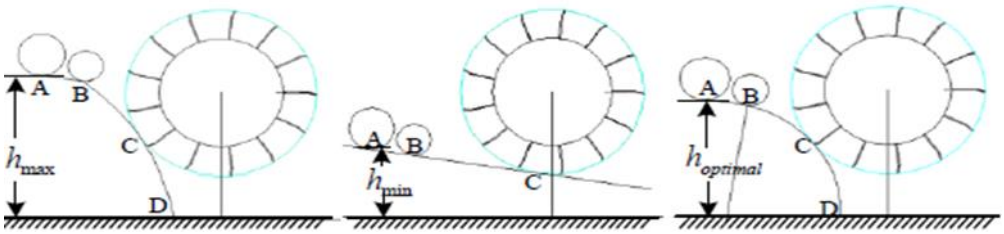

Figure 10. Functional diagram of passive wheels

Jin et al. carried out their work at 3 different forward speeds of the machine and 3 different rotational speeds of the mechanism. The best results were obtained at a forward speed of 2.52 km h^{-1} and a rotation speed of 138 rpm. As a result of the studies, they determined the loss ratio as 5.15%, the damage ratio as 3.22% and the harvesting efficiency as 99.15%. With this mechanism, 34.5 tons of products can be harvested per hour.

The helical mechanisms are shown in Figure 11.


Double helix


Triple helix

Figure 11. Types of spiral mechanism

Marshall et al. (1986) have produced double and triple spiral harvesting mechanisms with different diameters. In their work, they determined the beat number to the unit height of the plant depending on the number of spirals, the number of rotations of the helix and the forward speed. They have suggested that the fruit damage and the plant's uproot rate is less than 150 of the beat number to minimize. This beat number also reduces the amount of foreign material entering the system. The damage to the product of a double helix 150 mm in diameter was found to be less than the others.

Abernathy et al. (2006) produced two different harvesting mechanisms.


Figure 12. Helix mechanism (left) and helical mechanism with fingers (right)

The first one is made up of two spirals with three spaced helixes mechanisms, and the second one with helixed fingers arranged at 30° angles on a cylinder. They tested both mechanisms at 300, 400 and 500 rpm and forward speed of 1.61-2.24 km h^{-1}.

In the first mechanism; at high speeds (500 rpm) both plant and ground losses are reduced. However, lower revolutions of number (300 and 400 rpm) resulted in higher product damage. With the second mechanism, the number of revolution was increased while the rate of foreign material and the rate of pepper damage were observed to increase.

Kang et al. (2016) developed an important concept of the harvesting activity for the pepper harvester. In their work, they developed a screw-type collection head to test factors such as type, direction of rotation and speed of rotation of the screw. Force was transmitted to the mechanics by using bevel gears to rotate each helix with a different direction of rotation. In order to adjust the angle of the spirals, they placed at both ends of a rail. In this study, helices formed from double and triple helixes were tried at different rotational speeds and in two different directions of rotation. They reached the best harvesting efficiency at 400 rpm. They determined that the helix consisting of two spirals is more suitable. They have reported that there is no mechanical effect of rotation speed and helical type.

A study was conducted to compare finger and helix harvesting mechanisms (Funk and Walker 2009). Compared to others, the helical mechanism gave the best result with 89% harvesting efficiency. The product damage rate of this mechanism is around 10.5%.

Result

There are many researches on pepper harvesting mechanism in the world and different machines are manufactured by some companies. However, these machines still have not achieved satisfactory sufficiency. These harvesting mechanisms can not distinguish the different maturity peppers. This case arises classification problems. In addition, additional mechanisms are needed to clean foreign materials in these machines. This increases energy consumption and machine size. Peppers falling on the field surface during harvesting arises again the picking problem. For this reason, different solutions have been tried on some machines. Harvest mechanisms, plant and crop damage are still at high rates. The harvest activity results obtained ranged from 70 to 100%.

In our country, there are almost no studies on this subject. Given the amount of production in our country, it will be useful to conduct more work.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Dripline Uniformity in The Irrigation with Different Reclaimed Wastewaters

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Abstract: In this research, it was investigated the effects of treated wastewater on the performance of the drip lines used cauliflower and red cabbage irrigations for two years. The experiment was conducted in a randomized complete block design with three replicates using four different water (fresh water, filtered wastewater, filtered and aerated wastewater and the mix of fresh water with filtered wastewater named diluted water). It was determined the changes in the dripper performances by calculating the statistical uniformity coefficient from the drippers flows measured at the harvests. Langelier Saturation Index (LSI) values of the waters were also calculated to evaluate the chemical clogging effects of irrigation waters on the drippers. The uniformity coefficients of driplines for all waters in first years end was calculated approximately 97%. End of second year, the uniformity coefficients in the filtered and filtered and aerated wastewater applications was in poor class due to the between 60-70%. The uniformity coefficients were determined as fair (70-80%) and excellent classes (> 90%) for diluted and fresh water conditions, respectively. It could be concluded that positive LSI values determined for both the filtered wastewater and filtered and aerated wastewater pointed out to an extra chemical risk in clogging of drippers.

Key words: Dripline, clogging, uniformity coefficient, wastewater

Özet : Bu araştırmada, arıtılmış atık suların tarımsal sulama amaçlı yeniden kullanım olanaklarının araştırılması amacıyla yürütülen iki yıllık bir çalışmada kullanılan damlatıcı laterallerinin performansları belirlenmeye çalışılmıştır. Tesadüf bloklar deneme desenine göre 3 tekrarlı yürütülen çalışmada, temiz su (normal sulama suyu), filtre edilmiş atık su, filtre edilmiş ve havalandırılmış atık su ve filtre edilmiş, 1:1 oranında temiz su ile seyreltilmiş atık su olmak üzere dört farklı kalitedeki su, damla sulama yöntemiyle karnabahar ve kırmızı lahana bitkilerinin sulamalarında kullanılmıştır. Her iki deneme yılında hasat sonrası ölçülen damlatıcı debilerinden istatistiksel yeknesaklık katsayısı belirlenerek damlatıcı performanslarındaki değişimler belirlenmeye çalışılmıştır. Bununla birlikte sulama sularının kimyasal açıdan sistem üzerindeki etkisini belirleyebilmek için LSI değerleri hesaplanmıştır. Elde edilen yeknesaklık katsayıları incelendiğinde, denemenin ilk yılı tüm sulama konularında % 90 dan daha büyük değerler elde edildiği için konular çok iyi sınıfına girmişlerdir. Denemenin ikinci yılında atık su ve havalandırılmış atık su konuları % 60-70 arasında değerler alarak zayıf sınıfına girerken, seyreltilmiş atık su konusu % 70-80 arası değerler alarak orta sınıfına temiz su konusu ise % 90 dan daha büyük değerler alarak çok iyi sınıfına girmişlerdir. Atık suların pozitif LSI değerleri de dikkate alındığında tıkanmada kimyasal risklerinde söz konusu olduğu söylenebilir.

Anahtar Kelimeler: Damlatıcı, tıkanma, üniformite katsayısı, atık su

Introduction

Freshwater is a scarce resource and it is unevenly distributed on the world. More than half of the world's population live water scarcity. The worldwide population is increasing continuously and it is expected that it will be between 9 and 11 billion reached in 2050. Moreover, wastewater production and the number of people vulnerable to the impacts of wastewater pollution will increase (Corcoran et al. 2010).

Urban wastewater is a combination of the grey and black waters coming from homes, commercial establishments and institutions including hospitals, industrial effluent if present, storm water and other urban runoff (Raschid-Sally and Jayakody 2008). Therefore, wastewaters may contain harmful organisms and heavy metals causing environmental and health problems. One of the important reasons for heavy metal contamination in agricultural lands is irrigation with wastewaters (Jafarian and Alehashem 2013; Tunc and Sahin 2017).

Agriculture is the biggest water consuming sector comparing with the commercial and domestic use sectors. The water use ratio for agriculture aims is approximately 70% of the global total freshwater (Corcoran et al. 2010). Population increasing and water scarcity have required the reuse of wastewater in semi-arid and arid agricultural areas of many countries. Although there are many risks related to the public health under wastewater irrigation, it can provide different contributions such as reducing the demand for freshwater, rising the soil fertility and preventing pollutants from being discharged into the waterways. Wastewater has been widely used in agricultural irrigation due to containing especially macro nutrients need for plant growth (Kiziloglu et al. 2008; Demir and Sahin 2016).

Clogging of emitters in drip irrigation systems is important problem caused less driplines uniformity (Sahin et al. 2012). Clogging of emitters is closely related to the quality of the irrigation water, and occurs as a result of many biotic and abiotic factors, including physical, biological and chemical agents (Şahin et al. 2005; Eroglu et al. 2012).

The main objective of wastewater users in the agriculture is to improve their profit. However, suspended solids and some chemical matters in wastewater under drip irrigation conditions can effect system performance negatively. Therefore, we used the urban wastewaters reclaimed with primary and secondary processes in the cauliflower and red cabbage irrigations and examined the effects of treated wastewaters on the performance of the driplines.

Materials and methods

Site description

The experiment was conducted in 2010 and 2011 at the Agricultural Research Station of Ataturk University, Erzurum, Turkey. The region has a semi-arid climate. Some meteorological data are given in Table 1 for growing periods.

Table 1. Some monthly meteorological data in the experimental region during growing periods

		Temperature, ºC	Relative humidity, %	Wind speed, m s^{-1}	Daily sunshine, h	Evaporation, mm	Precipitation, mm
2010	May	11.7	65.7	2.9	9.1	32.5	1.1
	June	15.9	60.1	2.8	9.1	171.3	51.5
	July	19.5	56.0	3.3	9.9	220.1	59.0
	August	20.3	44.8	3.7	10.1	254.2	12.8
	September	17.9	45.1	3.3	9.3	131.8	4.0
2011	June	14.6	63.4	2.7	10.9	174.5	52.7
	July	19.6	53.3	4.0	8.1	236.5	15.0
	August	19.4	48.2	3.8	6.4	259.4	16.0
	September	14.2	53.5	3.1	5.3	151.7	15.0

Experimental design

The experiment was conducted in a randomized complete block design with three replicates. Four different water (fresh water, filtered wastewater, filtered and aerated wastewater and the mix of fresh water with filtered wastewater named diluted water) were used in the experiments. Royale F1 type red cabbage (Brassica oleracea L. var. rubra) and Snowball F1 type cauliflower (Brassica oleracea var. botrytis) were used as plant material. Red cabbage and cauliflower seedlings planted by hand on May 25 in 2010 and July 1 in 2011. Each experimental plot consisted of 5 red cabbage or 5 cauliflower rows with a plant row spacing of 50 cm (Fig 1).

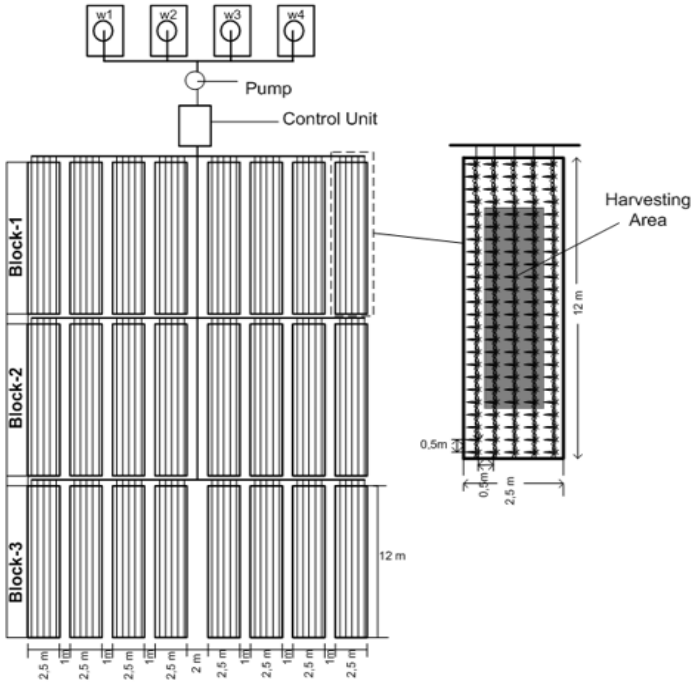

Fig 1. Experiment scheme

Wastewater reclamation processes and irrigation applications

In this study, primary reclamation was made using 2 mm diameter mesh filters for the removal of coarse objects in raw-wastewater. In the secondary reclamation process, raw-wastewater firstly was filtered and then filtered wastewater was filled into polyethylene tanks and continuously aerated by circulation using a pump. Freshwater (FW) for irrigation of the control plots were used. Many properties of different quality waters used in irrigations are shown in Table 2.

Drip irrigation system was consisted of storage tanks, pump, hydrocyclone, disc filter, valves and pipelines. PE driplines with 16 mm diameter were installed at the soil surface to be 50 cm between two adjacent driplines. The in-line type emitter spacing along the dripline was 0.50 m. The flow rate for each emitter was 2 L h^{-1} under at 0.1 Mpa operation pressure.

Table 2 Some properties of irrigation waters used in experiments.

		2010				2011			
Parameter	Unit	W1	W2	W3	FW	W1	W2	W3	FW
pH	-	7.95±0.04	7.86±0.02	7.46±0.20	7.13±0.05	7.86±0.03	7.83±0.02	7.49±0.14	7.15±0.08
EC	dS m^{-1}	1.85±0.04	1.86±0.03	1.25±0.05	0.46±0.01	1.87±0.02	1.86±0.03	1.20±0.01	0.40±0.01
Ca	me l^{-1}	4.10±0.16	4.17±0.32	2.97±0.10	2.35±0.14	4.07±0.33	4.51±0.40	2.88±0.64	1.52±0.21
Mg	me l^{-1}	1.03±0.08	1.50±0.19	0.93±0.10	1.15±0.03	1.03±0.07	1.11±0.36	1.05±0.17	1.18±0.08
Na	me l^{-1}	6.67±0.50	7.78±0.40	4.87±0.43	0.39±0.02	7.79±0.69	7.01±0.59	4.03±0.57	0.37±0.02
K	me l^{-1}	3.25±0.10	4.15±0.38	3.16±0.34	2.67±0.05	3.87±0.44	4.32±0.18	2.39±0.47	1.64±0.12
CO_3	me l^{-1}	0.66±0.15	0.60±0.20	0.11±0.11	-	0.56±0.05	0.54±0.02	0.27±0.03	-
HCO_3	me l^{-1}	6.23±0.44	6.33±0.23	4.67±0.29	3.43±0.20	6.67±0.13	6.30±0.10	4.50±0.21	3.17±0.03
SO_4	me l^{-1}	5.28±0.60	5.82±0.28	3.54±0.23	0.51±0.03	5.52±0.18	5.64±0.21	3.17±0.12	0.48±0.02
Cl	me l^{-1}	1.01±0.07	1.06±0.14	0.62±0.12	0.28±0.03	1.59±0.04	1.56±0.13	0.59±0.08	0.30±0.05
Total N	mg l^{-1}	13.8±0.22	13.9±0.09	6.95±0.10	-	13.7±0.40	13.8±0.64	6.84±0.48	-
Total P	mg l^{-1}	7.64±1.19	6.96±0.94	5.70±0.37	-	8.48±0.37	8.07±0.73	4.50±0.31	-

The dripline uniformity and Langelier saturation index (LSI)

The driplines uniformity was determined by calculating the statistical uniformity coefficient using the emitters flows measured at the end of growing periods. Langelier Saturation Index (LSI) values of the waters were calculated to evaluate the chemical clogging effects of irrigation waters on the drippers.

In both trial years, a dripline was selected from each treatment and flow measurements were made from a total of 9 drippers from the beginning, the middle and the end of the laterals. Then irrigation uniformity, which is the most important indicator of the performance of the drip irrigation system, was determined by Equation of Statistical Uniformity (Bralts and Kesner 1983);

$Us = 100x(1 - \frac{Sq}{q})$

Us : The statistical uniformity coefficient (%)
Sq : The standard deviation of dripper flow (L/h)
q_{ort} : Mean dripper flow (L/h)

The LSI is basically a way to determine if water is **corrosive** (negative LSI) or **scale-forming** (positive LSI). It is calculated based on the difference between the actual pH of the water and the saturation pH.

Results and Discussion

The findings of dripline flows, statistical uniformity coefficient and LSI values in two trial years are shown in Table 3. Bralts et al. (1985) classified the limits of uniformity coefficient accepted in drip irrigation systems as follows.

- Very good for > 90%
- Good for 80-90%
- Medium for 70-80%
- Weak for 60-70%
- Unacceptable for < 60%
-

When the coefficients of uniformity obtained are examined, the results in the first year showed that uniformity coefficients are very good due to obtained of values over of 90% are obtained in all the irrigation subjects (Table 3). In the second year of the experiment, the wastewater (W1) and the aerated wastewater (W2) entered the weaker class, while the diluted wastewater (W3) was in the middle class. Fresh water (FW) was in very good class with values of greater than 90%.

When the LSI values are examined waste water (W1) and the aerated waste water (W2) LSI positive value indicates the finding of lime precipitation (Table 3). Chlorination in irrigation practices has reduced biological risks. Therefore, the main component of the clogging is primarily physical clogging. Taking into account the positive LSI values of wastewater (W1 and W2), it can be said that the chemical risks are also related to the clogging.

Table 3 Mean flow, statistical uniformity coefficient and LSI values for 2010 and 2011 and mean flow variance analysis results

Year	Treatment	Mean Flow (L/h)	Uniformity coefficient (%)	LSI
2010	W1	1.89±0.05 **b**	97.25	0.68
	W2	1.88±0.05 **b**	97.13	0.61
	W3	1.86±0.04 **b**	97.63	-0.01
	FW	1.92±0.05 **a**	97.34	-0.47
	F	*8.322*		
	P	*0.001*		
2011	W1	1.60±0.59 **b**	63.13	0.62
	W2	1.60±0.58 **b**	63.75	0.61
	W3	1.73±0.36 **ab**	79.19	-0.01
	FW	1.93±0.07 **a**	96.37	-0.66
	F	*3.028*		
	P	*0.033*		

Conclusions

As a conclusion, there was no important difference in the first year uniformity test, while the negative results were determined which affect the performance of the system in the dripline uniformity coefficients in the second year. This shows that system performance can be reduced if the waste water applies by drip irrigation system. In this case, it coud be said that the need of changing the lateral lines of the drip irrigation system has been required in short periods such as 2-3 years.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of Different Tillage and Soil Residual Nitrogen on Chickpea Yield under Wheat-Chickpea Rotation System in Central Anatolia Condition

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Abstract: The aims of the study were to determine, the effects of tillage system and soil residual nitrogen on chickpea plant height, first pod height, biological yield and grain yield in rotation with wheat under dry land condition of Central Anatolia Region. In this study, two tillage methods (conventional and reduced tillage), three crop rotations (wheat-wheat; wheat-fallow; wheat-chickpea) and four N levels (0, 50, 100, 150 kg ha^{-1}) were evaluated four years duration (2012-2015). Soil tillage methods were placed in to main plots, crop rotation in to subplots and N levels in to sub-sub plots. The effect of crop rotation was investigated only in wheat results. Only chickpea results were examined in this study. Therefore, the variance analyses were performed according to split plot. There was no statistically significant difference in investigated properties between the two soil tillage methods for chickpea but chickpea grain yield was higher for CT than RT. Residual fertilizer N was significant affected chickpea grain yield and some investigated properties. 100 and 150 kg N ha^{-1} applied on wheat were increased grain yield in chickpea. Results also indicated that; conventional tillage may be more appropriate for chickpea in rotation with wheat. Different N doses applied to wheat influenced the grain yield of chickpea following it.

Keywords: Chickpea, tillage, nitrogen, crop rotation, rain-fed conditions

Özet: Bu çalışmanın amacı, Orta Anadolu Bölgesi'nin kuru tarım alanlarında farklı toprak işleme yöntemleri ve buğdaya uygulanan azotun nohutta bitki boyu, ilk bakla yüksekliği, biyolojik verim ve tane verimi üzerindeki etkilerini incelemektir. Çalışmada, iki farklı toprak işleme yöntemi (geleneksel ve azaltılmış toprak işleme), üç ekim nöbeti sistemi (buğday-buğday, buğday-nadas; buğday-nohut) ve dört farklı azot dozu (0, 50, 100, 150 kg ha^{-1}) dört yıl süresince (2012-2015) denenmiştir. Toprak işleme yöntemleri ana parsellere, ekim nöbeti sistemleri alt parsellere ve azot dozları alt-alt parsellere yerleştirilmiştir. Ekim nöbetinin etkisi buğday sonuçlarında incelenebilmektedir. Bu çalışmada sadece nohut sonuçları incelenmiştir. Bu nedenle, varyans analizleri bölünmüş parseller deneme desenine göre yapılmıştır. Nohut için farklı toprak işleme yöntemleri arasında incelenen özellikler açısından istatistiksel olarak anlamlı bir fark bulunmamakla birlikte, tane verimi geleneksel toprak işlemede azaltılmış işlemeye göre daha yüksek olmuştur. Buğdaya uygulanan azotun kalıcı etkisi incelenen bazı özellikleri ve nohutta tane verimini etkilemiştir ve 100 ve 150 kg ha^{-1} azot dozu nohutta tane verimini arttırmıştır. Elde edilen sonuçlara göre; buğday ile rotasyona giren nohut için geleneksel toprak işlemenin daha uygun olabileceği saptanmıştır. Buğdaya uygulanan farklı azot dozları, onu takiben nohutta tane verimini etkilemiştir.

Anahtar kelimeler: Nohut, toprak işleme, azot, ekim nöbeti, kuru koşullar

Introduction

In the semi-arid area of the Central Anatolia water availability is the most limiting factor for crop production under rain-fed agriculture. Traditionally, farmers in the area used fallow to capture out of season rainfall to supplement that of the growing season. The effectiveness of fallow for moisture conservation depends on soil type, tillage practices (McDonald and Fischer 1987) rainfall ability and soil water storage capacity (French 1978a; French 1978b; Connor and Loomis 1991). Long-term studies should show that crop rotations with or without legumes are essential to maintain high production levels (Mitchell et al. 1991). Legume crop residues contribute to organic N and after decomposition by soilmicrobes, through mineralization, add available N for the next crop (Chu et al. 2004), and improve the nutrient status of the soil. Legumes in rotation contributed to reduction of plant diseases occurred under continuous cereal production and improved soil physical properties

and also release of plant growth stimulants from decomposing legume residues (Ries et al. 1977; Rice 1983; Russelle et al. 1987).

Tillage is defined as the mechanical manipulation of the soil for the purpose of crop production affecting significantly the soil characteristics such as soil water conservation, soil temperature, infiltration and evapotranspiration processes (Busari et al. 2015).Although the decrease in the use of mouldboard ploughs can reduce negative environmental effects, such as erosion or compactness of the soil (Soane and Van Ouwerkerk 1994), this operation is still widely used in mechanised agriculture in many countries. The main reason is that this tillage operation is very efficient for improving soil structure, burying fertilisers and residues of the preceding crop and controlling weeds (Moss and Clarke 1994). According to CTIC (2004), conservation tillage is tillage system that leaves at least 30% of the soil surface covered with crop residue after planting to reduce soil erosion by water. Conservation tillage may improve soil structure, increases oil organic carbon, minimize soil erosion risks, conserve soil water, decrease fluctuations in soil temperature and enhance soil quality and its environmental regulatory capacity (Busari et al. 2015).

The aims of the present study were to determine the effects of tillage system and soil residual N on yield and yield components of chickpea in rotation with wheat under Central Anatolia condition.

Materials and Methods

Study site and soil

The field experiment was conducted during the growing periods of 2012-2013 and 2014-2015 under dryland conditions at the experimental area of Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39º48' N; 30º31' E, 798 m above sea level). Eskişehir province has a cold rainy winters and hot dry summers. Climatic data for long term and experimental years illustrated shown in Figure 1. Long term annual total precipitation is 329.7 mm and it were 338.5 and 546.1 mm in the experimental years, respectively. Annual average temperature was 12.65 ºC in 2012-2013 and 11.13 ºC in 2014-2015. Physical and chemical properties of the soil at the experimental areas are presented Table 1.

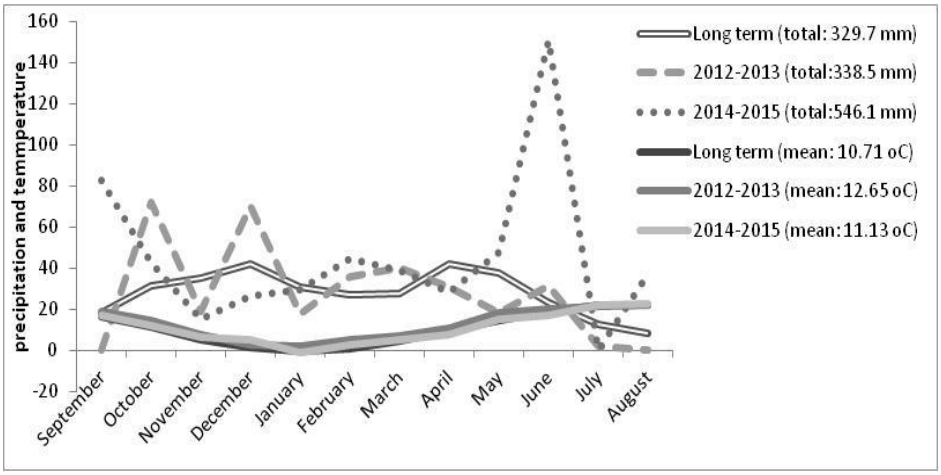

Figure 1. Total rainfall and monthly mean temperature for two season at Eskişehir, Turkey.

Table 1. Soil physical and chemical proporties of the experimental area.

Year	Depth (cm)	Texture	Ph	Total salt (%)	Lime (%)	Organic matter (%)	P_2O_5 kg ha^{-1}	K_2O kg ha^{-1}	N (%)
2012-2013	0-30	loamy	7.99	0.064	3.65	1.18	34.9	2258.6	0.05
2014-2015	0-30	loamy	7.46	0.020	5.40	1.63	65.3	3630.0	0.07

Experimental design and treatments

The field experiment was conducted 4 years duration (2012-2015). The experimental design was split split plot with three replicates. Soil tillage methods were placed in to main plots, crop rotation in to subplots and N levels in to sub-sub plots. The effect of crop rotation was investigated only in wheat results. Only chickpea results were given in this proceeding. Since effect of crop rotation could not be evaluated on chickpea, the results were evaluated according to split plot with three replicates.

Soil tillage: The conventional tillage included mouldboard ploughing followed by one passes of a sweep and/or rototiller cultivation to provide a proper seedbed. The reduced tillage included only sweep plowing and/or rototiller cultivation. Tillage depths for CT and RT were 25-30 and 8-10 cm, respectively. Tillage treatments were made in September and March in all years.

Crop rotation: Three crop rotations [wheat-wheat (WW); wheat-fallow (WF); wheat-chickpea (WC)] were considered in the experiment. Wheat was sown in all of the plots in the 2011-2012 and 2013-2014 years. In the 2012-2013 and 2014-2015 years, wheat, chickpea and fallow were sown on the research plots.

Fertilization: Nitrogen fertiliser was applied to wheat plots as ammonium nitrate. Half was applied at the sowing and the remaining N was topdressed at the beginning of the wheat stem elongation (pre-shooting stage). N fertilizer levels (0, 50, 100, 150 kg ha^{-1}) were applied to only wheat. Basal fertilizer application of 60 kg P_2O_5 ha^{-1} (for wheat) and 60 kg P_2O_5 ha^{-1} and 20 kg N ha^{-1}(for chickpea) were applied to each sub-subplot at the time of sowing.

Seeding: Each sub-subplot was 12 m^2 (4 m x 3 m). Chickpea varieties Gökçe was used research material. Sowing was made 30 cm row spacing at a seeding rate of 60 seeds m^{-2} on 01 April and 14 April in 2013 and 2015, respectively. No herbicide was applied and weeds were removed by hand. Chickpea was harvested on 29 July and 25 August in 2013 and 2015, respectively.

Crop yield measurements

Plant height (cm), first pod height (cm), biological yield (kg ha^{-1}) and grain yield (kg ha^{-1}) were measured for chickpea. Plant height and first pod height were evaluated on 10 randomly selected plants in each sub-subplot. Biological yield were estimated from a 0.25 m^{-2} area. Each sub-subplot was harvested, blended and grain yield was estimated (Tosun and Eser 1975; Aydin 1988).

Statistical analysis

All data were subjected to analysis of variance based on General Linear Model using the Statview package (SAS Institute). Means were compared by Least Significant Differences (LSD) test.

Results and Discussion

The effects of year on plant height, first pod height and biological yield were significant for chickpea. The plant height, first pod height and biological yield were higher for 2014-2015 growing season than 2012-2013 growing season. There was statistically significant difference in biological yield between N levels for chickpea (Table 2). In addition, interaction between year x N levels, soil tillage methods x N levels and year x soil tillage methods x N levels were significant for biological yield but year x N levels and year x soil tillage methods x N levels were significant for grain yield. The all of the values showed superior performance under 2014-2015 growing season for biological yield but all of the values had lowest biological yield in 2012-2013 growing season. Therefore, interaction between year, soil tillage methods and N levels was significant (Fig. 2A). The 100 N kg ha^{-1} showed superior performance under CT in the 2014-2015 growing season for grain yield but 150 kg ha^{-1} (soil residual N) N levels had lowest grain yield in same soil tillage methods and year. Thus, interaction between year, soil tillage methods and N levels was significant (Fig. 2B).

Plant height, first pod height, biological yield and grain yield was higher for 2014-2015 growing season than 2012-2013 growing season due to very high precipitation (Table 2). Total precipitation during the 2012-2013 growing season, 2014-2015 growing season and long term were 338.5, 546.1 and 329.7 mm, respectively. Mean temperature for 2014-2015 growing season was near the long term but total precipitation was very higher than 2012-2013 growing season and long term [especially June (151.1 mm)] (Figure 1). Plant height, first pod height

and biological yield of chickpea was particularly high than general in 2014-2015 growing season due to this high precipitation.

Table 2. Means of some characters chickpea under different tillage and residual N levels.

Treatments	**PH (cm)**	**FPH (cm)**	**BY kg ha^{-1}**	**GY kg ha^{-1}**
2012-2013	39,74 B	18,77 B	7757,2 B	1178,5
2014-2015	82,74 A	45,96 A	13566,7 A	1386,9
Mean	**61,24**	**32,36**	**10661,9**	**1282,7**
CT	62,67	33,31	10834,3	1331,9
RT	59,81	31,42	10489,2	1233,4
Mean	**61,24**	**32,36**	**10661,9**	**1282,7**
0 (kg ha^{-1})	59,16	31,73	10157,0 B	1211,2
50 (kg ha^{-1})	60,96	31,70	11388,0 A	1260,8
100 (kg ha^{-1})	61,92	33,38	10499,3 B	1351,9
150 (kg ha^{-1})	62,93	32,64	10603,3 B	1306,6
Mean	**61,24**	**32,36**	**10661,9**	**1282,7**
Year	**	**	**	ns
Tillage Methods	ns	ns	ns	ns
N levels	ns	ns	**	ns
Year x tillage met.	ns	ns	ns	ns
Year x N levels	ns	ns	**	**
Tillage meth. X N levels	ns	ns	**	ns
Year x til. met. x N levels	ns	ns	**	**

ns: non-significant, *: p≤0.05, **: p≤0.01. Means in the same column with different letters are significant. PH: plant height, FPH: first pod height BY: biological yield GY: grain yield.

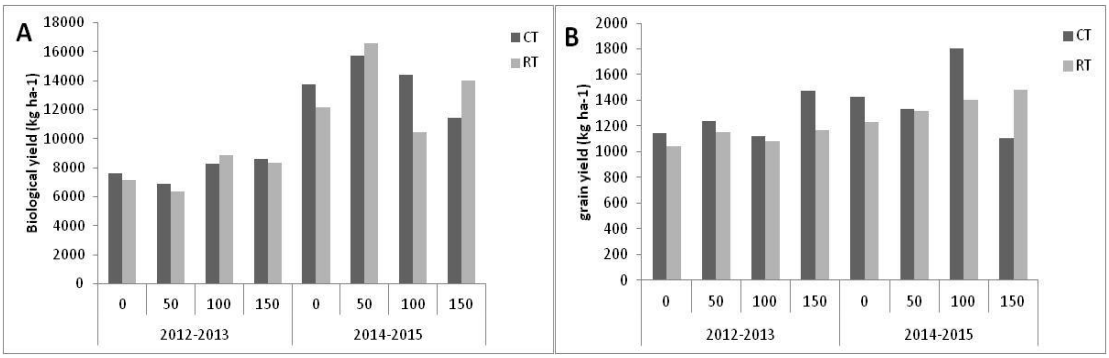

Figure 2. The interaction between year, soil tillage and N levels on biological yield (A) and between year, soil tillage and N levels on grain yield (B) of chickpea [LSD%1: 133,91 (A), 391,73 (B)]

There was no statistically significant difference in investigated properties between the two soil tillage methods for chickpea but chickpea grain yield was higher for CT than RT (Table 2). Effects of soil tillage should not occur in a short duration and there is a need for long-term research. Different types of tillage systems have different tillage depths and capacity to change soil physical and chemical properties that affect the crop yield and quality (Strudley et al. 2008). Hao et al. (2001) reported that higher grain yield was obtained in CT than minimum tillage for chickpea. Lopez-Bellido et al. (2004) reported that grain yield was higher in the CT than no-tillage for chickpea.

Increasing nitrogen levels (when N applied to the preceding wheat) increased plant height, first pod height and grain yield but there were no significantly different between N fertilizer levels for these properties. The highest biological yield was obtained 50 kg ha^{-1} N levels. Grain yield was higher 100 and 150 kg ha^{-1} N levels (Table 2). Lopez-Bellido et al. (2004) reported that 100 and 150 kg N ha^{-1} were increased chickpea grain yield when N levels applied to preceding wheat.

Conclusion

Plant height, first pod height, biological yield and grain yield was higher for 2014-2015 growing season than 2012-2013 growing season due to very high precipitation. There was no statistically significant difference in investigated properties between the two soil tillage methods for chickpea but chickpea grain yield was higher for

CT than RT. Effects of soil tillage might not occur in a short duration and there is a need for long-term research. Residual fertilizer N was significant affected chickpea grain yield and some investigated properties. 100 and 150 kg N ha^{-1} were increased grain yield in chickpea. Results also indicated that; conventional tillage may be more appropriate for chickpea in rotation with wheat. Fertilizer N applied to wheat influences the following chickpea grain yield.

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Morphogenetic, Ontogenetic and Diurnal Variability in Antioxidant Activity, Total Phenol and Flavonoids of *Foeniculum vulgare* Miller var. *vulgare* Extracts

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Abstract : Bioactive compounds, appearing at varying quantities and constituents in medicinal plants, act as natural antioxidants. The aim of this study was to monitor morphogenetic, ontogenetic and diurnal variability in antioxidant activity, total phenols and total flavonoids of bitter fennel extracts. Morphogenetic variability was assessed using leaf and root-bulb-stem samples of pre-, full- and post-flowering stages, flower samples of full flowering and seed samples of seed formation and harvest maturity. To monitor diurnal (3 times a day) and ontogenetic variability, whole plant samples were taken at pre-, full- and post-flowering. The plant extracts were obtained by methanol solution and radical scavenging effect of the extracts were determined spectrophotometrically. The root-bulb-stem extract produced the highest total phenol content. The highest radical-scavenging activity, with the lowest IC_{50} = 24.17 µg ml $^{-1}$, was obtained from the seed extract. Total phenols and flavonoids increased with the order of pre-flowering, full-flowering and post-flowering. Daily harvesting time (diurnal variability) caused no effect on antioxidant activity, total phenols and flavonoids. In conclusion, antioxidant activity of bitter fennel extracts significantly differs according to plant organs and growing stages.

Keywords: Bitter fennel, DPPH, phenolic compounds, radical scavenging activity.

Introduction

Medicinal and aromatic plants, used in traditional folk medicine since the ancient times, have gained a great deal of interest worldwide in the past few decades by virtue of their potential as a source of biologically active compounds. It has been well documented that the curative and aromatic properties of medicinal and aromatic plants are due to the presence of complex chemical substances, called secondary metabolites (Biesalski et al. 2009). Secondary metabolites are composed of various compounds and their biological activities can be attributed to the major components and/or to minor ones (Aazza et al. 2014). These bioactive compounds have long been used as a source of medicine, food, perfume and cosmetics. Recent researches revealed that the use of secondary metabolites in agricultural, pharmaceutical, medicinal and nutritional fields are due to their antioxidant and antimicrobial effects (Bakkali et al. 2008; Biesalski et al. 2009; Teixeria et al. 2013). Antioxidant and antimicrobial activities of any plant extracts are due to integrated action of different compounds they have. Polyphenols, including flavonoids and phenolic acids, are the most common classes of plant secondary metabolites and several thousand different compounds have been identified (Zujko and Witkowska 2011).

Bioactive compounds synthesized in medicinal and aromatic plants may vary greatly depending on a number of internal and external factors such as plant health and age, used plant part, growth stage and harvesting time (Figueiredo et al. 2008; Telci et al. 2009). The highest essential oil, for example, presents in leaves of certain plants, but in flowers of others. It has been suggested that the content and composition of bitter fennel essential oil obtained from different plant parts at different growth stages varied greatly (Açıkgöz et al. 2017a). On the other hand, soil and climatic conditions, production practices and postharvest operations play positive or negative effects on the amount and quality of bioactive compounds as well (Hussain et al. 2007; Figueiredo et al. 2008).

Common fennel (*Foeniculum vulgare* Mill.), with a long history of uses worldwide, widely grows in the Mediterranean area and temperate region of the northern hemisphere (Badgujar et al. 2014). Foeniculum vulgare has two commercially important fennel types: bitter fennel, *Foeniculum vulgare* Mill. subsp. vulgare var. vulgare, and sweet fennel, *Foeniculum vulgare* Mill. subsp. vulgare var. dulce (Telci et al. 2009). It is widely cultivated throughout the temperate regions of the world by virtue of its aromatic and flavorful fruits with

medicinal and culinary uses. Fennel, famous for its essential oil, has been extensively used in food, medical, perfumery and cosmetic industries. Fennel essential oil has been reported to contain several volatile compounds, which are accumulated in any of its parts, namely, roots, stem, shoots, flowers, and fruits (Özcan et al. 2006; Badgujar et al. 2014). Variation in content and constituents of essential oil bitter fennel as affected plant organs, growing stages and daily harvesting time was investigated and the seed extracts produced the highest content of essential oil with trans-anethol, methyl chavicol and fenchone as the main components (Açıkgöz et al. 2017a).

Recently, there has been a considerable interest in antimicrobial and antioxidant potential of fennel seed extracts and essential oil (Oktay 2003; Faudale et al. 2008; Anwar et al. 2009a; Miguel et al. 2010; Shatat et al. 2011; Dua et al. 2013; Diao et al. 2014). To the best of our knowledge, on the other hand, antimicrobial and antioxidant activity of bitter fennel essential oils and/or plant extracts with regard to the morphogenetic, ontogenetic and diurnal variability has just been reported in a few studies (Barros et al. 2009; Telci et al. 2009). Studying morphogenetic, ontogenetic and diurnal variability in antimicrobial activity of bitter fennel essential oil, Açıkgöz et al. (2017b) reported that antimicrobial activity of bitter fennel essential oil significantly varied based on used plant parts and growth stages, but daily harvesting hour caused no effect. In view of these, this study was undertaken to investigate the content of total phenols, total flavonoids and antioxidant activity of various bitter fennel extracts as affected by different plant organs, growing stages and daily harvesting times.

Material and Methods

Plant materials: The field part of the study was carried out in Ordu province located in the coastline of the central Black Sea Region of Turkey in 2012 and 2013 years. Bitter fennel plants (*Foeniculum vulgare* Mill. var. vulgare) were grown using standard production practices in a field experiment of completely randomized design with three replications. Seeds were sown in rows of 4 meters at a 40-cm row distance on a clay-loam soil. The soil was slightly acidic, with adequate level of nitrogen and average levels of potassium, phosphorous and organic matter. Basic fertilization was applied before planting at rates of 50 kg/ha N and 50 kg/ha P_2O_5.

Taking plant samples: To specify morphogenetic variability; leaf and root-bulb-stem samples were taken at three plant growth stages (pre-, full- and post-flowering), flower samples were picked up at full flowering and seed samples were gathered at two seed growth stages (at the beginning of seed formation and seed maturity). Whole plant samples were used to clarify ontogenetic (pre-, full and post-flowering) and diurnal (daily harvesting at 9:00 am, 1:00 pm and 5:00 pm) variability. A total of 20 plants were used in each sampling.

Preparing plant extracts: The plant extracts were prepared based upon the method described by Dalar at al. (2012). The plant samples were dried at 60 ºC in an oven until constant weight is attained and then grounded. Finely powdered samples were extracted with 96% methanol (1g/10ml) in a shaker at room temperature for 2 minutes and then kept in water bath at 45 ºC for one night. Collected extracts were filtered through double layered muslin cloth followed by centrifugation at 4000 rpm for 5 min. The filtrates were evaporated under vacuum in a rotary evaporator at 75 ºC to get clear dry supernatant. The supernatants were diluted in 1 ml methanol and collected in clean dry test tubes.

Analysis of total phenolic and flavonoid content: The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu reagent method as described by Wolfe et al. (2003). Absorbance was measured at 760 nm and standard gallic acid solution (5–1000 mg, 0.1 ml^{-1}) standard curve was obtained and the results were expressed in gallic acid equivalent mg/g. The total flavonoid analysis of the plant extracts was determined using aluminum chloride ($AlCl_3$) and quercetin as standards (Park et al. 2008). The results were expressed as mg quercetin (QE/g) after obtaining the standard curve (5–1000 mg, 0.1 ml^{-1}). All tests were run in triplicate and averaged.

Antioxidant activity: The antioxidant activity of bitter fennel extracts was assessed by measuring their free radical scavenging abilities to 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radicals. The DPPH assay was performed as described Shimada et al. (1992). Briefly, an 0.26 mM solution of DPPH in methanol was prepared. Afterwards, 1 ml of this DPPH solution was added to 300 µl plant extracts in 2700 µl ethyl alcohol. The mixture was vortexed and kept at dark condition. Then the absorbance values of reaction mixtures were measured at 517 nm using a UV-VIS spectrophotometer, lower absorbance values indicating higher free radical scavenging activity. IC_{50} values, which represent the concentration of plant extracts that cause 50% inhibition of DPPH radicals, were calculated from the plot of inhibition percentage against extract concentration. All the tests were performed in triplicates and the results were averaged.

Statistical analysis: Data were analyzed for completely randomized design by the analysis of variance (ANOVA) using Minitab 17 statistical program. Tukey test was used to determine the difference at the 5% significance level.

Results and Discussion

The results were given as the mean of the two years, since no significant differences were observed in terms of the study years. Total phenolic compounds, total flavonoids and IC_{50} values of bitter fennel extracts obtained from the whole plant samples taken at different growth stages are presented in Table 1. The contents of total phenolic compounds and total flavonoids and IC_{50} values of bitter fennel whole plant extracts affected by different growing stages varied significantly, indicating the presence of significant ontogenetic variability. The contents of total phenols and flavonoids showed a significant increase based on growing stages, following the order of before flowering<full flowering<after flowering. On the contrary, the whole plant extracts of before flowering showed greater radical-scavenging activity than those of full- and post-flowering stages, exhibiting IC_{50} values for 55.28, 64.28 and 61.05 µg/ml, respectively; as the lower the IC_{50} value, the higher is the antioxidant activity.

Total phenols, total flavonoids and IC_{50} values of bitter fennel extracts of the whole plant samples as affected by plant organs are given in Table 2. The content of total phenols, total flavonoids and IC_{50} values of bitter fennel extracts obtained from different plant organs were distributed unevenly, indicating the presence of significant morphogenetic variability.

Table 1. Total phenols, total flavonoids and IC_{50} values of bitter fennel extracts of whole plant samples taken at different growth stages.

Growing stages	Total phenols (mg/g)	Total flavonoids (mg/g)	IC_{50} (µg/ml)
Before flowering	28.35 c*	23.83 c	55.28 a
Full flowering	52.01 b	25.03 b	64.28 c
After flowering	59.73 a	26.20 a	61.05 b

*: The means with the same letters in the same column are not significantly different.

The highest amount of total phenols and total flavonoids were found in root-bulb-stem extracts (81.36 and 13.86 mg/g, respectively) which decreased significantly to 11.98 mg/g in the seed extracts and to 5.41 mg/g in the leaf extracts. On the contrary, the antioxidant activity of the seed extract, exhibiting the lowest IC_{50} value of 29.33 µg/ml, was greater than those of obtained from leaf, flower and root-bulb-stem samples.

Table 2. Total phenols, flavonoids and IC_{50} values of bitter fennel extracts obtained from different plant organs.

Plant organs	Total phenols (mg/g)	Total flavonoid (mg/g)	IC_{50} (µg/ml)
Leaf	44.05 c*	5.41 c	60.05 c
Root-bulb-stem	81.36 a	13.86 a	71.12 d
Seed	11.98 d	7.83 b	29.33 a
Flower	55.90 b	9.04 b	53.60 b

*: The means with the same letters in the same column are not significantly different.

Variation among the growing stage-based samples of the same organs were summarized in Table 3, indicating significant variations among the extracts taken at different growing stages from the same organs. The highest total phenols and total flavonoids were recorded in the extracts obtained root-bulb-stem samples harvested after flowering. However, the seed extracts obtained during seed formation produced the highest antioxidant activity, with the lowest IC_{50} value of 24.17 µg/ml.

Table 4 shows total phenols, total flavonoids and IC_{50} values of bitter fennel extracts of the whole plant samples as affected by growth stages and daily harvesting time. Daily harvesting time did not produce any effect on total phenol, total flavonoid and antioxidant activity of bitter fennel extract, indicating non-significant diurnal variability.

Antioxidant activity of plant extracts and/or essential oils, as well as total phenols and flavonoids, can vary significantly depending on a number of factors particularly plant organs and growing stages (Figueiredo et al. 2008; Roby et al. 2013). This variability may partly explain the contrasting properties and activities of the plant extracts and volatile oils obtained from the same species. It has been reported that the content of total phenols and total flavonoids were correlated with the antioxidant and antimicrobial activities of bitter fennel essential

oils and plant extracts (Dua et al. 2013; Salama et al. 2015). Studying to understand differences in the antioxidant potential of different plant parts, Barros et al. (2009) reported that the shoot had the highest radical-scavenging activity, which was in agreement with the highest content in phenolic compounds found in this part. On the contrary, this is not the case in our study in which the higher the content of total phenols and total flavonoids, the higher is the IC_{50} value, indicating lower antioxidant activity.

Table 3. Total phenols, total flavonoids and IC_{50} values of bitter fennel extracts obtained from the same plant organ harvested at different growth stages.

Plant organs	Growing stages	Total phenols (mg/g)	Total flavonoid (mg/g)	IC_{50} (µg/ml)
	Before flowering	44.70 a*	4.95	60.80 b
Leaf	Full flowering	47.40 a	5.08	57.30 a
	After flowering	40.05 b	6.21	62.04 b
	Before flowering	76.44 b	11.78 b	71.36
Root-bulb-stem	Full flowering	80.30 b	14.76 a	70.44
	After flowering	87.35 a	15.04 a	71.58
	Seed formation	14.45 a	8.25	24.17 a
Seed	Seed maturity	9.52 b	7.40	34.50 b

*: The means with the same letters in the same column are not significantly different, based on the evaluation within each plant organ.

Table 4. Total phenol, flavonoids and IC_{50} values of bitter fennel whole plant samples as affected by growth stages and daily harvesting time.

Growing stages	Daily harvest time	Total phenols (mg/g)	Total flavonoid (mg/g)	IC_{50} (µg/ml)
	09:00 am	28.37	23.84	54.30
Before flowering	13:00 pm	28.35	23.80	55.44
	17:00 pm	28.33	23.85	57.12
	09:00 am	52.20	25.05	64.12
Full flowering	13:00 pm	51.95	25.02	63.70
	17:00 pm	52.14	25.02	65.01
	09:00 am	59.73	26.22	59.94
After flowering	13:00 pm	59.75	26.18	65.01
	17:00 pm	59.71	26.20	61.78

Generally speaking, the antioxidant mechanism of plant extracts and essential oils has not been completely elucidated. In general, antioxidant activity can be attributed to the major compounds present, but some minor components with a synergistic effect may also play a significant role in antioxidant activity. Hussain et al. (2007) reporter that linalool, the major component of *Ocimum basilicum* essential oil, exhibited lower antioxidant activity than the entire oil with a conclusion that antioxidant activity of entire essential oil might be attributed to the presence of other compounds. Furthermore, the association between major and minor compounds may cause an antagonistic result (Aazza et al. 2014).

Despite the available reports on the content of total pehnolics and flavonoids along with antimicrobial and antioxidant activity of bitter fennel essential oil and plant exctracts, there are only limited references for the evaluation of morphogenetic, ontogenetic and diurnal variability. The contents of total phenolics and flavonoids and concequently antioxidant activity of the seed extracts in this study decreased from seed formation to the seed maturity, which shows a good agreement with the result of Telci at al. (2009), who repeorted that essential oil content decreased throguh the later seed growing stages. Anwar et al. (2009b) found the maximum (3.5%) and the minimum (2.8%) essential oil content in mature and immature fruit of sweet fennel with a significant maturity-based variation. Açıkgöz et al. (2017a) reported essential oil contents ranging from 0.22% to 5.52%, with the lowest and the highest obtained from the root-bulb-stem and seed samples of bitter fennel, respectively. Moreover, essential oil increased from 1.66% at pre-flowering to 2.21% at full-flowering and decreased sharply to 0.79% at post-flowering. In a previous study, Açıkgöz et al. (2017b) found that antimicrobial activity of bitter fennel essential oil significantly varied based on used plant parts and growth stages, but daily harvesting time caused no effect. Furthermore, inhibitory effect of seed essential oils extracted at the beginning of seed formation was higher than those of seed maturity, which is in a good agreement with the present study showing that antioxiant activity was higher at seed fromation than seed maturity.

In conclusion, total phenolos, total flavonoids and antioxidant activity of bitter fennel plant extracts significantly differed according to plant organs and growing stages, but daily harvesting time (diurnal variability) produced no significant effect. The plant extrats of pre-flowering showed higher antioxidant activity than those of full and post-flowering, suggesting significant ontogenetic variability. With regard to morghogenetic variability, the seed extract with the lowest IC_{50} value produced higher antioxidant activity than those of leaf, flower and root-bulb-stem extracts. However, further study is needed to determine how various different components interact to provide the antioxidant activity.

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Araştırma Makalesi/*Research Article (Original Paper)*

Plant Height Control of *Narcissus* cv. 'Ice Follies' by Gibberellin Inhibitors as Bulb Soak

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Abstract: In this study, effect of gibberellin inhibtors as preplant bulb soaks on plant height of *Narcissus* cv. 'Ice Follies' grown in pots were investigated. Bulbs of *Narcissus* cv. 'Ice Follies' were soaked in flurprimidol at 0, 10, 20 ppm and paclobutrazol at 0, 100, 200 ppm before planting. Effect of gibberellin inhibitors on the time of flowering, flower life, chlorophyll content of leaves, leaf length and plant height were determined. In addition, after narcissus were grown in pots in the greenhouse arrived at the sales stage to determinate the changes that occur in the plant height, plants were taken to the laboratory which was temperature held constant at 20 °C. The shortest plant height was obtained from the 20 ppm flurprimidol, 200 ppm paclobutrazol and 10 ppm flurprimidol treatment as given bulb soaks. In this treatments, plant heihgt was 7.11, 7.31, 7.75 cm and were 52%, 51%, 48% shorter than untreated control. Gibberellin inhibitors also shortened leaf lenght. Flurprimidol and paclobutrazol treatments were resulted higher chlorophyll content per unit area in the leaves than untreated controls. The highest chlorophyll content was obtained from the plants were treated 20 ppm flurprimidol with 60.6 CCI, while the control was 40.96 CCI. The effects of treatments on plant height has been maintained in lab conditions (home-office). The shortest plant height was obtained from 200 paclobutrazol treatment with 10.12 cm during post production period.

Key words: Flurprimidol, paclobutrazol, bulb soak, narcissus, plant height

Narcissus cv. 'Ice Follies' Çiçeğinde Soğandan Uygulanan Giberellin İnhibitörleri ile Bitki Boy Kontrolü

Bu çalışmada *Narcissus* cv. 'Ice Follies' çiçeğinin saksıda yetiştiriciliğinde dikim öncesi soğanlara uygulanan giberellin inhibitörlerinin bitki boyu üzerine olan etkileri incelenmiştir. *Narcissus* cv. 'Ice Follies' soğanları dikim öncesi 0, 10, 20 ppm dozlarında flurprimidol ve 0, 100, 200 ppm dozlarında paclobutrazol çözeltileri ile muamele edilmiştir. Giberellin inhibitörlerinin çiçeklenme zamanı, çiçek ömrü, yaprakların klorofil içeriği, yaprak uzunluğu ve bitki boyu üzerine olan etkileri incelenmiştir. Ayrıca serada saksıda yetiştirilen nergisler satış aşamasına geldikleri dönemde, bitki boyunda meydana gelen değişimleri inceleyebilmek amacıyla, sıcaklığı 20 °C' de sabit olan laboratuvara alınmıştır. En kısa bitki boyu dikim öncesi soğanlara uygulanan 20 ppm flurprimidol, 200 ppm paclobutrazol ve 10 ppm flurprimidol uygulamalarından elde edilmiştir. Bu uygulamalarda nergislerin bitki boyu 7.11, 7.31ve 7.75 cm ile kontrole kıyasla %52, %51 ve %48 daha kısa olmuştur. Giberellin inhibitörleri bitki boyu yanında yaprak boyunu da kısaltmıştır. Ayrıca flurprimidol ve paclobutrazol uygulamaları nergis yapraklarının birim alandaki klorofil içeriğini de arttırmıştır. En yüksek klorofil içeriği 60.6 CCI ile 20 ppm flurprimidol uygulamasının yapıldığı nergislerden elde edilirken, kontrol bitkilerinin klorofil içeriğinin 40.96 CCI olduğu saptanmıştır. Uygulamaların bitki boyu üzerine olan etkisinin laboratuvar koşullarında da (ev-ofis) devam ettiği belirlenmiş ve en kısa bitki boyu 10.12 cm ile 200 ppm paclobutrazol uygulamasından elde edilmiştir.

Anahtar kelimeler: Flurprimidol, paclobutrazol, soğan, nergis, bitki boyu

Introduction

Narcissus from the Amaryllidaceae family is perennial, herbaceous and bulbous plant. *Narcissus* flowers are commonly used in parks, gardens and refuges as ornamental plants. The narcissus flowers with long stems are used as cut flowers. In addition, *Narcissus* is also used as potted plant in the indoor. But the fact that their excessive

elongation after production at consumer conditions with low light makes it difficult to use as indoor plants. Elongation causes also downward curvature of the flower stem with heavy inflorescence. Therefore, plant height control is important for maintaining compactness and aesthetic appearance, as well as preventing damage during transportation and marketing due to stem elongation (Çelikel et al. 2016). We may control plant height either by physical methods with environmental factors (light, temperature, etc.), or by chemical methods with growth regulators mostly gibberellin (Çelikel et al. 2016, Demir and Çelikel 2013). These inhibitors are paclobutrazol, flurprimidol, ancymidol, uniconazole, chlormequat chloride and daminozide (Currey and Lopez 2017).

Flurprimidol as bulb soaks at 30 ppm controlled the plant height of different narcissus cultivars (Miller 2013). Plant height of 'Carlton' narcissus cultivar was controlled by flurprimidol and paclobutrazol as soil drench at 3 mg/pot concentration (Miller 2010). Flurprimidol preplant bulb soaks significantly controlled 'Tete a Tete' and 'Ducth Master' plant height during both greenhouse forcing and postproduction evaluation at concentration ≥ 25 ppm (Krug et al. 2006). Paclobutrazol preplant bulb soaks significantly controlled 'Tete a Tete' narcissus plant height during greenhouse forcing at concentration ≥ 150 ppm. Plant height of 'Tete a Tete' narcissus cultivar in the post-production period was controlled by paclobutrazol bulb soaks at concentration ≥ 202.8 ppm (Krug et al. 2006). Flurprimidol at 25 mg/L concentration controlled the plant height of 'Ice Follies' narcissus (Miller 2012). 30 ppm flurprimidol decreased the stem and leaf length of 'Ice Follies' at end of flowering (Miller 2013).

Effects of flurprimidol and paclobutrazol were investigated in narcissus cultivars and other species before. Effect of planting time on flowering period of 'Ice Follies' was investigated in our country (Kebeli and Çelikel 2013). However there is no previous study on height control of *Narcissus* cv. 'Ice Follies' in Turkey. Therefore, we investigated the effects of paclobutrazol and flurprimidol as preplant bulb soaks on plant height and other properties of *Narcissus* cv. 'Ice Follies' grown in pots.

Material and Method

Plant material
Narcissus cv. 'Ice Follies' bulbs with circumference of 10 cm from Asya Lale (Turkey) were used in this study. 'Ice Follies' are large flowered daffodils with a single flower per stem (Miller and Olberg 2016). 'Ice Follies' narcissus was obtained by crossing of *Narcissus poeticus* and *Narcissus pseudonarcissus* (Burnie et al. 1999).

Chemical materials
Gibberellin inhibitors of flurprimidol (Sigma-Aldrich) and paclobutrazol (25% Cultar; Syngenta) were used.

Treatments
Bulbs were soaked into flurprimidol solutions of 0, 10, 20 ppm or paclobutrazol of 0, 100, 200 ppm for 30 min before planting. Ethanol (2%) was used as a solvent of flurprimidol. Therefore a control for the solvent was included in these experiments. Bulbs were allowed to air dry and were planted into a 15 cm diameter plastic pots (1.6 volume) containing soil, peat and perlite (1:1:1) as one bulb per pot on the day of treatment (7 October).

Greenhouse
Plants grown in a polyethylene covered greenhouse were irrigated as needed with tap water.

Postproduction evaluation
When narcissus reached to the sale stage (one open flower in a stem with buds), four replicate plants randomly selected from each treatment were taken to the laboratory. Postproduction life and quality of pot plants were evaluated in this laboratory at 20 °C illuminated with Cool White Fluorescent light of 1000 lux at bench level, under a diurnal cycle of 12 h day, 12 h night as standard conditions (Çelikel and Karaçalı 1991; Çelikel 1993).

Flowering time and flower life
Flowering time was determined as number of days from planting time to opening of the flower. Flower life was calculated as the number of days from the opening of the flower to the wilting of the flower.

Chlorophyll content
Chlorophyll content of leaves was measure by chlorophyll meter (Apogee) at anthesis time (10 March). It was determined as Chlorophyll content index (CCI).

Plant height and leaf length
The leaf length (the longest leaf) and plant height (from the pot rim to the uppermost of the inflorescence) were started to measure respectively 115 days (31 January 2014) and 133 days (18 February) after planting, when they were started to emerge. Measurements were made weekly.

Data Analysis
Data were tested by one way analysis of variance (ANOVA) using a completely randomized design. The study was conducted with 10 replications except 3 replications for quantitative analyses and 4 replications for postproduction evaluation. The obtained data were analyzed statistically by using the SPSS package program. The mean and standard error ($\overline{X} \pm S\overline{x}$) values were determined. Differences between means were separated by Duncan's multiple range tests ($P \leq 0.01$).

Results and Discussion

Flowering time and flower life
Plant growth regulators delayed the flowering time of 'Ice Follies'. There was significant difference ($P \leq 0.01$) among treatments for flowering time (Table 1). The latest flowering was obtained from 200 ppm paclobutrazol and 20 ppm flurprimidol treatments with 155 days, while ethanol and control were 150 days, respectively. There was no difference among lower doses of these gibberellin inhibitors and control plants (Table 1). The lower doses of gibberellin inhibitors also shortened the plant height of 'Ice Follies' (Table 2). The gibberellin inhibitors delayed flowering time about 5 days in our study. A delay was observed in some *Iris* cultivars in the visible appearance buds in plants treated with paclobutrazol (Francescangeli 2009). The application of paclobutrazol delayed the appearance of the flower color in *Petunia* (Francescangeli and Zagabria 2009). Ancymidol treatments didn't delayed flowering time, but uniconazole delayed flowering time of 'Nellie White' lilium (Wilfret 1990). It was reported that flurprimidol at ≥ 25 ppm concentrations delayed the flowering about 3 days in tulips (Krug et al. 2005a). Flurprimidol application caused flowering delay and reduced the inflorescence and flower diameter of *Ornithogalum saundersiae* (Salachana and Zawadzińska 2013). Flowering time was not affected by lower rates of flurprimidol, but it was slightly delayed when flurprimidol was applied at higher doses in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder 2006). Blázquez et al. (1998) reported that the gibberellin class of plant hormones has been implicated in the control of flowering in several species. Su et al. (2001) reported that GA_3 promote the flower initiation and development. It was reported that exogenous GA_2 promote the switch from vegetative growth to flowering in a variety of plants by Wilson et al. 1992. Therefore the gibberellin inhibitors used in this study, effected flowering. Treatments affected the duration of the cycle. Uniconazole at 5 ppm and flurprimidol at ≥ 200 ppm concentration delayed the flowering of 'Star gazer' lilium (Krug 2005b). It was reported that plant growth regulators affect plant morphology and physiology positively or negatively (Eris 1990; Gündoğdu et al. 2017).

The lower dose of flurprimidol gave the longest flower life, with 15 days in 'Ice Follies' narcissus (Table 1). There was no difference among the control, ethanol, higher dose of flurprimidol and paclobutrazol treatments for flower life of 'Ice Follies'. Flower life of 'Ice Follies' changed as 14-15 days. Gibberellin inhibitors such as paclobutrazol, flurprimidol have acts in inhibition the enzymes have role in the gibberellin synthesis (Rademacher 2000). Changing in flowering caused by gibberellin inhibitor application may be associated with this.

Chlorophyll content
Effects of gibberellin inhibitors on chlorophyll content of leaves in 'Ice Follies' narcissus is given Table 1. There was significant ($P \leq 0.01$) difference among treatment for chlorophyll contents of leaves in 'Ice Follies' narcissus (Table 1). The highest chlorophyll content was 60.60 CCI, obtained from plants treated with 20 ppm flurprimidol, while control and ethanol applications were 40.96 and 40.16 CCI, respectively. Paclobutrazol and flurprimidol applications increased the chlorophyll content of narcissus. The use of flurprimidol resulted plants with an increased relative chlorophyll content of *Ornithogalum saundersiae* (Salachna and Zawadzińska 2013). It was reported that anti-gibberellin growth retardant of chlorocholine chloride increased the biomass of leaves, by decreasing the leaf length in *Lilium* Oriental hybrids 'Sorbonne' (Zheng et al. 2012). Similarly in our study treatments decreased the leaf

length. This decrease in leaf length may result in an increase of leaf thickness by increasing chlorophyll contents in unit area.

Table 1. The effects of flurprimidol and paclobutrazol on flowering time, flower life and chlorophyll contents of *Narcissus* cv. 'Ice Follies' Mean ± Standard Error ($\overline{X} \pm S\overline{x}$)

Treatments	Flowering time (days)	Flower life (days)	Chlorophyll content (CCI)
Control	150.00 ± 1.22 ab	14.25 ± 1.60 ab	40.96 ± 4.71 b
Ethanol (2%)	149.75 ± 1.97 b	13.75 ± 2.92 ab	40.16 ± 6.56 b
10 ppm flurprimidol	151.80 ± 1.02 ab	15.10 ± 1.52 a	45.99 ± 3.19 ab
20 ppm flurprimidol	154.60 ± 1.26 a	14.20 ± 1.09 ab	60.60 ± 6.59 a
100 ppm paclobutrazol	151.30 ± 1.38 ab	13.75 ± 0.94 ab	51.13 ± 4.37 ab
200 ppm paclobutrazol	155.10 ± 1.31 a	13.50 ± 1.23 ab	57.00 ± 6.90 ab
Significance	0.008	0.0059	0.009

**Different letters in the same column indicate differences among treatments according to Duncan multiple range test (1%)

Plant height and leaf length

The gibberellin inhibitors caused a decrease in plant height of 'Ice Follies' narcissus (Figure 1). The shortest plant height was obtained from 20 ppm flurprimidol, 200 ppm paclobutrazol and 10 ppm flurprimidol with 7.11, 7.31 and 7.75 cm, respectively, whereas the control plants and ethanol 14.88 and 13.17 cm, respectively (Table 2). There was significant ($P \leq 0.01$) difference among the application for plant height (Table 2). Plants applied 20 ppm flurprimidol, 200 ppm paclobutrazol and 10 ppm flurprimidol were 52%, 51% and 48% shorter than untreated control (Table 2). Flurprimidol, paclobutrazol and uniconazole preplant bulb soaks at ≥ 59.4, 50.0, 5.0 ppm, respectively, shortened the plant height of 'Prominece' tulips before (Krug et al. 2005a). Uniconazole is also a gibberellin inhibitor like flurprimidol and paclobutrazol. Flurprimidol preplant bulb soaks significantly controlled plant height of 'Tete a Tete' and 'Dutch Master' narcissus cultivars during greenhouse forcing at concentrations ≥ 25 ppm (Krug et al. 2006). *Narcissus* treated with flurprimidol and paclobutrazol preplant bulb soak in this study were shorter than control plants during post production evaluation (Figure 2). The height differences between control and treated plants were maintained in the post-production period. The shortest plant height (13.5 cm) was obtained from 200 ppm paclobutrazol treatment whereas the height of untreated control plants was 33 cm during the post-production life of pot plants (Figure 2). 'Tete a Tete' narcissus cultivar treated with flurprimidol were shorter than control at the end of the post production evaluation (Krug et al. 2006). It was reported that storage and transportation can have very deleterious effects on the ornamental quality of potted plants (Ferrante et al., 2015). Therefore it is important that maintaining compactness to prevent damage during transportation and marketing. We found that flurprimidol and paclobutrazol effectively controlled the plant height not only during production in greenhouse but also after harvest and there was no statistical difference between low and high doses (Figure 1, Figure 2).

There is significant difference ($P \leq 0.01$) among applications for leaf length (Table 2). The shortest leaf length (12.00 and 12.45 cm) was obtained from 200 and 100 ppm paclobutrazol, respectively, while the control and ethanol were 19.70 cm, the longest ones (Table 2, Figure 3). Continuing effects of chemicals application on leaf length during post production in lab (home-office) conditions are given in Figure 4. The shortest leaf length was obtained from 20 ppm flurprimidol and 200 ppm paclobutrazol application with 15.50 cm, while the control was 30 cm (Figure 4). The difference of leaf lengths among applications were maintained in post-production period. Uniconazole foliar spray caused to reduction in leaf length and width of *Fuchsia x hybrida* (Kim 1995). The tepal size, leaf size and pedicel length of plants applied flurprimidol were smaller than the control plants in 'Mona Lisa' lily cultivar (Pobudkiewicz and Treder 2006). The use of flurprimidol resulted with the shorter leaves in *Ornithogalum saundersiae* (Salachana and Zawadzińska 2013) and *Zantedeschia aethiopica* (Gonzalez et al. 1999). Similarly in our study flurprimidol and paclobutrazol were effective to shorten leaf length of narcissus both during greenhouse and post-production period. Our results showed that the effect of plant growth regulators applications on plant height and leaf length continued in the laboratory conditions during the post-production period (Figure 2, 4, 5, 6).

Table 2. The effects of flurprimidol and paclobutrazol on plant height and leaf length of *Narcissus* cv. 'Ice Follies' Mean ± Standard Error ($\overline{X} \pm S\overline{x}$)

Treatments	Plant height (cm)	Leaf length (cm)
Control	14.88 ± 1.59 a	19.70 ± 3.34 a
Ethanol (2%)	13.17 ± 5.07 ab	19.70 ± 1.81 a
10 ppm flurprimidol	7.75 ± 1.12 c	15.25 ± 0.51 ab
20 ppm flurprimidol	7.11 ± 1.46 c	13.30 ± 0.61 ab
100 ppm paclobutrazol	10.06 ± 0.72 bc	12.45 ± 1.14 b
200 ppm paclobutrazol	7.31 ± 0.78 c	12.00 ± 1.21 b
Significance	0.005	0.002

**Different letters in the same column indicate differences among treatments according to Duncan multiple range test (1%)

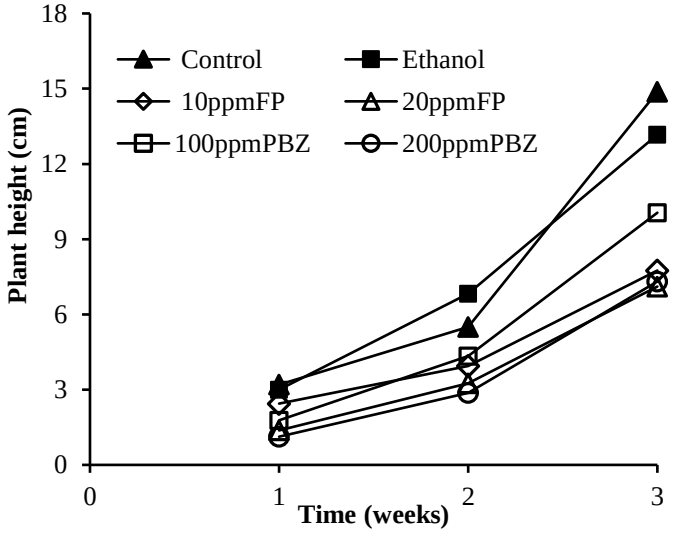

Figure 1. Effect of flurprimidol (FP) and paclobutrazol (PBZ) treatments on plant height of *Narcissus* cv. 'Ice Follies' during greenhouse production period**.** The plant height was started to measure 133 days after planting (1 week).

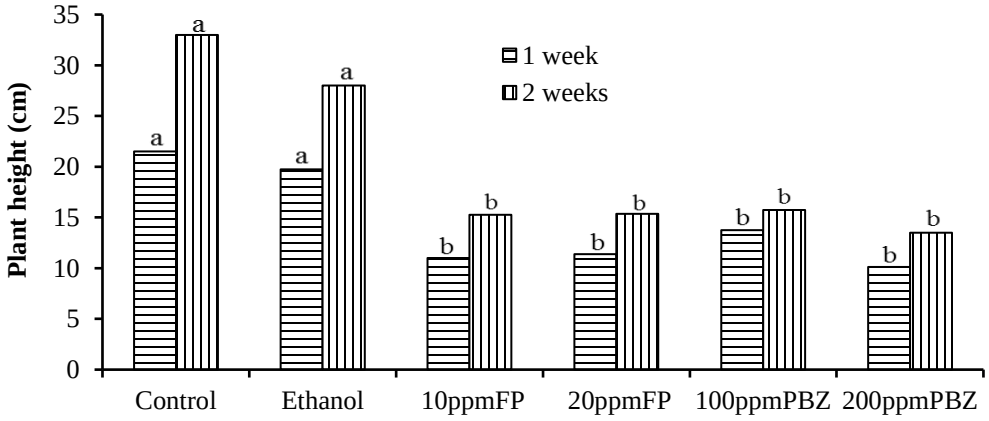

Figure 2. Effect of flurprimidol (FP) and paclobutrazol (PB) treatments on plant height of *Narcissus* cv. 'Ice Follies' during post production period in lab (home-office) conditions. Plant heigth was started to measure 149 days after planting (1 week)

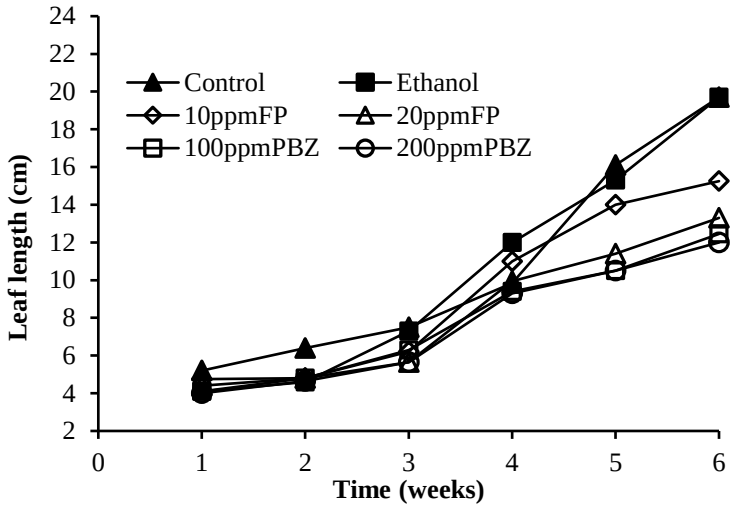

Figure 3. Effect of flurprimidol (FP) and paclobutrazol (PBZ) treatments on leaf length of *Narcissus* cv. 'Ice Follies' during greenhouse production period. The leaf length was started to measure 115 days after planting (1 week).

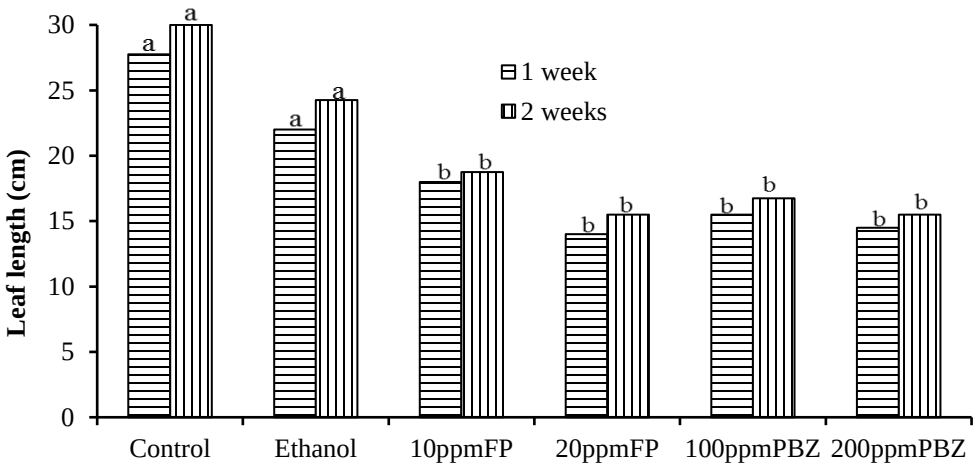

Figure 4. Effect of flurprimidol (FP) and paclobutrazol (PB) treatments on leaf length of *Narcissus* cv. 'Ice Follies' during post production period in laboratuary (home-office) conditions. Leaf length was started to measure 149 days after planting (1 week)

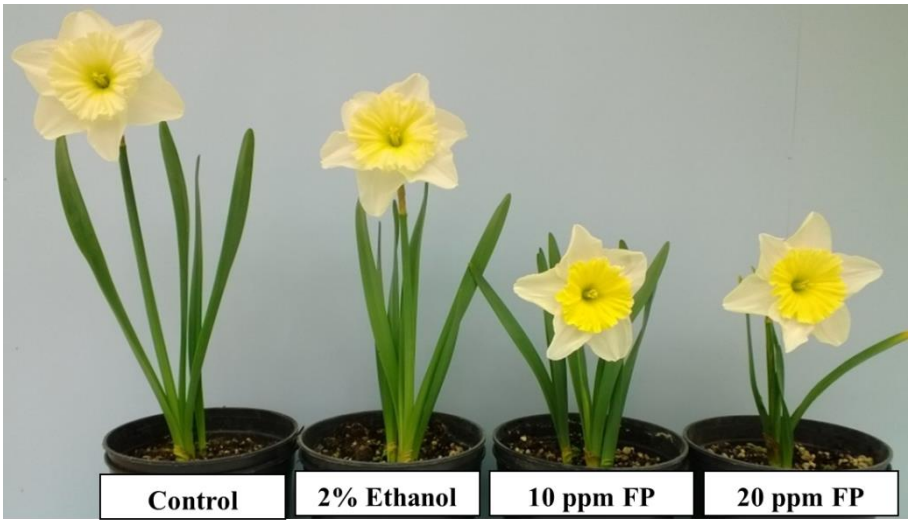

Figure 5. The effects of flurprimidol (FP) bulb soak on *Narcissus* cv. 'Ice Follies' (154 days after planting, fifth day in lab)

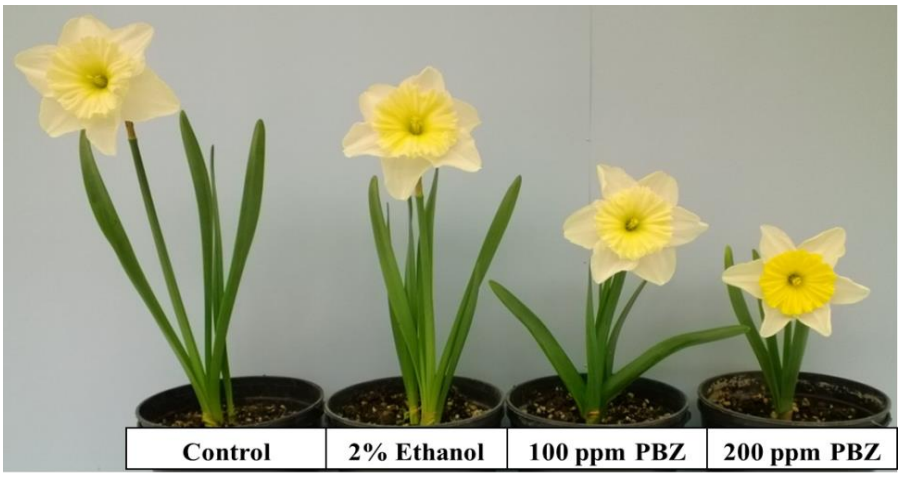

Figure 6. The effects of paclobutrazol (PBZ) bulb soak on *Narcissus* cv. 'Ice Follies' (154 days after planting, fifth day in lab)

In conclusion, gibberellin inhibitors controlled the plant height of 'Ice Follies' both during greenhouse and postproduction period. On the other hand paclobutrazol and flurprimidol decreased the leaf length and increased the chlorophyll content of leaves. As a result, we suggest 10 ppm flurprimidol or 100 ppm paclobutrazol, the lower concentration, treatment as preplant bulb soak in order to provide plant height control and maintain post production quality of *Narcissus* cv. 'Ice Follies' grown in pots.

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Araştırma Makalesi/*Research Article (Original Paper)*

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) İşletmelerinde Bazı Antioksidan Enzim Aktivitelerinin Ağır Metal İnhibisyonlarının Araştırılması

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Özet: Endüstriyel, tarımsal ve ticari kimyasalların, sucul ekosistemlerde meydana getirdiği kirlilik, giderek artan ciddi bir problemdir. Çevresel kirleticiler, sucul organizmalarda çeşitli zararlı etkilere neden olur. Balıklar, sucul organizmalar arasında ağır metallere en çok maruz kalan türlerdir. Ağır metallerin balıklarda birikmesi balık hastalıkları konusunda önemli bir sorun olmanın yanında, oksidatif stres sebebi olan reaktif oksijen türlerinin (ROT) artmasına da neden olmaktadır. Biyolojik sistemlerde oksidatif strese bağlı reaktif oksijen türlerindeki artış antioksidan savunma sistemlerinde hasara yol açar. Bu çalışmanın amacı gökkuşağı alabalık çiftliklerinde üretimi yapılan balıkların karaciğer, böbrek ve solungaç dokularındaki bazı antioksidan enzim aktiviteleri ile ağır metal iyonlarından kaynaklanan su kirliliği arasındaki ilişkiyi ortaya koymaktır. Bu amaçla; Su örneklerindeki ağır metal iyonlarının (Zn, Fe, Cu, Ni, Co, Hg, As, Mn) seviyeleri belirlenerek, bu iyonların balıklardan alınan kalp, karaciğer ve solungaç dokularındaki antioksidan enzimlerin (Süperoksid dismutaz (SOD), Katalaz (CAT), Glutatyon redüktaz (GR), Glutatyon S-transferaz (GST), Glutatyon peroksidaz (GPx)) aktiviteleri üzerine etkileri incelenmiştir.

Anahtar Kelimeler: Gökkuşağı alabalığı, oksidatif stres, ağır metal, antioksidan, enzim aktivitesi, Balık Hastalıkları

Investigation of Heavy Metal Inhibitions of Some Antioxidant Enzyme Activities in Rainbow Trout (Oncorhynchus mykiss) Farms

Abstract: The increasing pollution of industrial, agricultural and commercial chemicals in aquatic ecosystems is an increasingly serious problem. Environmental pollutants cause a variety of harmful effects in aquatic organisms. Fish are the species most exposed to heavy metals among aquatic organisms. Accumulation of heavy metals in fish is a major problem for fish diseases as well as an increase in reactive oxygen species (ROT), which is the cause of oxidative stress. The increase in oxidative stress-related reactive oxygen species in biological systems leads to damage in antioxidant defense systems. The purpose of this study is to establish the relationship between some antioxidant enzyme activities in the liver, kidney and gill tissues of fish produced in rainbow trout farms and the water pollution caused by heavy metal ions. For this purpose; The levels of heavy metal ions (Zn, Fe, Cu, Ni, Co, Hg, As, Mn) in water samples were determined and these antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR), glutathione S-transferase (GST), glutathione peroxidase (GPx)) activities.

Key words: Rainbow trout, oxidative stress, heavy metal, antioxidant, enzyme activity, Fish Diseases

Giriş

Balık yetiştiriciliği, dünyada hızla gelişen ve her geçen gün daha da önem kazanan önemli bir endüstri kolu haline gelmiştir (Özcan ve Önalan, 2017). Gökkuşağı alabalığı yetiştiriciliği, tatlı su balıkları içerisinde en yaygın olanıdır. Tüm organizmalarda olduğu gibi sucul organizmalarda da çevresel şartların sebep olduğu stres etkileri, oksidatif stres ile ilişkilendirilebilir. Oksijen, pek çok canlı için olduğu gibi balıklar için de hayati öneme sahip olmakla birlikte aynı zamanda çok tehlikeli toksik formlar olan serbest radikallere de dönüşebilmektedir. Serbest radikaller, eşleşmemiş elektron içeren atom, molekül veya iyonlardır (Halliwell ve Gutteridge, 1989; Zwart ve ark., 1999).

Serbest radikaller metabolik süreç esnasında endojen olarak üretilmesinin yanı sıra çevre kirliliği, güneş ışınları, sigara, radyasyon gibi ekzojen etkenler de serbest radikallerin oluşumuna neden olabilmektedir (Sarma ve ark., 2010). Reaktif bir yapıya sahip olan serbest radikaller başta proteinler, lipitler ve nükleik asitler olmak üzere tüm hücre bileşenleri ile etkileşebilme ve hasara sebebiyet verme potansiyeline sahiptirler.

Sağlıklı bir organizmada açığa çıkan ROT ile antioksidan savunma sistemi arasında hassas bir denge vardır (McCord., 1993). Oksidatif stres, hücresel metabolizma sırasında oluşan hidroksil radikali, süperoksit radikali ve hidrojen peroksit gibi ROT'nin artışı ile onları detoksifiye eden, antioksidanların yetersizliği sonucu oksidatif dengenin bozulması olarak tanımlanır (Lobo ve ark., 2010; Valko ve ark., 2007). Antioksidan savunma sisteminin en önemli özelliği, sistemin tüm bileşenlerinin ROT'ne karşı sinerji oluşturacak şekilde görev almasıdır (Chaudiere ve Ferrari-Illiou., 1999). Antioksidan enzimler, oksidatif stresin belirteçlerinden birisi olmasının yanı sıra bu enzimlerin aktivitelerindeki artış iyileşme sürecinin de bir göstergesidir (Rucinska ve ark., 1999; Mittler, 2002; Zembala ve ark., 2010). Bu nedenle, antioksidan enzimler hücre dengesinin düzenlenmesinde hayati bir öneme sahip olup ve indüksiyonları kirleticilere karşı verilen tepkinin bir sonucunu ifade etmektedir (Doyotte ve ark., 1997).

Ağır metaller, pestisitler, sıcaklık değişimleri, oksijen miktarı, parazitler gibi pek çok farklı çevresel faktör balıklarda serbest radikallerin sebep olduğu oksidatif stresin artmasına dolayısıyla da ekonomik kayıplara yol açmaktadır. Sucul ortama tarımsal ve endüstriyel faaliyetler, yağmur ve yüzey suları veya atık sular yoluyla taşınan ağır metaller başta olmak üzere tüm metaller sucul organizmalarda ROT'nin oluşumunu teşvik eden oksidatif stresin önemli indükleyicileridir (Banni ve ark., 2014; Alak ve ark., 2012). En önemli özelliği değerlik değiştirebilme yeteneği olan ağır metaller, ROT'nin üretimini iki farklı mekanizma ile artırabilir. Bunlardan ilki metallerle ilişkili süreçlerden kaynaklanırken, diğer mekanizma ise değişken değerliğe sahip iyonlar tarafından serbest radikal oluşturmasından kaynaklanmaktadır. Buna ilaveten değişken değerlikli iyonların etkisinin daha baskın olduğu göz önünde bulundurulduğunda ikinci mekanizmanın birinci mekanizmaya engel teşkil edebileceği önceki çalışmalarda bildirilmiştir. (Koutsogiannaki ve ark., 2014).

Bütün aerobik organizmalar gibi balıklarda da oksidatif stresi ve bunların meydana getirdiği hasarı önlemek için vücutta birçok savunma mekanizması gelişmiştir. Bunlar antioksidan savunma sistemleri olarak bilinir ve enzimatik karakterdeki süperoksid dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx), glutatyon redüktaz (GR), glutatyon S-transferaz (GST) ile enzimatik olmayan glutatyon, A, E, C vitaminleri, selenyum ve melatonin gibi maddelerden oluşurlar (Dautremepuits ve ark., 2003; Trenzado ve ark., 2006).

SOD, reaktif oksijen türlerine karşı ilk savunma hattını oluşturur (Sen ve ark., 2010; Sen ve Chakraborty., 2011). SOD, süperoksit radikalini (O_2^-) hidrojen peroksit (H_2O_2) ve moleküler oksijene (O_2) katalizleyen enzimatik bir antioksidandır. H_2O_2 daha sonra, CAT ya da GPx ile ortamdan uzaklaştırılır (Young ve Woodside., 2001).

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

CAT, her bir alt birimi, bir hem grubu ve bir NADPH molekülü içeren dört protein alt birimden meydana gelen enzimatik bir antioksidandır (Young ve Woodside., 2001; Kirkman ve ark., 1987). CAT, H_2O_2'nin, H_2O ve O_2'ye dönüşümünü katalize eder (Limon-Pacheco ve Gonsebatt., 2009). Süperoksit radikali ($O_2^{\cdot -}$), SOD aracılığıyla H_2O_2 dönüştürülür. Hidrojen peroksit (H_2O_2), bir radikal olmamasının yanı sıra biyolojik önemi olan moleküllerin çoğu ile reaksiyona girmemesine rağmen, Cu ve Fe iyonlarının katalizörlüğünde Fenton reaksiyonu ile en reaktif oksijen türü olan hidroksil radikali ($OH\cdot$) oluşumunda ön madde olarak rol oynamaktadır (Cheung ve ark., 2001; Larson., 1988).

$$2H_2O_2 \xrightarrow{\text{CAT}} H_2O + O_2$$

$$H_2O_2 + Cu^+ / Fe^{+2} \xrightarrow{\text{Fenton reaksiyonu}} OH\cdot + OH\cdot + Cu^{+2} / Fe^{+3}$$

Hücrelerin sitoplazmasında bulunan GPx, H_2O_2 'den $OH\cdot$ radikalinin oluşmasını engelleyerek H_2O_2 kaynaklı oksidatif hasara karşı hücreleri korur. Her bir alt birimi bir selenyum atomu içeren GPx, dört protein alt biriminden oluşur (Sen ve Chakraborty., 2011). GPx, elektron kaynağı olarak glutatyonu (GSH) kullanarak H_2O_2 'yi ve organik hidroperoksitleri (lipit hidroperoksitler, DNA hidroperoksitler) metabolize eden bir enzimdir. GPx enziminin selenyuma bağımlı ve selenyuma bağımlı olmayan iki tipi saptanmıştır. Aktif bölgesinde selenyum içeren selenyuma bağımlı glutatyon peroksidaz (Se-GPx), H_2O_2 ve organik hiperoksitlere karşı etkilidir. Selenyuma bağımlı olmayan glutatyon peroksidaz (GST) ise daha çok organik hidroperoksitlerin metabolize edilmesinde faaliyet gösterir (Cnubben ve ark., 2001; Reiter ve ark., 1995).

Bu metabolize etme reaksiyonları sırasında GSH, hidrojen verici olarak hareket ettiğinden dolayı H_2O_2 ve hidroperoksitler indirgenirken GSH okside olur (Reiter ve ark., 1995). Okside glutatyon, glutatyon disülfittir (GSSG). Glutatyon redüktaz (GR) enzimi varlığında okside glutatyon redükte glutatyon haline geri indirgenir. Bu indirgenme reaksiyonu esnasında GR elektron vericisi olarak NADPH'yi kullanır (Sen ve Chakraborty., 2011; Reiter ve ark., 1995).

GR, flavin adenin dinükleotid (FAD) içeren flavoprotein bir enzimdir. GR, NADPH'nin bir elektronunu okside glutatyonun disülfid bağlarına aktararak yeniden GSH'ye dönüştürülür. Bu nedenle NADPH serbest radikal hasarını engellemek için gereklidir ve en önemli kaynağı heksoz monofosfat (pentoz fosfat) yoludur (Sen ve ark., 2010; Özkan ve Fışkın., 2004).

Materyal ve Yöntem

Balık Örneklerinin Toplanması

Bayburt ilinde faaliyet gösteren 5 ayrı yerel gökkuşağı alabalık üretme çiftliğinde yetiştirilen yaklaşık olarak 200-250 gr ağırlığında işletme başına 6'şar adet Gökkuşağı alabalığı örneği işletmelerden Temmuz 2017 tarihinde satın alınmıştır. Balıklar işletme sahiplerinin yanında seçilerek ağ kepçe yardımı ile yakalanmıştır. Alınan balıklar %70'lik etil alkol ile dezenfeksiyon işleminin ardından işletme ve sıra kodları belirli olan steril poşetlere konularak +4 ºC'de aynı gün soğuk zincirde laboratuvara getirilmiştir (Ürkü and Önalan, 2018).

Doku Örneklerinin Alınması ve Nakli

Çalışmada toplanan ve laboratuvara uygun şartlarda getirilen balıklar aseptik şartlarda nekropsi işlemine tabi tutulduktan sonra solungaç, karaciğer ve böbrek dokuları enzim çalışmalarında kullanılmak üzere alınmıştır. Dokular soğuk %0,9'luk NaCl çözeltisiyle yıkanarak çalışmada kullanılıncaya kadar -20 ºC'de muhafaza edilmiştir.

Su Örneklerinin Alınması ve Analizi

Çalışmanın yapıldığı işletmelerdeki suların ağır metal içeriklerinin belirlemesi için su örneklerinin alınması aynı gün içerisinde gerçekleştirilmiştir. Su örnekleri balık yetiştiriciliği yapılan havuzlardan çıkış suyuna 1m uzaklıktan ve 25 cm derinlikten alınmıştır. Su örnekleri alımında polipropilen şişeler kullanılmıştır. Örnekler şişelendikten ve etiketlendikten sonra +4°C'de muhafaza edilerek laboratuvara getirilmiştir. Alınan su numunelerindeki ağır metal analizleri Bayburt Üniversitesi Merkezi Araştırma Laboratuvarı'nda AGILENT marka ICP-MS 7800 cihazı ile yapılmıştır.

Dokulardaki Antioksidan Enzimlerin Aktivitesinin Belirlenmesi

GST Enzim Aktivitesinin Ölçümü

Glutatyon s-transferaz, 1-kloro-2,4-dinitrobenzen (CDNB) ile glutatyonun – SH grubu arasındaki tepkimeyi katalizler. Enzim aktivitesi 340 nm'de 37 ºC'de GSH ve CDNB kullanılarak dakikada oluşan S-2,4-dinitrofenilglutatyonun 1 mikro molünü katalizleyen enzim miktarının ölçülmesiyle belirlendi (Habig ve ark., 1974).

GR Enzim Aktivitesinin Ölçümü

GR enziminin aktivite ölçümünde reaksiyona giren NADPH'ın 340 nm'de maksimum absorbans vermesi esasından yararlanıldı. GR enzimi katalizlediği reaksiyonda NADPH'ın azalmasına sebep olmaktadır. Bu azalma spektrofotometrik olarak 340 nm'de takip edilerek enzim aktivitesi belirlendi (Carlberg ve Mannervik., 1981).

GPx Enzim Aktivitesinin Ölçümü

GSH-Px tarafından katalizlenen reaksiyonda GSH'nın H_2O_2 ile oksidasyonu sonucu oluşan GSSG'nin glutatyon redüktaz (GSSG-Rd) kataliziyle tekrar GSH'a dönüşmesi sırasında tüketilen NADPH konsantrasyonu üzerinden 340 nm'de oluşan absorbans azalmasının 4 dakika boyunca izlenmesi prensibine dayanır (Jocelyn., 1970).

SOD Enzim Aktivitesinin Ölçümü

Oksidatif yolla enerji üretimi sırasında oluşan endojen ve eksojen kaynaklı toksik süperoksit radikellerinin suya ve moleküler oksijene dismutasyonunu hızlandıran SOD enzim aktivitesinin ölçüm prensibi, ksantin varlığında ksantin oksidazın açığa çıkardığı süperoksit radikallerinin nitroblue tetrazolium (NBT) ile 560 nm'de absorblanan rengin ölçülmesine dayanır (Kankaya ve ark., 2015; Sun ve ark., 1988).

CAT Enzim Aktivitesinin Ölçümü

Katalaz aktivitesi tayini Aebi tarafından tarif edilen yönteme göre yapılmıştır. Yöntemin esası, H_2O_2 substratının katalaz ile enzimatik yıkılmasının 240 nm de izlenmesidir (Aebi., 1974; Kankaya ve Kaptaner, 2017).

Protein Miktarının Ölçümü

Protein miktarının ölçümü Bradford metoduna göre 595 nm'de spektrofotometre kullanılarak yapılmıştır. Ölçümde bovin serum albümin proteini standart olarak kullanılmıştır (Bradford., 1976).

İstatistik Analiz

Beş farklı işletmedeki balık örneklerinden elde edilen solungaç, karaciğer ve böbrek dokularının antioksidan enzim düzeylerine ait veriler JMP istatistik paket programı kullanılarak (SAS, Institute Inc., 2007) ANOVA ile analiz edilmiştir. Her bir işletmeye ait ortalama değerlerin karşılaştırılmasında Tukey HSD ortalama karşılaştırma testi ve önemlilik düzeyi için $P<0.05$ değeri kullanılmıştır.

Bulgular

Balık kalp dokularında; GR, CAT, GST ve GPX enzim aktivitesi 1 nolu işletmede, SOD enzim aktivitesi ise 3 nolu işletmede en yüksek seviyede olduğu belirlenmiştir. Balık karaciğer dokularında; GR, CAT ve GPX enzim aktivitesi 4 nolu işletmede, SOD aktivitesi 1 nolu işletmede, GST aktivitesi ise 2 nolu işletmede en yüksek seviyede olduğu belirlenmiştir. Solungaç dokularında; SOD ve GST enzim aktivitesi 3 nolu işletmede, GR enzim aktivitesi 5 nolu işletmede, CAT enzim aktivitesi 4 nolu işletmede, GPx enzim aktivitesi ise 1 nolu işletmede en yüksek seviyede olduğu belirlenmiştir.

Farklı işletmelerden toplanan su numunelerinde toplam mineral düzeylerinin belirlenmesi amacı ile Zn, Fe, Cu, Ni, Co, Hg, As, Mn minerallerinin düzeyleri incelenmiştir. Elde edilen sonuçlar aşağıdaki tabloda verilmiştir.

Tablo 1. Farklı işletmelerden alınan su numunelerindeki toplam mineral düzeylerine ait en küçük kareler ortalaması ve standart hatası

İşletme Numarası	Toplam Mineral Düzeyi	SH	P Değeri
1	0,181[e]		
2	0,242[d]		
3	0,522[b]	0,04	<0,001
4	0,441[c]		
5	0,569[a]		

[a-e] Aynı sütundaki farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir ($P<0.05$)

Bayburt ilindeki gökkuşağı alabalığı işletmelerinin her birinden toplanan 6'şar adet balık örneğinde kalp, karaciğer ve solungaç dokularında GR, CAT, SOD, GST ve GPX enzim aktivite düzeyleri belirlenmiştir. Bu sonuçlar ışığında dokularda enzim aktivitelerine ait değerler ve en küçük kareler ortalaması ile standart hatalar aşağıda verilmiştir.

Tablo 2. Farklı işletmelerden alınan balık kalp dokularında GR, CAT, SOD, GST, GPX enzim aktivite düzeylerine etkisine ait en küçük kareler ortalaması ve standart hatası

İşletme	GR	CAT	SOD	GST	GPX
1	0,057[a]	124,59[a]	0,237[b]	0,068[a]	0,119[a]
2	0,043[c]	72,01[d]	0,087[d]	0,038[d]	0,074[d]
3	0,058[a]	112,43[b]	0,312[a]	0,057[b]	0,110[b]
4	0,053[b]	120,78[a]	0,147[c]	0,051[c]	0,079[c]
5	0,045[c]	10,79[c]	0,248[b]	0,039[d]	0,068[d]
SH	0,028	6,71	0,027	0,03	0,005
P değeri	<0,001	<0,001	<0,001	<0,001	<0,001

[a-d] Aynı sütundaki farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0.05)

Tablo 3. Farklı işletmelerden alınan balık karaciğer dokularında GR, CAT, SOD, GST, GPX enzim aktivite düzeylerine etkisine ait en küçük kareler ortalaması ve standart hatası

İşletme	GR	CAT	SOD	GST	GPX
1	0,029[e]	142,05 [c]	0,112 [a]	0,068 [b-c]	0,083 [d]
2	0,036 [d]	127,27 [d]	0,073 [b]	0,074 [a]	0,091 [c]
3	0,039 [c]	125,29 [d]	0,053 [c]	0,061 [d]	0,122 [b]
4	0,073 [a]	208,06 [a]	0,040 [d]	0,070 [b]	0,138 [a]
5	0,052 [b]	150,36 [b]	0,048 [c-d]	0,065 [c]	0,091 [c]
SH	0,002	7,363	0,009	0,03	0,002
P değeri	<0,001	<0,001	<0,001	<0,009	<0,001

[a-e]Aynı sütundaki farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0.05)

Tablo 4. Farklı işletmelerden alınan balık solungaç dokularında GR, CAT, SOD, GST, GPX enzim aktivite düzeylerine etkisine ait en küçük kareler ortalaması ve standart hatası

İşletme	GR	CAT	SOD	GST	GPX
1	0,127 [c]	87,08 [c]	0,347 [a]	0,057 [c-d]	0,101 [a]
2	0,114 [d]	71,65 [e]	0,343 [a]	0,055 [d]	0,066 [c]
3	0,133 [c]	100,91 [b]	0,367 [a]	0,070 [a]	0,080 [b]
4	0,143 [b]	109,69 [a]	0,165 [b]	0,060 [b-c]	0,066 [c]
5	0,171 [a]	80,97 [d]	0,133 [b]	0,062 [b]	0,065 [c]
SH	0,006	4,927	0,041	0,002	0,005
P değeri	<0,002	<0,001	<0,001	<0,002	<0,001

[a-e]Aynı sütundaki farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0.05)

Tartışma ve Sonuç

Serbest radikaller, hücre zar yapısında bozulma, enzim etkinliğinde değişiklikler, protein ve diğer moleküller ile kovalent bağlar oluşturma, koenzimlerin etkilerini yavaşlatma, sinir iletisini azaltma, DNA hasarı ve buna bağlı mutasyonlar oluşturma ve lipit peroksidasyonu gibi pek çok olumsuzluktan sorumludurlar. Günümüzde sucul ekosistemleri ve bu ekosistemlerde yaşayan organizmaları tehdit eden sorunların başında gerek doğal yollarla gerekse doğada insanoğlunun neden olduğu kaynaklardan su ortamına giren ve her geçen gün derişimi artan ağır metaller gelmektedir. Ağır metaller, ekosistemin önemli bir bileşeni olan balıkların hücresel mekanizmalarına zarar vererek hasara yol açabilmektedir (Basha ve Rani., 2003). Sucul ekosistemlerdeki ağır metal kaynaklı kirlilik, sadece bu ekosistemleri değil aynı zamanda besin zinciri yoluyla insana kadar pek çok canlıyı etkilemesi sebebi ile sucul ekosistemlerin kontrolü son derece önemlidir.

Ağır metaller deniz, göl ve akarsularda fazla miktarda bulundukları zaman sucul organizmalar tarafından organizmaya ağız, solunum ve deri yolu ile alınır fakat özel bir destek olmadan vücudun boşaltım yolları ile (böbrek, karaciğer, barsak, akciğer, deri) atılamazlar. Bu nedenle toksik ağır metallerin büyük bir bölümü sucul organizmaların özellikle metabolik olarak aktif organlarında birikme eğilimindedirler. (Akçalı ve ark., 2009; Richetti ve ark., 2011; Oliva ve ark., 2012). Sucul organizmalardaki bu metallerin varlığı, etkin dozlara ulaştıklarında ise ciddi hastalıklara ve hatta ölümlere dahi neden olabilirler.

Çalışmamızda Bayburt ilinde faaliyet gösteren 5 adet Gökkuşağı alabalık işletmesinin benzer noktalarından alınan su numunelerindeki Zn, Fe, Cu, Ni, Co, Hg, As, Mn metallerinin varlığı ve miktarı belirlenmiştir. Sonuçlar birlikte yorumlanarak işletme bazında toplam mineral düzeyleri ortaya konulmuş ve toplam mineral düzeyleri ile enzim aktiviteleri arasındaki ilişki incelenmeye çalışılmıştır. Farklı işletmelerden alınan balıkların aseptik şartlarda gerçekleştirilen nekropsi işleminin ardından kalp, karaciğer ve solungaç dokuları alınarak GR, CAT, SOD, GST ve GPX enzim aktivite düzeyleri belirlenmiştir. Balıklarda kirleticiler ile ilk etkileşen organ solungaçlardır. Kirleticiler hedef organ ve dokularda birikerek yapısal ve işlevsel mekanizmalara hasar verebilirler (Hsu ve ark., 2013; Pereira ve ark., 2013).

Yaptığımız çalışmada elde edilen veriler ışığında toplam mineral düzeyi en yüksek olan 5 nolu işletmede balık solungaç GR enzim aktivite düzeyinin diğer işletmelere göre daha yüksek seviyede olduğu görülürken, SOD ve GPx enzim aktivite düzeyinin diğer işletmelere kıyasla daha düşük olduğu belirlenmiştir. Toplam mineral düzeyinin en düşük olduğu 1 nolu işletmede ise balık solungaç GPx enzim aktivitesinin diğer işletmelere nazaran daha yüksek olduğu, balık karaciğer GR enzim aktivitesinin ise daha düşük olduğu belirlenmiştir.

Balıkların karaciğer ve böbrek dokuları, metaller tarafından oluşan oksidatif stresten korunmak amacıyla CAT ve SOD gibi antioksidan savunma sistemlerince zengin olduğu bildirilmektedir (Basha ve Rani, 2003; Lushchak, 2011). Bizim çalışmamızda toplam mineral düzeyinin en fazla olduğu 1 nolu işletmedeki balık karaciğer SOD enzim aktivitesinin diğer işletmelerden daha yüksek seviyede olması yönüyle bunu desteklemektedir.

Aşırı O_2 tüketiminin CAT aktivitesini inhibe edebilme özelliği ile CAT aktivitesini azaltabileceği belirtilmiştir (Suvetha ve ark., 2010). CAT, tüm biyolojik membranlardan geçerek, bazı enzimleri inaktivite edebilir. Metallerden kaynaklanan kirleticilere maruz kalan balıklarda doza bağlı olarak CAT enzim aktivite seviyesinin indüklenme ya da inhibe olma yönünde farklılık gösterdiği bilinmektedir. Bu nedenle CAT aktivitesinin oksidatif stres için önemli bir biyobelirteç olduğu kabul edilmektedir (Romeo ve ark., 2000; Gül ve ark., 2004). Bu konuyla ilgili yapılan bir çalışmada, Hindistan'ın Yamuna Nehri'nin ağır metallerce kirlendiği düşünülen Panipat ve Agra bölgelerinden yakalanan *Wallago attu* (Mully Catfish) türü balıkların karaciğer, solungaç ve böbrek dokularında CAT, GSH ve lipit peroksidasyon düzeyleri incelenmiştir. Bu balıklarda GSH ve lipid peroksidasyonunun yüksek olduğu, CAT aktivitesinin solungaç, karaciğer ve böbrekte önemli derecede azaldığı belirtilmiştir (Pandey ve ark., 2003). Ayrıca farklı dozlarda Cd uygulaması sonucunda çipura (*Sparus aurata*) türü balıklarda, CAT enzim aktivitesinde önemli düşüşler gözlendiği rapor edilmiştir (Goel ve ark., 2005).

Çalışmamızda balık kalp CAT enzim aktivitesi 1 nolu işletmede, balık karaciğer ve solungaç CAT enzim aktivitesinin 4 nolu işletmede en yüksek seviyede , balık kalp CAT enzim aktivitesi 5 nolu işletmede, balık karaciğer CAT enzim aktivitesi 3 nolu işletmede ve balık solungaç CAT aktivitesinin ise 2 nolu işletmede en düşük seviyede olduğu gözlemlenmiştir. Bir başka çalışmada ise bazı metal iyonlarının (Cu, Cd, Fe ve Ni) *Channa punctata*'nın solungaçlarının biyokimyasal ve morfolojik özelliklerine etkilerinin incelendiği , CAT, GST ve SOD gibi antioksidan enzim aktivitelerinde zamana bağlı azalmalar gözlendiği belirtilmiştir (Sanchez ve ark., 2005). Çalışmamızda bu iyonların diğer işletmelere nazaran nispeten daha fazla olduğu 2 nolu işletmede balık kalp GR, SOD ve GST, balık karaciğer GPx ve balık solungaç GR, CAT ve GST enzim aktivitelerinin diğer işletmelerden daha düşük olduğunu değerlendirdik. Buna benzer bir başka çalışmada ise üç grup halindeki gökkuşağı alabalığına (*Oncorhynchus mykiss*) yedi gün süreyle 1 ve 5 ppm Cd uygulanarak solungaç dokularında bazı antioksidan enzim aktivitelerindeki değişimler incelenmiştir. Deneyin üçüncü gününde SOD, GPx ve CAT enzim aktivitelerinin solungaç dokusunda önemli derecede arttığı, beşinci günden itibaren ise azaldığı belirtilmiştir. Subletal dozlardaki kadmiyumun, gökkuşağı alabalıklarında oksidatif strese sebep olduğu ve diğer antioksidan enzimlerin bu stresin önlenmesinde rol oynadıkları bildirilmiştir (Alak ve Hisar., 2008).

Sucul organizmalarda antioksidan sistemlerin içerdiği bazı enzim (SOD, GPx, CAT) gruplarının (Kehrer., 1993; Kerameti ve ark., 2010) ROT' ni yok edici etkilerinin olduğu ve pestisitlerden kaynaklanan serbest radikallerin zararlı etkilerine karşı hücresel sistemi koruyabildikleri (Banerjee ve ark., 1999; Yarsan ve ark., 1999; Banerjee ve ark., 2001) ve söz konusu enzimlerin kirlilik çalışmaları ve ekotoksikolojik risk değerlendirmelerinde uygun ve güvenilir biyobelirteçler olarak kullanılabileceği bildirilmiştir. Gerek elde ettiğimiz sonuçlar gerekse yaptığımız literatür taramaları sonucunda; sucul ortamda bulunan ağır metallerin, sucul organizmaların antioksidan savunma sisteminde birtakım değişikliklere sebebiyet verdiği ve bunun sonucunda hücresel düzeyde hasarlara sebep olduğu değerlendirilmektedir.

Çevre ve su kirliliğinin bir göstergesi olarak değerlendirilen ağır metal kaynaklı kirleticiler özellikle su ürünlerinde sıklıkla yüksek seviyelere ulaşabilir ve başta balıklar olmak üzere su ürünlerini aynı zamanda besin zinciri yoluyla insanların yanı sıra pek çok canlıyı olumsuz etkileyebilir. Günümüzde artan nüfus yoğunluğu ve besin ihtiyacı göz önünde bulundurulduğunda insan ve çevre sağlığı bu nedenle büyük önem arz etmektedir.

Sucul organizmalarda ağır metal birikimi ve buna bağlı hasarların incelendiği araştırmaların yapılması ve sayılarının artırılması, ağır metallere karşı duyarlılığı yüksek olan türlerin belirlenmesinin yanı sıra sucul organizmaların biyokimyasal ve fizyolojik parametrelerinde meydana gelebilecek değişikliklerin belirlenmesi açısından da oldukça önemlidir. Su kaynaklarına yakın yerlerdeki endüstriyel faaliyetler ilgili kurumlarca sıklıkla denetlenmelidir. Su ortamı ve sucul canlılarda kirlilik izleme programları ve ekotoksikolojik risk değerlendirme çalışmaları ağır metal düzeyinin yüksek olduğu düşünülen ortamlarda periyodik olarak izlenmelidir.

Balık hastalıkları açısından da su kalitesi çok önemli olduğu bilinmektedir. Zira hastalıkların meydana gelebilmesi için gerekli üç ana şarttan birisi olan çevre, su kalitesi ile doğrudan ilişkilidir. Su kalite kriterlerindeki değişimler, vitamin mineral düzeylerinin artması veya azalması gibi sonuçlar hastalık etkenlerinin belirtilerinin ortaya çıkmasına ve yüksek ölümler ile sonuçlanmasına sebep olabilmektedir. Sucul ortamda yaşayan tüm canlılar açısından su kalitesinin önemi düşünüldüğünde gerekli önlemlerin alınması ve yaşam alanlarının korunması gerektiği kanaatindeyiz.

Teşekkür

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Araştırma Makalesi/*Research Article (Original Paper)*

Generalized Linear Mixed Model versus Transformation on Bayesian Approach

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Abstract: Linear mixed effects models (LMM) have been widely used for nearly all analysis of animal breeding data. They are very powerful tools for the estimation of variance components and genetic parameters, and for the prediction of genetic merit of animals. These models can only be used when some specific assumptions are provided such as normality and constant of variances. However, if these assumptions are not provided, the data should be transformed and the statistical analysis is carried out with the transformed data. Generalized linear mixed-effect models (GLMM) provide a solution for this problem by satisfying normality assumptions without transformation. This allows differences among animals to be assessed properly using the data most appropriate to the researcher's theoretical context. The aim of this study is to estimate variance components, genetic parameter of birth weight (BW), weaning weight (WW) which have economic values in animal breeding with LMM_t (with transformed data), LMM_ut (with untransformed data) and GLMM based on Bayesian approach. The present study also intended to compare the both models and the estimated parameters values obtained with LMM_t and GLMM. The data is obtained from BW and WW of 4972 Awassi lambs were born between the years of 2012-2016. As a result, GLMM is the most suitable model for BW and WW according to DIC values. Although estimation of BW heritabilities does not change in all models, there are significant differences in estimation of WW heritabilities.

Keywords: Transformed Data, GLMM, Bayesian approach

Bayesyen yaklaşımında Genelleştirilmiş Linear Karışık Etkili Model ile Transformasyonun Karşılaştırılması

Özet: Linear karışık etkili modeler (LKM), hayvan ıslahı verilerinin neredeyse tüm analizlerinde yaygın olarak kulanılmaktadır. Varyans bileşenleri ile genetik parametrelerin tahmini ve hayvanların genetik değerini belirlemek için çok güçlü araçlardır. Bu modeler normallik varsayımı ve varyansların sabitliği gibi bazı varsayımlar sağlandığında kullanılabilen modellerdir. Ancak bu varsayımlar sağlanmazsa veriler transforme edilip analize transforme edilmiş verilerle devam edilir. Genelleştirilmiş linear karışık etkili modeller (GLKM) transformasyon olmaksızın normallik varsayımını iligili problemler için çözüm sağlar. Bu, araştırmacıların teorik bağlamda en uygun verileri kullanarak hayvanların uygun şekilde değerlendirilmesini sağlar. Bu çalışmanın amacı; Bayesyen yaklaşımı ile hayvan ıslahında önemli ekonomik değere sahip doğum ağırlığı (DA) ve sütten kesim ağırlığına (SKA) ait verileri transformasyon uygulanmış (LKM_t), transformasyon uygulanmamış (LKM_ut) haliyle LKM ve transforme edilmemiş haliyle GKLM modelleri kullanarak varyans unsurlarını ve genetik parameterini tahminlemektir. Bu çalışma aynı zamanda hem modelleri hem de LKM_t ve GLKM ile elde edilen tahmini parametre değerlerini karşılaştırmayı amaçlamıştır. Analizde kullanılan veri 2012-2016 yılları arasında doğan 4972 İvesi kuzularından elde edilmiş DA ve SKA' dan meydana gelmiştir. Sonuç olarak; GLKM model seçme kriteri DIC'ye göre DA ve SKA için en uygun model olarak belirlenmiştir. DA'na ait kalıtım derecesi tahminleri tüm modelerde değişmemesine rağmen SKA'na ait kalıtım derecesi tahminlerinde önemli farklılıklar vardır.

Anahtar kelimeler: Transforme edilmiş veri, GLKM, Bayesyen yaklaşımı

Introduction

The most known assumptions of linear models that are often used for the analysis of animal breeding data are independency of errors, having normal distribution with zero mean, and similarity between treatments (homogeneity of variance). These assumptions are the basis of parametric statistical methods. However, the

distribution of the data used to analyze the traits that are economically important in animal breeding may not be normal. In the analysis of these data, various transformation methods were preferred before nonparametric tests. Transformation of the data is usually not only the preferred method for alleviating heterogeneity, but results in loss of information. Many transformation methods are used for data analysis. Among these transformation methods, Rank transformation can act like a unified form of both parametric and nonparametric tests. Conover and Iman (1981), reported that the approaches of rank transformation are easier than both other transformation methods and other non-parametric tests. Although, the use of rank transformation has been the subject of researchers for many years. While some researchers (Conover and Iman, 1980; Crouse, 1968; Iman and Conover, 1979; Lemmer and Stoker, 1967; Leys and Schumann, 2010; MacDonald and Thompson, 1967; Scheirer, Ray, and Hare, 1976; Zimmerman, 1994) emphasized that it is useful in factorial design, some researchers (Hamilton, 1976; Quade, 1967; Nordstokke and Zumbo, 2010) said that it is useful in discriminant and covariance analysis. But Robert and Casella (2004), mentioned that it is not enough for complex model. Sawilowsky (1990) reported that a rank transformation's statistical power is three times higher than a factorial ANOVA in data that shows non-normal distribution. On the other hand, advancing computer technology, has provided a development of solution-oriented new methods in time. Generalized Linear Models (GLM), which is conceptually defined first-time by Nelder and Wedderburn (1972), do not need to provide the assumption of normality. GLM assumes that non-normal distributed dependent variable is from exponential distribution family, enables linear regression model (Koç, 2012). In non-normal distributed model, GLM has been expanded with the addition of the random effects to linear estimators, Generalized Linear Mixed Model (GLMM) has been developed. The disadvantages of GLM because of the its assumption, Williams (1982) has been eliminated with GLMM development. The presence of the random effects in the GLMM model causes the likelihood function to comprise a high-dimensional integral, thus make the maximum likelihood estimates difficult. This difficult situation in the GLMM model was solved by Markov Chain Monte Carlo (MCMC) techniques in the Bayesian approach. This approach allows to be solved of non-normally distributed data structures and provides great convenience to calculation of transformed estimates (such as heritability) by using posterior distributions (de Villemereuil et al., 2013). Bayesian approaches have been used to estimate quantitative genetic parameters providing better approximation for parameters' distributions (Waldmann et al., 2008; Apiolaza et al., 2011; Cappa et al., 2012; Aparicio et al., 2015). Barbosa et al. (2015), reported that Bayesian approaches reduce the bias of breeding value estimates even data is small. In addition, the accuracy of genetic parameter estimates depends on the factors in model, the number of observations, the statistical model used, and the estimation method of variance-covariance components (Barbosa et al., 2008). The aim of this study is to estimate variance components and genetic parameter of birth weight (BW) and weaning weight (WW) which have economic values in animal breeding with LMM_t (with transformed data), LMM_ut (with untransformed data) and GLMM based on Bayesian approach.

Material and Methods

In this study, birth weight and weaning weight of Awassi lambs records are used from 2012 to 2016. The pedigree consist of 4972 records from 80 different rams and 1917 dams. Birth weight and weaning weight have important economic value for breeders, so the records of these weights were used for estimate genetic parameters.

The mathematical model which was used in this study:

$$Y_{ijklmn} = \mu + year_i + birthtype_j + sex_k + dam\_age_l + flock_m + e_{ijklmn}$$

General Linear Mixed model equation is:
y= Xb+Za+e
If we transform our response variable, the resulting model may look like this:
f_t(y)= Xb+Za+e
wheref_t (y) is the transformation function applied to y.
Generalized Linear Mixed Model equation is

g(E(y|b,a, ϕ))= η =Xb+Za+e

where g (.) is a monotonic "link" function.
Where **y** is the vector of observation,
b is a vector of fixed effect and having constant prior

$$\beta \propto \text{constant}$$

a is a vector of random genetic effects and having Gaussian prior

$$a \mid G_a \sim N\left[0,(A \otimes G_a)\right]$$

Xand **Z**are design matrix for fixed and random effects,
e is the vector of random errors and having Gaussian prior

$$e \mid R \sim N\left[0,(I_n \otimes R)\right]$$

where A, G_a, R, I_n, relationship matrix, direct genetic, residual covariance, identity matrix, respectively. Additive variance and covariance matrices and residual matrix are derivate from Inverse Wishart (IW) distribution.

$$G_a \mid v_G, V_G \sim IW(v_G, V_G)$$
$$R \mid v_R, V_R \sim IW(v_R, V_R)$$

v_G,V_G, v_R and V_R parameters of prior values (Waldmann and Ericson, 2006).

Analyses were performed via MCMC package (Hadfield, 2010) in R software (R Development Core Team, 2012; http://www.r-project.org). Individual Animal Model is based on Bayesian approach. In this study, the values of total iteration number, burn-in and thinning interval 250000, 100000 and 1 were used, respectively. Variance and covariance components and genetic parameters were calculated according to Willham's (1972) ($h^2_a = \sigma^2_a / \sigma^2_p$) reported.

Results and Discussions

Descriptive statistics for birth and weaning weights of Awassi lambs has been shown in Table 1.

Table 1.Descriptive statistics

	Min	**Max**	$\overline{X} \pm S_{\overline{X}}$	***S***	**CV**
BW	1.66	6.83	3.79±0.01	0.75	19.75
WW	9.12	36.44	17.36±0.04	2.78	15.99

BW: Birth weight; WW: Weaning weight; Min: Minimum; Max: Maximum; $\overline{X} \pm S_{\overline{X}}$: mean±standarterror; S: standartdeviation, S^2 : variance and CV: covariance coefficient

The results of variance analysis for all fixed effects of these traits (transformed and untransformed) are statistically significant (p <0.001). ANOVA table for the traits with untransformed and transformed has been shown in respectively Table 2 and Table 3.

Table 2. ANOVA table for the traits with untransformed.

Source	**Dependent Variable**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Year	BW	324,141	3	108,047	274,259	,000
	WW	8778,268	3	2926,089	646,403	,000
Birth_year	BW	202,300	1	202,300	513,506	,000
	WW	886,388	1	886,388	195,812	,000
Sex	BW	326,714	1	326,714	829,309	,000
	WW	2479,043	1	2479,043	547,646	,000
Dam Age	BW	14,133	5	2,827	7,175	,000
	WW	64,492	5	12,898	2,849	,010
Flock	BW	13,480	2	6,740	17,108	,000
	WW	234,732	2	117,366	25,927	,000

BW: Birth weight; WW: Weaning weight

Before the parameter estimation, Markov chains are obtained by iterations autocorrelation and convergence control were made for MCMC algorithms accuracy. For this aim, effective sample size, trace graphs and Heidelberg stationary test has been controlled. According to this, effective sample size should be higher than 1000 and p values of Heidelberg stationary test higher than 0.05 in practice (Hadfield, 2010). The results of Heidelberg stationary test and effective sample size for birth weight and weaning weight in Linear mixed model and Generalized Mixed model have been shown in Table 4.

Table 3. ANOVA table for the traits with transformed.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Year	BW	561,967	3	187,322	265,962	,000
	WW	1154,195	3	384,732	658,221	,000
Birth_year	BW	373,451	1	373,451	530,229	,000
	WW	129,267	1	129,267	221,157	,000
Sex	BW	585,571	1	585,571	831,399	,000
	WW	304,425	1	304,425	520,827	,000
Dam Age	BW	26,100	5	5,220	7,411	,000
	WW	9,821	5	1,964	3,360	,005
Flock	BW	24,967	2	12,484	17,724	,000
	WW	30,309	2	15,154	25,927	,000

BW: Birth weight; WW: Weaning weight

Analysis of genetic parameter estimates for weaning weight, birth weight is used as an covariate. According to results, used iteration number, burn-in values and thinning interval values are suitable for parameter estimation.

Table 4. The results of effective sample size and Heidelberg stationary test

Models	Traits	Effective sample size Animal and units	Heidelberg stationary Animal and units
LMM_ut	BW	1420.522 - 2940.725	0.230 - 0.238
LMM_ut	WW	2033.835 - 3378.822	0.535 - 0.818
LMM_t	R_BW	1368.826 - 2785.447	0.471 - 0.515
LMM_t	R_WW	1218.535 - 1768.094	0.560 - 0.652
GLMM	BW	1354.525 - 2578.645	0.240 - 0.248
GLMM	WW	2002.688 - 2980.537	0.450 - 0.524

The estimates of genetic parameter with three model (LMM_ut, LMM_t, GLMM) based on Bayesian Approach were shown in Table 5. Deviance Information Criteria (DIC) values were used for compare the models.

Table 5.Genetic parameters estimations for birth and weaning weights

Trait:	**BW**				**WW**			
		High Density interval				High Density interval		
Models	h_a^2	Lower Limit	Upper limit	DIC	h_a^2	Lower Limit	Upper limit	DIC
LMM_ut	0.20	0.158	0.252	9090.386	0.35	0.292	0.418	19467.15
LMM_t	0.20	0.156	0.215	11975.83	0.42	0.359	0.490	19443.84
GLMM	0.20	0.158	0.253	9065.520	0.38	0.320	0.455	9136.212

The results of model comparison criterion (Deviance Information Criteria, DIC), the most suitable model for birth weight is the generalized linear mixed model (GLMM). Direct heritability of birth weight was found same in three models. The highest DIC value was found in LMM_t for BW.

As for Weaning weight, DIC values of LMM_ut and LMM_t are approximate, but GLMM has lowest DIC value. In the light of this, GLMM can be preferred. In addition, the highest direct heritability are obtained from LMM_t model. Although, estimates of direct heritability of WW with LMM_ut and GLMM models are found same. When compare to DIC values, it can be said that GLMM can be preferred. Generalized linear models are more flexible than transformations of the response, in that they allow a separate modeling of linearity and variance relationships. The results obtained from this study are similar to those of Henderson and MC (1990), which show that GLMM can be used as a substitute for transformation. Consequently, it is shown that the structure of data is very important in model selection and genetic parameter estimation can be performed without transformation when the pattern of non-normal distributed data.

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Araştırma Makalesi/*Research Article (Original Paper)*

Comparison of the Histological Changes in the Digestive Tract of Lake Van Fish (*Alburnus tarichi* Güldenstädt, 1814) During Reproductive Migration

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Abstract: Van Lake is the largest soda lake in the world. The Van Fish, which is a single fish species has adapted to the extreme conditions of the lake. The fish is anadrom and migrates from the lake to fresh water for reproduction and returns to the lake again. In the present study, the histological structure of the digestive tract of Van Fish in the lake and the freshwater environment was investigated. It was observed that the digestive tract was composed of a short oesophagus, a stomach-like structure, anterior intestine and posterior intestine, respectively. It has been determined that there is no obvious stomach structure as in some other fish. The digestive tract of Van Fish taken from fresh water and lakes were stained with Hematoxylin-Eosin, Periodic Acid Schiff and Alcian Blue pH 2.5. When the cross sections of digestive tracts were examined, it was observed that it consisted of mucosa, submucosa, muscularis and serous layers. Muscularis mucosa layer was not observed in this species. It was determined that the mucosa layer consisted of simple columnar epithelial cells, connective tissue and blood vessels of the submucosa layer, muscularis layer along the digestive tract, both transverse and longitudinal smooth muscle cells, and the serous layer were composed of thin connective tissue. It has been found that the thickness of these layers varies according to the parts of the digestive tract. Goblet cell that containing neutral and acidic glycoconjugate, sizes, number, and distribution in the mucosa layer were changed according to the parts of the digestive tract. It was observed that the Na^+, K^+, ATPase (NKA) containing cells, which play a role in ion regulation, are densely in the posterior intestine and rectum of the samples taken from the lake adapted fish. It was determined that the digestive tract of Van Fish showed similarity to the family of Cyprinidae.

Keywords: *Alburnus tarichi*, digestive tract, histology, Na^+, K^+, ATPase

Introduction

Lake Van is one of the largest soda lake of the world, which is located at 1650 m above mean sea level, maximum depth of 451 m and surface area of 3738 km^2 in the Eastern Anatolia region of Turkey. The lake water has high pH (9.8), alkaline (153 $mEqxl^{-1}$) and brackish waters (‰ 22). These unsuitable conditions greatly limit animal diversity in the lake. Only plankton and one fish species have been adapted to the lake (Danulat and Selcuk, 1992; Danulat, 1995; Reimer et al., 2009).

Van Fish, *Alburnus tarichi* is an endemic cyprinid species living in the Lake Van basin. The fish migrate to fresh water once a year for reproducing and return to the lake again (Danulat and Selcuk, 1992). The total catch of Van Fish was about 10.000 tons per year. It is a particularly important protein source in regions of Eastern Anatolia of Turkey. Due to overfishing and habitat loss, its population has undergone a decline in numbers. Furthermore, Van Fish is IUCN Red list. Despite the great economic importance for the region, there is limited information about fish biology.

The digestive tract shows morphological and histological differences in fish (Albrecht et al., 2001; Abdulhadi, 2005; Becker et al., 2010). These differences are usually due to the diet of the fish. There are also anatomical differences between the parts of the digestive tract in different fish species. The presence or absence of stomach, intestine lengths and folds may vary between species. Digestive tract histology is less than anatomic differences. When the wall of the digestive tract is examined, it is generally composed of mucosa, submucosa, muscularis, and serosa (Genten et al., 2009). These layers may differ in thickness or cell type in different parts of the digestive tract. The submucosa layer is the part responsible for the preservation of the underlying cells and the absorption of nutrients. In this layer, from multi-layered cubic cells to simple squamous epithelial cells, mucous cells and,

rarely, granulocytes, lymphocytes, and macrophages are seen. The submucosa layer is composed of connective tissue containing collagen fibrils. The muscularis layer consists of longitudinal and transverse smooth muscle cells. On the outermost side, there is a serosal layer formed of simple squamous epithelium surrounding the digestive tract (Chatchavalvanich et al., 2006; Cao and Wang 2009; Nascimento et al., 2015).

The digestive tract of teleost plays an important role in the digestion and osmoregulation. The saltwater adaptation of fish, a number of changes were observed in the digestive tract. Increased surface areas of intestinal and rectum parts, decreased mucus cell density and increased Na^+, K^+, ATPase (NKA) activity was determined. The presence and density of NKA in digestive tract or other tissues like gill and kidney is an indicator of ion transport activity (Giffard-Mena et al., 2008; Wilson and Castro, 2010).

Little is known about of the morphology and histology of the digestive tract in Van Fish. Many studies have attentive on the osmoregulatory role and histology of gill and kidney (Danulat and Kempe 1992; Oğuz 2013; Oğuz 2015a; 2015b). The objectives of this study, determinate of digestive tract morphology and histology, compare histological and histochemical structure both in the lake and freshwater acclimation, immunolocalization of the NKA anterior and posterior intestine, and rectum of the digestive tract in the Van Fish, *Alburnus tarichi*.

Material and Methods

Between April and August 2016, a total of 40 fishes were collected from Lake Van and freshwater (Karasu River) (Fig 1.). Van fishes were collected with net and kept alive in plastic containers supplied with oxygen to be transported to the laboratory for histological analysis. After the fish were anesthetized with MS-222 (40 mg/L, Sigma Chemical Co., St. Louis, MO), they were killed rapidly by decapitation. All animal experimental procedures were carried out in accordance with animal study protocols approved by the Animal Researchers Local Ethic Committee of Yüzüncü Yıl University (2017/).

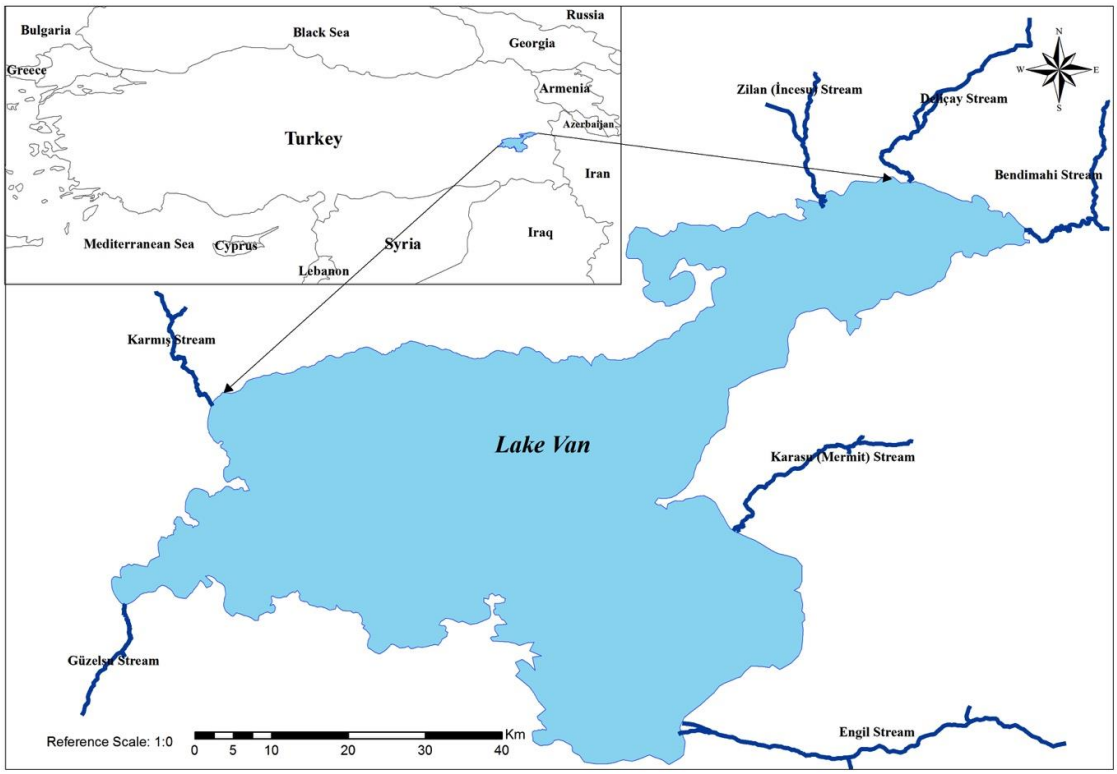

Figure 1. Map of the sampling locations in Lake Van basin, Turkey

Digestive tract samples were immediately placed into Bouin and 4% Paraformaldehyde solution for histology and immunohistochemistry studies. Fragments of digestive tract were embedded in paraffin blocks, sections of 7 µm were cut with a microtome (HM 325 Manual Microtome; MICROM International GmbH, Waldorf, Germany) from the paraffin blocks and mounted on glass slides for staining. All samples were stained with

hematoxylin and eosin (HE) for standard histological examination. Sections were stained with periodic acid-Schiff and Alcian Blue pH 2.5 for goblet cells. Finally, slides were mounted with Entellan (Entellan®, Merck-Millipore, Germany). Photomicrographs were taken using a Leica Inverted Light Microscope (DMI 6000B microscope (Leica, Germany) with a Leica DFC 490 camera.

For immunohistochemical localization of NKA, a fragment of digestive tract was fixed in 4% paraformaldehyde for 12 h and then placed in a 30% sucrose solution containing sodium azide. Sections (10 μm) were taken from tissues using a cryostat microtome (Leica CM 1100, Germany). Immunohistochemical staining was performed using the Histostain Plus kit (Invitrogen). The sections were washed in phosphate-buffered saline (PBS) and incubated for 10 min with a blocking solution. The slides were incubated overnight at room temperature with the specific monoclonal mouse antibody (1:10) raised against the α subunit of the chicken NKA (Developmental Studies Hybridoma Bank, University of Iowa, USA). The slides were washed in PBS and were then incubated with the biotinylated secondary antibody for 10 minutes in a humidified chamber. The slides were incubated with an enzyme conjugate, for 10 minutes then developed with diaminobenzidine chromogen (DAB) (DAKO, Germany). All slides were mounted with a coverslip and viewed with a digital camera attached to a microscope (Leica, Wetzlar, Germany). Null control sections were incubated under the same conditions without primary antibody and yielded no immunoreactivity.

Results and Discussion

The total length of Van Fish digestive tract was between 11.5-14.5 cm. The general view of the digestive tract in Van Fish was shown in figure 2. The digestive tract has two folded structures, the first fold extending towards the longest lobe of the liver then lengthens to the anterior of fish and makes the second fold and ends in the anus. Macroscobic observations revealed the digestive tract subdivided into four distinct regions: oesophagus, stomach like structure, anterior and posterior intestines and rectum (Fig. 2). The oesophagus was the shortest part of the digestive tract in the Van Fish. There was no stomach structure in Van Fish. The anterior intestine was found thicker then posteror intestine. No morphologic difference was observed in digestive tract between the lake fish and freshwater adapted fish.

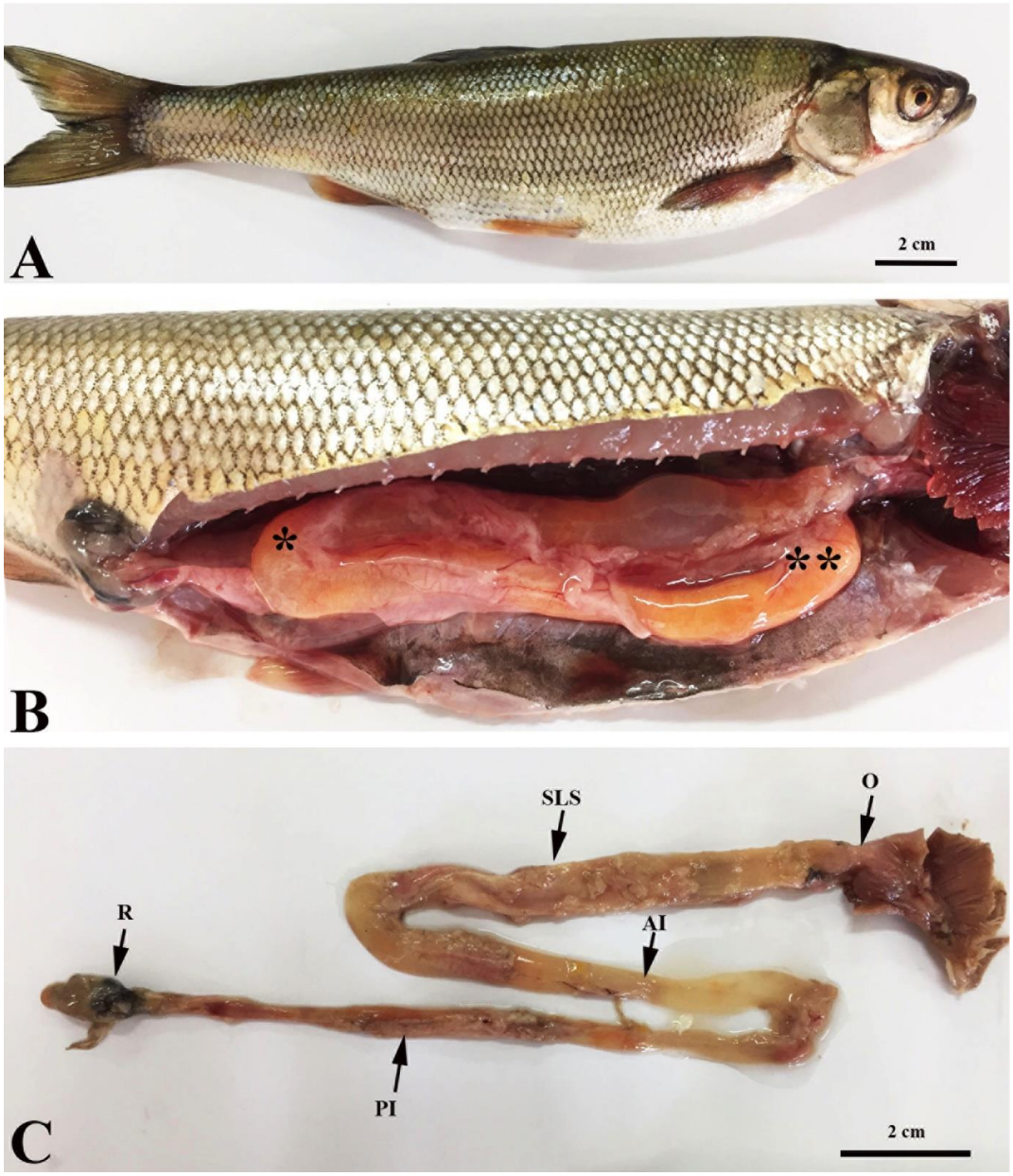

Figure 2. Van Fish (A). General view of digestive tract (*first **second) (B) External morphology of the digestive tract of Van Fish (C). O oesaphagus, SLS stomach like structure, AI anterior intestine, PI posterior intestine, R rectum.

Histologically, digestive tract wall consists four different layers mucosa, submucosa, muscularis, and serosa. Muscularis mucosa layer was not observed in Van Fish. The mucosa layer consisted of simple columnar epithelium and the submucosa layer consisted of connective tissue and blood vessels. Muscularis layer was observed to be composed of transverse and longitudinally arranged smooth muscle cells in different thicknesses in different parts of the digestive tract. The serous layer has consisted of a single layer of squamous cell and the thin layer and of the digestive tract. Oesophagus has many longitudinal folds and taste buds could not be identified in the mucosa layer. The thickest muscularis layer in the digestive tract was observed in the oesophagus. Mucus cells were not determined in the samples taken from the freshwater adapted fish (Fig 3). Mucus cells were observed in along digestive tract of lake-adapted fishes. But mucous cells were not seen in oesophagus of freshwater adapted fish. Mucous cells containing neutral and acidic glycoconjugate were numerous, larger and densely stained in freshwater adapted fishes (Fig 4.-5.).

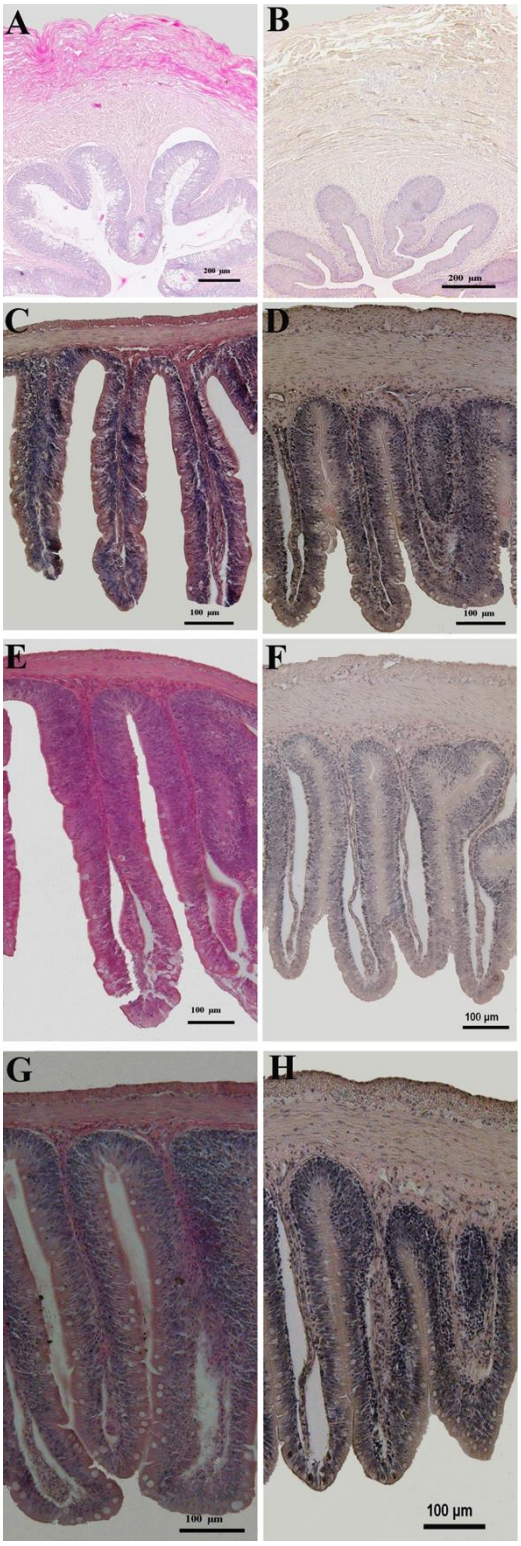

Figure 3. Photomicrographs of cross sections stained Hematoxylin Eosin of digestive tract of Van Fish (A, B oesophagus, C, D stomach like structure, E, F anterior and G, H posterior intestine. lake (A, C, E, and F) and freshwater (B, D, G, and H) adapted Van Fish. 1 serosa, 2 muscularis, 3 submukosa, 4 mucosa, L lumen.).

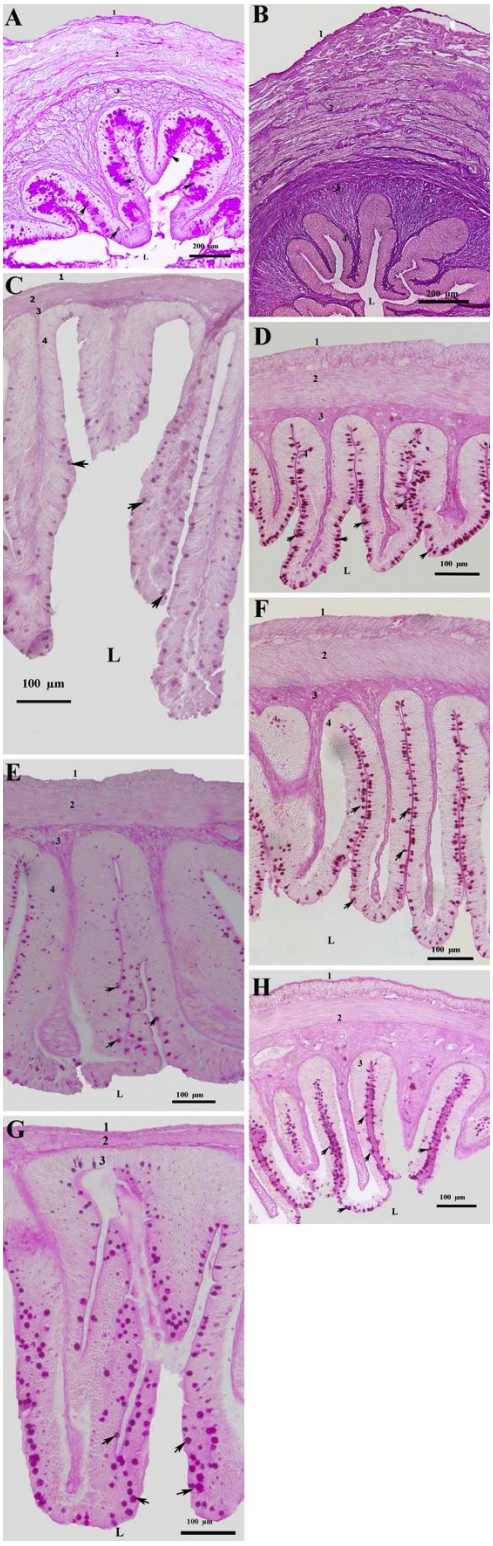

Figure 4. Photomicrographs of cross sections stained Periodic Acid Schiff of digestive tract of Van Fish. (A, B oesophagus, C, D stomach like structure, E, F anterior and G, H posterior intestine. lake (A, C, E, and F) and freshwater (B, D, G, and H) adapted Van Fish. 1 serosa, 2 muscularis, 3 submukosa, 4 mucosa, L lumen.).

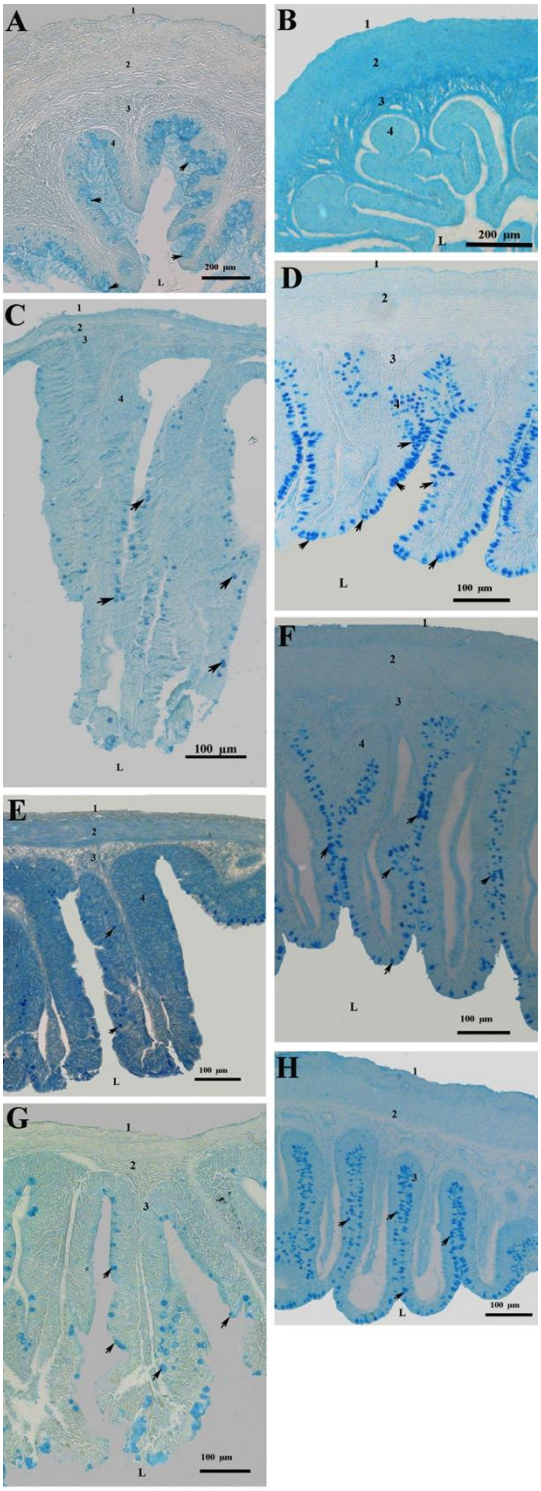

Figure 5. Photomicrographs of cross sections stained Alcian Blue of digestive tract of Van Fish (A, B oesophagus, C, D stomach like structure, E, F anterior and G, H posterior intestine. lake (A, C, E, and F) and freshwater (B, D, G, and H) adapted Van Fish. 1 serosa, 2 muscularis, 3 submukosa, 4 mucosa, L lumen. 1 serosa, 2 muscularis, 3 submucosa, 4 mucosa, L lumen).

As a result of NKA labeling, the basolateral parts of epithelial cells in the mucosa layer were positively stained (Fig 6.). The density of NKA was observed to be more intense in the intestines and rectum of lake samples when compared to freshwater samples. Immunostain were observed to be more intense in the intestines of lake adapted fishes when compared to freshwater adapted fishes. The rectum of the fish, which are intense according to other layer of digestive tract.

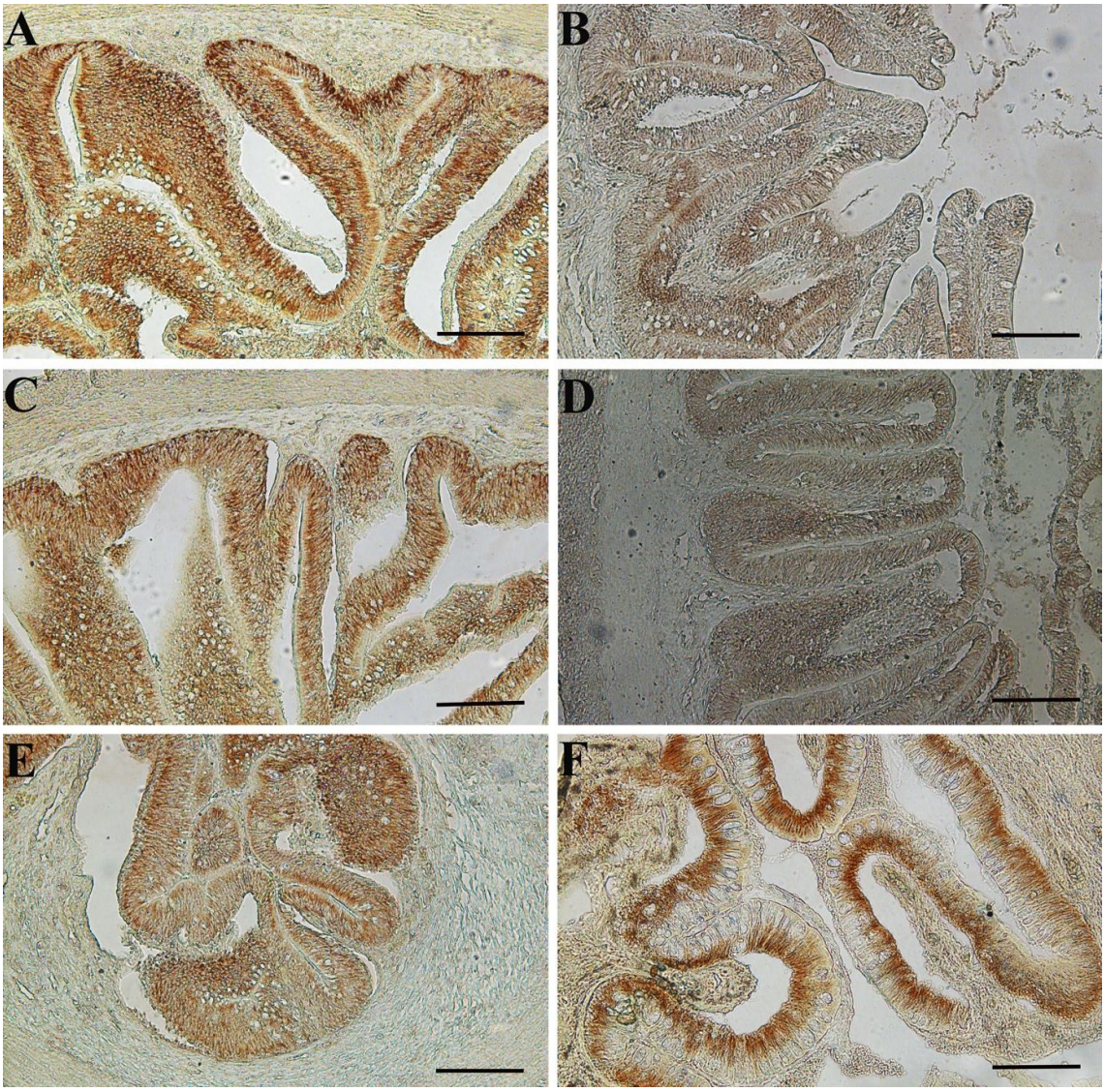

Figure 6. Localization of NKA in the anterior (A, B) and posterior (C, D) intestines and rectum (E, F) of lake (A, C and E) and freshwater adapted (B, D and F) Van Fish. Scale bar 100 µm.

In the fish, the digestive tract is composed of oesophagus, stomach, anterior and posterior intestine (Takashima and Hibiya, 1995; Oliveria-Riberio and Fanta, 2000; Ebrahimi 2015). The parts of the digestive tract may vary depending on the fish species and feeding types. When the digestive tract of the Van Fish was examined, it was observed that there were no stomach observed in other some fish species. This is the general characteristic of the Cyprinidae family which Van Fish is in. Stomach development in fish is related to carnivorous grade (Geldiay and Balık, 1988). The diet of Van Fish has phytoplankton, zooplankton, and chironomid in Lake. For this reason, it is expected to have a simple tube-like stomach structure of fish.

In fish, the digestive tract consists of four layers, mucosa, submucosa, muscularis and serous when examined histologically. The cellular structure in these layers is quite different from each other. When the histology of the digestive tract of Van fish was examined, it was determined four layes that the thicknesses of these layers were different between the regions. Furthermore, the submucosa layer in the posterior intestine was very difficult to distinguish. The layer of muscularis mucousa in digestive tract of Van Fish was not detected. It was also determined that this layer was not found in studies carried out in different fish species (Takashima and Hibiya, 1995; Veira Lopes et al., 2013; Andrade et al., 2017). The taste buds observed in eosophagus in some fish species such as nile tilapia and catfish (Morrison and Wright, 1999; Abd El Hafez et al., 2013). Ünal et al., (2001), despite mentioning the presence of the taste buds of the esophagus of the Van Fish larvae, could not be determined in this study. In the present study, the lack of taste buds in eosophagus may be due to the developmental period of the fish.

The digestive tract lengths differed among the sampled both freshwater and lake adapted fish. This difference may be caused by the age of the fish. When the thickness of the layers of digestive tract taken from the lake and freshwater adapted fish were compared, it was observed that there was no significant difference. Salinity and pH are known to affect many parameters on fish physiology (Boeuf and Payan, 2001). Van Fish are not found in fresh water as long as the lake environment, it is expected that there will not be a clear difference in the layer thickness of the digestive tract.

The mucus secreted by the goblet cells allows the surface of the mucosal layer of the digestive tract to be protected from physical and chemical influences of food and against bacteria and viruses. The size, density, and localization of goblet cells were investigated in many fish species (Hur et al., 2005; Domeneghini et al., 2005; Cao and Wang, 2009; Park and Kim, 2001; Diaz et al., 2003). In these studies goblet cell sizes, densities and localizations differed according to the feeding type and fish species. It was determined that the goblet cells of the sampled from lake and freshwater adapted fish differed in the concentration of the glycoconjugate, number and size and their localizations in the mucous layers. These differences may be due to the different physico-chemical effects of nutrients and water taken from the water.

Fish are constantly receiving water from outside because they are hypotonic in freshwater. Kidneys produce dilute urine for excess water. In seawater or brackish water, fish lose passively water from their body surface in hypertonic environments. Fish take water from the outside to the digestive tract to prevent water loss. Regulation of water-borne ions is carried out by enzymes such as Na^+, K^+, $2Cl^-$ and NKA in cells. NKA is located in the basolateral membrane of the cells. In Van Fish, NKA-containing cells were densely localized in the posterior intestine and rectum of the digestive tract from sampling lake adapted fish. It has been reported that intakes of Na and NKA activity are higher in the intestines and rectum than the gills (Fuentes et al., 1997; Grosell et al., 1999; Hogstrand et al., 1999; Giffard-Mena et al., 2008; Kim et al., 2008). In addition, NKA gene expression increased in seawater adapted fish from fresh water. Although it is not as salty as sea water, Van Lake has a salt content of 22 ‰. NKA, one of the basic enzymes in the ion transport, seems to be expected to be more intense in fish that are adapting to the lake.

As a result, we have identified morphologic and histologic structure in digestive tract during reproductive migration in two different habitats. it is similar to the other species of the Cyprinidae family, including Van Fish. So, in the Van Fish, the morphologic structure is not changed but mucous cell composition and NKA contained cell in mucous layer of the digestive tract is less varied compared lake and freshwater adapted fish. Further study is required to characterize their digestive enzyme in the stomach like structure and other transmembrane enzyme such as Na^+, K^+, 2Cl and CFTR for ion regulation in the digestive tract of Van Fish.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of Different Microbial Organic Fertilizers on Yield and Quality of Tomato Grown in Greenhouse Condition

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Abstract: Vegetables have protective and healing effects on human health due to the containing and the increase of these substances are associated with their grown conditions especially organic means. This study was carried out to determine the effects of LIFEBAC-NP (L), NSAH (N) and SALUS EXHILARE (S) microbial organic fertilizers consisting of microbial formulations and natural products of plant origin in different doses [1000 mL/100 L (A), 3000 mL/100 L (B), 5000 mL/100 L (C) and control (no application) (D)] on yield and quality of organic grown Cevahir F_1 tomato (*Solanum lycopersicum* L.) cultivar in greenhouse condition. The number of fruit per plant, fruit weight per plant, total yield, fruit width and length, total soluble solid, pH, ascorbic acid, color properties, fruit shape index, dry matter yield parameters were evaluated on tomato cultivar. The fruit weight per plant and total yield in doses A and C treatments were found the highest in N microbial organic fertilizer. The fruit width in dose A treatment was determined the highest in L microbial organic fertilizer. The highest fruit shape index was obtained in D treatment and dose B of L treatment. The fruit number per plant (respectively 24.71 and 23.56), the total solid contents (5.80 %) and ascorbic acid contents (261.67 mg/l usare) in doses A and C treatments were found the highest in L microbial organic fertilizer. It is concluded that both fruit quality and nutrient content could be significantly increased in tomato by using microbial organic fertilizers.

Key words: Fruit quality, microbial, organic fertilizers, *Solanum lycopersicum* L.

Introduction

Tomato (*Solanum lycopersicum* L.) that are the most produced, consumed and commercial subject in the world are one of indispensable vegetables in the human nutrition (Singh and Siddiqui 2015; Seymen et al. 2015; Özer 2017). The amount of tomato production in the world has been realized as 233 466 175 tons in 2016 (FAOSTAT 2018). This amount is 12 750 000 tons in 2017 in Turkey (TÜİK 2018). Vegetables have protective and healing effects on human health due to the containing and the increase of these substances are associated with their grown conditions especially organic means. As an increased in application day by day throughout the world, organic wastes composted or degraded by appropriate methods are used as components of the growing medium in mixtures at various ratios, sometimes pure, and sometimes in order to provide the desired properties in plant growth medium, according to the purpose. Using of microbial organic fertilizers is a new practice that uses to ensure the optimization of absorption of mineral nutrients by cultivated plants, containing viable microorganisms, obtaining high yields, reduction of chemical load of agricultural land and soil restoration in organic farming in many countries. Microbial organic fertilizers contain in especially plant growth-promoting rhizobacteria (PGPR) of micro-organisms (Tilak 1991), plant nutrients, low toxic element and high humic acid (Parr et al. 1994; Higa and Wididana 1991; Higa 1994). The using of a special microbial fertilizer for some kinds of crops has increased in yield of tomato (Ligong et al. 2006; Öztekin et al. 2015), potato, peanut and coffee (Dung et al.2009). The study was carried out to determine the effects of three different microbial organic fertilizers on growth and yield in tomato under greenhouse conditions.

Material and Method

This study was conducted under greenhouse conditions at Atatürk University, Turkey, in 2017. The tomato (*Solanum lycopersicum* L., cv. Cevahir F_1) were cultivated under natural light conditions. Tomato seedlings were planted in the second week of May in 2017. The experiment was ended on 25 September in the same year. Three different concentrations [1000 mL/100 L (A), 3000 mL/100 L (B), 5000 mL/100 L (C) and control (no application) (D)] of microbial organic fertilizers were used in this study and microbial organic fertilizers were

obtained from Professor Dr. Metin TURAN (Yeditepe University, Department of Genetics and Bioengineering, İstanbul, Turkey). LIFEBAC-NP (L), NSAH (N) and SALUS EXHILARE (S) microbial organic fertilizers are consisted of microbial formulations and natural product (Table 1). This experiment was consisted in a completely randomized design with 3 replications and each replication has 10 plants. The applications were treated after a week of planting of the seedlings. The solutions were given to the root zone of the plant and repeated 4 times at intervals of 10 days.

The measurements were taken including the fruit number per plant, fruit weight per plant, the fruit width and length, total soluble solid, pH, color properties (in CIE L*a*b* scale, including croma and hue angle) (McGuire 1992), fruit shape index, ascorbic acid, yield and dry matter (Kacar 1972). All data in the present study were processed by SPSS and the means were separated by Duncan's multiple range tests.

Table 1. Fertilizers and their properties

LIFEBAC-NP (L) (Anonymous 2018 a)	
pH	4,5-6,5
Guaranted content	*Baciullus subtilis* $1x10^9$ KOB/mL; *Baciullus megaterium* $1x10^8$ KOB/mL
NSAH (N) (Anonymous 2018 b)	
pH	7-9
Total organic matter (%)	15
Organic C (%)	6
Total Soluble Solid K2O (%)	1
SALUS EXHILARE (S) (Anonymous 2018 c)	
Included Organisms	*Bacillus subtilis, Geotrichum candidum, Brevibacillus spp, Azospirillum brasilense, Azotobacter spp.Pseudomonas spp, Bacillus subtilis, Bacillus megaterium, Bacillus licheniformis, Acinetobacter calcoaceticus, Bacillus polymyxa, Bacillus pumilus, Acinetobacter spp, Lactocoecus spp., Rhizobium spp, Cyanobacteria spp, Nitrosomonas spp, Nitrococcus spp, nitrobacter, Acinetobacter baumannii, Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis, Bacillus megaterium, Bacillus lentus, Bacillus thermophilus, Bacillus lentus, bacillus amyloliquefacians, Rhodotorula spp,Zygosaccharomyces spp, Micrococcus luteus and Burkholderia cepacia PGPR bacterial and Aspergillus niger and FF9 fungus.*
Total Live Organism	1 X 10^9 KOB/ml

Results and Discussion

The results of our study under organic growing conditions showed that treatment of different doses and microbial organic fertilizers significantly affected especially on yield parameters (Table 2).

The results showed that yield significantly increased in ($p<0,001$) N-A (10 480.67 kg m^{-2}) and N-C (10 781.99 kg m^{-2}) treatments compared to D treatment (6 670.23 g m^{-2}). Moreover, fruit weight per plant of plants fertilized with N-A (2620.17 g/plant) and N-C (2695.50 g/plant) treatments were higher than those fertilized with D (1667.56 g/plant) and other treatments ($p<0,001$). Increasing in soil productivity is one of the factors that can effect on production (Thorup-Kristensen 1999; Wang et al. 2017); the increase in yield obtained at the end of the study was in parallel with this information. Increased tomato yields were determined with organic fertilizer applications (Öztekin et al. 2015; Özer 2017; Wang et al. 2017) ın previous researches. When soil nutrient contents increased with microbial organic fertilizers, it has occurred increasing in yields. Compared with the control treatment, the number of fruits per plant in L-A (24.71) was high, followed by the treatment with L-C treatment (23.56) ($p<0.01$). In addition, L-C (5.80 ^{o}Brix) application gave the highest the total soluble solid, when compared to control treatments. The total soluble solid ranged from 4.67 to 5.80 ^{o}Brix. Özer (2017) also reported near values (varied between 3.20%-5.60%) for tomatoes. LIFEBAC-NP (L) microbial organic fertilizer used in this study could be able to enhance the fruit number per plant, the total soluble solid, the fruit width, ascorbic acid and fruit shape index by promoting natural organic acids, amino acids, auxins and cytokines with *Bacillus subtilis* and *Bacillus megaterium* isolates (Adesemoye et al. 2009; Sreenivasa et al. 2010). Also, Mena-Violante and Olalde-Portugal (2007) and Islam et al. (2017) have demonstrated that the PGPR strain *B. subtilis* has capacity to modify some tomato fruit quality and yield aspects.

Table 2. The effects of treatments on yield and fruit properties in tomato

Treatments	Number of fruits per plant	Fruit weight per plant (g/plant)	Total yield (kg/m^2)	Fruit diameter (mm)	Fruit height (mm)	Fruit shape index	**Dry matter (%)**	**Total soluble solid (°Brix)**
D (Control)	15.56 c**	1667.56 c***	6670.23 c***	53.12 d***	53.12 ns	0.82 a*	5.09 ns	5.13 ab**
S-A	21.11 ab	2461.72 ab	9846.88 ab	56.77 c	56.77	0.80 ab	3.87	4.97 ab
S-B	20.89 ab	2587.56 ab	10350.23 ab	55.29 ab	55.29	0.74 d	3.81	4.97 ab
S-C	18.78 bc	2542.67 ab	10170.67 ab	58.37 ab	58.37	0.77 bcd	3.44	4.67 b
L-A	24.71 a	1784.78c	7139.11 c	58.38 a	58.38	0.77 bcd	2.25	5.70 ab
L-B	19.39 b	2216.28 b	8865.11 ab	58.41 c	58.41	0.82 a	3.31	5.50 ab
L-C	23.56 a	2541.84 ab	10167.35 ab	56.18 ab	56.18	0.75 cd	3.24	5.80 a
N-A	21.28 ab	2620.17 a	10480.67 a	58.10 bc	58.10	0.80 ab	3.74	4.97 ab
N-B	19.61 b	2442.11 ab	9768.45 ab	58.57 ab	58.57	0.78 bc	3.24	5.17 ab
N-C	22.22 ab	2695.50 a	10781.99 a	55.96 abc	55.96	0.76 bcd	3.10	5.53 ab
Mean	20.71	2356.02	9424.07	56.92	56.92	0.78	3.51	5.25

* P <0.05; ** P<0.01; ** P<0.001; ns: not significant. The numbers in one column having the same letter are not significantly different.

The highest fruit shape index was obtained in dose B of L treatment and D treatment (Table 2). Ascorbic acid contents (261.67 mg/l usare) were found the highest in doses A of L treatment (Table 3). Xu et al. (2001) suggested that organic fertilization improved fruit quality by ascorbic acid content. The results of the present study exhibited that organic fertilization improved fruit quality in tomato. Also, Jackson et al. (1964), Tilak (1991) and Ünlü and Padem (2009) have reported similar findings confirming our data in the present study in tomato.

Table 3. The effects of treatments on fruit properties in tomato

Treatments	L*	a*	b*	Chrome value	Hue angle	Ascorbic asid	pH
D (Control)	33.90 ns	27.65 ns	15.05 ns	31,48 ns	28,55 ns	192,67 ab*	4,20 ns
S-A	33.58	28,38	14,57	31,90	27,14	190,33 ab	4,19
S-B	33.66	28,52	15,00	32,22	27,75	166,00 ab	4,04
S-C	34,36	28,26	15,35	32,16	28,50	193,33 ab	4,08
L-A	34,75	27,61	15,59	31,71	29,46	261,67 a	4,14
L-B	34,14	27,29	15,18	31,24	29,09	194,00 ab	4,05
L-C	34,13	27,44	16,42	32,01	30,82	197,67 ab	4,13
N-A	33,92	27,95	15,15	31,79	28,45	153,67 b	4,10
N-B	33,89	28,62	15,14	32,37	27,90	188,33 ab	4,15
N-C	33,98	28,10	15,19	31,95	28,39	209,67 ab	4,18
Mean	34,03	27,98	15,26	31,88	28,60	194,73	4,13

The results of our study showed that applications of different doses and microbial organic fertilizers did not affect (p>0.05) on pH, fruit height (mm), dry matter (%), L*, a*, b*, chrome value and hue angle. The average pH values ranged from 4.04 to 4.20 in our study (Table 3). In general, when the pH of sour products is low (around 2,0), the acidity of the sweet products is low. It is higher than pH 4.5 in many vegetable groups (Brown 2007). Fruit color is one of the most important and complicated fruit quality criteria. Color formation in the fruit varies depends on both genetic and environmental factors (Lopez Camelo and Gomez 2004). As the color becomes darker, the L value decrees. The mostlight color fruit crust formation was obtained by K-A application (Table 3). However, climate conditions are also influential on quality and yield characteristics (Dorais et al. 2001).

As a result of this study, microbial organic fertilizers used in this study especially A and C dose of NSAH (N) treatment had significant effects on yield parameters of tomatoes. In terms of the parameters evaluated, same doses of LIFEBAC-NP (L) microbial organic fertilizer were also considered for tomato quality and yield. The

microbial organic fertilizers can be considered as an important fertilizer input in in the production of organic tomatoes and can also be added environment friendly applications.

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Araştırma Makalesi/*Research Article (Original Paper)*

The incidence of *Barley Yellow Dwarf Viruses* (BYDVs) in Wheat Crops in Diyarbakir (Turkey) and Sequence Characterization of BYDV-PAV

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Abstract: To ascertain the presence of Barley/Cereal yellow dwarf viruses (BYDV-PAV, MAV, SGV, RMV, and CYDV-RPV), a total of 365 wheat leaf samples were randomly collected from wheat cultivation fields of Diyarbakir province in 2016 and were screened by using multiplex RT-PCR and RT-PCR methods. As a result of PCR tests the wheat samples were found to be infected by BYDV-PAV, BYDV-SGV and CYDV-RPV with the percentage of 3.5%, 2.4% and 1.3%, respectively. Detected mix infections of BYDV-PAV+BYDV-SGV, BYDV-PAV+CYDV-RPV, BYDV-SGV+CYDV-RPV, and BYDV-PAV+BYDV-SGV+CYDV-RPV were 2.2%, 0.8%, 0.8%, and 0.8%, respectively. A BYDV-PAV Diyarbakir isolate was chosen randomly, and its complete coat protein gene was characterized. The coat protein gene of BYDV-PAV virus (Genbank accession no. KX774424) was molecularly cloned and sequenced with the universal primers. The CP nucleotide sequence of BYDV-PAV Diyarbakir isolate was showed 81.4 - 98.3% identities with the other 21 world isolates. The present study is the first report for documentation of BYDV-PAV, BYDV-SGV and CYDV-RPV viruses in wheat fields of Diyarbakir province (Turkey).

Key words: Wheat, viral diseases, Multiplex RT-PCR, Characterization, Cloning, Turkey.

Introduction

Turkey has 3.5% of the world wheat area. Within the area of 23.9 million hectares of arable land, grains have the most significant share with 49% share. Wheat is in the first place with 67% share amongst in total grain areas. Barley follows wheat with 24%, corn with 6% and rice with 1%. Also, regarding the production of bread wheat, the Central Anatolia Region is the first with 33,5%, Marmara Region with the second with 17,3% and Southeastern Anatolia Region with the third with 14,3% (TUİK, 2016).

Wheat (*Triticum aestivum* L.) is a primary annual product belonging to the *Poaceae* family that widely cultivated in Turkey since ancient dates. Wheat contains organic and inorganic compounds as amino acids, minerals, vitamins, fiber source, etc. that is valuable to the human and animal diet (Shewry, 2009).

Many viral agents in wheat can cause diseases and cause different levels of quantitative and qualitative losses in grain yield or another kind of abnormalities in the host plants. *Tritium aestivum* is naturally affected to about 55 viruses (Wiese, 1987; Brunt et al., 1996).

C/BYD (*Cereal / Barley yellow dwarf*) disease is a significant virus disease, caused by (BYDV) strain PAV, PAS, MAV, GAV, SGV, RMV, and GPV or CYDV-RPV. These viruses are utmost prevalent and destructive, that are responsible heavily losses in *Poaceae* family including wheat, barley, corn, oat and rice and many other grain crops (Rochow and Miller, 1971; Ayala-Navarrete and Larkin, 2011; Lister and Ranieri 1995; Kundu, 2008; Rochow, 1970).

C/BYDVs has 25-28 nm in diameter isometric particles and the ssRNA genome belonging to the *Luteoviridae* family. BYDV is transmitted by about 25 aphid species nourishing on the phloem sap of an infected plant in a persistent, circulative manner, but not spread to via mechanically and seeds (Rochow, 1970; D Arcy and Burnett, 1995; Wiese, 1987; Nemeth, 1986). C/BYDV strains are named according to the species of aphids they carry (Fauguet and Mayo, 1999). The BYDV-PAV virus is transported with *Rhopalosiphum padi* and *Sitobion avena*, BYDV-MAV virus with *S. avenaee*, BYDV-SGV virus with *Schizaphis graminum*, BYDV-RMV virus with *Rhopalosiphum maidis* and CYDV-RPV virus with *R. padi* (Rochow, 1970).

The symptom manifestation of BYDVs is very varied depending on the time and the duration of infection, the physiological state, and variety of host plant, as well as environmental factors such as temperature, soil humidity, and soil fertility. BYDV infection causes regression in root length and development, leaf curling and dwarf, decreased heading and delayed heading time, bright yellow and redness patterns on infected wheat leaves (Perry et al., 2000; Nemeth, 1986; D'arcy 1995; Riedell et al. 2003).

There are no studies on BYDV viruses in the Diyarbakır provinces. In the present study here, techniques based on Multiplex Reverse Transcriptase Polymerase Chain Reaction (M-RT-PCR), which regulated for the simultaneous detection and discrimination of BYDVs, and RT-PCR were applied for the detection of BYDVs in wheat tissues.

Objectives of this report; to determinate infection rates of some BYDV species (1), to elucidate the phylogenetic evaluation of CP gene of BYDV-PAV Diyarbakir isolate with other BYDV species using gen sequence analysis (2), and to better understanding the molecular evolution of BYDV-PAV (3).

Material and Methods

Survey areas and sampling

Surveys were carried out in only four wheat district where intensively grown in Diyarbakir at booting- heading stage seasons during April-March, 2016. Due to some limited factors at the region, the other cereal growing regions of Diyarbakir did not surveyed (Figure 1).

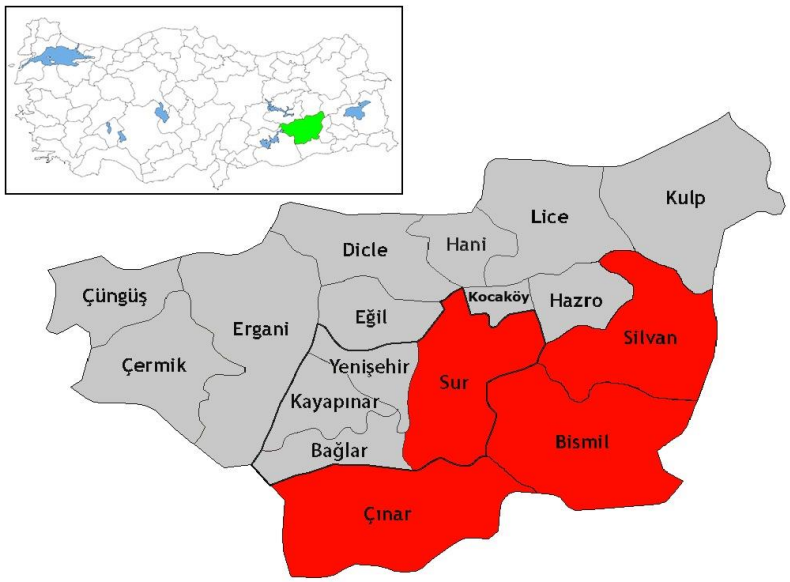

Figure 1. Surveyed regions of Diyarbakır province (survey areas are shown in red).

Samples, with or without BYDV's symptoms, were randomly collected from fields and were brought to laboratory in a cool chain. A total of 365 wheat samples, 160 from Bismil, 80 from Cinar, 70 from Silvan, and 55 from Sur were collected to determine the occurrence of BYDVs.

Total RNA extraction and cDNA process

Total RNA was extracted from approx. 100 mg of fresh wheat samples by silica-based method (Foissac et al., 2001). The purity level and the concentration of extracted total RNA were checked by spectrophotometer.

For the synthesis of complementary DNA (cDNA); 5 µl of TNA, 1 µl of 10 mM dNTP mix, 1 µl of 20 pmol/µl the Universal Yan Reverse primer (5'-TGTTGAGGAGTCTACCTATTTG-3') (Malmstrom and Shu, 2004) which allows the synthessis of cDNA for all strains, 5 µl of RNase free water were put into Eppendorf tube. The mixture was than incubated at 65 °C for 5 min and immediately placed in ice for 5 min. The reaction volume

was completed to 20 µl by adding 4 µl of 5X RT reaction buffer, 2 µl of 0.1M DTT, 1 µl of RNase inhibitor and 1 µl of reverse transcriptase enzyme into the reaction mix on ice and incubated at 42 °C for 50 min. To inactivate the reverse transcriptase enzyme the mixture was incubated at 70 °C for 15 min. cDNAs were stored in a deep freeze at -20 °C until needed.

Oligonucleotide primers and detection of BYDVs by Multiplex-RT-PCR

Primers used in the diagnosis of BYDVs in this research were published by previous studies (Table 1.) and were synthesized through Sentegen company (Ankara).

Table 1. Primer sets used for identifying BYDVs

Virus	Primer	Sequence	Amplicon size	References
Group1	YAN-R	TGTTGAGGAGTCTACCTATTTG	832bp	(Malmstrom and Shu, 2004)
BYDV-PAV,	SHU-F	TACGGTAAGTGCCCAACTCC		
MAV, SGV)				
Group 2	S2A-F	TCACCTTCGGGCCGTCTCTATCAG	372bp	(Malmstrom and Shu, 2004)
(CYDV-RPV,	S2B-F	TCACCTTCGGGGCGTCTCTTTCTG		
BYDV-RMV)				
BYD-SGV	SGV-R	ACATTTCTTCGTGTGTTGCG	254bp	(Malmstrom and Shu, 2004)
BYDV-PAV	PAV-F	ACCTAGACGCGCAAATCAAA	590bp	(Malmstrom and Shu, 2004)
BYDV-MAV	MAV2-F	AATAACCGCAGGAGAAATGG	590 p	(Malmstrom and Shu, 2004)
BYDV-SGV	SGV-F	ACCAGATCTTAGCCGGGTTT	237bp	(Deb and Anderson, 2008)
	SGV-R	CTGGACGTCGACCATTTCTT		
BYDV-RMV	RMV-F	ACGAGGACGACGACCAAGTGGA	365bp	(Deb and Anderson, 2008)
	RMV-R	GCCATACTCCACCTCCGATT		
CYDV-RPV	RPV-F	ATGTTGTACCGCTTGATCCAC	400bp	(Deb and Anderson, 2008)
	RPV-R	GCGAACCATTGCCATTG		

PCR reactions were performed by two main methods: basic multiplex RT-PCR and enhanced RT- PCR. In the first one, subgroup-specific primers: Shu-F, S2a-F and S2b-F with Yan-R were used to amplify two DNA bands, which subgroup I (832 bp, indicates probable BYDV-PAV, BYDV-MAV, BYDV-SGV infections) and subgroup II (372 bp, indicates probable CYDV-RPV, BYDV-RMV infections). In the enhanced RT-PCR test, species-specific primers were used to distinguish BYDV subgroup members (Malmstrom and Shu, 2004). TNAs of BYDVs from a previously detected and characterized isolates infecting cereals were used as positive source in PCR assays (Usta, 2013).

In multiplex-PCR, final volume of 50 µl mastermix composed of 31.6 µl RNase free water, 5µl 10X PCR Buffer, 3µl 25mM $MgCl_2$, 1µl 10mM dNTP, 1µl 20µM of each primer sets (Shu-F, Yan R, S2a-F S2b-F), 0.4 µl *Taq* DNA polymerase enzyme (5U/µl) and 5 µL cDNA. PCR cycling program was as follows: 5 min incubation at 94°C for initial denaturation followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s for annealing and 72°C for 7 min for final extension. Fifteen microliters of PCR yield was run by agarose gel containing ethidium bromide and were visualized under UV light (Syngene™ UV Transilluminator 2020LM).

Molecular characterization of BYDV-PAV-Diyarbakir isolate

Gene specific primers designed for the complete CP gene of BYDV-PAV (BYDV-PAV-F (5'-CAGTGGATCCATGAATTCAGTAGGTCGTAG-3' and BYDV-PAV-R 5'-CAGTAAGCTTGAGGAGTCTACCTATTTGGC-3') were used to characterize BYDV-PAV-Diyarbakir isolate, generating a 614 bp fragment (Usta, 2013).

PCR amplified CP gene of Diyarbakir isolate was ligated into pGEM T-Easy vector using T4 DNA ligase enzyme (Promega). Recombinant plasmids containing the BYDV- PAV CP gene were transformed into *E. coli* strain JM109 by electroshock. A colony positive from recombinant bacterial clone was selected and cultured in liquid LB medium, and then plasmid purification was performed by GeneJET Plasmid Miniprep Kit (Thermo) following manufacturer´s protocol.

DNA sequencing was performed by New Generation Sequencing (NGS) system and the phylogenetic relationship was studied by comparing other world isolates belonging to *Luteoviridae* family in NCBI using CLC

Main Workbench software. Multiple alignments were carried out using Clustal W and a phylogenetic tree was executed by the Neighbor-joining method with 1000 bootstrap by MEGA 5.1 program (Tamura et al., 2011).

Results

Field surveys

Between 2015 and 2016, a large scale survey was conducted in main wheat growing districts of Diyarbakir to determine the incidences of BYDVs infections. Viral symptoms, such as dwarfing, mosaic, reddening of flag leaves, and chlorotic streak patterns on leaves were rarely observed in the surveyed wheat fields during the surveys (Figure 2).

Figure 2. General view of viral symptoms in wheat plantations during the survey (a,b,c) red colors on wheat leaves in Bismil (d, e, f), chlorosis on wheat fields in Cinar and Bismil.

Determination of the incidence of BYDVs infection by M-RT-PCR method

As shown in Fig. 3, PCR experiments against BYDVs in wheat leaves yielded expected size of amplicons representing the subgroup I and subgroup II, 832 bp and 372 bp DNA bands, respectively.

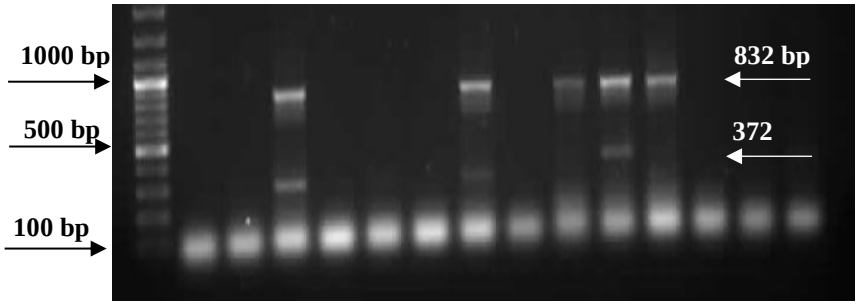

Figure 3. Multiplex-RT-PCR analysis on agarose gel for wheat leaf samples from Bismil (M:100-1000 bp), 3, 7, 9, 10, 11 lines: infected with at least one of the group I viruses; 3, 7, 10 lines: infected with at least one of the group II viruses

In surveyed regions of Diyarbakir three different BYDV identified: BYDV-PAV, BYDV-SGV, and CYDV-RPV. The overall infection rate in Diyarbakir was determined as 4.9%. Of the 365 plant samples collected (Table 1), 18 were identified as BYDV-positive. The highest rate of infection was recorded in Bismil district and the lowest rate of infection was found in Cinar district.

The most common BYDV was BYDV-PAV, detected in 3.5% of the samples (13 samples), followed by BYDV-SGV detected in 2.4 % (9 samples) and CYDV-RPV detected in 1.3% (5 samples). BYDV-MAV and BYDV-RMV viruses were not detected in any of the tested samples.

As a result of PCR tests with species-specific primers, as expected, 600 bp for the BYDV-PAV, 254 bp for the BYDV-SGV, and 400 bp fragment for the CYDV-RPV were observed on 1% agarose gel. No PCR yield was visualized from asymptomatic wheat leaves (Figure 4).

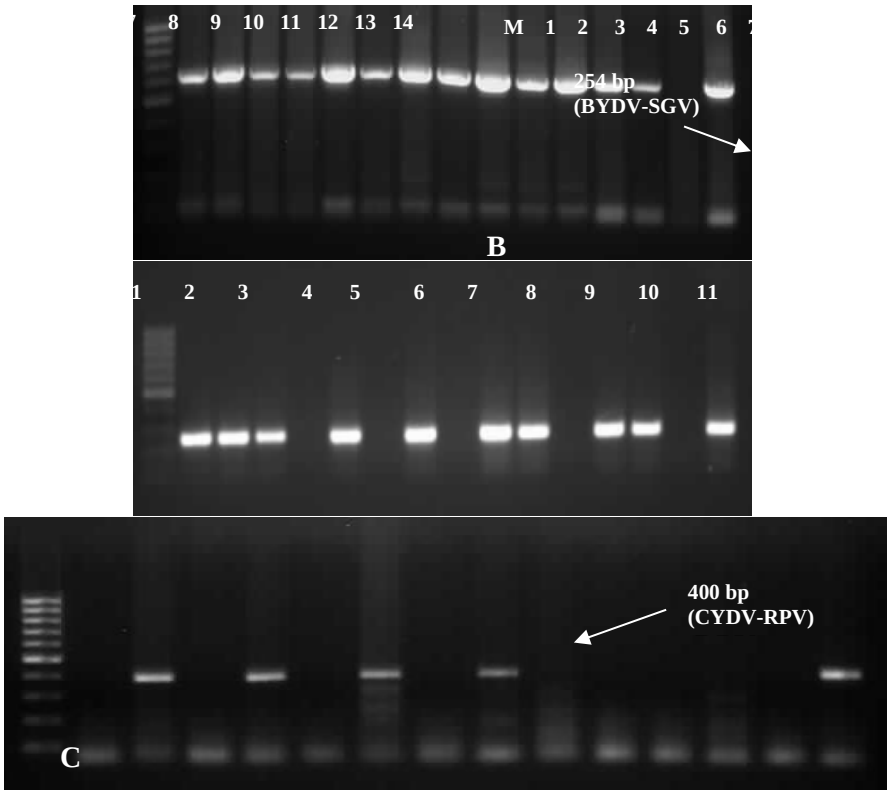

Figure 4. Determination of BYDVs by RT-PCR in Diyarbakir Panel A: Agarose gel image of BYDV-PAV obtained from Silvan M: Marker (100-1000 bp), Lane 1-13: positive samples, Lane 14: healthy wheat tissue Lane 15: positive control; Panel B: Agarose gel image of BYDV-SGV obtained from Silvan M:Marker (100-10000bp); Lane 1, 2, 3, 5, 7, 9, 10, 12, 13: positive samples, Lane 14: healthy wheat tissue; Panel C: Agarose gel image of CDV-RPV obtained from Bismil M: Marker (100-10000bp); Lane 2, 4, 6, 8: positive samples, Lane 13: healthy wheat tissue, Lane 14: positive control.

Mixed infections were also detected in wheat samples. PCR tests revealed that nine samples were infected with BYDV-PAV+BYDV-SGV (2.4%), three samples with BYDV-PAV+ CYDV-RPV (0.8%), three samples (0.8%) with BYDV-SGV+CYDV–RPV and three samples (0.8%) with BYDV-PAV+BYDV-SGV+CYDV-RPV. The data of tested samples are presented in Table 2.

Table 2. Geographic distribution of *Barley yellow dwarf virus* (BYDV) in wheat plants sampled in Diyarbakir

		BYDV-positive samples						
District	**Samples**	**SGV**	**PAV**	**RPV**	**PAV/SGV**	**PAV/RPV**	**SGV/RPV**	**PAV/SGV/RPV**
Bismil	160	5	8	5	4	3	3	3
Cinar	80	1	1	-	1	-	-	-
Silvan	70	3	3	-	3	-	-	-
Sur	55	1	1	-	1	-	-	-
Total	**365**	**9**	**13**	**5**	**9**	**3**	**3**	**3**

Phylogenetic tree and molecular characterization of the CP gene of Diyarbakır BYDV-PAV isolate

An isolate among positive BYDV-PAV detected by Multiplex RT-PCR in the wheat field survey of Diyarbakir was used in this research, and its complete CP gene was cloned and characterized. The sequence lenght of CP gene of BYDV-PAV Diyarbakır isolate was found as 603 bp. The recombinant plasmid containing the BYDV-PAV CP gene was subjected to bidirectional DNA sequencing (Sentegen Biotech- ANKARA). The DNA sequence of BYDV-PAV was submitted to the NCBI gene bank (http://www.ncbi.nlm.nih.gov) with the Accs. No. KX774424. The lenght of the CP gene of the isolatewas the same with the other isolates in the world.

Based on sequence data of selected BYDV-PAV isolate, phylogenetic tree and multiple comparisons were created with other 21 BYDV-PAV isolates previously identified in NCBI Gene Bank as shown in Table 3. For further rooting of the phylogenetic tree, antiviral protein gene of *B. spectabilis* Willd. (KP096226) was used as an outgroup.

Table 3. The access number, host, country, gene and length of the BYDV-PAV complete CP gene in NCBI Gene Bank

NO	Accs. No.	Host	Country	Gene	Lenght
1	KX774424	*Triticum aestivum*	Turkey	CP	603 bp
2	AJ007918	-	France	CP	603 bp
3	JX067849	*Triticum aestivum*	Brazil	CP	603 bp
4	AJ007926	-	France	CP	603 bp
5	AJ223587	*Lolium multiflorum*	France	CP	603 bp
6	AJ223588	*Hordeum vulgare*	France	CP	603 bp
7	JX067852	*Triticum aestivum*	Brazil	CP	603 bp
8	AJ295639	*Hordeum vulgare*	Greece	CP	603 bp
9	DQ285674	-	America	CP	603 bp
10	FJ875303	*Triticum aestivum*	China	CP	603 bp
11	JQ811487	*Triticum aestivum*	Pakistan	CP	603 bp
12	JX067842	*Hordeum vulgare*	Brazil	CP	603 bp
13	JX067845	*Lolium spp.*	Brazil	CP	603 bp
14	JX473288	*Lolium multiflorum*	Pakistan	CP	603 bp
15	JX067847	*Triticum aestivum*	Brazil	CP	603 bp
16	JX067850	*Zea mays*	Brazil	CP	603 bp
17	AY167109	-	France	CP	603 bp
18	JX067851	*Avena sativa*	Brazil	CP	603 bp
19	JX067846	*Triticum aestivum*	Brazil	CP	603 bp
20	JX473287	*Sorhgum halapense*	Pakistan	CP	603 bp
21	KC900900	*Triticum aestivum*	Turkey	CP	603 bp
22	KP096226	*Bougainvillea spectabilis*	Turkey	AVP	893 bp

Similarity of nucleic acid sequence of BYDV-PAV Diyarbakır isolate ranged from 81.41-98.34% with the world isolates. Among Turkish isolates BYDV-PAV Diyarbakir isolate showed a maximum similarity of 98.34%. In phylogenetic tree, created by Mega 7 software, the compared isolates clustered into six groups. Each group was divided into subgroups. BYDV-PAV Van (KC900900) and BYDV-PAV Diyarbakır isolates (KX774424) have the highest similarity rate (98.34%) and clustered in the in the same group.

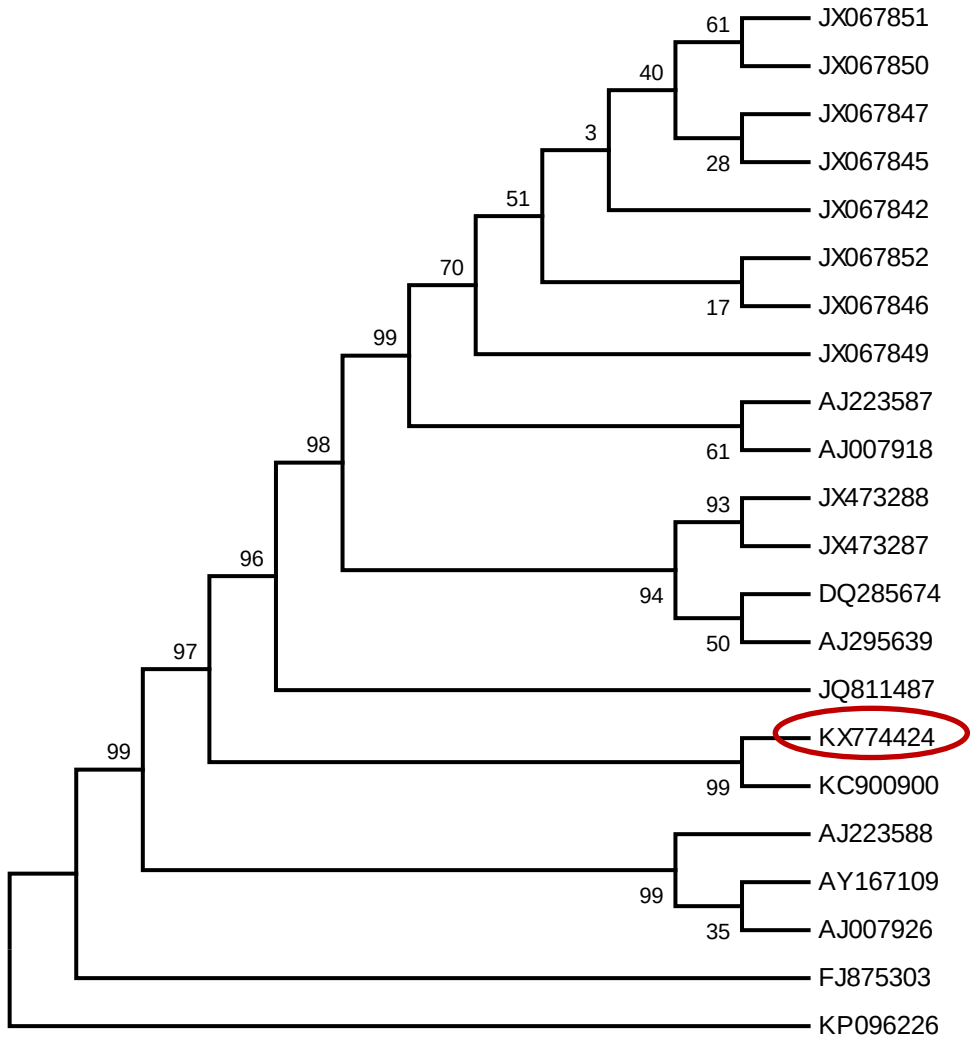

Figure 5. Phylogenetic tree showing the genetic relationships of BYDV-PAV Diyarbakır isolate (KX774424) with the other world isolates.

Discussion

Barley yellow dwarf viruses (BYDVs) are a grass- and cereal-infecting virus throughout the world and leading to economic losses ranging from 5 to 80% (average 30%), depending on infection period and plant's variety (Sutic, 1999; Miller and Rosachova 1997; Perry et al., 2000). There are many studies on BYDVs in Turkey and in the world's different regions. The distribution of BYDV varies from region to region, from year to year and from plantation to plantation (Lister and Ranieri, 1995).

Survey studies conducted in different sites of the world have shown that wheat viruses are present at different levels in cereal crops. Among them the most common virus is BYDV-PAV (Conti et al., 1990; El-Yamani and Hill, 1990).

Up to date, PCR testing, as an advantageous diagnostic method with high sensitivity, is preferred by researchers for the detection of wheat viruses. Especially multiplex PCR helps to diagnose more than one viruses quickly, economically, less labor and simultaneously as compared with serological methods (Saade et al., 2000; Nie and Singh, 2000; Vunsh et al., 1990).

In the present study, three different types of BYDV (BYDV-PAV, BYDV-SGV, CYDV-RPV) were concomitantly detected in wheat samples by M-RT-PCR. BYDV-MAV and BYDV-RMV viruses were not

detected in any of the 365 wheat samples tested. BYDV-PAV virus was detected as the most common virus (3.5%) in agriculture wheat in Diyarbakır.

In a previous study, BYDVs were investigated in 900 wheat samples in Eastern Anatolia (Turkey) in which the wheat crops were found infected with 5.5 % BYDV-PAV, 4.8 % BYDV-SGV and 0.4 % CYDV-RPV. No BYDV-MAV or BYDV-RMV viruses were reported in the region (Usta, 2013). The same viruses were identified in Diyarbakir province as recorded in the Eastern Anatolia Region. In a survey study conducted in Trakya region (Turkey), a total of 260 wheat samples were tested against BYDV in 2003. The incidence of infection levels were 25% for BYDV-MAV, 22.3% for BYDV-PAV and 8.5% were CYDV-RPV (Köklü, 2004). Likewise, in another study conducted in Edirne, Kırklareli, and Tekirdağ province reported that 63 out of 90 leaf samples (corresponding 70%) were infected with BYDV-PAV by ELISA in 2001 (Ilbagi et al., 2008). In addition, BYDVs have also been identified the Central Anatolia (Kayseri, Ankara, Eskişehir, Sivas, Nevşehir and Çankırı), in the Marmara (Çanakkale, Balıkesir), in Aegean (İzmir, Afyon and Kütahya) and in Black Sea (Çorum, Tokat, Amasya, Samsun) region (Yurdakul et al., 1987; Bremer and Raatikainen 1975; Ilbagi, 2003; Ilbagi et al.,2013; Pocsai et al., 2003; Ilbagi et al., 2005; Ilbagi, 2006: Ilbagi et al., 2006; Ilbagi et al., 2008; Ilbagi et al., 2011, Deligoz et al., 2011, Dayan and Ilbagi, 2014).

The rates of virus infections detected in Diyarbakir's samples are much lower than the rates of infection detected in other regions of Turkey and the world. The infection rates of the other agricultural crops in the Eastern Anatolia Region, have also been reported to be at a very low level (Sipahioğlu, 2011).

BYDV infections is also high in the world. In a study employing Dot-blot hybridization method in China, BYDV-PAV was recorded as 7.92 % in 2004, 27.50 % in 2005 and 31.82 % in 2006 (Liu et al., 2007). In Argentina, five strains of BYDVs (PAV, MAV, SGV, RPV, and RMV) and in Brazil five strains of BYDVs (PAV, MAV, and SGV) were recorded by ELISA test (Webby et al., 1993; Truol, 2002). In Pakistan, 45 of cereal samples were tested against BYDV (PAV, MAV, RPV, RMV, and SGV). According to the results of the ELISA, samples were found infected by BYDV-PAV with 64.4 %, 40 % BYDV-MAV and 4.4 % BDV-SGV (Bashir et al., 1997). In Hungary, the incidence of BYDV-PAV was investigated by PCR and the infection rate was determined as 58 % (Áy et al., 2008).

According to the results of the nucleic acid sequence analysis, the homogeneity of BYDV-PAV isolate ranged from 81.41% to 98.34% with wold isolates. BYDV-PAV Diyarbakir isolate exhibited highest similarity with BYDV-PAV Van isolates with 98.34% most probably due to close geographic proximity.

This report provides a basis for future research on wheat viruses. Findings from this study show that BYDV viruses (BYDV-PAV, BYDV-SGV, and CYDV-RPV) are present in wheat crops in Diyarbakır. Measures should be taken to reduce infection rate and to stop spreading. The use of BYDV tolerant or resistant varieties, late-sowing, remove of aphid vectors that may contribute to spread of BYDVs, eradication of weed species which serve as virus reservoir may help disease control.

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Araştırma Makalesi/*Research Article (Original Paper)*

Peach and Nectarine Affected with 16SrXII and 16SrIX Phytoplasma Groups in Northern Provinces of Iran

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Phytoplasmas are insect-transmitted, phloem-inhabiting, cell wall-less prokaryotes that cause numerous diseases in plants. A survey carried out during 2012-2013 in peach and nectarine orchards in three Northern provinces including Mazandaran (Sari, Neka, Babol, Behshahr and Babolsar), Golestan (Gorgan, kordkooy and Aliabad) and West Azerbaijan (Urmia, Khoy, Salmas, Oshnavieh and Makou) in Iran. A total of 103 Samples showing leaf roll, yellowing, purple colored of edges, leaf stunting and weak shoot growth symptoms were collected and tested by polymerase chain reaction (PCR) and Nested -PCR using primer pairs P1/P7, P1/Tint, PAF/PAR, R16F2n/R2, fU5/rU3 and NPA1F/NPA1R. The amplicons were purified, sequenced and the nucleotide sequences were analyzed by virtual restriction fragment length polymorphism (RFLP). The phytoplasmas associated with the peach and nectarine yellow leaf roll (PYLR) disease were identified as members of 16SrXII (Stolbur group) and 16SrIX (pigeon pea) groups and were placed in a unique subgroup close to 16SrXII-A (*Candidatus* Phytoplasma solani) and another one in 16SrIX-C (*Candidatus* Phytoplasma phoenciium) subgroups, respectively in constructed phylogenetic trees. The present study is the first report of a new subgroup in 16SrXII and first report of 16SrIX-C subgroup phytoplasma associated with peach and nectarine from North of Iran.

Keywords: Nested-PCR, *Candidatus* phytoplasma phoenicium, *Candidatus* Phytoplasma solani

Introduction

Phytoplasmas are wall-less prokaryotes classified in the class *Mollicutes*. In nature, phytoplasmas are transmitted from diseased to healthy plants by phloem-feeding insect vectors, mainly leafhoppers and psyllids (Tsai 1979). They are evolutionary descendants from the low G+C-containing, Gram-positive bacteria and through chromosome reduction, represent the smallest self-replicating life forms and are associated with diseases in several hundred plant species worldwide (Bertaccini et al. 2014). A wide variety of symptoms are associated with infection of these prokaryotes, including yellows, growth decline, stunting, witches' broom, leaf curl, phyllody and virescence (Bai et al. 2006). Since symptoms induced by a given phytoplasma can vary and on the other hand, different phytoplasmas may create similar symptoms, the identities of infecting phytoplasmas must be determined by molecular characterization. Phytoplasmas are usually present in low concentration; therefore, it is often necessary to use a nested PCR in two steps to obtain high sensitivity (Olmos et al. 1999). A phytoplasma group classification system has been established on the basis of restriction fragment length polymorphism (RFLP) analysis of 16S rRNA gene sequences (Lee at al. 2000; Seemüller et al. 1994). Iran is one of the countries that peach (*Prunus persica*) and nectarine (*Prunus persica* var. *nectarina*) grown in wide range in many provinces. However, there is no data on phytoplasma diseases of peach and nectarine in Mazandaran, Golestan and west Azarbaijan provinces of Iran. So the aim of this study was to verify the presence of phytoplasma diseases in symptomatic peach and nectarines in these three provinces using PCR assay. The detected phytoplasmas were characterized and classified using sequence analysis of PCR-amplified 16SrDNA and virtual gel RFLP.

Materials and Methods

Sources of phytoplasma strains

During surveys in summer and early autumn of 2012-2013, foliage samples of symptomatic peach and nectarine trees were collected from peach and nectarine orchards in the major peach and nectarine grown areas in Mazandaran (Sari, Neka, Babol, Behshahr and Babolsar), Golestan (Gorgan, kordkooy and Aliabad) and West

Azerbaijan (Urmia, Khoy, Salmas, Oshnavieh and Makou) provinces in the North of Iran. Asymptomatic trees samples were also collected and used in molecular analysis as negative controls. In total 103 samples were collected from different locations (53 from Mazandaran, 32 from West-Azerbaijan and 18 from Golestan provinces). Some of sequenced isolates with their characteristics have been listed in Table 1.

Table 1. Characteristics of peach and nectarine phytoplasma associated strains collected from peach and nectarine orchards in three Northern provinces of Iran

Isolate	Host	Region-Geographic Origin	Disease symptoms	Classification subgroup	**GenBank Accession No.** R16F2n/R2	fU5/rU3	NPA2F/R
PN53	peach	Oshnavieh-Urmia	Leaf roll with low yellowing	16SrXII-A	-	KF932288	-
PN115	Peach	Urmia	Leaf roll	16SrXII-A	KF263684	-	-
PN118	Nectarine	Gorgan-Golestan	Leaf roll	16SrXII-A	KF921621	-	-
PN119	Nectarine	Neka-Mazandaran	Leaf roll-bronzing of foliage	16SrXII-A??	KF739408	KF932292	-
PN131	Nectarine	Sari-Mazandaran	Leaf roll	16SrXII-A	KF923872	-	-
PN155	Nectarine	Aliabad-Golestan	Leaf roll	16SrXII-A	-	KF263685	-
PN168	Peach	Noshahr	Yellowing and little leaf	16SrIXI-C	KF923873	KF932287	KF932297
PN193	Nectarine	Sari-Mazandaran	Yellowing and leaf stunt	16SrIXI-C??	-	KF932284	KF932299
PN195	Peach	Salmas-Urmia	Yellowing Leaf roll with purple-colored margins	16SrIXI-C	KF923876	KF932285	-
PN196	Peach	Salmas-Urmia	Yellowing and reduce of internode	16SrIXI-C	KF923877	-	-
PN199	Peach	Babol-Mazandaran	Leaf roll with purple-colored margins	16SrIXI-C	KF923874	KF932286	KF932300
PN201	Peach	Neka-Mazandaran	Leaf roll with purple-colored margins	16SrIXI-C	KF923875	-	KF932301
PN203	Peach	Neka-Mazandaran	Leaf roll and proliferation	16SrXII-A	KF739407	KF932291	-

Nucleic acid extraction

Total DNA extraction was performed using Murray and Thompson procedure (Murray and Thompson 1980). Approximately 0.25g of leaf petioles and midribs from healthy controls and from naturally-infected samples was used for each extraction.

PCR condition and used primer pairs

Extracted DNA was subjected to PCR with P1/P7 and P1/Tint primers universal for 16S/23S rRNA region of phytoplasmas (Schneider et al. 1995; Deng and Hiruki 1991; Smart et al. 1996). The PCR products were diluted 1:30 with sterile deionized water and used as templates in nested PCR primed by the second universal primer pairs R16F2n/R2 (Gundersen and Lee 1996) or fU5/rU3 (Lorenz et al. 1995) as well as two primer pairs PA2F/R in PCR and NPA2F/R for Nested-PCR that amplified a region of 16S rRNA and a partial of spacer region of 16S/23S (Heinrich et al. 2001). The phytoplasma universal primer pairs P1/P7 and P1/Tint which amplifies a 1784bp and 1627bp DNA fragments, respectively and PA2F/R, which amplified a 1187 bp DNA fragment were used to initially detect phytoplasmas. The total volume of 20µl PCR reaction mixtures contained 20ng DNA, 0.2mM of each dNTPs (Cinnagen, Iran), 1.6mM $MgCl_2$, 1U of *Taq* DNA polymerase (Cinnagen, Iran), 0.5µl of each primer pair (20pmol/µl) and 1X polymerase buffer. The reaction mixtures were subjected to 35 cycles at the following conditions: First round PCR (35Cycles): 1 min (3 min for the first cycle) denaturation step at 94 ºC, 1 min for annealing at 57 ºC and 1.5 min (10 min for the last cycle) for primer extension step at 72 ºC. Second

round Nested PCR (35cycles): 2 min (5 min for the first cycle) denaturation step at 94 ºC, 1 min for annealing at 57 ºC and 2 min (10 min for the last cycle) for primer extension step at 72 ºC. The PCR products were analyzed by electrophoresis in a 1% agarose gel using Tris-Borate EDTA (TBE) buffer, and stained with 5µg/ml ethidium bromide. An ultraviolet (UV) transilluminator was used to visualize DNA band (Smart et al. 1996). The molecular weight of the PCR products was estimated using 1 Kb GenRuler^{TM} ladder (Fermentas).

Sequencing and phylogenetic analysis

PCR products of nested PCR were sent to sequencing directly. Sequencing was performed by Macrogen (South Korea) on both strands. Nucleotide sequence similarity and multiple alignments and phylogenetic tree construction using the neighbor-joining (NJ) method were done with MEGA5 software (Tamura et al. 2011) and subjected to bootstrap analysis using 1000 replicates. The *Acholeplasma laidlawii* isolate was used as an outgroup.

Virtual RFLP Analysis

Virtual RFLP analysis using iPhyclassifier (Zhao et al. 2009) was used to determine 16Sr group and subgroup affiliation of the detected phytoplasmas. RFLP profiles of detected phytoplasmas were compared to those of 16SrIX–subgroups A to G and 16SrXII-subgroups A to H using *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I enzymes.

Results

Phytoplasma symptoms

During summer and early autumn of 2012-2013, 103 peach and nectarine samples with phytoplasma symptoms were collected from different parts of Mazandaran, Golestan and West Azarbaijan provinces of Iran. The symptoms varied and the most characteristic symptoms were yellowing and inward rolling of thickened leaves with purple-colored margins of the adaxial surface and weaker shoot growth. The yield of fruits on infected peach and nectarine trees were reduced and they were small in size with low quality (the symptoms are summarized in Table-1 and some symptomatic plants were shown in Figure-1).

Figure- 1. Symptoms of phytoplasma diseases in peach and nectarine trees growing in the Northern regions of Iran. (a and b) yellowing, leaf rolling and purple colored (c) leaf rolling, (d) leaf rolling and reduce of coloration-bronzing e) yellowing f) rosetting, leaf rolling and malformation. c, d and f affected by a member of 16SrXII-A subgroup (*Ca*. P. solani) and a, b and e affected by a member of 16SrIX-C subgroup (Naxos virescence).

Sequencing and phylogenetic analysis

DNA fragments of approximately 1200bp, 850 and 480bp, respectively were amplified using phytoplasma universal and specific primer pairs R16F2n/R2, fU5/rU3 and NPA2F/NPA2R in nested-PCR from symptomatic plants whereas no DNA was amplified from asymptomatic plants. These obtained amplicons were sequenced and the nucleotide sequences of the isolates PN53, PN115, PN118, PN119, PN131, PN155 and PN203 from Mazandaran, Golestan and West Azarbaijan provinces were deposited in Genbank (Accession Nos. KF614623, KC417485, JN887313, JQ730750, JQ730744, JX311953, respectively). BLAST searches in NCBI database using phytoplasma 16SrRNA gene and partial sequences of IS region shared the highest homology (99-100%) to members of the 16SrXII group 99% to 100% identity shared (*Candidatus* Phytoplasma solani) a member of Stolbur group. Also, the isolates PN195, PN196, PN168, PN199 and PN201 from Mazandaran and West Azarbaijan provinces, respectively had 99 to 100% homology to *Ca.* P. phoenicium isolate PEYc2 (accession JX857827), *Ca*. P. phoenicium isolate NaxYc3 (accession JN791266), Periwinkle virescence phytoplasma strain NAXOS (accession HQ589191), Lactuca serriola phytoplasma strain P12 (accession AF515638), Khafr (Iran) Almond witches'-broom phytoplasma (accession DQ195209), Bushehr (Iran) eggplant big bud phytoplasma (accession JX483700) and Iranian Almond witches'-broom phytoplasma (accession FJ160959) in 16SrIX group. Phylogenetic trees on the basis of 16SrDNA gene were constructed using the maximum parsimony (MP) method that affirmed by neighbor-joining and maximum likelihood algorithm, with MEGA5.05 software (Tamura et al. 2011) comparing presence phytoplasmas in GenBank. The reliability of the tree was assessed by bootstrap analysis with 1000 replication (Figures. 2, 3 and 4).

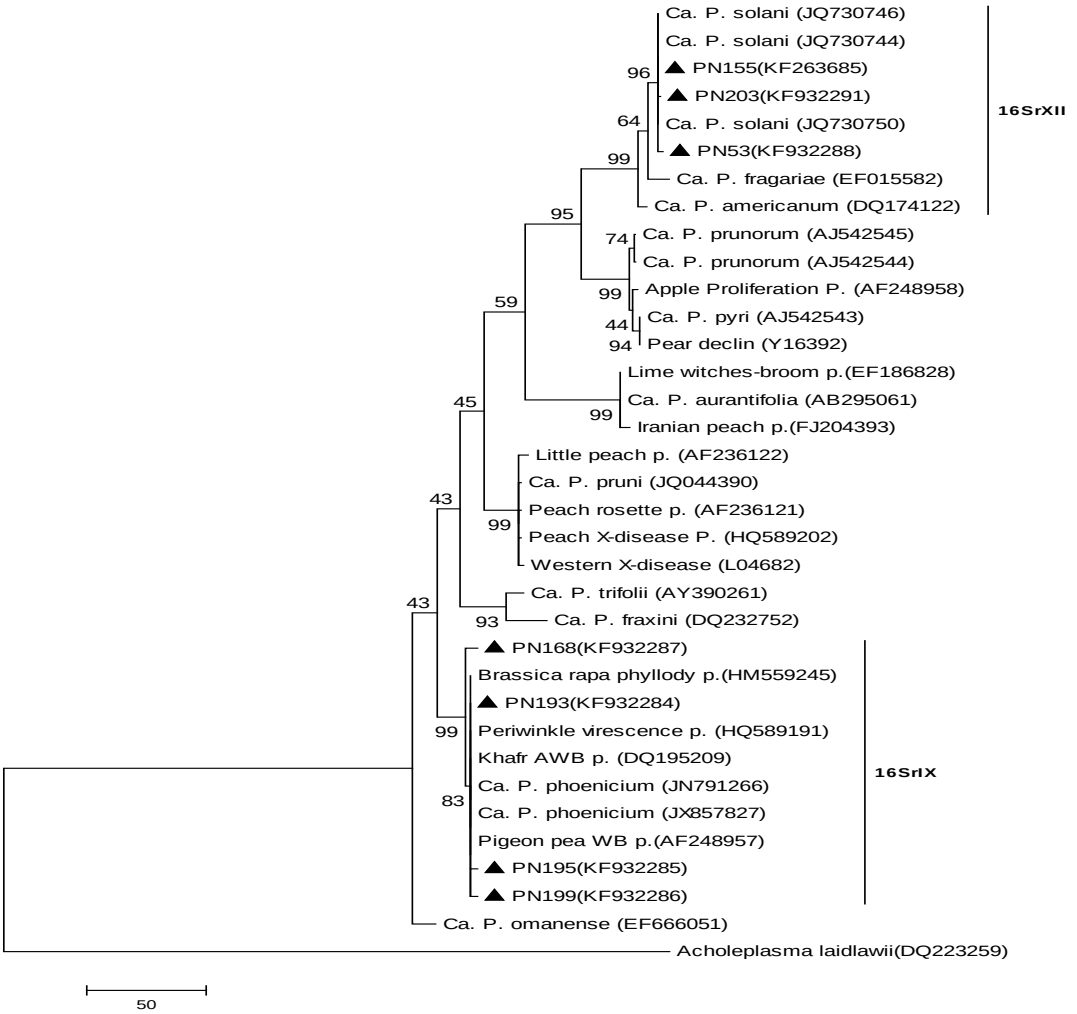

Figure- 2. Phylogenetic tree constructed by the maximum parsimony (MP) of 16SrRNA gene sequences using primer pairs fU5/rU3 from 28 phytoplasma and phytoplasmas identified from Peach and nectarine and *Acholeplasma laidlawii* as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

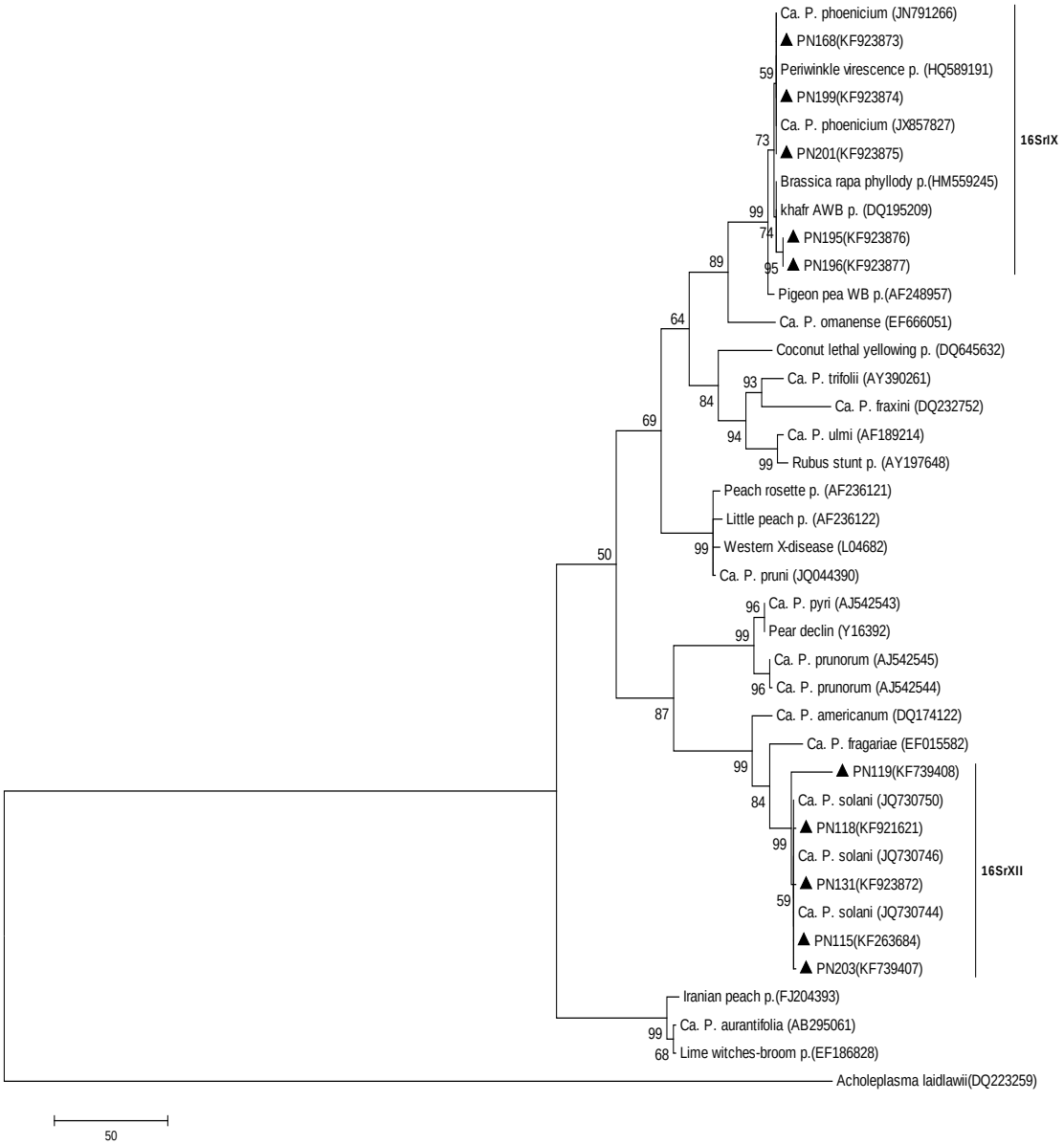

Figure- 3. Phylogenetic tree constructed by the maximum parsimony (MP) of 16SrRNA gene sequences using primer pairs R16F2N/R16R2 from 29 phytoplasma and phytoplasmas identified from Peach and nectarine and *Acholeplasma laidlawii* as outgroup. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

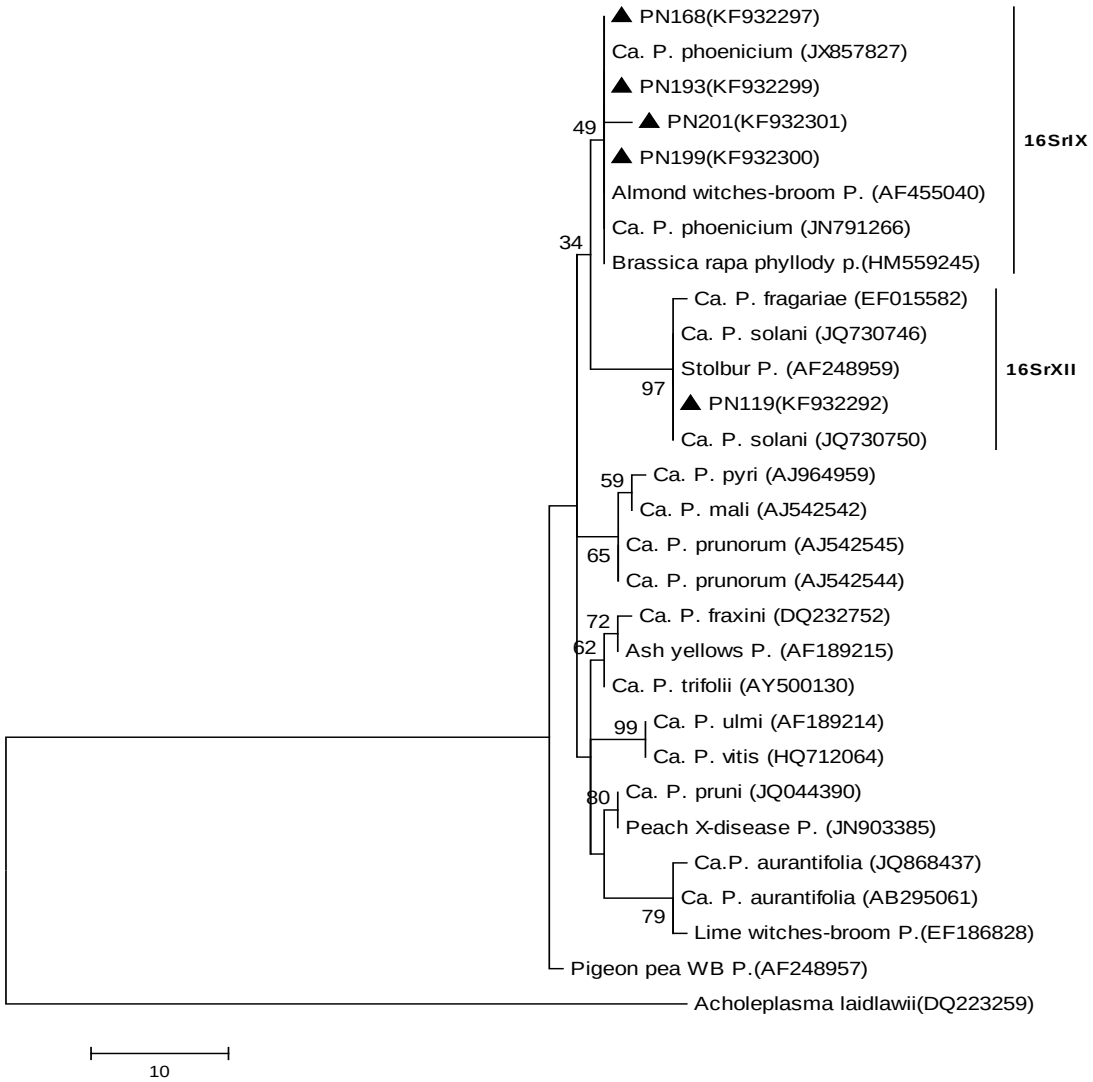

Figure- 4. Phylogenetic tree constructed by the maximum parsimony (MP) of 16SrRNA gene sequences using primer pairs NPA1F/NPA1R from 24 phytoplasma and phytoplasmas identified from Peach and nectarine and *Acholeplasma laidlawii* as outgroup. Direct PCR was performed using by primer pairs PA2F/PA2R. Numbers at the nodes are bootstrap values based on 1000 repetitions. GenBank accession numbers for sequences are given in parentheses.

Virtual RFLP Analysis

In silico digestion based on calculated RFLP pattern similarity coefficients and overall sequence similarity scores, after digestion with 17 different endonucleases and virtual electrophoresis pattern confirmed that PN168, PN195, PN196, PN199 and PN201 were most similar to members of the Pigeon pea witches'- broom group and 16SrIX-C subgroup. Also analysis of virtual electrophoresis pattern of the isolates PN115, PN118, PN119, PN131 and PN203 confirmed that these isolates were most similar to those previously published for 16SrDNAs from members of the Stolbur phytoplasma group and 16SrXII-A subgroup. (Figures 5 and 6).

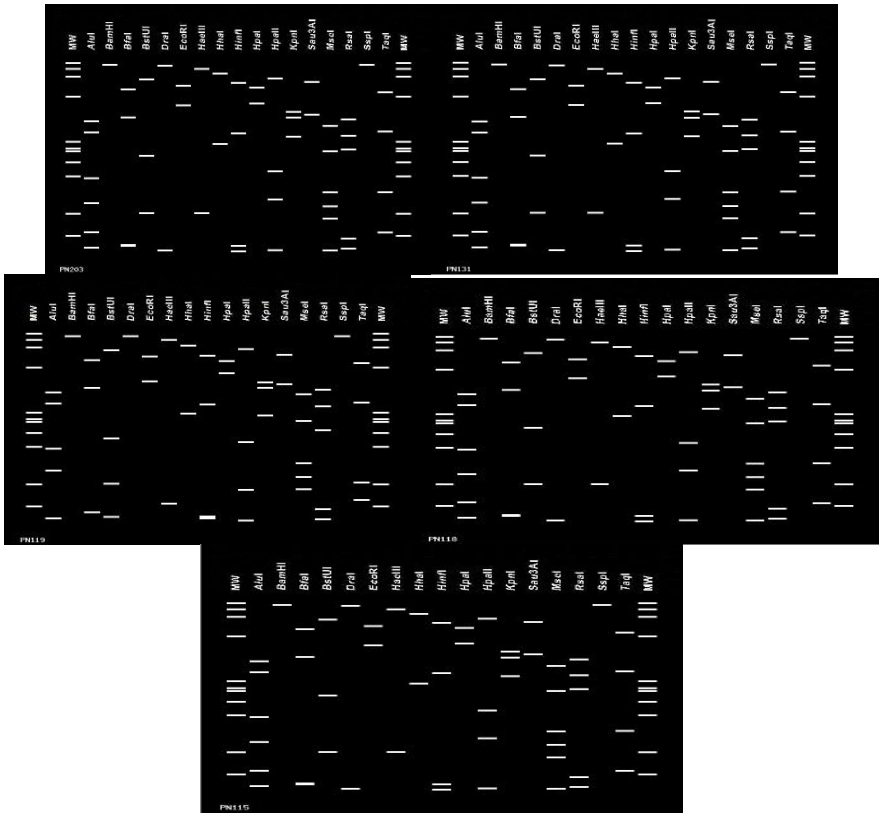

Figure- 5. Virtual restriction fragment length polymorphism (RFLP) pattern of $R_{16}F_{2n}/R_{16}R_2$ PCR product sequence from peach and nectarine phytoplasmas shows similarity with 16SrXII-A subgroup. Restriction sites for the 17 restriction enzymes were used in the simulated digestions: *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I.

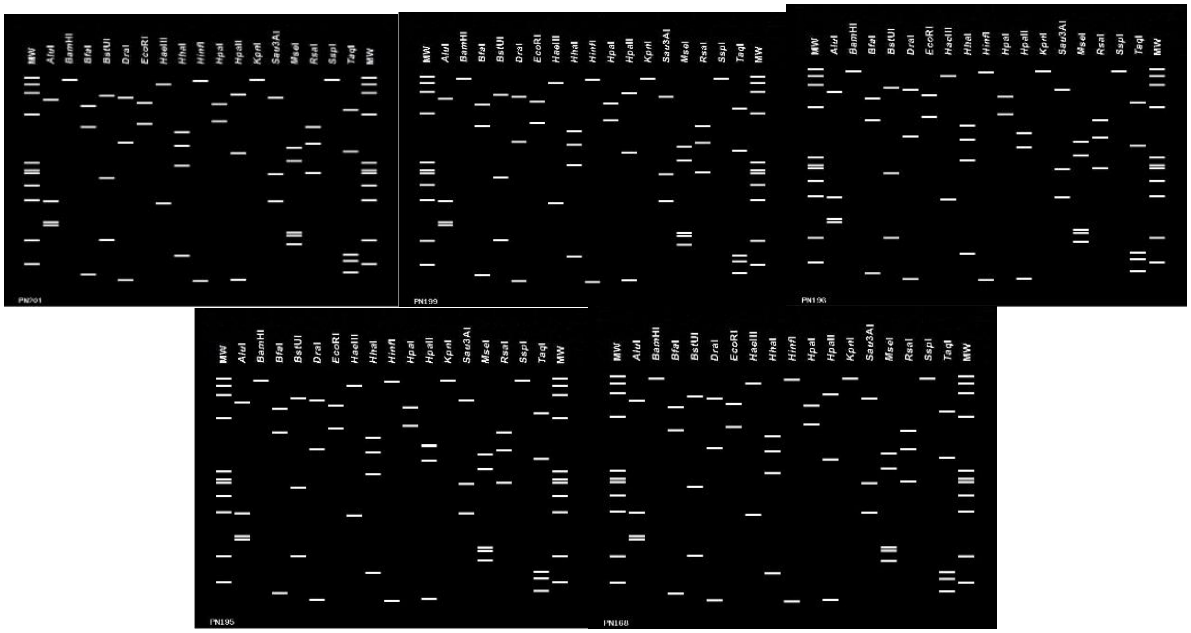

Figure-6. Virtual restriction fragment length polymorphism (RFLP) pattern of $R_{16}F_{2n}/R_{16}R_2$ PCR product sequence from peach and nectarine phytoplasmas shows similarity with 16SrIX-C. Restriction sites for the 17 restriction enzymes were used in the simulated digestions: *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I.

Discussion

The phytoplasmas on nectarine and peach plants with leaf roll, yellowing, bronzing and tattering symptoms in Mazandaran, Golestan and West Azarbaijan provinces were found to belong to phytoplasma groups classified in 16SrIX (pigeon pea witches'-broom group) and 16SrXII (Stolbur group) groups. The phytoplasmas identified from peach and nectarine with leaf roll, reddening and yellowing symptoms were classified in 16SrX (SrX-A, SrX-B, SrX-C), 16SrIII (SrIII-A)(Blomquist and Kirkpatrick 2002; Schneider et al. 1993; Gundersen et al. 1994; Paltrinieri et al. 2001; Navratil et al. 2001; Fialova et al. 2004; Seemuller and Schneider 2004) and 16SrV (SrV-A) (Thakur et al. 1998), 16SrVII (SrVII-A) and 16SrXII (SrXII-B) (Jones et al. 2005) previously. Phytoplasmas belong to ribosomal group 16SrIX (pigeon pea witches'-broom group) is associated with a lethal disease of almond, peach and nectarine named almond witches'-broom disease (AlmWB) (Abou-Jawdah et al. 2009) and it is shown that the Iranian phytoplasma strains associated with A1mWB and almond brooming shared >99% similarity with phytoplasmas of subgroup IX-C (Molino Lova et al. 2011). Several strains of this phytoplasma have previously been reported from different parts of Iran affecting a number of plants including *Lactuca serriola, L. sativa, Solanum lycopersicon, Sonchus* sp. [16SrIX-E], *Carthamus tinctorius*, *Chrysanthemum morifolium* cv. Paniz and *Prunus amygdalus* [16SrIX-B] (Bayat et al. 2013; Salehi et al. 2006; 2011). The 16SrXII group of phytoplasmas, especially '*Ca*. Phytoplasma solani', are widespread in Iran and have been reported on different crops including peach (Zirak et al. 2009; 2010). Our results suggest the potential threat of these two phytoplasmas (*Ca.* Phytoplasma phoenicium and *Ca.* Phytoplasma solani) for crops such as almond, nectarine, plum, peach and grapevine in these regions of Iran because of their wide host range in which they are maintained, perpetuated and transmitted by insect vectors to other commercial crops. This is, to our knowledge, the first report of a new subgroup in 16SrXII and first report of 16SrIX-C subgroup phytoplasma associated with peach and nectarine from North of Iran.

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Araştırma Makalesi/*Research Article (Original Paper)*

Domates Güvesi Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae)'nın Entomopatojen Nematodlar ile Biyolojik Mücadelesi

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Özet: Bu çalışmanın amacı *Steinernema affine* 46 (Bovien, 1937), *S. feltiae* 879 (Filipjev, 1934), *S. carpocapsae* 1133 (Weiser, 1955) ve *Heterorhabditis bacteriophora* 1144 (Poinar, 1976)'nın domates güvesi *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)'ya karşı virülensliklerini belirlemek ve karşılaştırmaktır. EPN'ler Türkiye'nin farklı bölgelerinden izole edilmiş, farklı gelişme dönemlerindeki *T. absoluta* bireyleri ise Çanakkale'deki domates tarlalarından toplanmış ve kitle üretimleri yapılmıştır. Pupa denemeleri, laboratuar koşullarında dört farklı sıcaklıkta (10, 15, 20 ve 25±1 ºC) 12 çukurlu kültür kaplarında yürütülmüş, her bir nematod türü için bir *T. absoluta* pupasına 30 infektif juvenil (IJ) inokule edilmiştir. Pupalar uygulamalardan sonraki 7. günde kontrol edilmiş ve ölüm oranları kaydedilmiştir. Çalışmada kullanılan tüm nematod türleri özellikle 25 ºC'de pupalarda ölüme neden olmuş, ancak *S. affine* 46 ve *S. feltiae* 879 düşük sıcaklıklarda da etkili iken, *H. bacteriophora* 1144 yüksek sıcaklıklarda daha etkili bulunmuştur. Çünkü sıcaklığın hem nematodun hem de onun simbiyotik bakterisinin aktivitesi ve patojenitesi üzerinde çok önemli bir rolü vardır. Bu çalışmadan elde edilen sonuçlar; EPN'lerin *T. absoluta*'yı infekte etmede önemli bir potansiyele sahip olduğunu ve bu zararlının mücadelesinde kullanılabileceğini ortaya koymuştur. Ancak EPN'lerin IPM programlarında kullanımlarının desteklenmesi için bunların domates üretim alanlarındaki etkinliği ile ilgili daha çok çalışma yürütülmelidir.

Anahtar kelimeler: Entomopatojen nematodlar, biyolojik kontrol, domates, *Tuta absoluta*, pupa

Biological Control of Tomato Leafminer Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) by Entomopathogenic Nematodes

Abstract: The present study aimed to determine and compare the virulence of *Steinernema affine* 46 (Bovien, 1937), *S. feltiae* 879 (Filipjev, 1934), *S. carpocapsae* 1133 (Weiser, 1955) and *Heterorhabditis bacteriophora* 1144 (Poinar, 1976) against tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). EPNs were isolated from different parts of Turkey and individuals of different developmental stages of *T. absoluta* were collected from tomato fields in Çanakkale and mass produced in climate room. Pupae experiments were conducted under laboratory conditions at four different temperatures (10, 15, 20 and 25±1 ºC) in the 12-well culture plates and 30 IJs were inoculated to a pupa of *T. absoluta* for each nematode species. After the treatments, pupae were checked on the 7th day and mortalities were recorded. All nematode species caused pupal mortality particularly at 25 °C but *S. affine* 46 and *S. feltiae* 879 were more effective at lower temperatures while *H. bacteriophora* 1144 was more effective at higher temperatures. Because temperature has an important role on both the activity and pathogenicity of the nematodes and its symbiotic bacteria. According to the results of this study, EPNs have significant potential for infecting *T. absoluta* and they can be used in the management of this pest but more studies on the efficacy of EPNs in tomato growing areas must be conducted to support their use in IPM programs.

Keywords: Entomopathogenic nematodes, biological control, tomato, *Tuta absoluta*, pupa

Introduction

Tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered as one of the most serious pest for *Solanum lycopersicum* L. and very challenging insect to control due to its biology and behavior (Lietti et al. 2005; Urbaneja et al. 2007). Tomato leafminer added to EPPO A1 List in 2004 and transferred to the A2 List in 2009 due to the distribution in EPPO region. In Turkey *T. absoluta* was first recorded in 2009 in Urla-Yağcılar Village and Çeşme-Ovacık Village (İzmir) (Kılıç 2010). It has also been reported in Çanakkale in July-August of the same year and caused significant crop losses in tomato production area in 2010 (Kasap et al. 2011).

Damage is caused by the larvae and without control methods, *T. absoluta* can result yield losses up to 100%, that occurs during the whole growing cycle of tomatoes, reduces the quality of fruits both in field and in greenhouse at high population and makes unsuitable tomatoes for marketing. It attacks all parts and stages of the tomato such as leaves, flowers, fruits and stems (Vargas 1970; Estay 2000; Desneux et al. 2010). The pest has four larval instars and pupation occurs in the soil, on surface of the leaf or in the mines, opened by the larva and it can overwinter as egg, pupa or adult (EPPO, 2005).

Young larvae penetrate the plant tissue, start feeding and produce large irregular mines in leaves, burrow into stalks, apical buds, green and ripe fruits. These mines cause a decreased rate of plant photosynthesis and enable attacks by pathogens (Cáceres 1992). Although *T. absoluta* mainly prefers tomato and other members of Solanaceae, it can also feed, develop and reproduce on the other hostplants by showing a high tendency for using various plants as alternative hosts.

Successful chemical control of tomato leafminer is difficult because it feeds internally within mesophyll tissues of the plant. Also it has the ability to develop resistance to some insecticides (Siqueira et al. 2000; Lietti et al. 2005) so this is the another remarkable difficulty in chemical control of tomato leafminer. Furthermore intensive use of pesticides inevitably results in fauna impoverishment and in devastation of the habitats that provide the food supply for many birds, mammals and other animals that inhabit farmland ecosystems. Due to these negative impacts of insecticides on biodiversity and on the waterbody quality of nearby biotopes, other reliable approaches need to be found for the managament of *T. absoluta*. Therefore, biological control can be considered as an alternative approach to chemical control. So entomopathogenic nematodes (EPNs) can be an alternative to chemicals due to having important potential as biological control agents. They are parasites of soil-dwelling organisms that infect pests that live in, on, or near the soil surface, can be used effectively to control serious pests and they have been reported as potential control agents for leafminers in recent years (Olthof and Broadbent 1990).

EPNs belong to the families Steinernematidae and Heterorhabditidae share a mutualistic relationship with bacteria in the genera *Xenorhabdus* (Thomas & Poinar, 1979) and *Photorhabdus* (Boemare, Akhurst & Mourant, 1993), respectively (Boemare et al. 1997; Burnell and Stock 2000). EPNs have many advantages as high reproductive potential, ability to kill hosts quickly, highly virulence, broad host range, easy mass rearing, safety to vertebrates, plants, and other nontarget organisms (Kaya and Gaugler 1993). In this study it was aimed to determine the efficacy of native EPNs against the pupae of *T. absoluta* under laboratory conditions.

Materials and Methods

Source and rearing of entomopathogenic nematodes

Four native EPNs; *S. affine* 46, *S. feltiae* 879, *S. carpocapsae* 1133 and *H. bacteriophora* 1144, isolated from different parts of Turkey in a previous project were reared at 25±1 ºC, 65±5% RH in the dark condition on the last instar of the greater wax moth larvae *Galleria mellonella* L. (Lepidoptera: Pyralidae), the most commonly used insect host to produce EPNs (Bedding and Akhurst 1975; Kaya and Stock 1997). Nematode-infected *G. mellonella* larvae were transferred to White traps (White 1927), IJs emerged from cadavers were harvested, these IJs were rinsed in distilled water and stored at 8-10 ºC in tissue culture flasks within a week. Before being used for bioassays their viability was checked by observing movements under a stereomicroscope.

Source and rearing of Tuta absoluta

Larvae, pupae and adults of *T. absoluta* were collected from different tomato fields in Çanakkale and maintained in wooden rearing cages (50x50x50 cm), covered with netting fabric for adequate ventilation on tomato plants in climate room at 25±1 ºC and 65±5% RH with a 16:8 h L:D photoperiod. Healty pupae were collected for laboratory bioassays.

Laboratory bioassays

The virulence of four native nematode species against pupa of tomato leafminer was evaluated in the laboratory. A single pupa was placed in each well (22,4 mm) of the 12 well plates (127,8×85,5×20 mm) and filled with moistened sterile sandy soil. The bioassays were conducted at 10, 15, 20 and 25±1 ºC by 30 IJs for each pupa with two replicates. EPNs were applied on the soil surface, only distilled water was added to the control plates. After the treatments, pupae were checked on the 7th day, the number of surviving and dead pupae were counted, mortalities were recorded and infected pupae were transferred to White traps.

Statistical analysis

Data on EPN species and temperatures were analysed by one-way ANOVA followed by Duncan's multiple range test ($P < 0,05$).

Results

The results revealed that the pupae of tomato leafminer were susceptible to four EPN species tested in the study. Different mortalities were obtained on the pupae based on the temperatures (Table 1).

Table 1. Mortality of *Tuta absoluta* pupae caused by EPNs at four different temperatures Mean (%)±SE

EPN Species	10 ºC	15 ºC	20 ºC	25 ºC	
S. affine 46	12,50±4,17 Aa	16,66±0,00 ABa	29,16±7,98 Ba	45,83±4,17 Ca	$F_{(3,15)}$= 9,12*
S. feltiae 879	4,17±4,17 Aab	12,50±7,98 Aa	29,16±7,98 ABa	45,83±14,23 Ba	$F_{(3,15)}$= 3,93
S. carpocapsae 1133	8,33±4,81 Aab	12,50±4,17 Aa	16,66±0,00 Aa	33,33±0,00 Ba	$F_{(3,15)}$= 11,87
H. bacteriophora 1144	0,00±0,00 Ab	4,17±4,17 ABa	16,66±6,80 Ba	45,83±4,17 Ca	$F_{(3,15)}$= 21,12
	$F_{(3,15)}$= 2,00**	$F_{(3,15)}$= 1,12	$F_{(3,15)}$= 1,42	$F_{(3,15)}$= 0,66	

*Means in the row followed by the same capital letter for the EPN species are not significantly different ($P < 0,05$).
**Means in the column followed by the same small letter for the temperatures are not significantly different ($P < 0,05$).

Influence of EPN species

Significant differences in the mortality of *T. absoluta* pupae were observed among four nematode species at 10 ºC, the lowest temperature in the study. *S. affine* 46 caused 12,5, *S. feltiae* 879 caused 4,17, *S. carpocapsae* 1133 caused 8,33 and *H. bacteriophora* 1144 caused no mortality on the pupae ($F_{3,15}$=2,00; $P < 0,05$). Similar mortalities were observed on the *T. absoluta* pupae by *S. affine* 46 (45,83), *S. feltiae* 879 (45,83) and *H. bacteriophora* 1144 (45,83) at 25 ºC, the highest temperature in the study and the lowest mortality were occurred on *S. carpocapsae* 1133 (33,33) ($F_{3,15}$=0,66; $P < 0,05$) (Table 1).

Influence of temperatures

S. affine 46 caused 12,5, 16,66, 29,16 and 45,83% ($F_{3,15}$=9,12; $P < 0,05$), *S. feltiae* 879 caused 4,17, 12,5, 29,16 and 45,83% ($F_{3,15}$=3,93; $P < 0,05$), *S. carpocapsae* 1133 caused 8,33, 12,5, 16,66 and 33,33% ($F_{3,15}$=11,87; $P < 0,05$), *H. bacteriophora* 1144 caused 0, 4,17, 16,66 and 45,83% mortality by the temperatures 10, 15, 20 and 25 ºC respectively ($F_{3,15}$=21,12; $P < 0,05$) (Table 1).

Discussion

More studies on the efficacy of EPNs against the larvae of *T. absoluta* have been conducted all over the world, but less studies have been conducted on the efficacy of EPNs against pupae of this pest. In this work we aimed to determine the virulence of four native EPNs against the pupae of *T. absoluta* in the laboratory.

All nematode species used in this study caused different mortalities on the pupae of tomato leafminer. Findings indicate an increase in mortality as the temperature rises. *S. affine* 46, *S. feltiae* 879 and *S. carpocapsae* 1133 showed higher efficacy than *H. bacteriophora* 1144 at lower temperatures. *H. bacteriophora* 1144 did not cause any mortality at 10 ºC. Many studies clearly show that *H. bacteriophora* needs high temperatures for successful development because it is less cold tolerant and requires at least 12 ºC (soil temperature) to infect and kill the host successfully, reproduce and produce viable IJs in the tissues of the pest (Grewal et al. 1994; Susurluk and Ehlers 2008). In similar studies; high larval (78,6-100%) and low pupal mortality (<10%) (Batalla-Carrera et al. 2010), high larval and no pupal mortality (Garcia-del Pino et al. 2013), and low pupal mortality (7%) of *T. absoluta* were reported (Türköz and Kaşkavalcı 2016). Our results are consistent with the results of these studies.

Although high efficacy were occurred with the same isolates on the larvae of *T. absoluta* by *S. feltiae* 879 90,7, 94,3%; *S. affine* 46 39,3, 43,7%; *S. carpocapsae* 1133 43,7, 49,3% and *H. bacteriophora* 1144 81, 83% in 2012 and 2013, respectively (Gözel and Kasap 2015), EPNs are not able to penetrate and infect pupae so pupal mortalities were generally low because of pupal of resistance. Major cause of pupal resistance to EPN infections is explained by the closure of all openings as spiracles, mouth and anus owing largely to sclerotization and thickening of the cuticle into puparial cells with many researches. Due to the lack of these entry ways in this stage, pupae were hardly infected by nematodes compared to the larvae (Grewal et al. 2005; Rohde et al. 2012; Foelkel et al. 2016; Minas et al. 2016).

We conclude that EPNs have potential to control tomato leafminer. Four native isolates tested in this study showed efficacy at different rates against *T. absoluta* pupae especially at high temperatures. Therefore, further studies should be conducted in the field as soil applications against to emerging adults of *T. absoluta* because EPNs could be an important biological control agents for decreasing the population of this pest in nature.

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Araştırma Makalesi/*Research Article (Original Paper)*

Dietary Enrichment of Eggs with DHA Using Different Sources of Fatty Acids

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Abstract: Ω-3 polyunsaturated fatty acids (PUFAs), first of all α-linolenic (ALA, C18:3), eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids are necessary for the brain development, the eye function, the prophylaxis of cardiovascular diseases etc. One hundred and forty ISA Brown hens (n=20/treatment), 60 weeks old were fed with diets containing sunflower (2.5%), soybean (2.5%), fish oil (1.5 and 2.5 %) or marine algae (1.5 and 2.5 %) in a period of 45 days. There was a significant (P<0.05) influence of the fatty acids' sources (fish oil and marine algae) on the total egg weight, albumen weight and yolk weight. DHA content in one yolk was significantly higher (p<0.01) in the groups fed on diet supplemented with 1.50% of fish oil (120.94 mg), 2.5% of fish oil (162.91mg), 1.50% of marine algae (130.73 mg) and 2.5% of marine algae (243.44 mg). Although with the diet supplemented with 2.5 % marine alga the amount of DHA in yolk was eight times higher (15.58 mg/g yolk) in comparison with DHA in the control (1.70 mg/g yolk).

Keywords: laying hen, fatty acids' sources, egg's structure, DHA deposition, yolk

Introduction

Of special interest for the dietologists are the alimentary products rich in Ω-3 polyunsaturated fatty acids (PUFAs), first of all α-linolenic (ALA, C18:3), eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids, which are necessary for the brain development (Swanson et al., 2012), the eye function, the prophylaxis of cardiovascular diseases (Van Elswik 1997; Mata Lopez and Ortega 2003), etc. Some physiologically important fatty acids – docosahexaenoic (DHA, C22:6Ω-3) and γ-linolenic (GLA, C18:3Ω-6) belong in the conditionally essential PUFAs because they are not synthesized in the organism, except in a certain stage of the ontogenesis or during some illnesses (Kavtarashvili et al. 2017).

Generally, the recommended minimal daily intake/dose for healthy adults is 250–500 mg of EPA and DHA together. However, higher amounts are often recommended for certain health conditions (Global Recommendations for EPA and DHA Intake (Rev 16 April 2014). In most countries in the world, including Macedonia, Omega 3 fatty acids are deficient in the human diet, including the insufficient consumption of fish which are an important source of these acids but are an expensive nutritional product (https://ec.europa.eu/fisheries/6-consumption_en).

Laying hens have limited ability to convert alpha-linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in their organism, and for this reason it is recommended to supplement the feed with these fatty acids in order to enrich the egg n-3 PUFA content effectively (Kralik et al. 2007; Kralik et al. 2008; Škrtić et al. 2007; Škrtić et al. 2008).

The aim of our research was to evaluate the effect of different sources and dietary level of PUFA in the laying hen's diet on the eggs' quality and yolk DHA content.

Material and Methods

Animals, housing and diets

One hundred and forty ISA Brown hens, 60 weeks old, were housed in laying cages (2 birds per cage (400x350x380mm) with average live weight (1960 g) in a standard poultry house with a light regime of 16H

light and 8H darkness and feed according the requirements of the hybrid before the experiment. The hens were divided in one control group (20 birds) and six experimental groups (20 birds per group). The experiment lasted 45 days. Feed consumption was 120g/day/bird. Water was supplied by 2 nipple drinkers in each cage. The test ingredients composition and the composition and nutrient content of the experimental diets are presented in Table 1 and 2.

Table 1. Lipids' and fatty acids' content of the test ingredients

Content (%)	*Sunflower oil*	*Soybean oil*	*Fish oil*	*Marine algae*
ME, KJ/kg	36990	36990	37740	
Total lipids (fat)	100	100	100	45.3
Fatty acids, saturated, total	9.75	15.34	21.29	
Fatty acids, monounsaturated, total	83.59	21.71	56.56	
Fatty acids, polyunsaturated, total	3.80	58.21	15.60	

Table 2. Composition and nutrient content of experimental diets

Ingredient (%)	*Treatments*						
	Control Basal Feed (BF)	Sunflower oil 2.5	Soybean oil 2.5	Fish oil 1.50	Fish oil 2.50	Marine algae 1.50	Marine algae 2.50
Corn	51.96	51.96	51.96	51.96	51.28	51.96	51.28
Wheat middlings	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sunflower meal	16.64	14.14	14.14	15.14	14.14	15.14	14.14
Soybean meal	10.18	10.18	10.18	10.18	10.86	10.18	10.86
Fish oil	-	-	-	1.50	2.50	-	-
Sunflower oil	-	2.50	-	-	-	-	-
Soybean oil	-	-	2.50	-	-	-	-
Marine algae	-	-	-	-	-	1.50	2.50
DL methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.08
L lysine	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Limestone	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Premix [1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Premix (1 kg) contains: vitamin A 3,000,000 i.u., vitamin D_3 700,000 i.u., vitamin E 6,000 i.u., vitamin K_3 600 mg/kg, vitamin $B_1$800 mg/kg, vitamin B_2 1,200 mg/kg, nicotinic acid 8,000 mg/kg, calcium pantothenate 2,400 mg/kg, vitamin B_6 1,000 mg/kg, vitamin B_{12} 2,000 μg/kg, folic acid 200 mg/kg, biotin 40 mg/kg, iodine (I)160 mg/kg, manganese (Mn) 16,000 mg/kg, zinc (Zn) 16,000 mg/kg, cobalt (Co) 50 mg/kg, iron (Fe) 12,000 mg/kg, copper (Cu) 1,800 mg/kg, selenium (Se) 60 mg/kg, canthaxanthin 6,000 mg/kg, ethoxyquin 24,000 mg/kg and plant base up to 1 kg.

Egg's quality parameters

The egg's structure parameters (eggwhite/albumen weight, yolk weight and eggshell weight) and their percentage in the total egg weight was measured in 6 randomly selected eggs, 3 times during the experiment (every 15th day) on a scale with 0.1 g accuracy. The eggs were weighed, then the yolks were separated with an egg separator. The albumen residuals were eliminated from the yolk using blotting paper and then weighed. The shell was wiped clean and weighed. The albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The viteline membrane of the yolk was removed using tweezers, then mixed manually with a spatula and stored at –20°C prior to analyses.

Total lipids and DHA content in the yolk

The total fat in the yolk was measured using Soxhlet extraction method. The concentrations of docosahexaenoic (DHA, C22:6n-3) fatty acid in egg's yolk were measured. Six yolks were mixed, then dried with sodium sulphate, mixed with DI (deionized) water and hexane and centrifuged 2–3 minutes at 2500 rpm. DHA was determined using gas chromatography (AOCS –Ce 1f – 96) adapted by Abril and Barclay (1999), with identification of fatty acids by comparing their retention times and quantified by area's standardization.

Statistical analysis

Statistical analysis was performed using Statgraph 3 software package. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan's Multiple Range Test was performed. All results are presented as means with their standard deviations (SD).

Results and discussion

The eggs' parameters, such as total egg weight, shell weight, albumen weight, yolk weight, proportion and edible portion and yolk:white ratio are presented in Table 3. The egg weight, albumen weight and yolk weight were significantly affected by the supplementation of different sources of fatty acids in different doses ($P<0.05$).

The egg weight of the group fed with a supplement of marine algae (1.5 and 2.5%) and soybean oil significantly decreased (59.85 g, 60.10 g and 60.29 g, respectively) in comparison to the control group. In addition, the lower egg weight found/measured in some investigations may be a consequence of the lower feed consumption (Gonzalez-Esquerra and Leeson 2000). In our investigation the feed was limited on 240 per cage (2 hens in cage), so the intake maybe was lower on one hen then the other in the same cage. This conclusion is in accordance with the results obtained regarding egg's albumen weight. The albumen weight and yolk weight of the aforementioned experimental groups have significantly ($P<0.05$) lower values in comparison to the control group.

Table 3. Egg's quality parameters at the end of the supplementation period

	Control Basal Feed (BF)	*Sunflower oil 2.5*	*Soybean oil 2.5*	*Fish oil 1.50*	*Fish oil 2.50*	*Marine algae 1.50*	*Marine algae 2.50*
Egg weight, g	67.74±3.95a	63.73±3.11a	60.29±4.83b	70.46±4.42a	68.30±4.25a	59.85±5.18b	60.10±5.35b
Albumen weight, g	40.51±3.48a	41.16±2.14a	37.59±3.57b	42.93±4.13a	40.81±3.91a	36.62±4.31b	36.96±4.35b
Yolk weight, g	19.07±2.03a	17.72±1.56a	15.74±1.27b	19.35±1.46a	19.51±1.83a	15.75±1.06b	15.63±1.25b
Shell weight, g	8.16±0.69	8.48±0.54	6.93±0.60	8.32±0.81	8.14±0.61	7.56±0.67	7.51±0.57
Albumen weight %	59.77±3.06	61.11±1.65	62.28±1.56	60.80±2.95	59.41±2.97	61.04±2.36	61.37±2.25
Yolk weight %	28.19±3.00	26.28±1.64	26.14±1.46	27.54±2.58	28.66±2.85	26.41±1.67	26.07±1.73
Shell weight %	12.04±0.67	12.61±0.87	11.51±0.70	11.81±0.93	11.93±0.65	12.67±1.11	12.55±1.02
Edible portion, %	87.96±0.67	73.72±1.64	73.43±1.34	88.34±1.61	88.07±0.65	72.88±1.16	73.36±1.17
Yolk : white	47.52±7.61	43.09±3.70	42.85±2.84	45.57±6.22	48.58±7.04	45.36±2.41	43.65±3.10

The values are means ± S.D

a, b - Significant differences respect to the control for the f-tests run to compare means ($P<0.05$)

Total lipids and DHA content of the eggs are shown in Table 4. The total lipids' content varied from 26.09 to 27.86% in the examined eggs. There were no significant differences between the group fed with basal feed and the groups fed with supplemented feed regarding lipids' content in the eggs ($P>0.05$). Our results are in accordance with the results obtained by Grigorova et al. (2006) on Bovans brown laying hens fed on diet supplemented with 2% and 10% of dry biomass from green algae of Chlorella genus.

Table 4. Lipids and DHA content in experimental eggs at the end of the supplementation period

	Control Basal Feed (BF)	*Sunflower oil*	*Soybean oil*	*Fish oil 1.50*	*Fish oil 2.50*	*Marine algae 1.50*	*Marine algae 2.50*
Lipid content in egg, %	26.09±0.60	27.30±0.27	26.59±0.21	27.82±0.98	27.86±1.36	26.85±0.91	27.42±0.89
Lipid content in yolk, g	4.97±0.11	4.84±0.05	4.18±0.03	5.38±0.19	5.47±0.25	4.21±0.14	4.28±0.14
DHA mg/g yolk	1.70±0.28A	2.05±0.07A	2.05±0.07A	6.25±0.14B	8.35±0.07B	8.30±1.44B	15.58±0.8B

The values are means ± S.D
A, B – Significant differences respect to the control for the f-tests run to compare means (P<0.01).

DHA content in the egg yolk was significantly different (P<0.05). The highest content was noticed in the group fed with supplemented feed with 2.50 marine algae (15.58 mg/g egg yolk) in comparison with the control group (1.70 mg/g egg yolk). The content of DHA was also significantly affected by the nutrition supplemented with 1.50% of fish oil, 2.50% of fish oil and 1.50% of marine algae. The calculated content of DHA in one average egg yolk was significantly (P<0.05) highest (243.44 mg/yolk) in the group/fed with 2.50% of marine algae. These results are presented in figure 2.

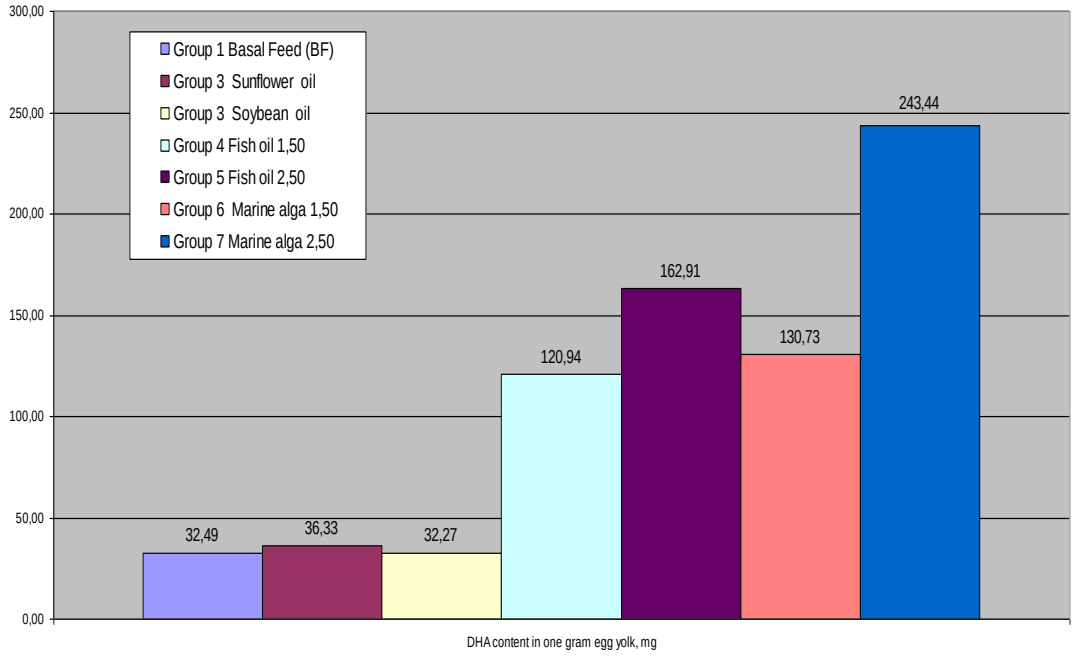

Figure 2. DHA content in yolk, mg

Researchers, during the last decade, have elucidated the importance of Omega 3 fatty acids for the human health. In most countries in the world, including Macedonia, Omega 3 fatty acids are deficient in the nutrition, including the insufficient fish consumption (https://ec.europa.eu/fisheries/6-consumption_en). Also, there are some discussions about the safety of the fish consumption as a good source of Omega 3 fatty acids. The pollution results in an increase in exposure to methyl-mercury and other contaminants in the fish tissue. (Castano et al., 2015; Taylor et al., 2016). The price of fish meat, its availability on the market, consumers' habits, make the researchers take into consideration other modes for satisfying the requirements for PUFA in the human nutrition. One mode is foodstuffs enrichment with PUFAs, and the most used animal product with low price and enriched with PUFAs is eggs enriched with Omega 3 fatty acids (Kralik et al. 2017). Further studies are required to investigate the effect of enriched eggs, on one hand, and fish with high content of Omega 3, on the other hand, as sources of Omega 3 fatty acids, on its digestibility and absorption and also on some biochemical parameters related to human health.

Conclusions

Supplemented diets with different sources of fatty acids fed to ISA Brown laying hens have produced eggs enriched with DHA fatty acid. Our results show that the diet supplemented with 1.50% of fish oil, 2.5% of fish oil, 1.50% of marine algae or 2.5% of marine algae as a sources of omega-3 fatty acids obtain acceptable level of DHA in table eggs. These table eggs are products with a significantly higher DHA content. It is possible to produce health promoting enriched eggs by manipulation of hens' diet.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Potassium Fixation Capacities of Different Textural Soils

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Abstract: In this study, potassium fixation capacities of clay, loam and loamy sand soils were determined by Langmuir and Freundlich isotherms, and relationships between isotherm coefficients and soil properties were investigated. While the Langmuir isotherm gave the best fit for K adsorption of clay and loam soils, Freundlich isotherm gave the best fit for K adsorption of loamy sand soil. Potassium adsorption capacities of the soils ordered as clay > loam > loamy sand soil according to the b and Kf absorption values of the isotherms, Clay soil had more ability to supply K ion to soil solution than loam and loamy sand soils. Potassium absorption capacity coefficients gave significant positive correlations with clay, organic matter, cation exchange capacity, exchangeable K, Ca and Mg contents and significant negative correlations with pH, CaCO3 and sand content. The potassium fixation mechanisms of soils varied depend on soil textural and other physicochemical properties. In sustainable and productive soil management, K absorption capacities of soils should be determined for correct K fertilization programme.

Key words: Potassium fixation, Langmuir, Freundlich, adsorption.

Farklı Tekstürdeki Toprakların Potasyum Fiksasyon Kapasiteleri

Özet: Bu çalışmada, kil, tın ve tınlı kum tekstüre sahip toprakların potasyum fiksasyon kapasiteleri Langmuir ve Freundlich izotermleri ile belirlendi ve izoterm katsayıları ile toprak özellikleri arasındaki ilişkiler araştırıldı. İzotermlerin b ve K*f* absorpsiyon değerlerine göre, toprakların potasyum adsorpsiyon kapasiteleri kil > tın > tınlı kum şeklinde sıralanmıştır. Kil bünyeli toprak tın ve tınlı kum topraklardan daha fazla toprak çözeltisine K iyonu sağlama kapasitesine sahipti. Potasyum adsorpsiyon kapasitesi katsayıları kil, organik madde, katyon değişim kapasitesi, değişebilir K, Ca ve Mg içerikleri ile önemli pozitif korelasyonlar, pH, CaCO3 ve kum içeriği ile önemli negatif korelasyonlar vermiştir. Toprakların potasyum fiksasyon mekanizmaları toprakların tekstür ve diğer fizikokimyasal özelliklerine bağlı olarak değişmiştir. Sürdürülebilir ve üretken toprak yönetiminde, toprakların K adsorpsiyon kapasitelerinin doğru gübreleme programı için belirlenmesi gerekir.

Anahtar kelimeler: Potasyum fiksasyonu, Langmuir, Freundlich, adsorpsiyon.

Introduction

Potassium is one of the most important macro elements for plant growth. The most studies showed that plants adsorb a large amount of potassium in the soil (Syers, 2003; Hu and Wang, 2004; Wang et al., 2010). Although most of the soils are thought as rich in total potassium reserves, plants cannot benefit from all this potassium reserve. Potassium, depending on its availability to plants in soil, is generally classified into four groups: water-soluble, exchangeable, non-exchangeable and structural forms (Tisdale et al., 1984). The water-soluble and the exchangeable potassium are about 0.1–0.2 % and 1–2% of the total K in soil, respectively (Sparks, 1987). Potassium fixation in soils, is known as a transformation of available K forms into unavailable ones, has a direct effect on K availability and on the degree of fertilizer K uptake by plants. The fate of added K fertilizers differs between soils due to different adsorption characteristics of K by various soils. The rate of K adsorption on illite and vermiculite was much slower than that on montmorillonite and kaolinite (Ogwada and Sparks, 1986; Metha and Singh, 1986). Knowing the soil's ability to fix and release potassium is important in terms of improving potassium fertilization practices and modeling the transport of potassium fertilizer in the soil (Sparks and Huang, 1985).

The potassium fixation mechanism is important in terms of K fertilization, conservation of productivity, understanding of chemical processes in the soil, and sustainable soil fertility. Sorption, is one of the most important chemical processes, affects the mobility and fate of nutrients in the soil. Sorption isotherm represents

the relation between equilibrium amount of a substance in soil solution and the amount adsorbed by the solid phase of the soil (Wajid et al., 2013). The Langmuir and Freundlich isotherms have been generally used to describe the adsorption of cations by the colloidal phase of the soil. Many studies have reported that Freundlich and Langmuir isotherms are more suitable than other isotherms for determining the mechanism of potassium sorption and adsorption in the soil (Oskay, 1986; Kou, 1988; Quang et al., 1996; Jin et al., 2005; Chitrakar et al., 2006; Kang et al., 2011). The main objectives of this study were to determine the potassium fixation capacities of different textural soils using the Langmuir and Freundlich isotherms, and to investigate the relationships between the coefficients of isotherms and the soil properties.

Material and Methods

In this study, three different surface soil samples (0-20 cm) with clay, loamy and loamy sand textural soils were used. Clay soil sample was taken from the experimental field of Agricultural Faculty in Ondokuz Mayıs University, Samsun. Loamy soil and Loamy Sand soil were sampled from Çetinkaya village and near the coast of Red River in Bafra District of Samsun, respectively.

Some chemical and physical characteristics of the soil samples were determined as follows; particle size distribution by hydrometer method, bulk density (BD) by undisturbed soil core method (Demiralay, 1993), soil pH, 1:1 (w:v) soil:water suspension by pH meter, electrical conductivity (EC_{25^oC}) in the same suspension by EC meter, lime content by Scheibler Calcimeter method, organic matter content by Walkley-Black method and cation exchange capacity (CEC), exchangeable cations by ammonia acetate extraction (Kacar, 1994).

To determine the potassium fixation in the soil, KCl was used as the K carrier to prepare the solution. The soil samples were shaken with 1:5 soil:KCl solutions including 2, 4, 8, 16, 24, 32, 40 and 60 meq K /L for 24 hours and filtered. The amount of potassium in the soil solutions was determined using the Atomic Adsorption Spectrophotometer.

The potassium adsorption of soils is showed by the following isotherm equations.

$S=bkC/(1+kC)$ or $\frac{1}{S}=\frac{1}{b}+\frac{1}{bkC}$ Langmuir (1918);

$S=K_fC^{(1/n)}$ or $\lg S=\lg K_f+\frac{1}{n}\lg C$ Freundlich (1930);

Where, S is amount of potassium adsorbed on the soil (me/100g), b (me/100g), k and (1/n) are the coefficients expressing the potassium adsorption capacity of soil, C (me/L) is the concentration of potassium in the equilibrium soil solution or in the soil extract after shaking, K_f is the equilibrium coefficient which expresses the potassium holding capacity of the soil. The amount of K adsorbed by soil particles (S) was estimated subtracting the K concentrations in equilibrium solutions (C) from the initial K concentrations of the solutions used in the experiments.

Correlation analyses of the experimental data were done using the SAS software package (SAS Institute, 1988).

Results and Discussion

The soil physical and chemical properties are given in Table 1. These results can be summarized as; the clay, loam and loamy sand soil samples are none saline, neutral, moderately alkaline and strongly alkaline in pH, medium, low and very low in organic matter content, respectively (Soil Survey Staff, 1993).

The results of K adsorption experiments done using the different initial K concentration solutions are given in Table 2. While the initial added concentration of K solution increased from 2 to 60 me/L, K concentration in equilibrium solution and also K adsorption in all soil samples increased. When the soil particle size distribution decreased from loamy sand to clay soil, K concentrations in equilibrium solutions also decreased. It means that K adsorption mostly occurred by the surface of organic or inorganic colloidal size particles in the soil. The K adsorption amount (S) by the soil samples varied from 0.79 to 14.22 me/100g in clay, from 0.78 to 9.86 me/100 g in loam and from 0.25 to 4.01 me/100 g in loamy sand soil. Similarly, Auge et al. (2018) studied on the K adsorption on Sidama zone soils of Southern Ethiopia and reported that K adsorption increased in clay soil with the initial added K concentration increased, but it slightly increased in the other textural soils. In this study also,

K adsorption in clay soil increased with all initial added concentration of K, and it slightly increased in loam and loamy sand soils after 32 me/L and 16 me /L of K concentrations, respectively (Figure 1).

Table 1. Some physical and chemical properties of soils

Soil Properties	**Soil Samples**		
	1 (Clay Soil)	**2 (Loam Soil)**	**3 (Loamy Sand Soil)**
Clay, %	62.74	26.00	8.63
Silt, %	25.13	34.00	10.00
Sand, %	12.13	40.00	81.27
Texture	Clay	Loam	Loamy Sand
pH (1:1)	6.78	8.35	8.85
EC, dS/m	0.600	0.970	0.357
$CaCO_3$, %	3.40	2.98	9.20
Organic Matter, %	2.42	1.50	0.44
CEC, me/100g	53.0	28.4	12.8
Na, me/100g	0.42	0.63	0.36
K, me/100g	0.72	0.50	0.10
Ca, me/100g	22.03	17.90	6.46
Mg, me/100g	19.74	7.93	4.29

Table 2. The experimental results of K concentration in equilibrium solution and K adsorption by clay, loam and loamy sand soils.

Initial solution	C, me/L			S, me/100g		
K conc., me/L	Clay	Loam	Loamy Sand	Clay	Loam	Loamy Sand
2	0.41	0.46	1.50	0.79	0.78	0.25
4	0.88	1.26	2.94	1.56	1.37	0.53
8	1.86	2.76	6.68	3.07	2.62	0.66
16	2.60	5.03	13.14	6.70	5.43	1.43
24	8.09	11.97	20.56	7.95	6.30	1.72
32	12.90	16.59	27.32	9.55	7.80	2.34
40	17.78	23.08	34.02	11.11	8.78	2.99
60	31.57	46.39	51.98	14.22	9.86	4.01

C: The amount of K in equilibrium solution, S: The amount of K adsorbed by soil particles.

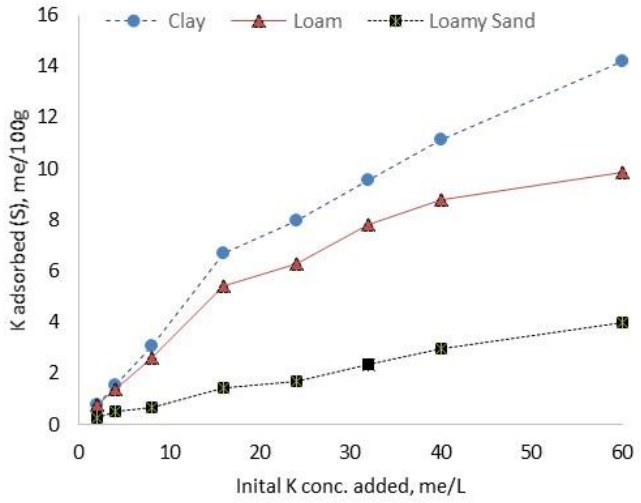


Figure 1. Potassium adsorption of different textural soils.

Langmuir and Freundlich isotherms obtained using the linear relationship between S and C adsorption data for different textural soils are given in Figure 2. The coefficients of the both isotherm (b, k, K*f* and 1/n) and determination values (R^2) were found from the linear relationships (Table 3). Many studies indicated that Freudlich isotherm gave a better fit of equilibrium K adsorption data for clay soil due to having unlimited adsorption sites with heterogeneous surfaces (Hutson and Yang, 2000; Horuz et al., 2017; Auge et al. 2018). On the other hand Langmuir equation had a better fit of equilibrium K adsorption data for coarse textured soils du to the homogeneity of sorption sites in the soil that allows only complete monolayer adsorption of solutes (Auge

et al., 2018). However in this study, the determination values (R^2) indicated that better fits of equilibrium K adsorption data were obtained for clay and loam soils by Langmuir isotherm and for loamy sand soil by Freundlich isotherm (Table 3).

The coefficient of b in Langmuir isotherm indicates the maximum absorption and can be used to estimate of fertilizer amount for unfertilized soil (Rehman, 2004). K*f* coefficient of Freundlich isotherm, which is the ratio of the amount of K in the solid phase to the amount of K solution, is a capability factor indicating that a soil having a higher K*f* has more adsorption capacity than a soil having a lower K*f* (Shayan and Davey, 1978). In this study, b and K*f* coefficients, varied for the soils. According to the b and K*f* values, K adsorption capacity of the soils ordered as clay > loam >loamy sand soil (Table 3). Rehman (2004) reported that k or affinity coefficient of Langmuir isotherm indicates that how easily the added potassium is adsorbed on or release from the adsorbing surface. Mehandi and Taylor (1988) showed that smaller k values had more amount of adsorbed potassium converted to non-exchangeable from either by the formation of crystalline K or by occultation through K ions. In this study, smaller k value (0.09) of clay soil showed that adsorbed K from the added solution converted more tightly bonded on the adsorption sites on clay minerals. Assimakopoulos et al. (1986) reported that if potassium remained more tightly bonded on the adsorption sites, a large portion of adsorption sites of soil occupied and it would tend to produce less steep slope in the log plot. In this study, lower 1/n coefficients for loam (0.59) and clay (0.62) soils indicated that relatively big change in the K solution concentration. However, higher 1/n value for loamy sand (0.76) soil had relatively small change in the K solution concentration. Gregory et al. (2005) reported that a more heterogeneous system would have smaller 1/n value approaching zero. In this study, loam soil having the lowest 1/n value was more heterogeneous than the other soil. Maximum buffering capacity (MBC) of a soil which is a product of the Langmuir constant (k) and adsorption maximum (b), measures the ability of the soil to replenish K ion to soil solution as they tend to be depleted (Holford, 1979; Rehman, 2004). According to the MBC of soils given in Table 3, the ability of soils to supply K ion to soil solution was ordered as clay>loam>loamy sand soil.

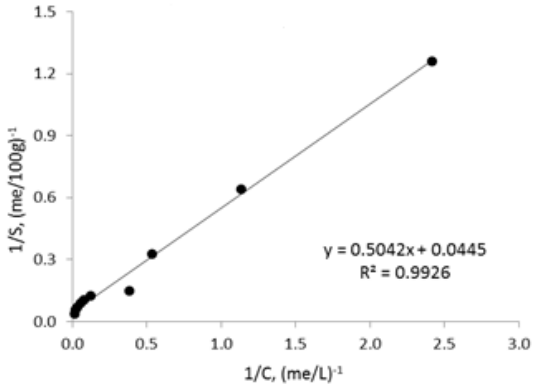
Table 3. The coefficient and determination values of Langmuir and Freundlich Isotherms.

Soils	Langmuir Isotherm				Freundlich Isotherm		
	b	k	MBC*	R^2	K*f*	1/n	R^2
Clay	22.47	0.09	2.02	0.992	1.92	0.62	0.943
Loam	7.85	0.24	1.88	0.963	1.40	0.59	0.960
Loamy Sand	3.76	0.05	0.19	0.975	0.19	0.76	0.983

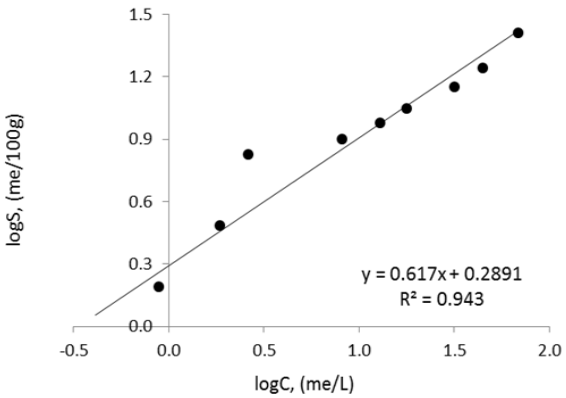
*MBC: Maximum Buffering Capacity.

The correlations between the soil properties and the coefficients of adsorption isotherms are given in Table 4. There was a significant positive correlation (0.894**) between b and Kf coefficients. Similarly, Borling (2003) reported that Langmuir b coefficient and Freundlich proportionality constant (K*f*) values gave a high correlation (r=0.99**). The K absorption capacity coefficients (b and K*f*) of both isotherm similarly gave significant positive correlations with clay, OM, CEC, exchangeable K, Ca and Mg contents and significant negative correlations with pH, sand, $CaCO_3$ contents of the soil samples. However, Loannou et al. (1994) reported positive correlation between pH and K adsorption as a result of formation of new sites available both H and K adsorption. Auge et al. (2018) similarly found that there were significant positive correlations among percent K adsorbed, CEC, clay and pH values. Increasing clay and OM contents in soil also improved soil physicochemical properties (Gülser, 2006; Candemir and Gülser, 2010; Gülser et al. 2015; Gülser and Candemir, 2015) and increased the specific surface area and number of negative sites which absorb and or fix K from soil solution. While high exchangeable K, Ca, Mg and CEC of soils indicate a large number of negative sites in soil for adsorption, high sand and $CaCO_3$ contents indicate a low number of negative sites in soil. It was thought that clay soil having lower pH and higher K adsorption capacity than loam and loamy sand soil caused the significant negative correlation between pH and K adsorption capacity in this study. On the other hand slope of the isotherms represented with k and 1/n had significant positive correlations with EC, loam and exchangeable Na content of soil samples.

Langmuir Isotherms **Freundlich Isotherms**



Clay Soil



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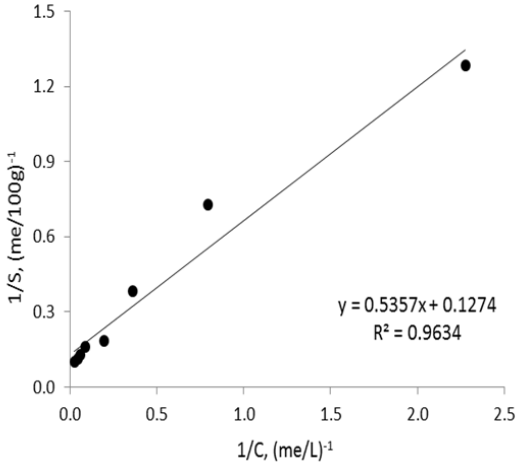
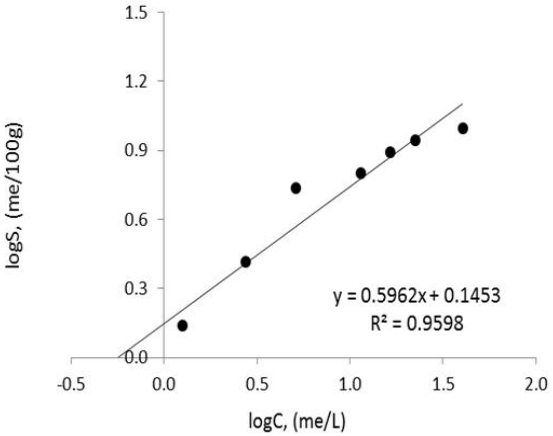
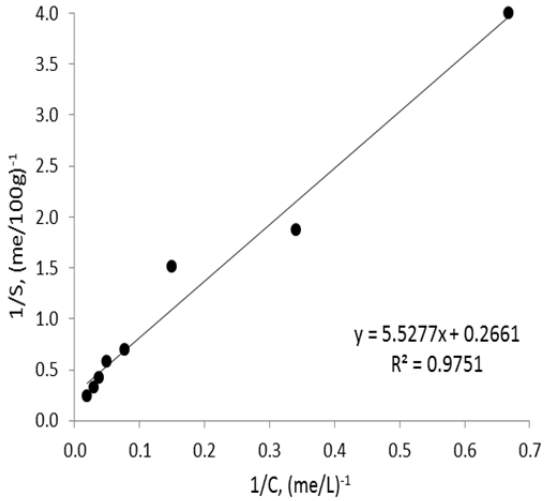


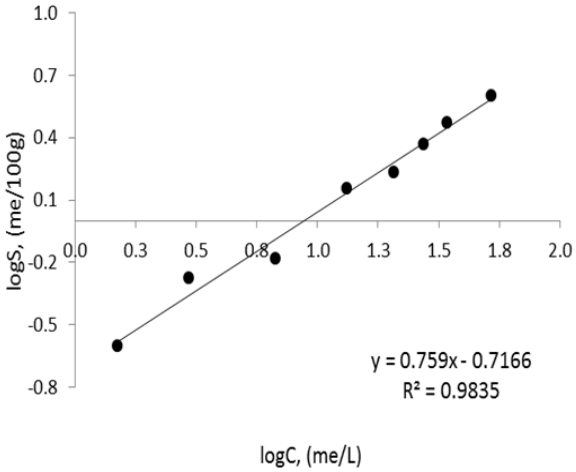
Loam Soil



Loam Soil



Loamy Sand Soil



Loamy Sand Soil

Figure 2. Langmuir and Freudlich Isotherms in different textural soils.

Table 4. Correlation matrix of soil properties

	b	k	K_f	1/n
b	1	-0.094	**0.894****	-0.012
k	-0.094	1	0.353	0.493
K_f	**0.894****	0.353	1	0.163
1/n	-0.012	0.493	0.163	1
Clay	**0.987****	0.006	**0.937****	-0.039
Loam	0.361	**0.882****	**0.716****	**0.577***
Sand	**-0.929****	-0.272	**-0.995****	-0.142
pH	**-0.990****	0.065	**-0.909****	0.087
EC	0.045	**0.969****	0.454	**0.649***
$CaCO_3$	**-0.667***	**-0.674***	**-0.918****	-0.424
OM	**0.949****	0.212	**0.989****	0.101
CEC	**0.979****	0.079	**0.960****	0.010
Na	-0.181	**0.968****	0.241	**0.658***
K	**0.911****	0.320	**0.998****	0.175
Ca	**0.870****	0.406	**0.996****	0.234
Mg	**0.990****	-0.070	**0.907****	-0.090

Conclusions

Potassium adsorption capacities of three different soil samples were analyzed with Langmuir and Freundlich isotherms. The best fit for equilibrium K adsorption data was determined for clay and loam soils using Langmuir isotherm and for loamy sand soil using Freundlich isotherm. According to the K absorption capacities represented with b and K*f* values of the isotherms, K adsorption capacities of the soils ordered as clay > loam > loamy sand soil. Maximum buffering capacity of the soils showed that clay soil had more ability to supply K ion to soil solution than loam and loamy sand soils. Clay soil having smaller 1/n and k coefficients had relatively big change in the K solution concentration and strong K absorption from the added solution on the adsorption sites. Potassium absorption capacity coefficients of the both isotherm had significant positive correlations with clay, OM, CEC, exchangeable K, Ca and Mg contents. Increasing the specific surface area or number of negative sites in soil caused more K absorption from soil solution. Therefore, the potassium fixation mechanisms in different soil types should be taken into consideration for correct and rational K fertilization in sustainable soil management.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Possibilities of Using Stereo Satellite Datas on Soil Surveys

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Abstract: Topographic maps, air photos and satellite data are cartographic material for the soil mapping, which is an important subdivision of the soil science. This material varies or changing depends on current and available technology. Although air photos was used in previous years, satellite data have been using in recent years due to technological advantages and increase in their resolution. Satellite data having high resolution and stereo image advantages supply significant ease for the interpreting the physiographical units. In this study, WorldView-2 satellite data, Microstation V8 and Leica Photogrammetry Suite PRO600 softwares were used and evaluated for the possibilities of using stereo satellite images of detailed soil survey in Adana Yumurtalik province. Using 3D view obtained by stereo satellite data, different soil series and different soil phases especially slope classes could have been delineated by surveyor for determining physiographic unit in large area before field work. Significant similarity between interpreted boundaries using a stereo data and actual soil boundaries was observed. It was concluded that, WorldView-2 satellite data's which have high spatial resolution and stereo view are extremely useful cartographic materials which can be used in a detailed soil survey.

Keywords: Adana, detailed soil survey, stereo satellite data, WorldView-2.

Stereo Uydu Görüntülerinin Toprak Etdülerinde Kullanılma Olanakları

Özet: Toprak biliminin önemli bir kolu olan toprak haritalama çalışmalarında kullanılan altlık materyaller güncel teknoloji ve olanaklara göre değişmekle birlikte topoğrafik harita, hava fotoğrafı ve uydu görüntüleridir. Önceki yıllarda daha çok hava fotoğrafları kullanılmasına karşın son yıllarda teknolojik gelişmelere bağlı olarak uydu görüntüleri kullanılmaya başlanmıştır. Toprak etütlerinde yersel çözünürlüğün üst düzeyde olduğu uydu görüntülerinin stereo görüntü verebilme özelliğinin de olması araştırıcılara fizyoğrafyanın yorumlanmasında önemli kolaylıklar sağlamıştır. Çalışmada, stereo uydu görüntülerinin detaylı toprak etütlerinde kullanımının Adana ili Yumurtalık örneğinde değerlendirilmesi amacıyla WorldView-2 uydu görüntüsü ve Microstation V8 ve Leica Photogrammetry Suite PRO600 yazılımları kullanılmış ve avantajları değerlendirilmiştir. Stereo uydu görüntüsünden yararlanılarak, geniş alanlarda fizyoğrafik birimlerin belirlenmesi ile farklı toprak serileri ve başta eğim olmak üzere önemli toprak fazları arasındaki sınırlar stereo görüş sağlanarak arazi öncesi çizilebilmiştir. Üç boyutlu görüş altında laboratuvarda çizilen geçici sınırlar ile arazi çalışması sonucu kesinleştirilen toprak sınırları arasında önemli düzeyde uyum elde edilmiştir. Sahip olduğu yüksek yersel çözünürlük ve stereo görüntü sağlaması açısından WorldView-2 uydu verilerinin, detaylı toprak etüt haritalama çalışmalarında kullanılabilecek önemli bir kartografik materyal olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Adana, Detaylı toprak etüdleri, Stereo uydu görüntüleri, WorldView-2.

Introduction

An important use of Remote Sensing (RS) and Geographic Information Systems (GIS) techniques is soil survey and mapping studies. Soil survey is a systematic study of the soil of an area including classification, determination of the properties and the distribution of various soil units (Soil Survey Division Staff, 1993; Dinc and Senol, 2009). In soil survey studies, the basic philosophy of periodic processes and applications remained unchanged, but material and/or methodology improved depending on the used technology.

Soil mapping studies have three different periods. Soil mapping studies began a new turn in the early 1980s, driven by improvements in computer technology. Digital mapping has begun with the use of computer aided cartographic materials and GIS technology. In this period, mapping studies using only topographical maps and partly aerial photographs have been predominantly left to satellite images, Global Positioning System (GPS) and GIS. Since 1980, digital mapping techniques have been developed along with technological developments and

modeling has started to be used. At the beginning of the 2000s, high-resolution satellite imagery began to be used on almost all of the detailed studies on the land with the acquisition of satellite images down to a resolution of less than 1 m. However, the most important development in recent years has been the launched of stereo satellites. The current WorldView-2 stereo satellite image used in the study has a high sensor resolution of 0.46 m; as well as the technology that will allow stereo viewing as the most important distinction from some other satellites. Many of the studies in this context have been based on either digital elevation model (DEM) extraction or erosion modeling. This development enabled satellite imagery to be used instead of aerial photographs in soil mapping studies.

The need of the detailed soil survey and mapping of soils of Turkey at the level of soil series and phase, is unavoidable. But, nowadays there is no any institutions responsible to do this work, lack of infrastructure and technical staff were taken into consideration. For this reason, there is urgent need to develop new techniques and methods that can be used at every stage of soil study, which will reduce the need for specialists, decrease the time spent on the ground, improve the quality of soil studies by using the latest techniques in remote sensing and geographic information systems. In this study, it is aimed to, using remote sensing and GIS and 3D technology, minimizing the specialist initiative and increasing the quality of detailed soil survey mapping studies.

Materials and Methods

Materials

The study area is the Yumurtalık province and its surrounding area of Adana City (Figure 1). An actual 3D WorldView-2 satellite image on November was used. The most important material is the 3D satellite image as a panchromatic and multispectral of the Yumurtalık (Figure 2). Erdas IMAGINE 9.1, ArcGIS 10.0, Microstation V8 and Leica Photogrammetry Suite (LPS) PRO600 software were used for the transfer, identification and evaluation of the data in the Geographic Information Systems. NVIDIA 3D display that allows 3D viewing, hardware compatible mouse and glasses are used as hardware.

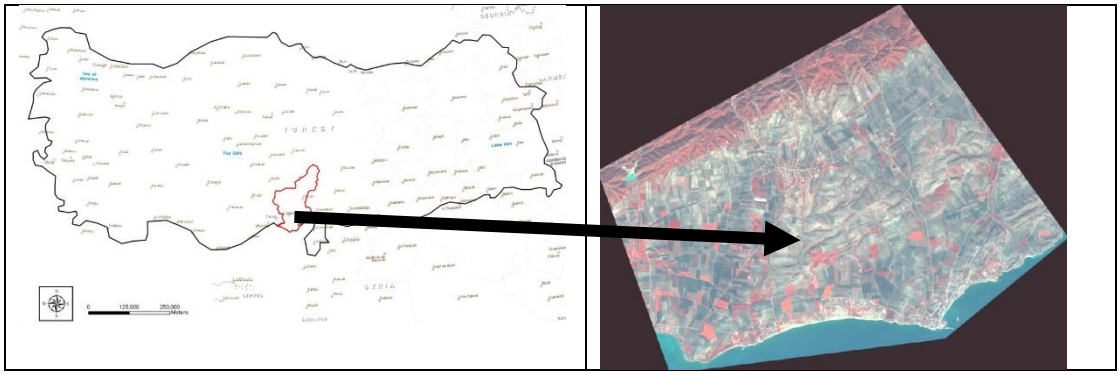
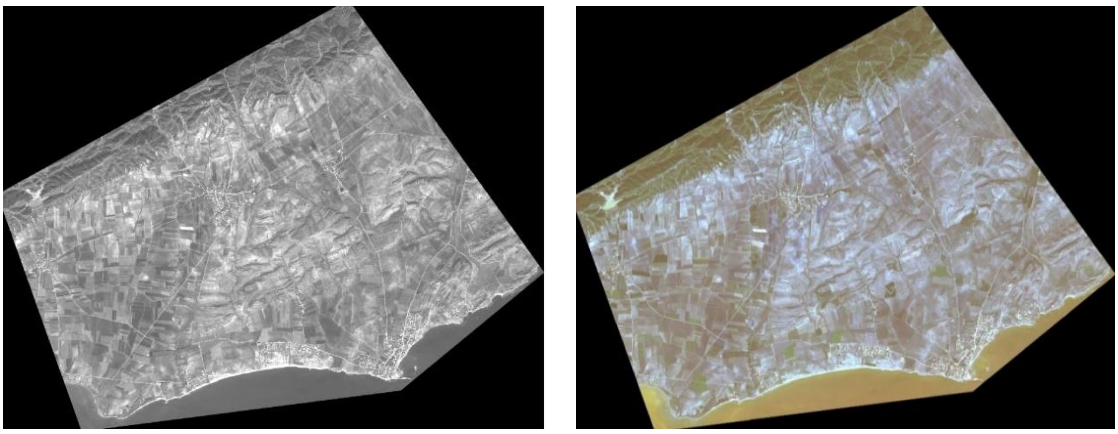


Figure 1. Location of the study area



(a) (b)

Figure 2. WorldView-2 images of the study area (a: Panchromatic, b: Multispectral)

General properties of study area

Yumurtalık province is a seaside town located 81 km southwest of Adana City. The district has an area of 501 km^2; of which 191 km^2 consists of non-agricultural land (settlement, forest, stony, sand dune, etc.). There is a total of 55 km of coastline (Anonymus, 2018).

Agriculture is one of the most important economic sectors (maize, sunflower, cotton, soybean and citrus) of the study area. The natural vegetation in study area: Myralus Comminus, Potersum Spinopsum, Phylera Latiblia, Nerium Oleander, Spartium Junseum, Pistacia Terebintus, Pistacia Lentiscus, Quercus Coccifera (Senol and Alagöz, 1979).

Method

First of all, the suitable time for satellite images to be acquired for the study area is determined. Due to the climate characteristics and agricultural activities of the area, the most suitable period for the acquiring satellite data has been decided to be between 15 October and 15 November. The activities for land use in this period are the soil surface is usually empty, on between sowing and seedling stage. The winter products do not cover the soil surface. In addition to this, the period in which there is a minimum of cloudy weather and precipitation.

Microstation V8 and Leica Photogrammetry Suite (LPS) PRO600 were used to create a stereo satellite image and mono image transferred 3D image blocks.

Results and Discussion

Aerial photographs were often used as the most crucial cartographic material in recent years. The soil survey specialist uses the assumption that the soil properties change in the areas where the slope changes, and identifies possible soil boundaries and transfers them to the aerial photograph (Lillesand and Kiefer, 1979). At this stage, the use of stereoscopes and the interpretation of photographs require a certain level of knowledge and experience. Moreover, it has several problems and difficulties, such as the hard-copy of aerial photographs, the lack of geographical correction of photographs, the relatively large scale errors, and the many numbers of aerial photographs, depending on the size of the working area. Mainly working with many numbers of aerial photographs under the stereoscope brings with it significant difficulties in preparing the print map.

However, in recent years, high-resolution satellite images have also begun to provide stereo vision, and these advantages of aerial photographs have come to an end. Rather than interpret many numbers of aerial photographs under a stereoscope, it is easier to see a single satellite image in 3D and to interpretation it in as a digital. According to the classical methodology, a less experienced person can also obtain more accurate possible boundary on the satellite image. Since the possible boundaries are made directly in the GIS, various sources of error also go away. Besides, the possible boundary can be determined more efficiently and accurately, for the convergence of the image can be made in the course of interpretation of the satellite image.

The WorldView-2 satellite data used in the study has been utilized at the highest level of advantage in this study of work because it allows both high resolution and stereo image. Stereo satellite data is created in a stereo computer environment with the aid of suitable hardware and software. This image allows the easy identification and interpretation of the physiographic units in the study area.

In this phase, firstly, the boundaries of the physiographic units are drawn on the outline on the 3D image which is worked on a small scale. Then, depending on the approximation of the "absolutely varying soil properties everywhere the slope changes" in the image, the places where the slope changes on the scaled-up image are interpreted with other image characteristics and digitized by creating polygons directly on the computer. (Fig. 3).

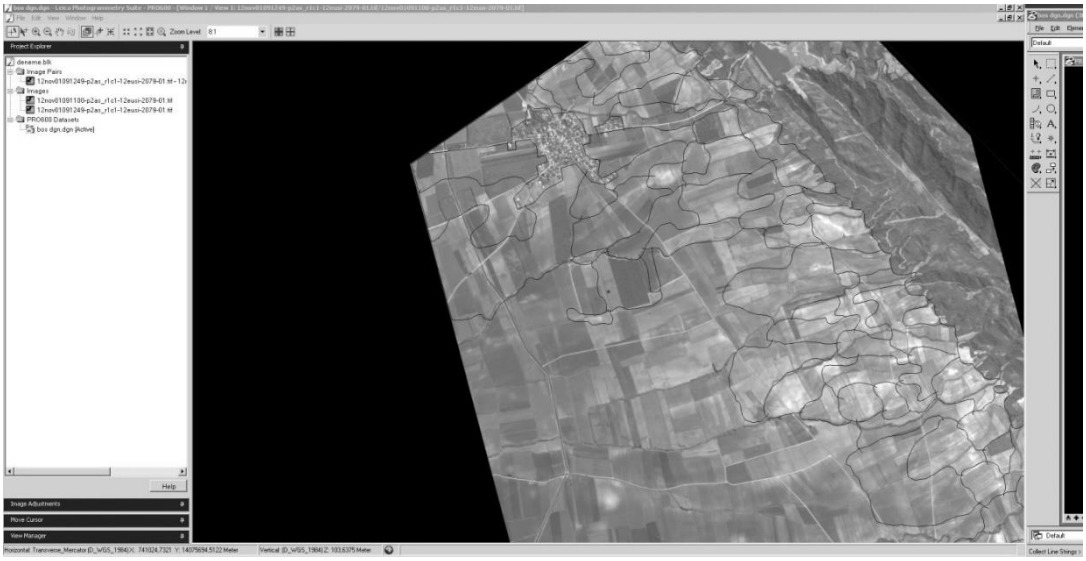


Figure 3. Screenshot on soil boundary on determination with Microstation and LPS

Conclusion

The actual stereo WorldView-2 satellite image of November 2012, used as the primary cartographic material in the study, allows for a high level of use in detailed soil survey and mapping studies regarding technical specifications. One of the most favorable views of the WorldView-2 satellite image is the possibility of stereo imaging. In a detailed soil survey, the effect of topography is too high on soil boundaries, so it is essential for this property. A considerable part of our land is located in sloping land. The topography and slope are interpreted very quickly by the surveyors who have land experience together with the necessary geomorphology information on the stereo satellite image, and possible soil boundaries are determined. In this study, the results obtained by interpretation of satellite data in the laboratory have reached to the result that the boundaries comply with to the soil boundaries with high accuracy even in the area. Thus, dramatically shortens the time spent on land and allows for the temporal sawing in an area.

The visualization of the stereo image of the Virtual GIS module in Erdas IMAGINE software used for interpretation of stereo satellite images is sufficient. However, with this version of the software drawing soil boundaries is not possible on satellite data. The Microstation and LPS software, which have the highest performance in the study, are both viewed as stereo and interpreted. Also, the ability to scale up and scale down when necessary in the software also provides great convenience in interpretation. With this feature, the topography can be seen and defined as a whole on a small scale, while drawing the soil boundaries can be reduced to a minimum by working on a large scale.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effect of Drought Stress on Sensitivities and Yields of Chickpea (*Cicer arietinum* L.) Cultivars

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Abstract: In the selection researches, under the changing climatic conditions, it should be studied in which growth stages of plants and how they will react to drought. The yield of the cultivated plant is one of the most important traits affected by drought. In this study, it was aimed to determine the sensitivities of eleven chickpea cultivars against drought and to compare their yield traits in different growth stages by establishing two field experiments (conditions, FEs) in 2007-2008. A group of cultivars was grown under natural rainfall conditions, while the other was irrigated before the blooming stage due to early drought in 2007 and during the blooming stage due to late drought in 2008. The morphological traits and drought susceptibility index of cultivars were investigated. In experiments, yields of cultivars decreased substantially and their susceptibilities changed according to the growth stages exposed to drought. The pod and seed numbers of plants, seed yield and biological yield traits of cultivars gave more reliable results than 100 seeds weight and harvest index traits in the selection of susceptible and resistant cultivars. It was concluded in this study that a parameter among the selection criteria accepted for a plant species or variety could prevent from reaching a clear judgment due to the severity and duration of stress exposed in different growth stages, and also due to the genetic characteristics of the plant. It is recommended to make the description of susceptible/resistant plant species, variety or genotype, according to the growth stage of plants exposed to the stress in the following selection studies.

Keywords: Drought, chickpea, drought susceptibility index, yield traits.

Nohut (*Cicer arietinum* L.) Çeşitlerinin Verim ve Hassasiyetleri Üzerine Kuraklik Stresinin Etkisi

Özet: Tarımsal açıdan tüm bitkilerde olduğu gibi nohut bitkisinde, kuraklık stresinden etkilenen en önemli parametre verimdir. Değişen iklim koşullarında bitkilerin kuraklık stresine, gelişimlerinin hangi evresinde ne tepki vereceği bilinmelidir. Bu çalışma ile, 2007-2008 yıllarında iki tarla denemesi kurularak farklı dönemlerde oluşabilecek olan strese karşı nohut çeşitlerinin hassasiyetlerinin belirlenmesi ve verim parametrelerinin karşılaştırılması amaçlanmıştır. Bir grup nohut çeşidi doğal yağış koşullarında sulama yapılmaksızın yetiştirilmiş, diğer bir grup ise 2007 yılında kuraklığın erken dönemde yaşanması nedeniyle çiçeklenme öncesinde, 2008 yılında ise kuraklığın geç yaşanması nedeniyle hem çiçeklenme öncesi hem de çiçeklenme döneminde sulanarak yetiştirilmiştir. Nohut çeşitlerinde bitkide bakla sayısı, bitkide tane sayısı, biyolojik verim, tane verimi, 100 tane ağırlığı, hasat indeksi ve kuraklık hassasiyet indeksi incelenmiştir. Her iki denemede de, nohut çeşitlerinin verimlerinde kuraklık stresi sonucu büyük oranda azalmalar görülmüştür. İncelenen tüm parametreler, her iki yılda da sulu ve kuru koşullarda yetiştirilen çeşitler arasında önemli farklılıklar göstermiştir. Kuraklığa maruz kaldığı dönemler açısından çeşitlerin kuraklığa hassasiyetlerinin değiştiği belirlenmiştir. Kurağa hassas ve dayanıklı çeşitlerin seçiminde 100 tane ağırlığı ve hasat indeksi parametrelerine göre bitkide bakla sayısı, bitkide tane sayısı, tane verimi ve biyolojik verim parametrelerinin daha güvenilir sonuçlar verdiği belirlenmiştir. Bu çalışmada, seleksiyon kriterleri arasında bir bitki türü ya da çeşidi için kabul gören bir parametrenin, bitkilerin farklı dönemde karşılaşacağı stresin şiddeti ve süresi ile birlikte farklı genetik özellikleri nedeniyle net bir yargıya varmayı engelleyebileceği sonucuna varılmıştır. İlerleyen dönemlerde seleksiyon çalışmalarında bir bitki türü için kuraklığa dayanıklı ya da hassas olduğu tespit edilirken, maruz kaldığı stresin dönemine dikkat edilerek sınıflandırılması tavsiye edilmektedir.

Anahtar kelimeler: Kuraklık, nohut, kuraklık hassasiyet indeksi, verim parametreleri

Introduction

Chickpea (*Cicer arietinum* L.) is an important food source worldwide and is the fourth place in terms of both production and yield among the legume plants, an according to the latest statistics set in 2016 (FAO 2018). The drought stress is one of the most limiting environmental factors for chickpea because it is grown under rainfed condition in major cultivated areas, and also is traditionally planted towards either the end or middle of the rainy season (March or April). The development of vegetative biomass for chickpea is very sensitive to drought. So, chickpea production is seriously affected by drought conditions due to the very short growing season, especially during the bloom and pod filling stages due to generally declining soil moisture and increasing temperature in these periods (Ganjeali et al. 2011). It is known that the reduction in chickpea production due to drought stress is quite severe. Globally, it is estimated to be around 33% according to Kashiwagi et al. (2015).

It is scientifically accepted as a result that chickpea yield loss due to drought effects is due to changes in many morphological, physiological and biochemical activities, especially the decrease in photosynthetic rates of plants. Reductions in yield due to drought may occur due to decreasing of photosynthesis or leaf expansion rate (Turner and Begg 1981), or due to decreasing of the stoma resistance and transpiration rate (Llorens et al. 2003; Sardans et al. 2008) or together. These facts directly affect the photosynthetic performance and therefore the productivity levels of the plants. Chickpea has genetic differences in the accumulation and distribution of photosynthetic dry matter, seed filling rate and duration (Davies et al. 1999) under drought stress condition or non-stressed. These issues have been demonstrated by previous studies about the drought-related yield and yield traits of chickpea. (Turner et al. 2001; Stoddard et al. 2006; Toker et al. 2007). Bruckner and Frohberg (1987) have identified that drought susceptibility index (DSI) could be used as a measure of drought tolerance. According to Blum (1988), screening for drought resistance among cultivars must be conducted based on high performance in stressed and non-stressed conditions, so genotypes that have a high yield in both stressed and non-stressed conditions are drought resistant. According to researchers, varieties with low DSI are expected to be more resistant to drought, because resistant cultivar shows the minimal reduction in yield caused by unfavorable compared with favorable environments. However, it is argued that only the DSI has a very serious limitation in terms of measuring the reactivity of the varieties to drought, because this index is due to the most reduced yield reduction in stress conditions (Denćić et al. 2000). For this reason, the DSI should be evaluated together with other selection parameters.

Despite the identifications of drought resistance physiological and biochemical mechanisms in many crops (Sairam and Saxena 2000; Turner et al. 2001; Nayyar et al. 2005; Nayyar et al. 2006; Gunes et al 2007; Guneri Bagci 2010), plant breeders are still largely guided by seed yield and its stability under dry conditions in selecting for drought resistance, because of low budget. Cekic (2007) suggested that DSI could be used for large breeding materials in arid conditions due to the convenience of application and economics in studying the effects of drought stress on wheat cultivars under field conditions. The consequence, this paper concentrates on the yield, yield characteristics and DSI results of cultivars, due to the differences in genetical and yield potential. Moreover, under the changing climatic conditions, it should be known how they will react to drought in which growth stages of plants. This may be assisted breeders to combine selectively some of these attributes into high yielding chickpea cultivars under drought stress. Therefore, the aim of this study was to determine the sensitivities of chickpea cultivars against drought and to compare some morphological traits in different growth stages by establishing two field experiments.

Materials and Methods

Eleven chickpea (*Cicer arietinum* L.) cultivars (Table 1) widely preferred in Turkey were grown under a natural rainfed condition in the Haymana Research and Application Farm of Ankara University Faculty of Agriculture during two growing seasons of 2007-2008 in between April and July. The experiments were carried out based on a randomized complete blocks design with four replications. For basal fertilization, 2.17 kg da^{-1} of diamoniumphosphate (DAP) per parcel (3x4 m) was applied before one week from sowing. In the FES, two groups of chickpea cultivars were used for treatments: First group (11 cultivars) cultivars were grown under natural rainfall conditions in both years. The second group (11 cultivars) was irrigated in the vegetative stage and in the starting of blooming stage (totally 34 mm m^{-2}) in 2007. But it was irrigated once during the blooming stage (32 mm m^{-2}) in 2008, because of enough participation in the vegetative stage.

The morphological traits and drought susceptibility index of cultivars were investigated at the end of the growing season. Some of the traits including the number of pods and seeds per plant, hundred (100) seeds weights, seed yield, biological yield, harvest index were measured in 50x60 cm of every parcel in each years and yield was

converted to kg da^{-1}. Drought susceptibility index (DSI) was calculated according to Fischer and Maurer (1978) for each genotype as: DSI = (1-Yds/Yno) / (1-Xds/Xno), where Yds: the biological yield under drought, Yno: the biological yield under near optimum conditions, Xds: the average biological yield of all cultivars under drought, Xno: the average biological yield of all cultivars under near optimum conditions. Analyzes of variance were performed on the data and were analyzed by ANOVA. The significant differences among treatment means were compared by descriptive statistics (±SE).

Table 1. Some characteristics of chickpeas used in the experiment (Anonymous 2008)

Cultivars	***Improvement Center***	***Registry year***	***Hundred (100) seeds weight (g)***	***Sensitivity Status****
Akçin	Field Crops Central Research Institute	1991	40	Anthracnose (T)
Küsmen	Field Crops Central Research Institute	1999	49-53	Anthracnose (MR)
Canıtez-87	Eskisehir Transitional Zone Agricultural Research Institute	1987	45-55	Anthracnose (S) Wilt, rust (R)
Gökçe	Field Crops Central Research Institute	1997	44-46	Anthracnose (MR)
Sarı	Aegean Agricultural Research Institute	1998	46-54	Anthracnose (MS)
Uzunlu-99	Field Crops Central Research Institute	1999	47-53	Anthracnose (T)
Er-99	Field Crops Central Research Institute	1999	45-50	Anthracnose (MR)
ILC-195	ICARDA	-	32-34	Anthracnose (T) Drought (R)
Menemen-92	Aegean Agricultural Research Institute	1992	38-45	Anthracnose (T) Wilt, rust (T)
İzmir-92	Aegean Agricultural Research Institute	1992	38-45	Anthracnose (T) Wilt, rust (T)
Aydın-92	Aegean Agricultural Research Institute	1992	34-39	Anthracnose (R) Wilt (MR)

* (T)=Tolerant , Resistant (R), Medium Resistant (MR), Susceptible (S), Medium Susceptible (MS)

Results and Discussion

The climatic data during periods of FEs in 2007 and 2008 were taken from the research station and compared to the precipitation and temperature averages of the region for many years (19 years). According to this, the precipitation amount decreased by 48.5 % and 32.3 % in 2007 and 2008, respectively. The precipitation amounts were 19.9 and 66.2 mm m^{-2} in the vegetative stage, and 39.4 and 15.9 mm m^{-2} during and after the blooming stage, respectively in 2007 and 2008. The air temperature compared with averages for many years increased by 4°C in the vegetative stage, 2°C during the bloom stage, 2.4°C in the harvest stage in 2007. In 2008, it decreased in the vegetative stage, but increased by 1.2°C and 0.5°C during and after the blooming stage, respectively. In the vegetation period, the total amount of precipitation in natural rainfall conditions (drought) was around 85 mm m^{-2} and 134 mm m^{-2} in 2007 and 2008, respectively. In this fact that it was observed that the drought season was severely in a vegetative stage in 2007, and plants were exposed to stress for a longer time. In other words, drought conditions in 2007 were more extreme than in 2008.

In experiments, yields of cultivars decreased substantially and their susceptibilities changed according to the growth stages exposed to drought (Table 2 and 3). In the FEs carried out in 2007 and 2008, a number of pods and seeds per plant and seed yield showed significant differences and decreasings among chickpea cultivars grown in both watered and drought conditions. Similar results reported for chickpea by Davies et al. (2000). As a result of comparison between years, it was seen that the number of pods per plant, number of seeds per plant and seed yield were higher in the drought conditions in 2008 than in 2007 (respectively, Figure 1 and 2). This can be explained by the fact that there was no rainfall in the flowering and pod-binding period (in May) in 2007, but the precipitation was by around 30 mm in 2008. During the seed filling period, it was thought to be effective in loss of yield in chickpea because of the reduced seed size due to limited water (Leport et al. 1998).

Table 2. The number of pods and seeds per plant and seed yields of cultivars in 2007 and 2008 growing season (W, watered condition; D, drought condition).

Cultivars	Number of pods per plant			Number of seeds per plant			Seed yields, kg da^{-1}		
2007	**W**	**D**	**Means**	**W**	**D**	**Means**	**W**	**D**	**Means**
Akçin	$6.29^{\pm0.05}$	$5.03^{\pm0.46}$	$\mathbf{5.66^{\pm0.32}}$	$5.58^{\pm0.16}$	$4.64^{\pm0.47}$	$\mathbf{5.11^{\pm0.29}}$	$130^{\pm5.35}$	$114^{\pm12.0}$	$\mathbf{122^{\pm6.77}}$
Küsmen	$1.51^{\pm0.29}$	$1.19^{\pm0.21}$	$\mathbf{1.35^{\pm0.18}}$	$1.04^{\pm0.17}$	$0.66^{\pm0.28}$	$\mathbf{0.85^{\pm0.17}}$	$20.0^{\pm3.63}$	$14.9^{\pm6.47}$	$\mathbf{17.5^{\pm3.56}}$
Canıtez-87	$1.08^{\pm0.17}$	$0.80^{\pm0.14}$	$\mathbf{0.94^{\pm0.12}}$	$0.79^{\pm0.14}$	$0.28^{\pm0.04}$	$\mathbf{0.53^{\pm0.15}}$	$16.9^{\pm1.96}$	$4.80^{\pm1.45}$	$\mathbf{10.8^{\pm2.55}}$
Gökçe	$0.91^{\pm0.08}$	$0.20^{\pm0.05}$	$\mathbf{0.56^{\pm0.14}}$	$0.51^{\pm0.08}$	$0.05^{\pm0.00}$	$\mathbf{0.28^{\pm0.10}}$	$10.9^{\pm2.23}$	$0.68^{\pm0.27}$	$\mathbf{5.78^{\pm2.19}}$
Sarı	$0.98^{\pm0.23}$	$0.63^{\pm0.21}$	$\mathbf{0.80^{\pm0.16}}$	$0.61^{\pm0.14}$	$0.34^{\pm0.09}$	$\mathbf{0.48^{\pm0.09}}$	$14.6^{\pm3.98}$	$6.58^{\pm1.85}$	$\mathbf{10.6^{\pm2.53}}$
Uzunlu-99	$2.11^{\pm0.21}$	$1.20^{\pm0.28}$	$\mathbf{1.66^{\pm0.24}}$	$1.74^{\pm0.19}$	$0.88^{\pm0.22}$	$\mathbf{1.31^{\pm0.21}}$	$37.4^{\pm3.14}$	$15.7^{\pm4.39}$	$\mathbf{26.6^{\pm4.80}}$
Er-99	$2.96^{\pm0.29}$	$0.60^{\pm0.11}$	$\mathbf{1.78^{\pm0.47}}$	$2.35^{\pm0.38}$	$0.35^{\pm0.08}$	$\mathbf{1.35^{\pm0.41}}$	$54.6^{\pm9.03}$	$6.89^{\pm1.49}$	$\mathbf{30.8^{\pm9.97}}$
ILC-195	$11.3^{\pm0.77}$	$1.79^{\pm0.33}$	$\mathbf{6.56^{\pm1.84}}$	$11.0^{\pm0.61}$	$1.03^{\pm0.29}$	$\mathbf{5.99^{\pm1.90}}$	$168^{\pm9.28}$	$15.0^{\pm4.49}$	$\mathbf{91.3^{\pm29.2}}$
Menemen-	$10.5^{\pm0.94}$	$0.90^{\pm0.46}$	$\mathbf{5.69^{\pm1.88}}$	$9.30^{\pm0.83}$	$0.65^{\pm0.39}$	$\mathbf{4.98^{\pm1.69}}$	$196^{\pm15.7}$	$11.1^{\pm6.83}$	$\mathbf{104^{\pm35.9}}$
İzmir-92	$3.25^{\pm0.68}$	$0.28^{\pm0.04}$	$\mathbf{1.76^{\pm0.65}}$	$2.70^{\pm0.56}$	$0.09^{\pm0.03}$	$\mathbf{1.39^{\pm0.56}}$	$54.7^{\pm13.6}$	$1.13^{\pm0.59}$	$\mathbf{27.9^{\pm11.9}}$
Aydın-92	$6.99^{\pm0.79}$	$1.30^{\pm0.20}$	$\mathbf{4.14^{\pm1.14}}$	$5.86^{\pm0.92}$	$0.80^{\pm0.19}$	$\mathbf{3.33^{\pm1.05}}$	$117^{\pm19.7}$	$14.7^{\pm3.79}$	$\mathbf{65.8^{\pm21.5}}$
Means	$\mathbf{4.35^{\pm0.57}}$	$\mathbf{1.26^{\pm0.21}}$		$\mathbf{3.77^{\pm0.55}}$	$\mathbf{0.89^{\pm0.20}}$		$\mathbf{74.5^{\pm10.0}}$	$\mathbf{18.7^{\pm4.86}}$	
F treatment	316**			320**			278**		
F cultivar	60.7**			63.3**			56.3**		
Finteraction	38.5**			42.5**			32.4**		
2008									
Akçin	$7.43^{\pm0.50}$	$3.03^{\pm0.88}$	$\mathbf{5.22^{\pm0.96}}$	$6.43^{\pm0.55}$	$2.49^{\pm0.75}$	$\mathbf{4.46^{\pm0.86}}$	$160^{\pm11.2}$	$69.4^{\pm19.6}$	$\mathbf{114^{\pm20.0}}$
Küsmen	$1.16^{\pm0.19}$	$0.73^{\pm0.13}$	$\mathbf{0.94^{\pm0.14}}$	$0.29^{\pm0.11}$	$0.08^{\pm0.03}$	$\mathbf{0.18^{\pm0.07}}$	$5.95^{\pm2.86}$	$0.87^{\pm0.45}$	$\mathbf{3.41^{\pm1.65}}$
Canıtez-87	$6.30^{\pm1.05}$	$3.03^{\pm0.24}$	$\mathbf{4.66^{\pm0.79}}$	$5.64^{\pm0.99}$	$2.54^{\pm0.23}$	$\mathbf{4.09^{\pm0.75}}$	$172^{\pm34.3}$	$78.1^{\pm6.37}$	$\mathbf{125^{\pm23.9}}$
Gökçe	$7.38^{\pm0.99}$	$4.48^{\pm0.46}$	$\mathbf{5.93^{\pm0.75}}$	$6.29^{\pm0.93}$	$3.73^{\pm0.33}$	$\mathbf{5.01^{\pm0.67}}$	$158^{\pm15.8}$	$103^{\pm11.2}$	$\mathbf{131^{\pm13.8}}$
Sarı	$8.34^{\pm0.69}$	$7.71^{\pm0.77}$	$\mathbf{8.03^{\pm0.49}}$	$7.19^{\pm0.60}$	$7.09^{\pm0.74}$	$\mathbf{7.14^{\pm0.44}}$	$229^{\pm23.5}$	$224^{\pm22.1}$	$\mathbf{227^{\pm15.0}}$
Uzunlu-99	$6.89^{\pm0.76}$	$6.14^{\pm0.43}$	$\mathbf{6.51^{\pm0.43}}$	$5.24^{\pm0.65}$	$4.89^{\pm0.45}$	$\mathbf{5.07^{\pm0.37}}$	$148^{\pm19.1}$	$138^{\pm12.6}$	$\mathbf{143^{\pm10.7}}$
Er-99	$7.69^{\pm0.53}$	$1.74^{\pm0.83}$	$\mathbf{4.71^{\pm1.21}}$	$6.79^{\pm0.40}$	$1.10^{\pm0.62}$	$\mathbf{3.94^{\pm1.13}}$	$179^{\pm10.8}$	$27.9^{\pm16.4}$	$\mathbf{103^{\pm29.9}}$
ILC-195	$5.93^{\pm0.33}$	$6.70^{\pm0.39}$	$\mathbf{6.31^{\pm0.28}}$	$6.50^{\pm0.83}$	$4.41^{\pm0.61}$	$\mathbf{5.46^{\pm0.62}}$	$108^{\pm13.7}$	$69.9^{\pm9.54}$	$\mathbf{88.9^{\pm10.5}}$
Menemen-	$2.10^{\pm0.19}$	$1.54^{\pm0.22}$	$\mathbf{1.82^{\pm0.17}}$	$1.04^{\pm0.13}$	$0.21^{\pm0.10}$	$\mathbf{0.63^{\pm0.17}}$	$21.0^{\pm2.31}$	$3.36^{\pm1.97}$	$\mathbf{12.2^{\pm3.61}}$
İzmir-92	$3.89^{\pm0.65}$	$1.63^{\pm0.49}$	$\mathbf{2.76^{\pm0.57}}$	$2.18^{\pm0.38}$	$0.54^{\pm0.25}$	$\mathbf{1.36^{\pm0.37}}$	$47.4^{\pm8.01}$	$10.9^{\pm5.23}$	$\mathbf{29.2^{\pm8.19}}$
Aydın-92	$1.40^{\pm0.36}$	$0.00^{\pm0.00}$	$\mathbf{0.70^{\pm0.31}}$	$0.53^{\pm0.21}$	$0.00^{\pm0.00}$	$\mathbf{0.26^{\pm0.14}}$	$8.98^{\pm3.45}$	$0.00^{\pm0.00}$	$\mathbf{4.49^{\pm2.33}}$
Means	$\mathbf{5.32^{\pm0.43}}$	$\mathbf{3.34^{\pm0.40}}$		$\mathbf{4.37^{\pm0.43}}$	$\mathbf{2.46^{\pm0.36}}$		$\mathbf{112^{\pm12.2}}$	$\mathbf{66.0^{\pm10.6}}$	
F treatment	64.5**			70.2**			58.5**		
F cultivar	35.5**			40.2**			49.2**		
Finteraction	5.96**			5.59**			5.51**		

**: $p<0.01$

The highest number of pods and seeds per plant and seed yields in drought conditions were obtained from cvs. Akçin and ILC-195 in 2007 and cvs. Sarı, Uzunlu-99, ILC-195, and Gökçe in 2008. Whereas, the reduction of these traits due to the drought effect was highest in cvs. ILC-195, Menemen-92, Aydın-92, and İzmir-92 in 2007 and in cvs. Aydın-92, Er-99, Küsmen, Menemen-92, and İzmir-92 in 2008.

In the FEs carried out in 2007 and 2008, hundred seeds weight of cultivars showed significant differences and generally decreasings (Figure 2). Similar results reported by Ali et al. (1998). These researchers concluded that 100 seeds weight was the best indicator of seed yield and seed growth which were the most important yield determining factors and that the 100 seeds weight of varieties under water-stressed was very low when compared to the watering conditions and that the reduction of the 100 seeds weight in stress conditions might be due to the fact that the photosynthetic translocation was much lower in developing seed in pod and that the different responses of the varieties to water stress could be attributed to their genetic properties.

Table 3. The hundred (100) seeds weight, biological yields and harvest indices of cultivars in 2007 and 2008 growing season (W, watered condition; D, drought condition).

Cultivars	**Hund. (100) seeds weight, g**			**Biological yields, kg da^{-1}**			**Harvest index, %**		
2007	**W**	**D**	**Means**	**W**	**D**	**Means**	**W**	**D**	**Means**
Akçin	34.9$^{\pm0.91}$	36.8$^{\pm0.57}$	**35.9$^{\pm0.62}$**	269$^{\pm8.42}$	225$^{\pm18.4}$	**247$^{\pm12.5}$**	48.4$^{\pm2.35}$	50.4$^{\pm1.30}$	**49.4$^{\pm1.30}$**
Küsmen	28.6$^{\pm1.72}$	33.4$^{\pm1.07}$	**31.0$^{\pm1.30}$**	114$^{\pm12.0}$	104$^{\pm7.40}$	**109$^{\pm6.83}$**	17.1$^{\pm1.96}$	13.4$^{\pm4.75}$	**15.3$^{\pm2.48}$**
Canıtez-87	33.9$^{\pm3.56}$	24.6$^{\pm3.54}$	**29.3$^{\pm2.91}$**	78.4$^{\pm5.73}$	79.0$^{\pm3.03}$	**78.7$^{\pm3.01}$**	21.5$^{\pm1.59}$	5.92$^{\pm1.58}$	**13.7$^{\pm3.11}$**
Gökçe	31.1$^{\pm1.77}$	20.5$^{\pm8.06}$	**25.8$^{\pm4.31}$**	71.0$^{\pm10.2}$	39.5$^{\pm2.68}$	**55.2$^{\pm7.69}$**	15.0$^{\pm1.57}$	1.68$^{\pm0.66}$	**8.34$^{\pm2.64}$**
Sarı	34.6$^{\pm2.15}$	29.2$^{\pm1.23}$	**31.9$^{\pm1.53}$**	90.8$^{\pm11.0}$	75.3$^{\pm3.31}$	**83.0$^{\pm6.08}$**	15.3$^{\pm2.30}$	8.75$^{\pm2.49}$	**12.0$^{\pm2.00}$**
Uzunlu-99	32.6$^{\pm1.55}$	26.3$^{\pm1.10}$	**29.5$^{\pm1.48}$**	118$^{\pm4.95}$	71.2$^{\pm7.57}$	**94.5$^{\pm9.72}$**	32.2$^{\pm3.22}$	20.9$^{\pm4.02}$	**26.5$^{\pm3.18}$**
Er-99	34.9$^{\pm0.55}$	31.2$^{\pm2.60}$	**33.0$^{\pm1.41}$**	152$^{\pm11.1}$	65.0$^{\pm2.45}$	**108$^{\pm17.2}$**	35.3$^{\pm3.36}$	10.7$^{\pm2.32}$	**23.0$^{\pm5.03}$**
ILC-195	23.0$^{\pm0.67}$	22.2$^{\pm1.45}$	**22.6$^{\pm0.76}$**	329$^{\pm15.9}$	59.6$^{\pm7.18}$	**194$^{\pm51.5}$**	51.0$^{\pm0.98}$	23.5$^{\pm5.24}$	**37.5$^{\pm5.74}$**
Menemen-	31.8$^{\pm1.03}$	25.0$^{\pm1.41}$	**28.4$^{\pm1.52}$**	460$^{\pm48.3}$	68.6$^{\pm13.4}$	**264$^{\pm77.5}$**	43.1$^{\pm1.48}$	13.0$^{\pm5.43}$	**28.1$^{\pm6.25}$**
İzmir-92	29.9$^{\pm2.28}$	13.4$^{\pm5.74}$	**21.6$^{\pm4.23}$**	202$^{\pm26.7}$	63.4$^{\pm3.10}$	**133$^{\pm29.1}$**	26.7$^{\pm4.36}$	1.69$^{\pm0.84}$	**14.2$^{\pm5.15}$**
Aydın-92	29.8$^{\pm0.53}$	27.2$^{\pm1.24}$	**28.5$^{\pm0.79}$**	340$^{\pm34.9}$	104 $^{\pm5.51}$	**222$^{\pm47.4}$**	34.2$^{\pm3.61}$	13.7$^{\pm2.89}$	**23.9$^{\pm4.43}$**
Means	**31.4$^{\pm0.69}$**	**26.4$^{\pm1.30}$**		**202$^{\pm19.7}$**	**86.8$^{\pm7.51}$**		**30.9$^{\pm2.01}$**	**14.9$^{\pm2.17}$**	
F treatment	18.9**			273**			157**		
F cultivar	4.99**			40.8**			33.4**		
Finteraction	2.43*			31.1**			6.14**		
2008									
Akçin	37.4$^{\pm0.79}$	42.1$^{\pm2.16}$	**39.7$^{\pm1.40}$**	320$^{\pm16.1}$	137$^{\pm43.6}$	**228$^{\pm40.8}$**	49.7$^{\pm1.75}$	52.7$^{\pm8.29}$	**51.2$^{\pm3.96}$**
Küsmen	31.0$^{\pm4.55}$	15.0$^{\pm3.54}$	**23.0$^{\pm4.04}$**	170$^{\pm8.79}$	145$^{\pm8.74}$	**157$^{\pm7.37}$**	3.47$^{\pm1.62}$	0.58$^{\pm0.30}$	**2.03$^{\pm0.94}$**
Canıtez-87	45.1$^{\pm1.61}$	46.2$^{\pm0.73}$	**45.6$^{\pm0.85}$**	339$^{\pm45.6}$	194$^{\pm12.2}$	**266$^{\pm35.0}$**	49.4$^{\pm4.00}$	40.1$^{\pm1.39}$	**44.7$^{\pm2.62}$**
Gökçe	38.7$^{\pm2.32}$	41.1$^{\pm0.94}$	**39.9$^{\pm1.25}$**	316$^{\pm27.4}$	252$^{\pm12.0}$	**284$^{\pm18.4}$**	50.1$^{\pm1.51}$	40.5$^{\pm2.59}$	**45.3$^{\pm2.28}$**
Sarı	47.5$^{\pm1.18}$	47.6$^{\pm0.60}$	**47.5$^{\pm0.61}$**	451$^{\pm34.3}$	424$^{\pm42.3}$	**441$^{\pm25.5}$**	50.4$^{\pm1.59}$	52.0$^{\pm1.11}$	**51.2$^{\pm0.94}$**
Uzunlu-99	42.4$^{\pm0.79}$	42.5$^{\pm0.39}$	**42.5$^{\pm0.41}$**	376$^{\pm30.2}$	367$^{\pm22.5}$	**372$^{\pm17.5}$**	38.9$^{\pm2.51}$	37.6$^{\pm1.91}$	**38.2$^{\pm1.48}$**
Er-99	39.5$^{\pm0.54}$	35.7$^{\pm2.01}$	**37.6$^{\pm1.20}$**	389$^{\pm20.4}$	172$^{\pm29.8}$	**281$^{\pm44.4}$**	45.8$^{\pm1.34}$	13.4$^{\pm5.92}$	**29.6$^{\pm6.73}$**
ILC-195	24.9$^{\pm0.24}$	23.8$^{\pm0.41}$	**24.3$^{\pm0.31}$**	250$^{\pm27.3}$	194$^{\pm13.3}$	**222$^{\pm17.6}$**	43.0$^{\pm0.82}$	35.6$^{\pm2.77}$	**39.3$^{\pm1.93}$**
Menemen-	30.8$^{\pm1.97}$	24.2$^{\pm6.47}$	**27.5$^{\pm3.37}$**	175$^{\pm9.95}$	149$^{\pm11.5}$	**162$^{\pm8.60}$**	12.2$^{\pm1.83}$	2.13$^{\pm1.12}$	**7.17$^{\pm2.15}$**
İzmir-92	32.7$^{\pm1.24}$	22.1$^{\pm7.63}$	**27.4$^{\pm4.10}$**	182$^{\pm15.7}$	162$^{\pm16.7}$	**172$^{\pm11.2}$**	25.9$^{\pm3.30}$	6.19$^{\pm2.67}$	**16.1$^{\pm4.22}$**
Aydın-92	25.9$^{\pm1.48}$	0.00$^{\pm0.00}$	**12.9$^{\pm4.94}$**	136$^{\pm6.58}$	91.3$^{\pm5.59}$	**114$^{\pm9.40}$**	6.29$^{\pm2.06}$	0.00$^{\pm0.00}$	**3.15$^{\pm1.52}$**
Means	**36.0$^{\pm1.20}$**	**30.9$^{\pm2.37}$**		**282$^{\pm16.7}$**	**209$^{\pm16.3}$**		**34.1$^{\pm2.78}$**	**25.5$^{\pm3.21}$**	
F treatment	18.9**			50.9**			47.6**		
F cultivar	32.8**			32.7**			87.5**		
Finteraction	5.75**			4.60**			6.00**		

*: $p<0.05$ and **: $p<0.01$

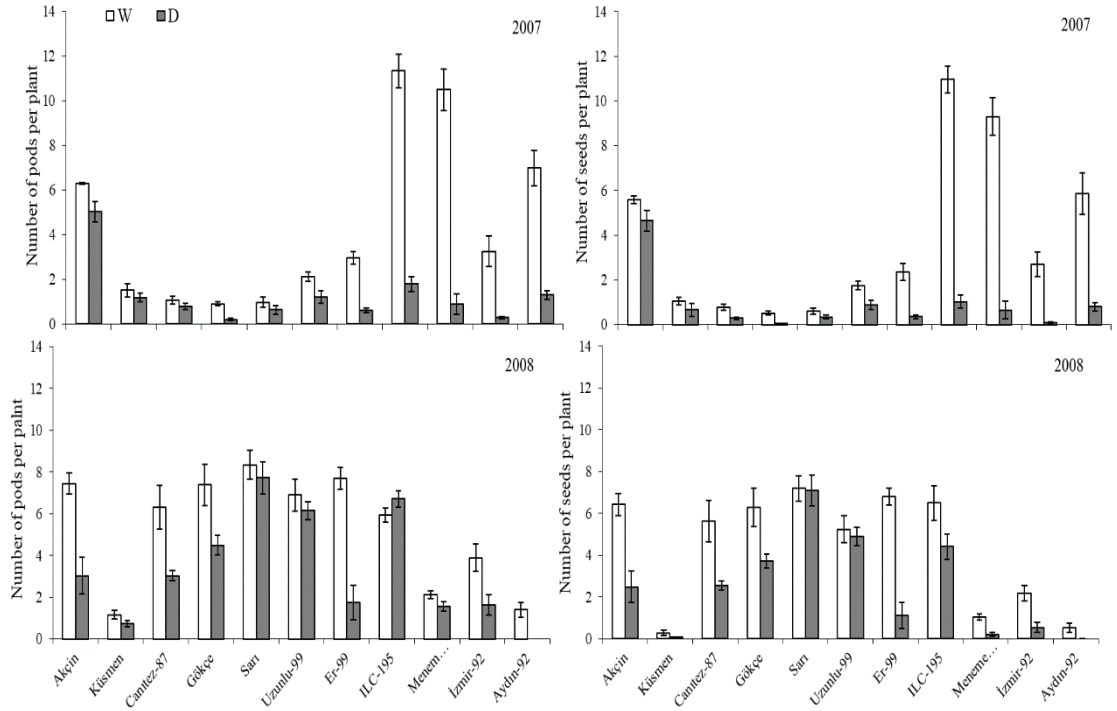

Figure 1. The number of pods and seeds per plant of chickpea cultivars (W, watered condition; D, drought condition).

In 2007, the 100 seeds weight of other cultivars, except cvs. Akçin and Küsmen, decreased due to drought stress (Figure 2). In 2008, the 100 seeds weight of cv. Akçin increased under dry conditions, whereas the declines in cvs. Küsmen, Er-99, ILC-195, İzmir-92, and Aydın-92 were significant. Ghassemi-Golezani et al. (2008) reported that there was a very high reduction in the average the number of seeds per plant, hundred seeds weight, and seed yield per unit of chickpea exposed to severe water stress, varying according to cultivars.

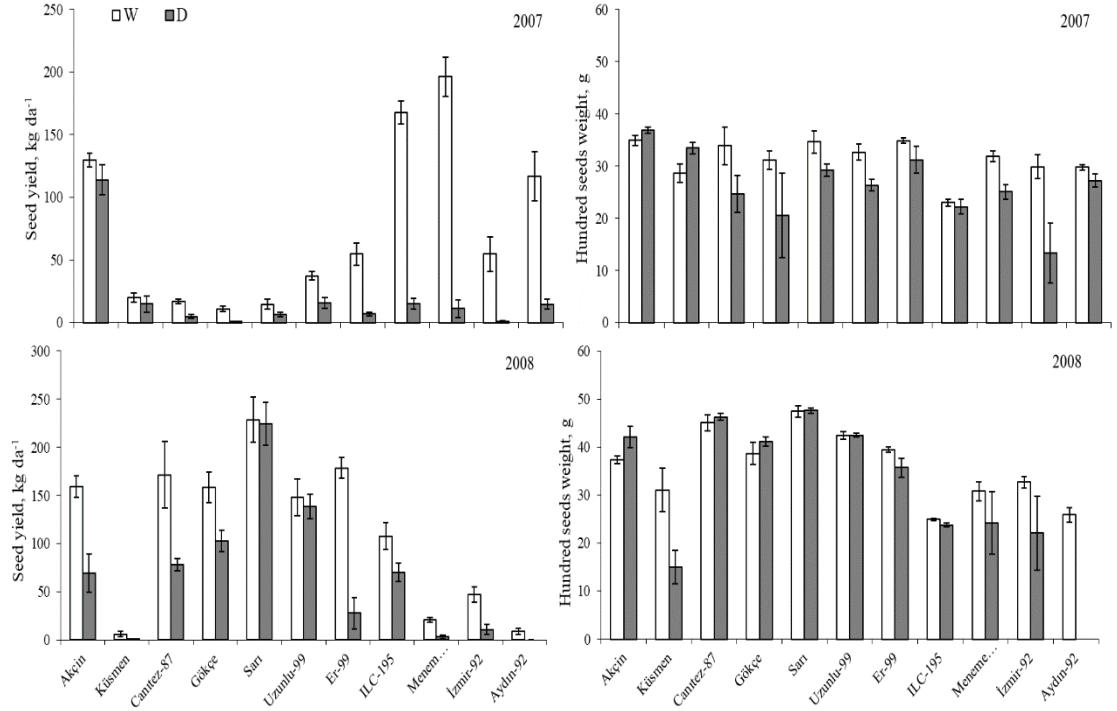

Figure 2. Seed yields and hundred seeds weight of chickpea cultivars (W, watered condition; D, drought condition).

The drought stress reduced biological yields in all cultivars in 2007, except cvs. Küsmen, Canıtez-87 and Sarı, and also the biological yield reduction were higher in Menemen-92, ILC-195, Aydın-92 and İzmir-92 cultivars

(Figure 3). In 2008, all cultivars decreased in their biological yields except cvs. Sarı, Uzunlu-99, Menemen-92 and İzmir-92, and the greatest reduction were in cvs. Akçin, Canıtez-87, Er-99, and Aydın-92.

In 2007, the decrease in harvest index was proportionally higher in cvs. Gökçe, Er-99, Menemen-92 and İzmir-92, and lower in cvs. Sarı and Uzunlu-99. In contrast, the harvest indices of Küsmen, Aydın-92, Menemen-92 and İzmir-92 cultivars under drought conditions in 2008 were close to zero or zero. Additionally, the highest reduction of harvest indices among the other cultivars was observed in Er-99 and İzmir-92 cultivars (Figure 3). Similar results have been achieved in different plant species. It was reported by Shao et al. (2008) that the drought decreased seed yield, number of seeds per plant and seed size of sunflower and reduced the harvest index of the plant due to the drought during the generative period.

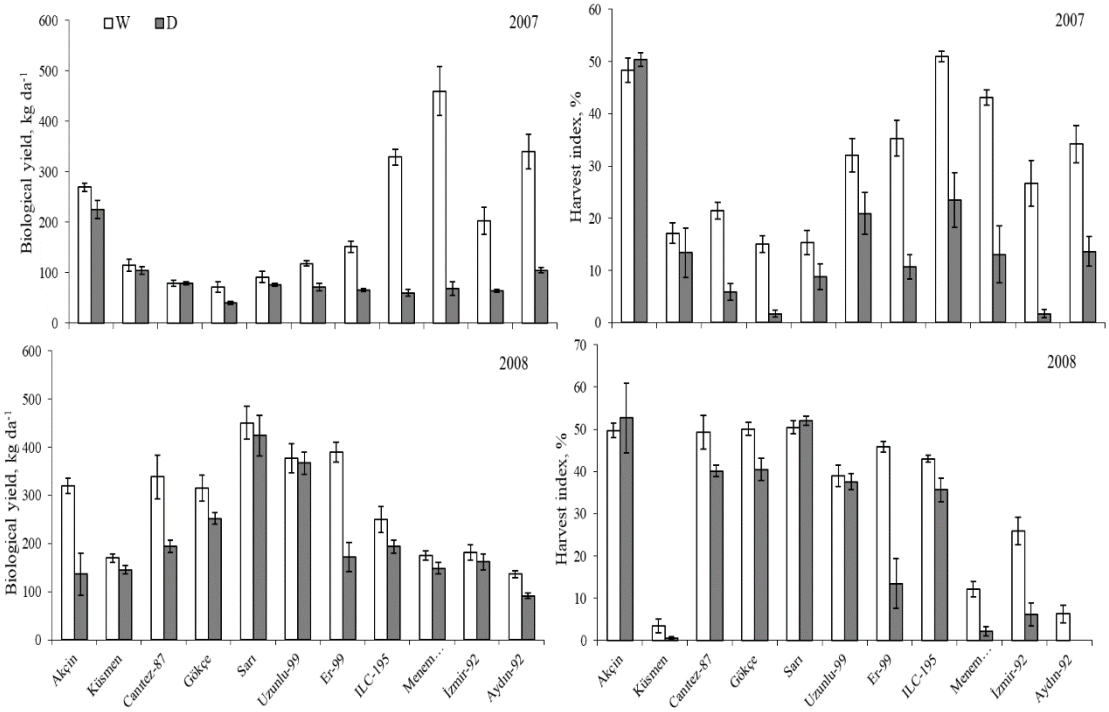

Figure 3. Biological yields and harvest index of chickpea cultivars (W, watered condition; D, drought condition).

Previous studies have suggested that the reduction in harvest indices of drought sensitive varieties is more serious (Rahman et al. 2006). Ali et al. (1998) reported that the water stress during the period from flowering to maturity of chickpea affected the seed yield per plant significantly and the harvest index of varieties varied seriously in both watered and drought conditions. Also, they declared that the number of pods per plant and seed yield instead of the harvest index under extremely arid conditions were higher and two traits might be more suitable to use in selection of resistant varieties. Leport et al. (1999) emphasized that not only dry matter production was high in water shortage caused by low rainfall or inadequate irrigation, but also high pod and seed forming cultivars should be identified in the unit area. Similarly, Amede et al. (1999) reported that drought-resistant broad bean lines had a relatively higher number of pods and number of seed per plant, and harvest indexes of them were higher.

Differences in the yields of plants exposed to drought at different periods of development have also been demonstrated with other studies. Gunes et al. (2008) found that responses to early and late drought cultivars of chic were quite different. Anyia and Herzog (2004) emphasized that moderate drought caused to a decrease in plant function and biomass yield, and that plant development in pre- and post-drought due to density and intensity of drought was much more effective in determining the total biomass yield than the response of the plant during drought.

DSIs of chickpea cultivars were given in Figure 4. It was determined that there are significant differences among cultivars in terms of sensitivity to drought. Akçin, Küsmen, Gökçe, Sarı, Canıtez-87, Uzunlu-99 and Er-99 cultivars, which had DSI values below 1.00, were found to be resistant to drought in 2007, and it was determined that the highest sensitivity to drought was cv. Menemen-92, which had DSI values above 1.00, followed by ILC-195, Aydın-92, and İzmir-92 cultivars.

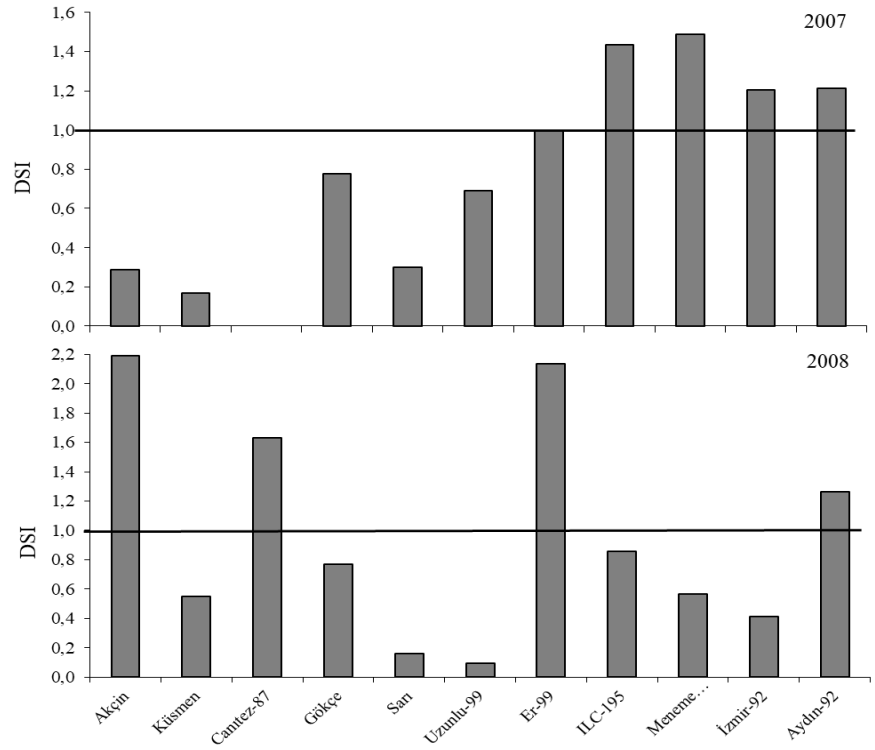

Figure 4. Drought susceptibility indexes (DSI) of chickpea cultivars (DSI<1.0 Resistant, DSI>1.0 Susceptible)

On the other hand, it was determined that in 2008 the drought sensitivity indices of the cultivars were to be higher than the previous. While the Küsmen, Gökçe, Sarı, Uzunlu-99, ILC-195, Menemen-92 and İzmir-92 cultivars entered in the drought-resistant group, cultivars of Er-99, Akçin, Canıtez-87, and Aydın-92 were in the susceptible group. The Küsmen, Gökçe, Sarı, and Uzunlu-99 were the cultivars in the two-year joint reaction and the drought-resistant group. But a similar result could not be reached among sensitive cultivars in two years. In another study investigating the effect of seasonal drought stress with the same chickpea cultivars in greenhouse conditions (Gunes et al. 2008), cultivars were exposed to the same severe stress during different growth stages (vegetative and generative) and were subjected to optimal irrigation after the stress period. As a result, it was determined that cultivars responded differently exposed to drought in the growth stages and exhibited different DSI values, while cultivars showed lower DSI in the vegetative stage than in the generative stage. In addition, it was evaluated in both stress periods that gave the joint response the Canıtez-87, Küsmen, Sarı and Gökçe cultivars were as drought-sensitive and the Aydın-92, Akçin and Uzunlu-99 cultivars were as drought-resistant (Gunes et al. 2008).

These past findings have been consistent with some of the results obtained in this study, but it has been determined that it is not appropriate to evaluate drought-tolerant varieties with only a trait. It has come to the conclusion that the differences in drought sensitivities of chickpea cultivars exposed to drought can emerge because of the different response is given depending on growth stages, growing conditions (controlled or field conditions), severe and duration of stress.

Conclusion

In this study, it was determined the sensitivities of chickpea cultivars against drought and to compare their yield traits in different growth stages by establishing two field experiments. In experiments, yields of cultivars decreased substantially and their susceptibilities changed according to the growth stages exposed to drought. It was determined that there were significant differences in the sensitivity and yield of chickpea cultivars. From the data obtained, it was concluded that the yield and its traits of chickpea were very much affected by drought stress, which was a result of the decrease in a number of pods and seeds per plant. DSI with together the number of seeds per plant, seed yield and biological yield traits of cultivars gave more reliable results than the number of pods per plant, 100 seeds weight and harvest index traits in the selection of susceptible and resistant cultivars. When the cultivars exposed to drought in both years were evaluated together with the cultivars of cvs. Küsmen, Gökçe, Sarı, and Uzunlu-99 were observed that they gave the joint responses and entered the resistant groups.

On the other hand, according to all results, a parameter accepted among the selection criteria for a plant species or variety can prevent from reaching a clear judgment due to the severity and duration of stress exposed in different growth stages and growing conditions and also due to the genetic characteristics of plant, even if it is the same kind. In the following selection studies, it is recommended to make the description of susceptible/resistant plant species, according to the growth stage of plants exposed to the stress, also the severity and duration of stress.

Acknowledgments

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Araştırma Makalesi/*Research Article (Original Paper)*

Plant and Soil Characteristics of Rangelands Improved with Different Methods

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Abstract: Annual plant species and thorny shrubs like Christ's thorn (*Paliurus spina-cristi*) are common in Mediterranean rangelands because of climate and heavy grazing. Mediterranean rangelands (Biga-Çanakkale/Turkey) with invasive Christ's thorn (CT) populations improved with various practices through removing shrubs from the rangelands and seeding the places with forage crops. Improvement practices were as follows; (C): Control treatment, (E): CT's were removed 15 years ago, rangelands were seeded with forage crops; (H): CT's shrubs were treated with herbicides; (M): CT's shrubs were removed through grubbing with dozers and rippers; (F): The rangelands were used as agricultural field and reseeded to create a rangeland. All treatment plots were seeded with perennial ryegrass, orchard grass and alfalfa. Effects of these improvement practices on soil and plant nutrients and some soil characteristics were investigated. Soil pH; decreased in herbicide-treated rangelands. The electrical conductivity of the soil did not change in the first year but changes have been occurred among the applications in second year of the study work. The amount of soil nitrogen varied with the applications, while there was no change in the amount of plant nitrogen. Treatments resulted in significant changes in soil and plant macro (K, Ca, Mg) and micro (Fe, Cu, Zn) nutrients and only the phosphorus (P) contents were not significant. The applied improvement methods in rangelands have brought certain changes the nutrients of soil and plant, but these variations did not mean to any negativity. It was concluded based on present findings that any methods used in this study could be used to eleminate Christ's thorn from the rangelands, but the method which is more economical and sustainable for the relavent rangeland should be preferred in reclamation works.

Keywords: *Paliurus spina-cristi*, rangeland improvement, soil, herbage

Farklı Yöntemlerle Islah Edilen Meraların Bitki ve Toprak Özellikleri

Özet: Akdeniz tipi meralarda iklim ve ağır otlatmadan dolayı tek yıllık türler ve karaçalı (*Paliurus spina-crist)* gibi dikenli çalılar oldukça yaygındır. Akdeniz tipi mera özelliğinde olan Türkiye'nin Çanakkale ilinin Biga ilçesinde karaçalı (CT) çalılarını yok etmek ve merayı ıslah etmek için çeşitli uygulamalar yapılmış ve yem bitkileri ekilmiştir. Merayı ıslah etme uygulamaları (C): Kontrol, (E): karaçalı çalıları 15 yıl önce yok edilmiş ve mera yem bitkileri ile tohumlanmıştır, (H): karaçalı çalısına herbisit uygulanmıştır, (M): karaçalı çalısı dozer ve ripel ile sökülmüştür, (F): mera uzun yıllar tarla alanı olarak kullanılmış daha sonra yem bitkileri ekilerek mera oluşturulmuştur. Yapılan bütün uygulamalarda çok yıllık çim, domuz ayrığı ve yonca bitkileri ile tohumlama yapılmıştır. Yapılan bu uygulamaların toprak ve bitkilerin besin elementi içeriğine ve toprağın bazı özelliklerine etkisi ortaya konulmuştur. Toprak pH'sı herbisit uygulanan parsellerde azalmıştır. Toprağın elektiriksel iletkenliği ilk yıl değişmemiş fakat ikinci yıl önemli bir değişim belirlenmiştir. Meraya yapılan iyileştirme çalışmaları ile topraktaki azot miktarında da değişim meydana gelmiştir. Yapılan uygulamalarla toprak ve bitkideki makro (K, Ca, Mg) ve mikro (Fe, Cu, Zn) besin elementlerinde önemli değişim olurken fosfor miktarında herhengi bir değişim meydana gelmemiştir. Meranın ıslahı için yapılan çalışmalar toprak ve bitki besin elementlerinde değişim meydana getirmiştir, fakat bu değişim olumsuz olmamıştır. Bu çalışma sonucunda meralarda bulunan karaçalının yok edilmesinde bu merada kullanılan yöntemlerin hepsi uygulanabilir fakat ıslah çalışması yapılacak mera için hangi uygulama daha ekonomik ve sürdürülebilirse o yöntem tercih edilmelidir.

Anahtar kelimeler: *Paliurus spina-cristi*, mera ıslahı, toprak, ot

Introduction

Christ's thorn is common over the rangelands without goat grazing. It is a defoliate species and provides high quality feed source in spring, summer and autumn. Year-long average protein content of Christ's thorn is 114,6 g/kg, NDF is 474,8 g/kg and digestibility value (DMD) is around 62,54% (Özaslan-Parlak 2011). However, it is grazed only by goats because of thorny nature of the shrub. Thorns may create injuries in mouths and udders of the other animals. Regional people usually deal with livestock activities and they never use the rangelands with Christ's thorn cover. Such a case then results in ever-increasing Christ's thorn population over these rangelands. In the erodication of invasive species in rangeland mechanical, chemical methods are used. In addition, the rangeland is completely removed from the vegetation. Rangeland is being processed and artificial pastures are established (Link et al. 2017). The plant of Christ's thorn has been harvested at bottom with mechanic methods or adopted the way of keeping away the bush using ripel like tools. During the removal of top soil is lower stem stolen. The herbicide is also applied by chemical methods. The seeds of forage are sown in the gaps formed by the destruction of the invasive species, leading to the improvement of the rangelands (Endress et al. 2012).

The Ministry of Food Agriculture and Livestock of Turkey have been implementing comprehensive rangeland improvement and management projects in accordance with 4243-numbered and 28.02.1998-dated Rangeland Law in almost every province of Turkey. Remarkable success was achieved in some of these works, but desired outcomes were not achieved in some others. However, success of reclamation or improvement works have usually assessed visually, scientific approaches haven't been used to assess the success of such practices.

The present study was conducted to scientifically assess the improvement practices implemented by Çanakkale Provincial Directorate of Agriculture over the rangelands of Hacıpehlivan village of Biga town. For this purpose, two-year data on plant and soil macro and micro nutrients of rangeland plots with five different improvement processes were assessed. Soil reaction (pH) and salinity (EC) were also determined to assess changes in soil chemical composition.

Materials and Methods

Description of Research Site

The research was conducted in Hacıpehlivan village with 113,2 decare rangeland area. The village is 17 km far from Biga town and 120 km from Çanakkale province. Çanakkale located in northwest part of Turkey. Rangeland site is adjacent to village settlement area and lies along East-West direction. Rangeland boundaries are surrounded with 40˚ 15′ 09″ N / 27˚ 24′ 24″ E - 40˚ 15′ 16″ N / 27˚ 24′ 58″ E coordinates from the North and with 40˚ 14′ 20″ N / 27˚ 25′ 27″ E - 40˚ 14′ 25″ N / 27˚ 25′ 36″ E coordinates from the South. Coordinates were measured with "Magellan 310" portable Global Positioning System (GPS) device.

Animals of the village (400 cattle, 55 goats and 40 sheep) are grazed over the rangelands. The region has a typical Mediterranean climate (Türkeş et al. 2002). Çanakkale have Mediterranean precipitation regime with the highest precipitation in winter and the least precipitation in summer.

Average temperature in Biga was above the long-term averages in both years. Precipitations were quite low in 2008 and were almost half of long-term averages, but the precipitations of 2009 were above the long-term averages (Figure 1).

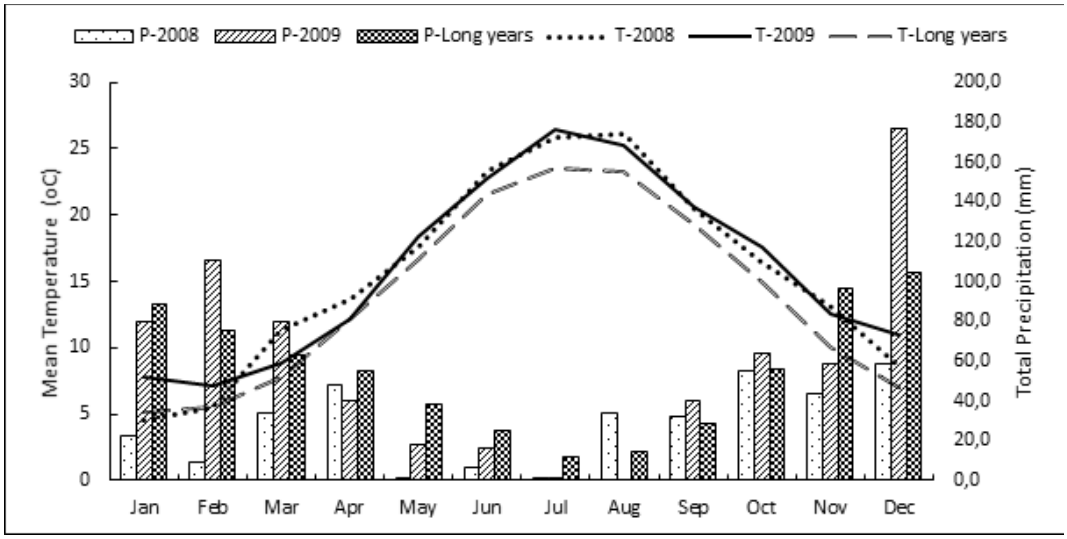

Figure 1. Avarage climatic data of experimental period and long years of Biga distict (Canakkale/Turkey)

Experimental Design

Experiments were conducted over five different sites with different previous improvement practices. The rangeland practices were: 1) Control (no improvement practices, C) these rangelands are dominated Christ's thorn (*Paliurus spina-cristi* Mill.); 2) Removal of Christ's thorns followed by forage crop planting (E), 3) Use of herbicides (glyphosate-containing) to treat Christ's thorn shrubs (H), 4) removal of Christ's thorn shrubs by grubbing with dozers and rippers (25-30 cm depth) (M), and 5) The shrubs were cut out in the rangelands dominated with Christ's thorn. The field has been plowed deep by a tractor. Then, it had been ased as field for 10 years (sown with wheat). At the end, it has been turned back in the form of rangelands by sowing forage crops (F).

The spaces created through removal of shrubs with mechanical and chemical methods were seeded with forage crops on 01.12.2016. In seeding treatments, 40% legumes and 60% grasses quadruple mixture (Plato alfalfa - *Medicago sativa* L., G.S. Gabriele bird's foot trefoil - *Lotus corniculatus* L., Verdi perennial ryegrass - *Lolium perenne* L. and orchardgrass - *Dactylis glomerata* L.) were used. In mixtures, 15% alfalfa, 25% bird's foot trefoil, 30% perennial ryegrass and 30% orchardgrass were used. Grazing did not performed for a year for better rooting of seeded species. During the sowing and after sowing, 4 kg/da nitrogen (N), 4 kg/da phosphorus (P) and 2 kg/da potassium (K) were applied with 15-15-15 and 20-20-0 composed fertilizers. Fertilization was not performed in subsequent years.

Investigated Traits

*Soil analyses***:** Soil samples were taken from 8 different locations (0-30 cm soil profile) of each rangeland plot. Coordinates of each sampling location were taken with a GPS device and sampling was performed from the same locations in subsequent years. Samples were transported to laboratory in polyethylene bags and air-dried in the laboratory. Then they were sieved through 2 mm sieves and made ready for analyses (Jackson 1958; Müftüoğlu et al. 2014). Soil samples were subjected to following analyses with the methods specified in Table 1.

Table 1. Soil Analyses and methods

Analyses	***Explanation***	***Methods***
Soil reaction (pH)	In soil-water suspension (1:2.5) with a glass-electrode pH meter	Jackson (1958)
Soil salinity (EC)	In soil-water suspension (1:2.5) with an EC meter	Richards (1954)
Total N	In LECO C-N elemental analysis device with dry-ashing method	Kirsten (1983)
Lime ($CaCO_3$)	In Scheibler calcimeter through volumetric measurement of CO_2 released by HCl acid	Allison and Moodie (1965)
Fe, Cu, Zn	In AAS-OES device after DTPA extraction	Lindsay and Norvell (1978)
Available P	In a spectrophotometer through readings from colored P with 0.5 M $NaHCO_3$ (pH=8.5) solution	Olsen et al (1954)

Nutritional composition of the herbage: Samples were ground and total nitrogen content of 1 g ground sample was determined with Kjeldahl method. Then total nitrogen content was multiplied by 6.25 to get crude protein content (AOAC 1990). NDF, ADF and ADL ratios were determined in accordance with Van Soest et al. (1991) and crude ash was determined in accordance with AOAC (1990). Ground herbage samples were subjected to microwave (Velp Scientifica DK 6 Heating Digester) digestion to get P, K, Ca, Mg, Na, Fe, Cu and Zn contents (Jones et al. 2001) and readings were performed in an ICP-OES (Perkin Elmer Optima 7000 DV) device (Isaac and Kerber 1971). Phosphorus readings were performed with a spectrophotometer (Shimadzu UV-1201V) as specified by Kitson and Mellon (1944) from the wet-digested and yellow-colored samples (Barton 1948).

Data Analysis

Resultant data were subjected to one-way ANOVA and means were compared with Duncan's multiple range test. Statistical analyses were performed with SPSS software (Winer et al. 1991). The relationships among rangeland yield, soil parameters and macro and micro nutrients were analyzed with Pearson's correlation tests.

Results and Discussion

Soil Chemistry

Soil pH levels were lower in H-plots (herbicide-treated and seeded) than the others in both years (Figure 2). Because of these low values, H-plots were classified as "slightly acidic" and the other plots were classified as "neutral" and "slightly alkaline". (Richards 1954; Grewelling and Peech 1960). In a previous study carried out over the same rangelands, Türkmen et al. (2013) reported the lime content of H-plots as 0,66% and such a value was lower than the other plots. Thus, low pH levels might have resulted from those lower lime contents of H-plots. Soil pH is directly related to lime content (Richard 1954). Such soils are not considered as problematic soils with regard to nutrient uptake of plants (Kacar 2009).

Soil salinity (EC) did not change significantly with the treatments in the first year, but significant changes were observed in F and M-plots in the second year (Figure 1). Despite the significant differences in soil salinity of the second year, values did not reach to harmful levels neither in different treatment nor in control treatment. All soils of improved plots were classified as "unsaline" (Maas 1986; Müftüoğlu et al. 2014).

The changes in Ca and K contents of rangeland soils in different years were found to be significant. Ca and K had similar characteristics also based on improvement practices (Figure 2). The highest values were observed in control plot and the lowest values were seen in H-plots. Such findings may be explained with low lime levels of H-plots as indicated by Türkmen et al., (2013). Low lime-originated carbonates may also reduce especially potassium and alkaline cation levels as carbonates ($CaCO_3$ and KCO_3) (Jones et al. 1998; Tan 2011).

The highest soil Mg content was observed in F-plot and the lowest value was seen in control plot (C). Such a case may be resulted from different soil characteristics of improved plots (Türkmen et al. 2013) and differences in herbage yields and diversity. Different plants uptake different amount of nutrients from the soil (Jones et al. 1998), thus nutrient composition of soils may greatly vary (Kacar 2009; Tan 2011; Kabata-Pendias 2011).

Similarly, significant differences were observed in N contents of the years. In the first year, the highest N content was observed in H-plots and the lowest value has been seen in E-plots. In the second year; E, H and M-plots had similar nitrogen contents and the lowest value was found in F-plots. Low pH levels of H-plots treated with herbicides might have resulted in excessive exhaustion or leaching of alkali cations (Kacar 2009; Tan 2011). On the other hand, positive and/or negative influences of herbicides on soil microorganisms were also reported by Buscot and Varma (2005). It has also been reported in another study carried out in the USA that glyphosate treatments reduced the microbial activity in soilless cultivation, but such treatments had insignificantly affected the microbial respiration with soil cultivation. It was also reported in another study that long-term high-dose glyphosate treatments increased microbial respiration in forest soils since microorganisms used this compound as carbon source (Busse et al. 2001).

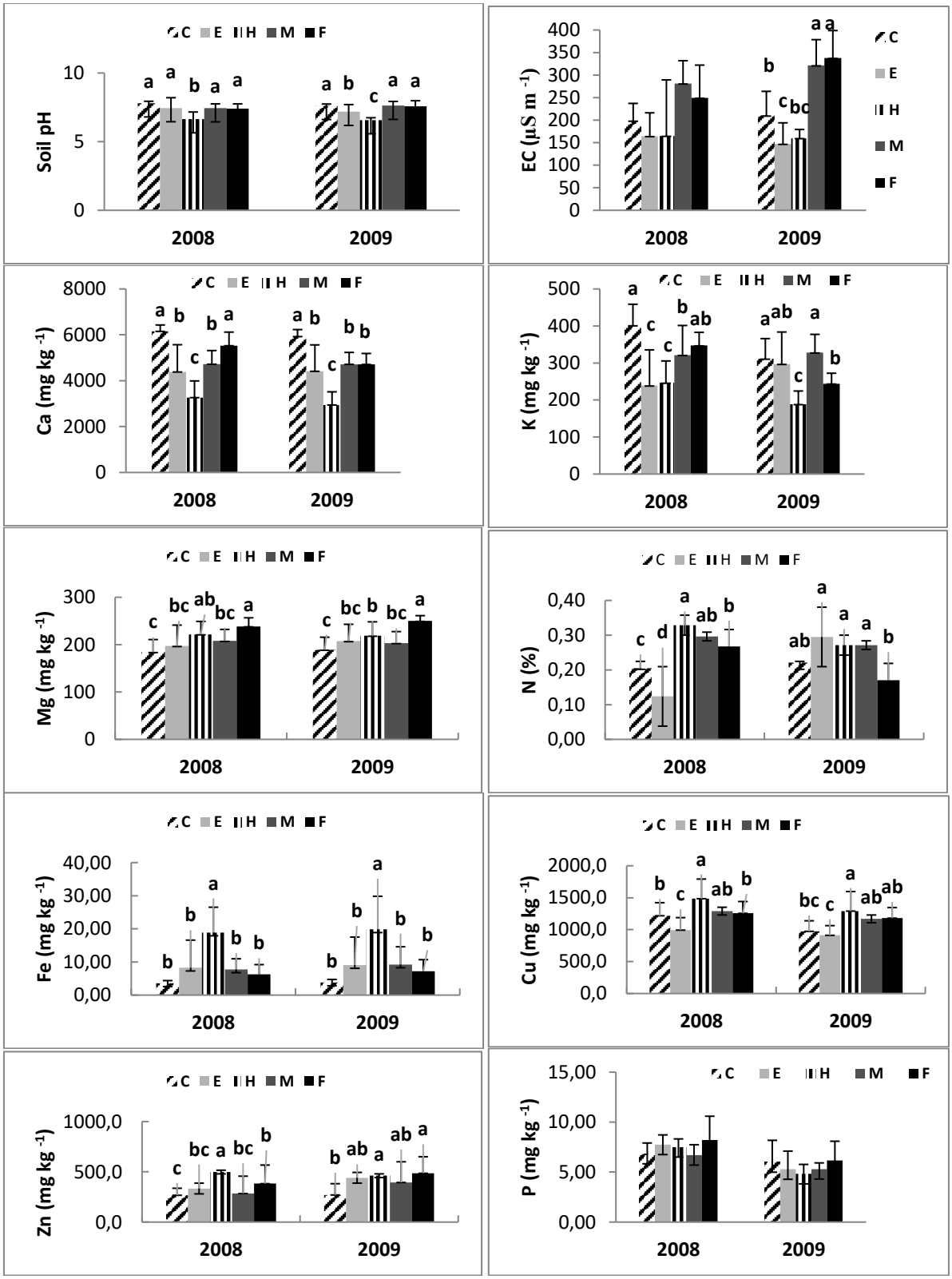

Figure 2. Soil macro and micro nutrients of rangeland plots improved with different methods (C): Control (no improvement practices; (E): Removal of Christ's thorns followed by forage crop planting; (H): Christ's thorn shrubs were treated with herbicides; (M): Removal of Christ's thorn shrubs by grubbing with dozers and rippers; (F): The shrubs were cut out in the rangelands dominated with Christ's thorn. The field has been plowed deep by a tractor. Then, it had been ased as field for 10 years (sown with wheat). At the end, it has been turned back in the form of rangelands by sowing forage crops. The differences in means indicated with different letters are significant, (a > b > c > d), Duncan test, P < 0,05).

Significant differences were not observed in phosphorus contents of improved plots. Available forms of micro nutrients (Fe, Cu and Zn) were also investigated in this study and significant differences were observed in micro nutrient contents of treatments in both years. The highest Fe, Cu and Zn contents were observed in H-plots in both years. Again low pH and the least lime levels of that plot increased micro element extraction of the soils and bioavailability of these elements (Hinsinger 2001; Jones 2012). Fe levels of the experimental plots were similar to each other. The lowest Cu content was observed in E-plots and the lowest Zn content was observed in control plot (Figure 2). Current findings comply with the results of Jones (2001) reporting different micro and macro nutrient uptakes of different high plants; with the results of indicating high variations in soil nutrient contents of different sites and with the findings of Kabata-Pendias (2011), Lindsay and Norvell (1978) and Jones (2012) indicating changing nutrient uptakes with soil characteristics.

Chemical Composition of Rangelands

Chemical composition of rangeland herbages significantly changed with improvement practices. Only the phosphorus did not have significant changes with improvement practices showed in Figure 2. The rangeland plot (F) has been used as agricultural filed for a long time and reseeded in 2006 had the highest Ca, Mg and Zn contents. The lowest herbage Ca, Mg, Fe, Cu and Zn contents were observed in control plot. While there were non-significant changes in K and Na contents in the first year, the changes in the second year were found to be significant. Correlations of soil nutrients with plant nutrients and yields were also investigated in this study. There was a significant positive correlation between soil EC ($P<0,05$) and yield ($r = 0,44$) in 2008 and 2009. Since the experimental soils were unsaline and increased salinity levels enriched salt-forming nutrients, except for sodium, such increases positively reflected on yields (Kabata-Pendias, 2011, Jones, 2012).

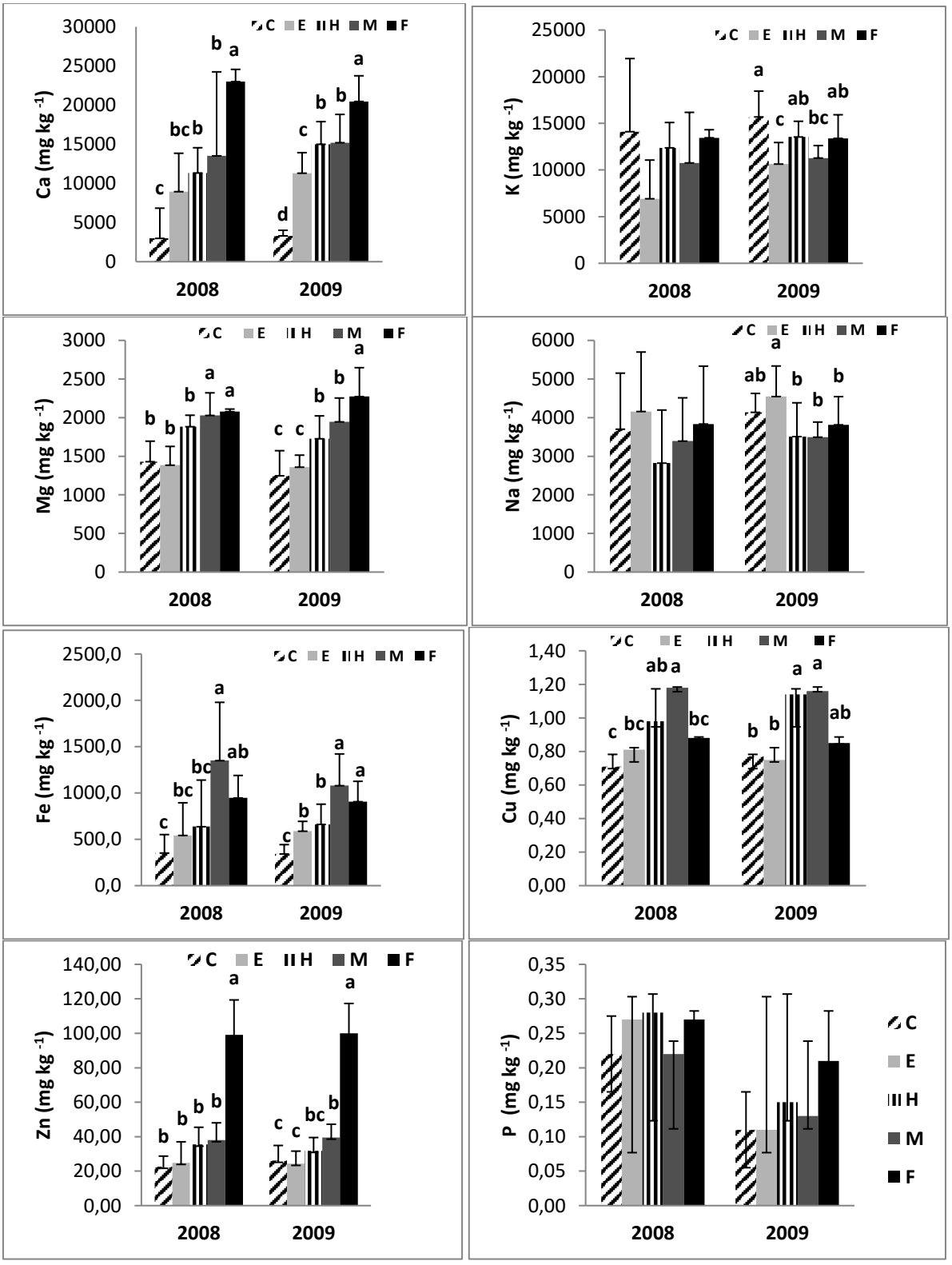

Figure 3. Herbage macro and micro nutrient contents of rangeland plots improved with different methods (C): Control (no improvement practices; (E): Removal of Christ's thorns followed by forage crop planting; (H): Christ's thorn shrubs were treated with herbicides; (M): Removal of Christ's thorn shrubs by grubbing with dozers and rippers; (F): The shrubs were cut out in the rangelands dominated with Christ's thorn. The field has been plowed deep by a tractor. Then, it had been ased as field for 10 years (sown with wheat). At the end, it has been turned back in the form of rangelands by sowing forage crops. The differences in means indicated with different letters are significant, (a > b > c > d), Duncan test, P < 0,05). Soil Mg content positively correlated with plant Mg (r = 0,47), Ca (r = 038), Fe (r = 0,39) and Zn (0,52) contents. There was also positive correlations between soil K

content and plant ash content (r = 0,34); between soil Cu content and plant Cu content Cu (r = 0,41); between soil P content and herbage crude protein content (r = 0,38).

Conclusions

The present study was conducted to assess soil and plant characteristics of rangelands improved with the application of different practices. Improvement practices were primarily implemented to eliminate the invasive Christ's thorn shrub from the rangelands of Mediterranean region in Turkey. These practices were (C): control treatment without any improvement practices, these rangelands are intensely covered with Christ's thorn (*Paliurus spina-cristi* Mill.); (E): Removal of Christ's thorns followed by forage crop planting (H): Christ's thorn shrubs were treated with glyphosate-containing herbicides (Roundup) and rangelands were seeded with forage crops (perennial ryegrass, orchardgrass, alfalfa, bird's foot trefoil); (M): Christ's thorn shrubs were removed through grubbing from 25-30 cm depth with dozer + rippers and rangelands were seeded with the same forage crops and (F): The shrubs were cut out in the rangelands dominated with Christ's thorn. The field has been plowed deep by a tractor. Then, it had been ased as field for 10 years (sown with wheat). At the end, it has been turned back in the form of rangelands by sowing forage crops. Significant differences were observed in soil macro and micro nutrients of treatment plots. Different treatments had similar pH levels, but the plot with herbicide treatments had lower pH and lime levels as compared to the other plots. Different treatments did not create any significant changes in both soil and plant P contents. There were significant differences in some plant macro and micro nutrients (Ca, Mg, Fe, Cu and Zn). While the differences in K and Na contents were not significant in the first year, they were found to be significant in the second year. In both years, soil Mg content positively correlated with plant Mg, Ca, Fe and Zn contents. There were also positive correlations between soil K content and plant crude ash, between soil Cu content and plant Cu content. Experimental plots had quite similar herbage quality parameters. It was concluded that mechanical control and herbicide treatments were successful in sites intensely covered with Christ's thorn. However, heavy grazing should definitely be prevented to sustain this success in shrub control.

Acknowledgment

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Araştırma Makalesi/*Research Article (Original Paper)*

Phenolics Content and Antioxidant Capacity of Mung Bean (*Vigna radiata* L.) Seed

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Abstract: Mung bean (*Vigna radiata*) is a summer growing legume and widely consumed in the Asian cuisine. In recent years, the functional properties of mung bean have received attention, particularly with respect to antioxidant, antitumor, anti-diabetic effects. In this research investigated the antioxidant capacity and phenolic compound profiles of dried mung bean seeds. The total phenolic content, DPPH$^{\bullet}$ scavenging activity, ferric-reducing antioxidant power (FRAP), ABTS$^{\bullet+}$ scavenging activity were determined after methanol and acetone extractions. HPLC analyse was used to identification mung bean phenolic compounds. The total phenolic content of mung bean seed was determined as 47.16 mg GA eq / g extract (504.65 mg / 100 g seed) and 66.05 mg GA eq / g extract (526.41 mg / 100 g seed) after methanol and acetoneuse as extractans, respectively. The dominant phenolic compounds of seeds were hydroxybenzoic acid derivatives. The radical scavenging activity of mung bean extracts against ABTS$^{\bullet+}$ was 1.093 mmol Trolox / g acetone extract. This study compared the antioxidant capacity of mung bean with literature data of antioxidant properties widely consumed different bean varieties such as red and white beans. Obtained results suggest that mung bean can be evaluated as functional ingredient with high antioxidant activity in several foods therefore larger field productions can be achieved for this legume.

Keywords: Phenolic content, *Vigna radiata,* DPPH$^{\bullet}$ scavenging activity, FRAP, ABTS

Maş Fasulyesinin (*Vigna radiata* L.) Fenolik Bileşikleri ve Antioksidan Kapasitesi

Abstract: Maş fasulyesi (*Vigna radiata*) Asya mutfağında yaygın tüketilen yazlık bir baklagil çeşididir. Son yıllarda maş fasulyesi özellikle antioksidan, antitumor, antidiyabetik gibi fonksiyonel özellikleri açısından dikkat çekmektedir. Bu araştırmanın amacı kurutulmuş maş fasulyesi tohumlarının antioksidan kapasitesini ve fenolik bileşik profilini araştırmaktır. Bu amaçla metanol ve aseton extratlarında toplam fenolik madde, DPPH$^{\bullet}$ radikal giderim aktivitesi, ferrik iyon indirgeme kapasiteleri**,** ABTS$^{\bullet+}$ katyon radikali giderim aktivitesi belirlenmiştir. Maş fasulyesinin fenolik bileşiklerini belirlemek amacıyla HPLC analiz yöntemi kullanılmıştır. Maş fasulyesinin toplam fenolik madde miktarı metanol ve aseton ektratlarında sırasıyla 47.16 mg GA eq / g ekstrat (504.65 mg / 100 g tane) and 66.05 mg GA eq / g ekstrat (526.41 mg / 100 g tane) olarak belirlenmiştir. Çalışmada, dominat fenolik bileşiklerin hidroksibenzoik asit ve türevleri olduğu bulunmuştur. Maş fasulyesinin aseton ekstresinde ABTS$^{\bullet+}$ katyon radikali giderim aktivitesi 1.093 mmol Trolox / g extract olarak belirlenmiştir. Bu çalışmada ayrıca maş fasulyesinin antioksidan kapasitesi, literetürde kırmızı ve beyaz fasulye gibi yaygın tüketilen fasulye çeşitlerinin antioksidan kapasiteleri ile karşılaştırılarak değerlendirilmiştir. Elde edilen bulgular sonucunda maş fasulyesinin yüksek antioksidan kapasitesi gibi çeşitli gıdalarda fonksiyonel ingredient olarak değerlendirilebileceği ve bu nedenle bu baklagilin daha geniş alanlarda üretilebileceği düşünülmektedir.

Anahtar kelimeler: Fenolik bileşikler, *Vigna radiata,* DPPH$^{\bullet}$ radikal giderim aktivitesi, FRAP, ABTS

Introduction

Recently studies conducted on potential health benefits of beans due to the presence of some bioactive phenolic constituents. These bioactive constituents able to keep from reactive oxygen species, which are capable some reactions causing many serious diseases (Amarowicz and Weidner, 2009). Plant Phenolics are secondary metabolites involved in the defence mechanisms against microbial pathogens, various environmental stresses and insect herbivores (Kumar et al. 2014). Plant-derived these components have played an important role in the

treatment and avoid human diseases. Therefore, the biological screening provides a scientific basis for validating the traditional utilization of medicinal plants. These bioactive constitutes of grain legumes make them suitable for creating new functional foods (Aguilera et al. 2011). Antioxidant activity of phenolic compounds present in edible grain legume seeds have been investigated in recent studies (Karamać et al. 2004, Amarowicz et al. 2008, Orak et al. 2016).

Mung bean (Vigna radiata L.) included to Fabaceae family and known as green gram or moong bean, as well as. Mung bean has been consumed as traditionally worldwide for more than 3500 years, especially in Asian countries (Kumar and Xu, 2018). Mung bean has high nutrient value similarly to soybean and kidney bean, and it is richness for proteins, minerals and vitamins and essential amino (Mubarak, 2005). Besides these nutrients, mung beans possess certain bioactive food components, which contain polyphenols. Based on the including to these components and efficacy of these bioactive components, mung beans have a great role in respect to antioxidant activity, detoxification, and also exhibits chemo-preventive effects (Ganesan and Xu 2017). Mung bean consumed in several cuisines as traditionally, therefore health promoting effects of mung bean seeds associated with the anti-inflammatory effects of diets in Asian countries (Luo et al. 2016). Dried mung bean seeds can be eaten in several ways such as cooked, fermented in western cultures, its sprouts are generally used as salad vegetable (Lambrides, 2007).

In this study, we aimed to determine the antioxidant capacity and phenolic compounds of mung bean seeds, which potentially functional properties. Identification of bioactive compounds such as phenolic compounds, flavonoids from plants have received attention for discover of therapeutic agents and to knowledge new sources of phytocompounds for the synthesis or to understand the actual significance of traditional remedies.

Materials and Methods

Plant materials

In this study, mung bean obtained from Field Crops Department, Agricultural Faculty of Namık Kemal University as material. Seeds were obtained from harvested plants at dry maturated stage at July.

Chemicals and reagents

Gallic acid, *p*-coumaric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), and trifluoroacetic acid (TFA)were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Apigenin was purchased from Extrasynthese S.A. (Genay, France) The solvents and other chemicals, if not otherwise specified, were acquired from Avantor Performance Materials (Gliwice, Poland).

Preparation of extracts

Extraction

Dried seed samples were milled with a laboratory mill and polyphenol extraction were done by using methanol 80 % (v/v) and acetone 80 % (v/v) as solvent. Sample : extractan ratio were 1:10 (v/w), and samples were shaken by using a shaking water bath (SW22, Julabo, Seelbach, Germany) at 70 °C for 15 min. The extractions were repeated three times and solvents were evaporated under vacuum using a rotary evaporator (Rotavapor R-200 (Büchi Labortechnik, Flawil, Switzerland). Samples were lyophilisated with a Labconco freeze dryer (Lyph Lock 6 freeze-dry system, Labconco, Kansas City, MO, USA) for analyses.

Total phenolic compounds content

The total phenolic content (TPC) of mung bean extracts and seeds was determined by spectrophotometric method with Folin-Ciocalteu's reagent (Karamać et al., 2015). The TPC was expressed as mg gallic acid equivalents (GA eq) per g of extract or per 100 g of seeds.

Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) of mung bean was determined using the ABTS discoloration assay described by Re et al (1999). The absorbance was measured at 734 nm. The results were expressed in terms of mmol Trolox equivalents per g of extract or seeds

DPPH radical scavenging activity

The method described by Brand-Williams et al. (1995) was used to determine DPPH• radical scavenging activity of mung bean extracts. The extracts were dissolved in range 2-10 mg/mL. These solutions (0.1 mL) were mixed with methanol (2 mL) and 1 mM DPPH (0.25 mL. The samples were allowed to stand in dark for 20 min and next the absorbance was measured at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration. EC_{50} values, defined as the amount of extract needed to scavenging 50% of the DPPH•, were estimated from the curves of absorbance versus extract concentration.

Ferric-reducing antioxidant power

Ferric-reducing antioxidant power (FRAP) of mung bean was determined according method previously described by Benzie and Strain (1996). Fe^{3+}-TPTZ complex was generated at pH 3.6 (300 mM acetate buffer) by mixing 10 mM TPTZ in 40 mM HCl and 20 mM ferric chloride (1:1, v/v). Then, mung bean extract solution (75 µL) and water (225 µL) was added to complex solution (2.25 mL). The absorbance was read at 593 nm. Ferrous sulfate was used to prepare calibration curve. The results were expressed as µmol Fe^{2+} equivalents per g of extract or seeds.

Phenolic compounds analysis

The phenolic compounds of mung bean extracts were analysed with Shimadzu HPLC system (Kyoto, Japan) containing two LC-30AD pumps, CBM-20A system controller, DGU-20A5R degassing unit, SIL-30AC autosampler and SPD-M30A photodiode array detector (PAD). Separation was performed using Luna C8(2) (4.6 × 150 mm, particle size 3 µm, Phenomenex, Torrance, CA, USA) column and gradient system of mobile phase (A - acetonitrile-water-trifluoroacetic acid, 5:95:0.1, v/v/v and B - acetonitrile-trifluoroacetic acid, 100:0.1, v/v) with flow rate of 1 mL/min. The PDA scanned through the wavelength range 200-400 nm. The quantification of phenolic compounds was carried out base on calibration curves of corresponding standards.

Statistical analysis

Antioxidant activity assays and HPLC analyse were performed in triplicate. Results were reported as means ± standard deviations. Analyse of variance (one-way ANOVA) followed by the least significant difference (LSD) test was conducted using statistical package of MSTAT-C software. Differences were considered to be statistically significant when $p < 0.05$.

Results and Discussion

Extraction yield and total phenolic content

The extraction yield and total phenolic contents of mung bean in extracts and seeds are shown in Table 1. The yield of mung bean seed extractions were 10.7 % from methanol extract and 7.97 % from acetone extract (Table 1). The total phenolic content (TPC) of mung bean seed was 47.16 mg GA eq/g extract (505 mg GA eq/100 g seed) and 66.05 mg GA eq/g (527 mg GA eq/100 g seed) in methanol and acetone extracts, respectively (Table 1). Although total phenolic content of acetone extract of mung bean found higher than methanol extract, there was no significant difference between seed TPCs.

Table 1. Extraction yield and total phenolic content in mung bean

Extract	Extract yield (%)	TPC (mg GA eq/ g extract)	TPC (mg GA eq/ 100 g seed)
MeOHext	10.70	47.16 ± 0.23b	505 ± 2a
ACEText	7.97	66.05 ± 2.09a	526 ±16a

* Data are reported as the mean ± standard deviation (n=3). In the same column values having different letters differ significantly (P<0.05).

Zhang et al. (2013) determined that acetone-water extract of mung bean had the higher TPC (5.07 mg GA eq/g) than methanol extract with indicating that acetone-water was a better solvent for extraction of phenolics from mung bean. In recent years, the functional properties of beans have received attention, particularly with respect to antioxidant effects and their total phenolic content. Therefore we compared the phenolic content of mung bean

with literature data of widely consumed different bean varieties along with mung bean and the reported studies were summarized in Table 2. The total phenolic content of mung bean determined in our study with value of 526 mg GA eq/100 g seed was higher than results showed by Marathe et al. (2011), Shi et al. (2016), Zhao et al. (2014), and similar to that presented by Zhang et al. (2013), Krishnappa et al. (2016), Khang et al. (2016) as seen Table 2. Lee et al. (2011) showed that mung bean contained a higher level of phenolics (about 4.01 GA eq/ g) than the soy beans (1.17 GA eq/g). According to Marathe et al. (2011) categorisation, the legumes depending on their phenolic content into three groups, as low (<1.0 mg GA eq/g), moderate (1.0–2.0 mg GA eq/g) and high (>2.0 mg GA eq/g). By this categorization, mung bean took placed in high content legume class. When compared the our results related to widely consumed white and red beans (*Phaseolus vulgaris* L.), mung bean exhibited similar total phenolic content to red bean, however nearly 8 and 26 fold more total phenolic content than white and kidney bean varieties, respectivly (Orak et al., 2015, 2016). Summarising, by comparing the results of the present investigation with

Table 2. The total phenolic content and main phenolic compounds of some different bean seeds reported in literature

Genotypes	TPC	units	main phenolic compounds	References
mung bean	1.83	mg GA/g seed	nd	Marathe et al. 2011
twenty Chinese mung bean cultivars	2.05-2.38	mg GA/g seed	phenolic acids (syringic, caffeic, p-coumaric, and ferulic acids)	Shi et al. 2016
ten commercial dry mung bean, China	5.07	mg GAE/g seed	salicylic acid, p-coumaric acid, ferulic acid, vitexin isovitexin	Zhang et al. 2013
green gram seeds from local market, India	4.86	mg GAE/g seed	Free phenolic acids, tannic acid, gallic acid,ferulic acid and sinapic acid	Krishnappa et al. 2016
commercial mung bean, Vietnam	5.80	mg GAE/g dry sample	nd	Khang et al. 2016
Mung bean	9.94	mg/g dry weight (DW)	vitexin and isovitexin	Peng et al. 2008
green mung bean sprout	2.09	mg GAE/ g (DW)	Gallic acid, p-coumaric acid, catechin, rutin, vitexin and isovitexin	Gan et al. 2016
56 mungbean genotypes from genebank of Korea	1.61 to 3.46	mg/g dry weight (DW)	Thirty types of phenolic compounds, including 11 flavonoids, 16 phenolic acids, pyrogallol, resveratrol, and vanillin	Kim et al. 2013
sixty mung bean genotypes	nd	-	catechin, chlorogenic acid, caffeic acid, p-coumaric acid, t-ferulic acid, vitexin, isovitexin, myricetin, quercetin and kaempferol	Meenu et al. 2016
mung bean **pinto bean** **black kidney bean** **red kidney bean**	26.7 33.4 32.9 27.1	mg GAE/g extract		Zhao et. al. 2014
red bean	55	mg CA /g extract.		Amarowicz and Troszynska, 2008
common red bean	3.58	mg GA /g of seed	nd	Marathe et al. 2011
two red bean varieties	1.69-4.85	mg GA /g seed	caffeic acid and rutin equivalent compounds	Orak et al. 2015
twenty nine white, red and	5.87–14.14	mg GA /g seed	nd	Akond et al. 2011

black common beans				
seven improved Brazilian common beans genotypes	4-0	mg GA/g seeds	nd	Rezende et al. 2017
kidney beans varieties	0.25 to 35.11	mg GAE/g DW	Pelargonidin, cyanidin, petunidin, delphinidin, malvidin	Kan et al. 2016
ten white bean varieties	0.33-0.63	mg GA /g of seed	caffeic acid equivalent compounds	Orak et al. 2016

those of earlier reports, it is evident that mung beans contained relatively high amounts of TPC than those reported in other types of beans and mung beans (Table 2).

Identification and quantification of phenolic compounds

Phenolic profiles of mung bean extracts in methanol and acetone extracts were screened by using DAD-HPLC technique. The HPLC chromatograms of mung bean extracts recorded at 290 nm are characterised by the presence of eight (1–8) peaks with a retention time of 1.8, 2.4, 6.8, 8.7, 9.3, 9.5, 10.6 and 15.1 min (Fig. 1) corresponding to phenolic compounds. Based on retention time and UV spectrum compounds 1-4 were classified as hydroxybenzoic acid derivatives. Compound 5 was assumed as hydroxycinnamic acid derivative. Spectra of compounds 6, 7 and 8 were comparable to that of apigenin and compounds were identified as apigenin derivatives. Contents of compounds 1–8 in the extracts and seeds of mung bean are given in Table 3. Gallic acid and *p*-coumaric acid were used as standards for quantification of hydroxybenzoic and hydroxycinnamic derivatives, respectively. Content of compounds 6-8 was expressed as apigenin equivalents

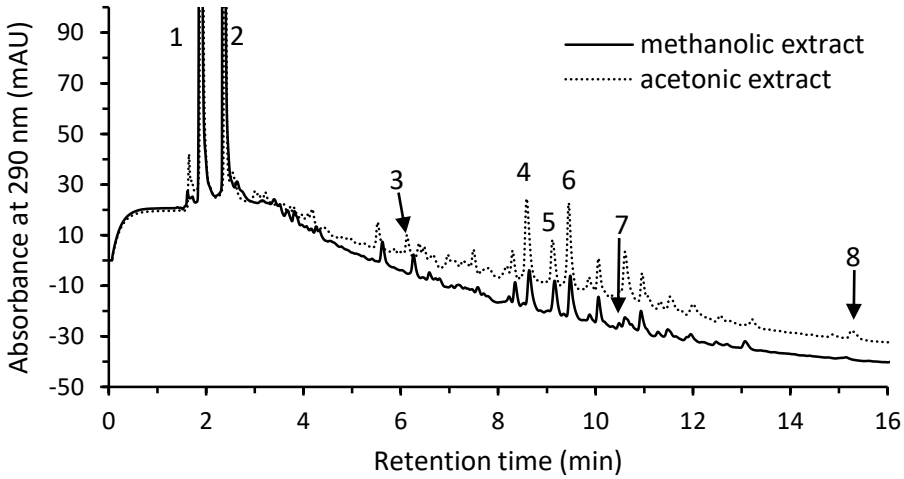


Figure 1. HPLC chromatograms of phenolic compounds of mung bean extracts recorded at 290 nm**.**

According to Table 3, compounds 1 and 2 were the most dominant phenolics in the extracts of mung bean. Compound 1 was found in the highest amount in methanol extract (5.74 mg/ g extract and 614.2 µg/g seed). The compound 2 followed it with high value of 3.54 mg/g (378.8 µg/g seed) in methanol extract. Acetone extracts explored lower content with value of 5.14 (409.6 µg/g seed) and 2.19 mg/g (174.5 µg/g seed) for these compounds, respectively (Table 3). Two other compounds expressed as gallic acid equivalents (compounds 3 and 4) were presented in mung bean in lower amounts (Table 3). Gallic acid is a hydroxybenzoic phenolic compound and the common phenolic acid found in beans (Wang et al. 2016). The seed coat (hull) of legume seeds primarily contains *p*-hydroxybenzoic, protocatechuic, gallic, vanillic and syringic acids (Amarowicz & Pegg, 2008). In reported studies we found only a few study which detected the gallic acid in mung bean. Kim et al. (2013) determined the gallic acid below the limit of quantification for 56 mung bean of Korean varieties. Nair et al. (2015) also determined low gallic acid content (from 9 to 45 µg/g) in mung bean lines grown in India. On the other hand, Krishnappa et al. (2016) determined higher gallic acid content (14.6 mg/g seed) from our findings. In comparison with other bean varieties, red sword bean and black sword bean coats contained high amounts of gallic acid (987 and 543 mg/100 g DW (dry weight), respectively) (Gan et al., 2016).

Compound 5; hydroxycinnamic acid derivative, was determined only in acetonic extracts. The content of this compound was 0.08 mg/g in extract and 6.4 µg/g in seed (Table 3). Shi et al (2016) and Gan et al. (2016) also reported the presence of *p*-coumaric acid in mung bean.

In our study it was not determined the presence of caffeic or ferulic acids that are typical hydroxycinnamates for beans (Amarowicz et al., 2008). In turn, Shi et al. (2016) between hydroxycinnamic acids identified three phenolic acids (caffeic acid, *p*-coumaric acid, and ferulic acid) in twenty Chinese mung bean cultivars.
Compounds 6, 7, 8 were classified as flavones and their content was expressed as apigenin equivalents. The content of compound 6 was the highest in these class phenolics at 0.21 mg/g extract (22.5 µg/g seed) and 0.37 mg/g extract (29.5 µg/g seed) in methanol and acetone extracts, respectively. Compound 7 determined only in methanol extract at level 0.016 mg/g extract (1.71 µg/g seed). The content of compound 8 was 0.07 mg/g in acetone extract and it was not detected in methanol extract. In recent studies the presense of vitexin (apigenin 8-C-glucoside) and isovitexin (apigenin 6-C-glucoside) in mung bean were recorded by Peng et al. (2008), Yao et al. (2011) and Zhang et al. (2013) (Table 2). The presence of apigenin or its derivatives in mung bean seeds is highlighted the value of mung bean, because studies have shown several beneficial health effects of these compounds, including antioxidant, anti-inflammatory, hypoglycaemic and hypocholesterolaemic activities (Arnoldi et al., 2015; Shukla and Gupta, 2010).

Table 3. Content of individual phenolic compounds in mung bean

Compounds	MeOHext (mg/g extract)	MeOHext (*µ*g/g seed)	ACEText (mg/g extract)	ACEText (*µ*g/g seed)
compound 1*	5.74 ± 0.22a	614.2 ± 2.3a	5.14 ± 0.24a	409.6 ± 10.1a
compound 2*	3.54 ± 0.14b	378.8 ± 14.9b	2.19 ± 0.10b	174.5 ± 7.9b
compound 3*	0.08 ± 0.01d	8.6 ± 1.1d	0.08 ± 0.04e	6.4 ± 3.1e
compound 4*	0.23 ± 0.01e	24.6 ± 1.0e	0.53 ± 0.02c	42.2 ± 1.6c
compound 5**	nd	nd	0.08 ± 0.00e	6.4 ± 0.1e
compound 6***	0.21 ± 0.01cd	22.5 ± 0.9cd	0.37 ± 0.01d	29.5 ± 0.9d
compound 7***	0.016 ± 0.01e	1.71 ± 0.8e	nd	nd
compound 8***	nd	nd	0.07 ± 0.01e	5.58 ± 0.7e

*Contents of compounds expressed as gallic acid equivalents. ** expressed as *p*-coumaric acid equivalents. ***expressed as apigenin equivalents. Data are reported as the mean ± standard deviation (n=3). In the same column values having different letters differ significantly (P<0.05).

Reported studies reveal that the mung bean is a good source of **phenolic acids**;
*such as **p-coumaric acid, ferulic acid;** (Shi et al 2016, Nair et al 2015, Zhang et al 2013, Khang et al 2016, Meenu et al 2016) **caffeic acid;** (Silva et al 2013, Meenu et al 2016) which are belong to **hydroxycinnamic acids**;
*such as **gallic acid**, (Nair et al 2015, Krishnappa et al 2016, Peng et al 2008) **syringic acid** (Shi et al 2016 Khang et al 2016) are belong to **hydroxybenzoic phenolic acids**;
*and **flavonoids** such as **catechin** (Peng et al 2008, Meenu et al 2016, Nair et al 2015), **quercetin** (Nair et al 2015, Meenu et al 2016) **vitexin and isovitexin** (Zhang et al 2013, Meenu et al 2016,Yao et al 2011, Peng et al 2008), one study detected the presence of **resveratrol** in mung bean (Kim et al 2013).

Antioxidant potential of mung bean seeds

The antioxidant activities of mung bean extracts and seeds determined as TEAC and FRAP are given in Table 2. The acetone extract exhibited much higher $ABTS^{\bullet+}$ scavenging activity (1.093 mmol Trolox/g extract; 0.087 mmol Trolox/g seeds) than methanol extract (0.742 mmol Trolox/g extract; 0.077 mmol Trolox/g seeds). Shi et al. (2016) determined the lower $ABTS^{\bullet+}$ radical-scavenging capacity from our findings that ranged from 3.82 ± 0.25 to 13.44 ± 1.76 µmol/ g seed in twenty Chinese mung bean cultivars. Higher TEAC values were determined by Zia-Ul-Haq et al. (2013) (21.2–31.1 µmol Trolox/g) for four mung bean (*Vigna radiata* L. Wilczek) varieties indigenous to Pakistan. When compared $ABTS^{\bullet+}$ scavenging activity of mung bean with that of other legumes, TEAC capacity of mung bean was higher than those of broad bean (0.58 mmol Trolox/g extract), red lentil (0.68 mmol Trolox/g extract), red bean (0.149–0.493 mmol Trolox/g extract) (Amarowicz et al., 2004; Orak et al., 2015), , however lower than adzuki bean (1.76 mmol Trolox/g extract) (Amarowicz et al., 2008). According to our recent research mung bean exhibited two or three fold more TEAC capacity compared to widely consumed white bean varieties (*Phaseolus vulgaris* L.) (27- 43 µmol Trolox/g extract) (Orak et al., 2016). Ferric reducing antioxidant power (FRAP) of extracts and seeds of mung bean were shown in Table 3. Acetone extract showed

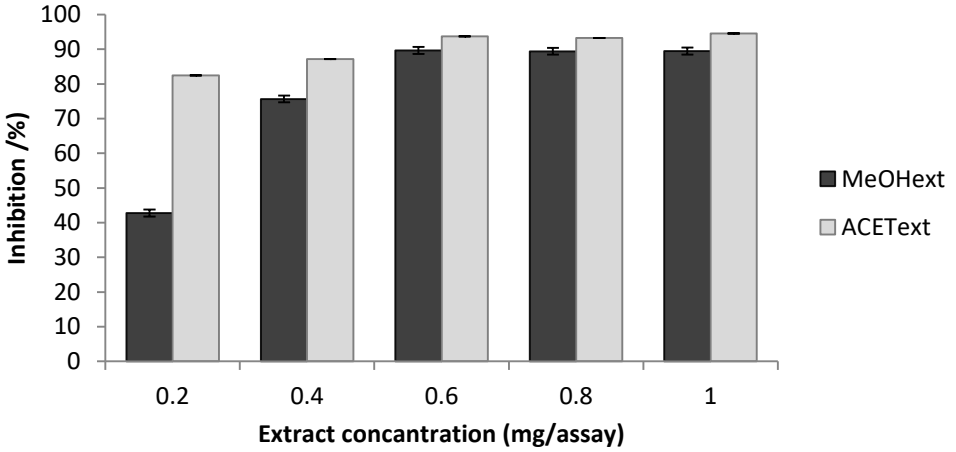
higher antioxidant activity (632.5 µmol Fe^{2+}/ g extract) than methanol extract (271.82 µmol Fe^{2+}/g). The ability of mung bean to reduce Fe^{3+} was found similar to red common bean (Orak et al., 2015) however nearly 3 or 4 fold higher than widely consumed white bean varieties (66 -89 µmol Fe^{2+}/g) (Orak et al., 2016). According to a previous research (Lee et al., 2011) the FRAP of the mung bean extracts (31.85 µmol Fe^{2+}/g defatted seed) was significantly higher than that of the soy bean extracts (8.23 µmol Fe^{2+}/g defatted seed). Also Djordjevic et al. (2011) found that mung bean extract had significantly greater reducing power than that of the soy bean extract.

Table 4. Trolox equivalent antioxidant activity (TEAC) and ferric-reducing antioxidant power (FRAP) of the in mung bean

Extract	TEAC (mmol TE/ g extract)	TEAC (mmol TE/ g seed)	FRAP (µmol Fe^{2+}/ g extract)	FRAP (µmol Fe^{2+}/ g seed)
MeOHext	0.742 ± 0.055	0.077 ± 0.001	271.8 ± 1.26	29.08 ± 0.13
ACEText	1.093 ± 0.026	0.087 ± 0.002	632.5 ± 5.16	50.43 ± 0.41

* Data are reported as the mean ± standard deviation (n=3). In the same column values having different letters differ significantly (P<0.05).

DPPH radical scavenging activity is widely used method to test antioxidant activity of samples. This radical react with hydrogen donors such as phenolic compounds and fade colours in assay solutions. As shown in Fig 3, the mung bean extracts showed significantly higher radical scavenging activities even at lower concentrations, however acetone extract demonstrated higher antiradical activity against DPPH• than the methanol extract. In the case of acetone extracts, phenolic compounds of mung bean were more active. The DPPH• scavenging activity of mung bean obtained in this study was stronger than that of the extract of the red bean (Orak et al., 2015). Anwar et al. (2013) determined lower IC_{50} value (between 16.4 µg/mL and 42.9 µg/mL) for mung bean seed in different extraction methods.



MeOHext (EC 50) = 0.089 mg/mL; ACEText (EC 50) = 0.053 mg/mL

Figure 3. Antiradical activity of mung bean extracts against DPPH• radical (n=3).

Conclusions

In recent years, the functional properties of mung bean have received attention, and this study evaluated the antioxidant capacity and phenolic compound profiles of mung bean seeds in methanol and acetone extracts and these constituents of mung bean compared with literature data of antioxidant properties widely consumed different bean varieties. According to investigations, mung beans showed strong antioxidant capacity with high total phenolic content. In each of extracts of mung bean, several phenolic compounds were detected and classified as hydroxybenzoic and hydroxycinnamic acids derivatives and as flavones. The hydroxybenzoic acid derivatives were dominant. Obtained results suggest that mung bean can be used as functional ingredient with high antioxidant activity in foods such as soup, pasta and can be increasing of consume in human diet. Therefore larger field productions can be achieved for this legume.

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Araştırma Makalesi/*Research Article (Original Paper)*

Some Growth Parameters of 'Red Globe' Grafted on 140 Ru (Ruggeri) Rootstock Grown on Silty Clay Loam Soil under Diluted Brackish Water Irrigation

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Abstract: Application of brackish water mixed with a local water source used for irrigation in ratios to keep the salinity of the irrigated soils below the threshold of the grapevines could be an urgent water management practice in areas suffering from scarce water resources such as Jordan. The objective of this research was to evaluate some growth parameters of 'Red Globe' grafted on 140 Ru (Ruggeri) irrigated with three water salinity levels: 3.0, 5.0 and 7.0 dS m^{-1} in addition to 1.0 dS m^{-1} as the control treatment. A complete randomized design was used with five replicates. 'Red Globe' grafted on 140 Ru maintained good performance at only 3.0 dS m^{-1} irrigation water salinity level in terms of shoot length, leaf area, pruning weights and chlorophyll content following bud break. Proline content per leaf area increased with the increase of the salinity levels. This experiment reveals that grapevine rootstocks that have *V. berlandieri* x *V. rupestris* in their parentage are good salinity tolerant rootstocks.
Keywords: salinity, shoot length, leaf area, proline, pruning weights, alternate irrigation.

Introduction

Precipitation is the main source of water in Jordan. However, Jordan suffers from water shortage due to climatic factors; aridity and high evapotranspiration and the rapid growth of the population. Jordan is considered a poor country in water resources. In fact, Jordan is now ranked as the world's second water-poorest country in terms of water resources per inhabitant (FAO 2015).

The annual reports of the Ministry of Agriculture and Ministry of Water and Irrigation in Jordan indicate that the agricultural sector portion of the total available water is about (70-75%). This portion has decreased during the last ten years and reached about (60-65%) (Ministry of Agriculture and Ministry of Water and Irrigation 2015) and is continuing to show reduction. This situation will increase the demand of using wastewater as well as brackish water to become potential alternative irrigation sources.

Salinity is considered one of the most important abiotic stresses that determine crop growth and productivity. Many studies indicated that salinity affect photosynthetic enzyme activities, chlorophyll content and cause osmotic stress (Baghalian et al. 2008; Jelali et al. 2011). Baghalian et al. (2008) considered water availability a major limitation in crop production especially in arid and semi-arid regions making brackish water an alternative water resource.

Worldwide, grapes are one of the major fruit crops planted and harvested. In Jordan, grapes are ranked in second place after olives regarding the total area planted (Department of Statistics 2016). The total area planted with grapes is 3888.5 hectares. More than half of that area is under irrigation. Grapes in Jordan are planted in the Jordan Valley, up high in the mountains and even in small areas in the eastern plains of the country (Badia). However, some areas in the Jordan Valley are salt-affected with relatively high organic matter due to the common agricultural practices applied in the Jordan Valley.

Among the most important rootstocks used by Jordanian grape growers are: 41B, 1103P, and 110R. The growth performance, antioxidant defense genes and stomatal resistance of 'Superior Seedless' cultivar; which is one of the main grape cultivars planted in the Jordan Valley, grafted on 41B, 1103P, and 110R under diluted brackish water

irrigation have been studied by Qrunfleh et al. (2017a), Qrunfleh et al. (2017b) and Qrunfleh (2018). The rootstock 140 Ru is one of the promising rootstocks to be used in Jordan. This rootstock has the same parentage of 1103P (*V. berlandieri* x *V. rupestris*). One of the main cultivars grafted on 140 Ru is 'Red Globe' which is one of the most popular grape cultivars in the Middle East because it has a long growing season. Therefore, the objective of this research was to evaluate some growth parameters such as shoot length, leaf area, proline content, pruning weights, chlorophyll content following bud break of 'Red Globe' grafted on 140 Ru irrigated with three levels of diluted brackish water in addition to a control treatment as irrigation water.

Materials and Methods

The grape rootstock evaluated in this study was 140 Ru (*V. berlandieri* x *V. rupestris*). The rootstock was purchased from France in 2016 and 'Red Globe' bud cultivar was grafted on the imported rootstock in a local nursery during the winter season of 2016. The grafted materials were planted in polyethylene bags filled with peatmoss and perlite (2:1 v/v) and kept at the nursery for one year until the winter of 2017.

In February 28, 2017, plants were transplanted into pots filled with 30 kg soil, which was previously crushed and sieved through a 1 cm sieve. The soil properties are presented in Table 1. Grapevine transplantation to the plastic pots was done after bud break. In order to unify grapevine growth before applying the treatments, the root system was cut back to about 15 cm in length and the vegetative system was cut back to five buds before transplanting the grapevines to the pots. The transplanted material was grown in a controlled greenhouse at 25 °C ± 1. Composite fertilizers, urea, ammonium sulphate and fungicides were applied during the growing seasons (spring and summer) of 2017 to obtain healthy grapevines before the beginning of treatments.

The source of the brackish water was a local dam located in the Jordan Valley. Brackish water was stored in a galvanized tank. Water properties are presented in Table 2. The three salinity water levels; in terms of electrical conductivity (EC), were: 3.0 (S1), 5.0 (S2) and 7.0 (S3) dS m^{-1} in addition to the control water 1.0 dS m^{-1} as the control (C) treatment. A portable conductivity meter (Model Cond 3210, WTW, Germany) was used to measure the EC and to obtain the assigned treatments. A complete randomized design was used with five replicates.

Vegetative growth was again unified before applying the assigned treatments. Starting date of applying the assigned treatments was the beginning of September 2017and the end of the experiment was by the end of December 2017. All pots received the same amount of water whenever irrigation was applied. Each pot received a total amount of irrigation water equal to 13.47 mm/month. Irrigation was scheduled according to evaporation readings from free water surface (in mm) taken every 48 hours and corrected using proper grapevine crop coefficient of 0.30 (according to Food and Agriculture Organization).

Growth Parameters

Shoot length was recorded three times during the growing season of 2017 in the end of September, October and December. Leaf area was also determined in November, 2017 by using a leaf area meter (AM300, Bioscientific Ltd., UK). On March 8, 2018 pruning weights of the whole treated vines were cut from the base and weighed using an upright balance scale. After bud break, a SPAD-502 purchased from Minolta CO., LTD, Japan, was used to measure the chlorophyll content of fully expanded mature leaves in March, 2018.

Proline Extraction

Free proline was extracted and colorimetrically estimated in fresh leaf samples after two weeks of the treatments and in November, 2017 following the procedure described by Bates et al. (1973). Half g of leaves was homogenized in 10 ml of 3% (w/v) aqueous 5-sulfosalycilic acid and the extract was filtered through Whatman No. 2 filter paper. Two ml samples of the filtrate were reacted with 2 ml acid-ninhydrin (prepared by dissolving 1.25 g ninhydrinein 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid with agitation) and 2 ml glacial acetic acid in test tubes; the mixture was heated using boiling bath (100 °C) for 45 minutes to allow color development. Thereafter, the reaction was stopped by placing the mixture in an ice bath. To extract the chromophore, 4 ml of toluene were added and vortex vigorously for 20 seconds. The upper chromophore was aspirated and absorbance was compared with toluene

blank using WPA Biowave II Spectrophotometer (Biochrom, UK) at 520 nm. Stock solution of pure proline (Sigma) at a concentration of 100 µg/ml in 3% (w/v) 5-sulfosalicylic acid was prepared to construct a standard curve.

Results and Discussion

One-year old grapevine rootstocks were grown on a silty clay loam soil. Soil physical and chemical properties are presented in Table (1). Plants were regularly irrigated with diluted brackish water at three salinity levels. The water chemical properties of the brackish water are presented in Table (2).

Brackish water used in the current study contained total chloride ions of approximately 1662 mg l^{-1} with an EC value of 7.56 (dS m^{-1}). Chloride formed approximately the majority of the ionic composition of the brackish water (before dilution).

Table 1. Some properties of the soil brought from the southern Jordan Valley.

Soil Property	**Value**
Soil Texture	Silty clay loam
pH 1:1	8.25
EC 1:1 (dS m^{-1})	3.56
Organic matter %	2.44

Table 2. Chemical analysis of brackish water before diluted for irrigation in the current research.

Water Properties	Value
pH	8.62
EC (dS m^{-1})	7.56
Cl (mg l^{-1})	1662
Ca (mg l^{-1})	120
Mg (mg l^{-1})	175

Shoot length was measured and recorded in the end of September 2017 (after one month from the beginning of the treatments). Shoot length was affected particularly at S3 (Figures 1 and 2). However, shoot length was not affected compared to the control particularly at salinity levels S1 and S2. The trend was the same in October (Figure 2) and November 2017 (since the shoot length was very similar to the data in October, the data is not shown).

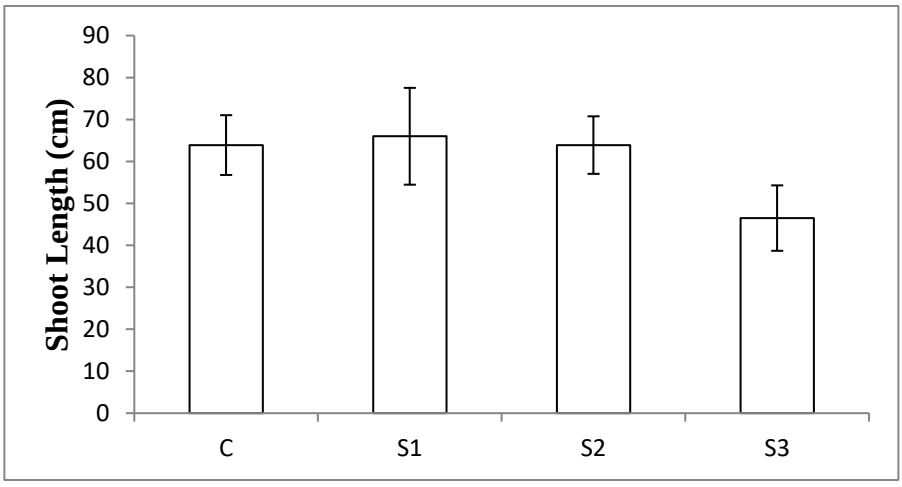

Figure 1. 'Red Globe' shoot length (cm) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in September 2017 n=5.

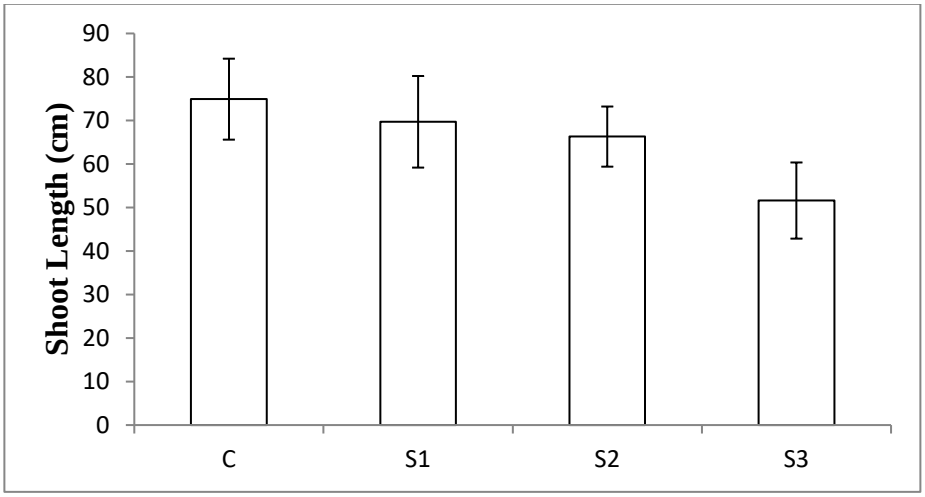

Figure 2. 'Red Globe' shoot length (cm) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in October 2017 n=5.

The results from Figures (1 and 2) show that the 'Red Globe' growth was affected negatively with the increase of the salinity levels. However, the tolerance level threshold of the grapevine rootstock (140 Ru) seems to be 3.0 dS m^{-1} which is considered slightly higher compared to other rootstocks (Walker 2010). This coincides well with the finding of Tregeagle et al. (2006) and Walker et al. (2010) as a Cl^- rootstock excluder.

Leaf area was another growth parameter measured in this study. As expected, leaf area was negatively affected at the highest salinity level (Figure 3). The results also support that the tolerance level threshold of the rootstock seems to be 3.0 dS m^{-1}.

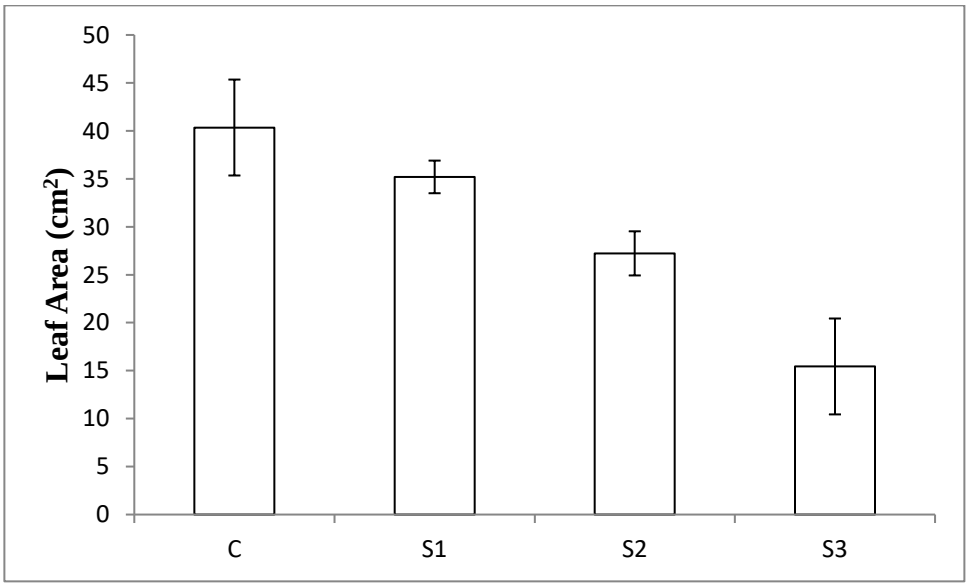

Figure 3. 'Red Globe' leaf area (cm^2) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in November 2017 n=5.

As a compatible osmolyte, proline is accumulated in response to salt stress and drought conditions (Shao et al. 2006). Proline was reported to stabilize proteins and membranes (Solomon et al. 1994). Moreover, proline participates in defense responses and signaling that mediate stress-responsive genes (Khedr et al. 2003). In this regard, proline content was quantified after two weeks in leaves of 'Red Globe' grafted on 140 Ru rootstock, when irrigated with water of different salinity levels (Figure 4). After two weeks of S1 (3.0 dS m^{-1}) salinity treatment, leaf proline content

reached almost 14 µg/g proline, while control plants had about 7 µg/g proline. The proline content almost doubled in the 'RedGlobe' leaves after two weeks from irrigating with diluted brackish water. However, at S2 (5.0 dS m^{-1}) and S3 (7.0 dS m^{-1}) the proline content was higher than the control but less than S1 (3.0 dS m^{-1}).

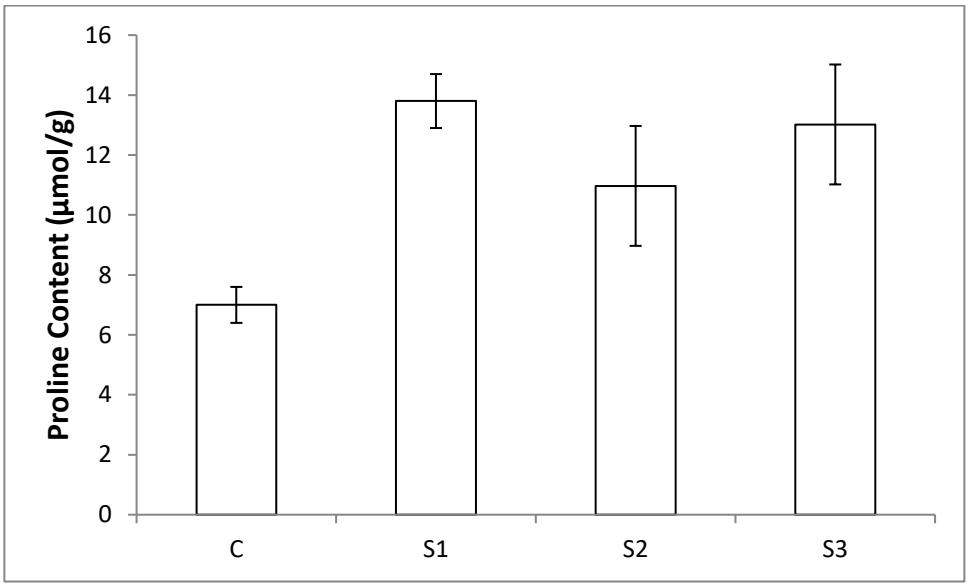

Figure 4. 'Red Globe' leaf proline content (µg/g) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} after two weeks irrigation n=5.

Taking into consideration the negative effects of the salinity level treatments on leaf area, proline content was quantified again in November and the proline content per unit area was calculated and is presented in Figure (5). The results shown in Figure (5) clearly show that more porline accumulated (µmol) per unit area (cm^2) with the increase level of salinity. The results imply that grapevine rootstock 140 Ru was attempting to tolerate the increase level of salinity by accumulating more proline. However, the attempt did not compensate the negative impact of salinity on the growth parameters discussed earlier particularly at S2 (5.0 dS m^{-1}) and S3 (7.0 dS m^{-1}), but did assist 'Red Globe' to tolerate S1 (3.0 dS m^{-1}). This assistance is clearly shown in the two other growth parameters, specifically the pruning weights and the chlorophyll content following bud break in spring 2018.

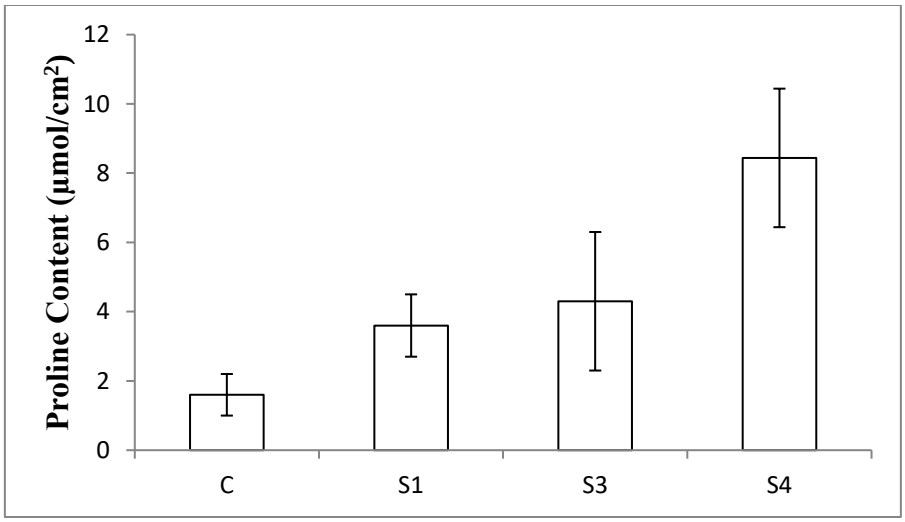

Figure 5. 'Red Globe' leaf proline content per leaf area (µmol/cm^2) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in November 2017 n=5.

Two growth parameters were measured to study accumulative effects of the treatments; pruning weights were cut and weighed during the dormant season (winter of 2018) (Figure 6) as well as leaf chlorophyll content following bud break (Figure 7). Regarding the pruning weights, at S1, the pruning weights were similar to the control treatment. However, at S2 and S3 pruning weights were negatively reduced. Again, the results also support that the tolerance level threshold of the rootstock seems to be 3.0 dS m^{-1}.

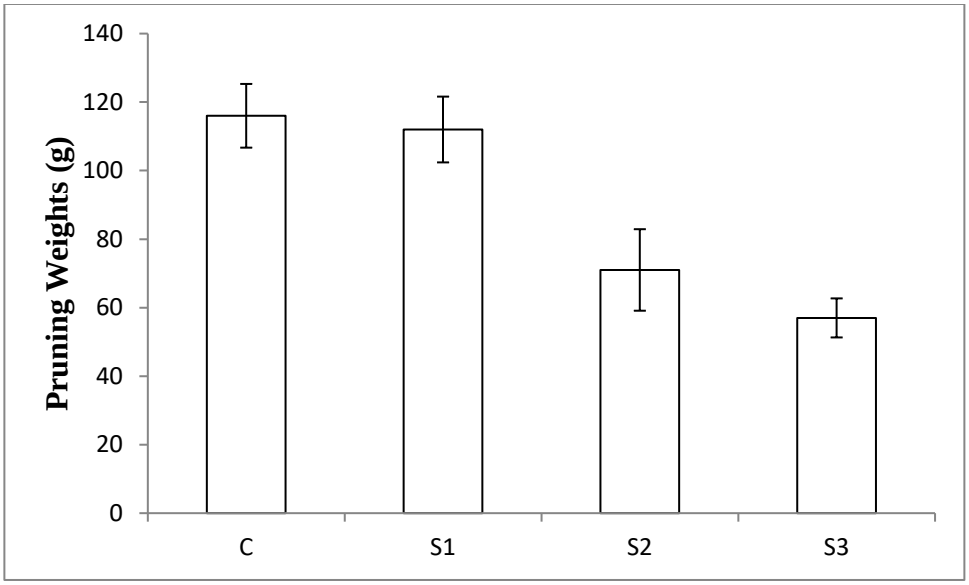

Figure 6. 'Red Globe' pruning weights (g) grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in March 2018 n=5.

Regarding the leaf chlorophyll content following bud break, the results are shown in Figure (7). As expected, the chlorophyll content decreased with the increase in the salinity level treatments. However, at S1 (3.0 dS m^{-1}) the results were not significantly different from the control providing further support to the salinity level tolerance threshold of the 140 Ru rootstock.

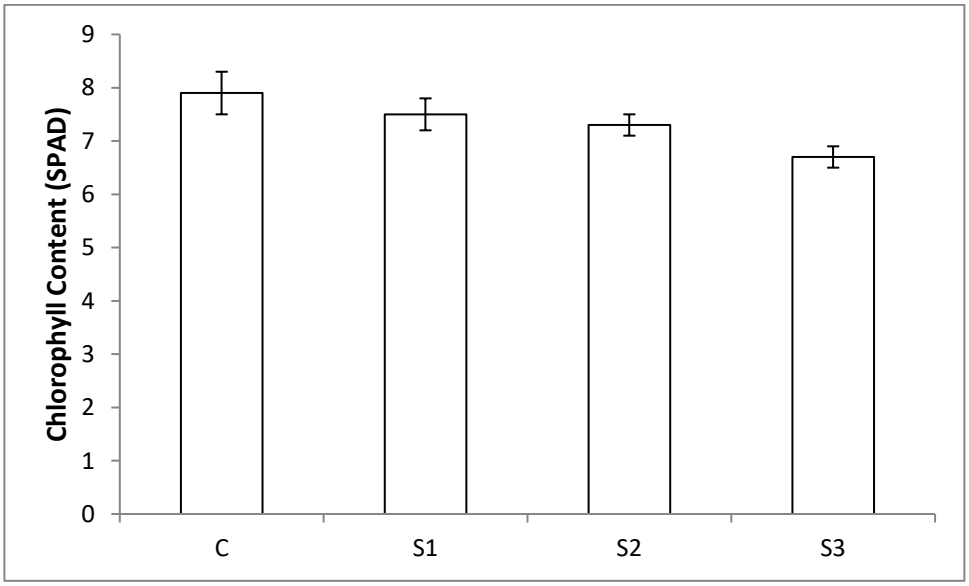

Figure 7. 'Red Globe' leaf chlorophyll content (Spad Readings) after bud break grafted on 140 Ru rootstock at the four treatments: C: control (1.0), S1: 3.0, S2: 5.0 and S3: 7.0 dS m^{-1} measured in March 2018 n=5.

Conclusions

This experiment shows and supports that grapevine rootstocks which hold *V. berlandieri* x *V. rupestris* in their parentage are among good salinity tolerant grapevine rootstocks. It also shows that 'Red Globe' grafted on 140 Ru maintained good performance at only 3.0 dS m^{-1} irrigation water salinity level. It can be also concluded that mixing diluted brackish water with irrigation water to be followed by irrigation with good quality water in order to flush excessive salts out of the root zone could be practiced in areas suffering from scarce water resources**.**

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Araştırma Makalesi/*Research Article (Original Paper)*

Management of the hairy rose beetle, *Tropinota squalida* (Coleoptera: Scarabaeidae) by mass trapping in apple orchards

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Abstract: The potential of mass trapping for the management of the hairy rose beetle, *Tropinota squalida* (Scopoli) was evaluated in commercial apple orchards in Jordan. A funnel trap baited with floral attractants was tested for effective capture of the beetle. Efficacy of the floral attractants compared to apple flowers was tested using an olfactometer. Factors affecting trap effectiveness including: trap color, placement, and baiting with floral attractants were evaluated in apple field trials. The potential of mass trapping using different densities of traps/h. was examined on large scale field trials over two growing seasons. Results showed that dispensers with flower attractants were more attractive to the beetles than apple flowers. Field trials showed that the beetles were highly attracted to blue, white and yellow traps compared to green and orange ones. The traps placed on the ground attracted more beetles than traps suspended on the tree. Moreover, traps baited with attractants were more attractive than traps with no bait. In the mass trapping trials, percentage of damaged flowers by the beetles was reduced to 3.5% when 50 traps/h were used in the treated field plots compared to 8.3% in the controls with no traps in the first growing season and to 4.7% in the treated plots compared to 15.3% in the control plots in the second growing season. These results indicated that mass trapping technique has the potential to be incorporated in an Integrated Pest Management (IPM) program to reduce the damage caused by the hairy rose beetle in apple orchards.

Key words *Tropinota squalida*, Mass trapping, Floral attractants, Apple.

Introduction

The hairy rose beetle, *Tropinota squalida* (Scopoli) (Coleoptera: Scarabaeidae) is a serious pest of fruit trees in many parts of the world. The beetle is widespread in the Mediterranean region and Europe (Toth et al. 2009) and is polyphagous, attacking several members in the Rosaceae and Asteraceae families (Hussien et al. 2005). Adult beetles are the most destructive stage causing severe damage by attacking trees during bloom, when feeding on the flowers of economically important trees such as apples and pears (Al-Alawi 2014). Feeding of this pest causes destruction of the flowers reproductive parts and results in reduced flower pollination and fruit set (Ateyyat and Al-Alawi, 2017). Conventional control of the hairy rose beetle relies primarily on applications of insecticides. Besides the harmful effects of insecticides on the environment, their application during the flowering period of the crop should be restricted because of the presence of pollinators foraging the crop during bloom (Schmera et al. 2004). In addition, application of pesticides using pressure sprayers during bloom usually results in flower dropping before pollination. Therefore, safe and pollinator friendly control measures are required for effective management of the target pest.
Mass trapping of pests is an approach that has been used successfully for other agricultural and forestry pests (El-sayed et al. 2006). This strategy relies on species-specific synthetic chemical lures, such as sex and aggregation pheromones and food/host attractants, to attract insects to a trap where they would be confined and die (El-sayed et al. 2006). Mass trapping has been used as an effective control method against a variety of pests such as the codling moth, *Cydia pomonella L.* (Madsen and Carty 1979), the goat moth, *Coses coses* L. (Faccioli et al. 1993), the pink bollworm, *Pectinophora gossypiella* (Saunders) (Mafra Neto and Habib 1996), the Chinese tortrix, *Cydia trasias* (Meyrick) (Zhang et al. 2002) and the leopard moth, *Zeuzera pyrina* Fab. (Hegazi et al. 2009).

A chemical attractant for *Epicometis hirta* Poda, a closely related and morphologically similar species to *T. squalida* was developed (Toth et al. 2004). The attractant was composed from cinnamyl alcohol and trans-anethole at a ratio

of 1:1 (Schmera et al. 2004; Toth et al. 2004). The same attractant has shown to be effective in attracting *T. squalida* (Toth et al. 2009). Thus, we hypothesized that these floral attractants could be incorporated as a component in an Integrated Pest Management (IPM) program against *T. squalida* using the mass trapping technique. In Jordan, hairy rose beetle is a monovoltine pest that overwinters in soil as adults and emerges in early to mid Feb. to attack flowers of fruit trees (Ateyyat and Al-Alawi 2017) . Deployment of effective hairy rose beetle traps might remove a large proportion of the emerging beetle population before they inflict damage on trees. Therefore, the objective of the current study was carried out to evaluate the efficiency of an on-farm built trap in capturing *T. squalida* adults and the potential of mass trapping in reducing damage caused by the target pest to apples.

Materials and methods

Experimental site

The field trials were carried out in a commercial apple orchard located in Alshoubak area in the southern part of Jordan, nearly 220 km from Amman. The area of the farm is about 40 ha planted mainly to the apple variety Golden Delicious. Few sections in the farm (about 1-1.5 ha) are planted to plums, cherries and peaches.

The trap

The hairy rose beetle trap (HRBT) consisted of three main parts: a landing and collecting platform, a container and an attracting dispenser. The landing platform is a plastic funnel (21 diameter X 25 cm height). The container serves to hold the captured insects that fall into the trap. It was made from empty pesticides plastic bottles (1L. in size). The screw cap of the bottles constituted the base of the container so that it could be opened and emptied if the trap were full of beetles. The landing platform and the container were assembled using plastic glue. The baiting dispenser was prepared with floral attractants used for trapping other *Tropinota* species and *E. hirta*. The attractants were (E)-cinnamyl alcohol (3-phenyl-2-propenyl alcohol) and (E)-anethole ((E)-4-propenylmethoxybenzene) in 50% dichloromethane solution (Sigma Aldrich, Eu.) at a blend ratio of 1:1 (Toth et al. 2004). One hundred mg of each attractant were added to 2 x 2 x 1 cm cotton pieces. The cotton pieces were then placed in 150µm polyethylene bags and the bags were heat sealed. The dispensers were individually wrapped in aluminum foil and stored at -20ºC until used. When used in the field, the dispensers were stuck to the inner surface of the funnel using grey duct tape and then punctured five times using size 2 insect pin.

Factors affecting trapping efficiency

To improve trapping efficiency of hairy rose beetles, color of landing and collecting surface, trap placement and number of supplied dispensers were evaluated in apple orchards. Five different colors for the funnel used as the landing and collecting surface were tested. The colors were blue, white, yellow, orange and green. For each tested color, three traps were placed between rows of apple trees. Traps from the same color as well as from different colors were separated by approximately 100 m. The traps were fastened on 100 cm wooden stakes so that the base of the trap was approximately 20 cm above ground. Each trap was supplied with one dispenser. Numbers of captured adults of the hairy rose beetle were recorded in each trap one week post placement of traps. The experiments were repeated with new dispensers so totally six replicates were performed for each color.

A trial was designed to evaluate the efficiency of capture as influenced by the placement of the trap either on the ground or hanging on blooming apple trees. Traps on the ground were placed as above, while traps on the tree were hung on apple trees approximately 1.5 m above ground. Only white traps were used and supplied with one dispenser. Numbers of captured adults of the hairy rose beetle were recorded in each trap one week post placement of the traps. Three traps were used for each placement and the experiment was repeated with new dispensers.

To test the effect of amount of attractant, white traps supplied with 0, 1, 2 and 3 dispensers placed on the ground between rows of apple trees as above. Numbers of hairy rose beetle adults captured in each trap was recorded for one week. There were 3 traps for each number of dispensers.

Effectiveness of the attractive dispenser compared to the natural host

The effectiveness of the floral attractive mixture of (E)-cinnamyl alcohol and (E)-anethol was compared to apple flowers as the natural source of attraction by using an olfactometer choice chamber in the lab. The olfactometer consists of a central chamber of a 500 mL spherical flat bottom glass flask. Four openings were made on each side of the flask and 10 cm length and 2 cm diameter glass tubes were sealed on each opening. Polyethylene tubing 100 cm in length was fastened at the end of each glass tube. Three 250 mL flasks individually contained a dispenser with (E)-cinnamyl alcohol and (E)-anethol (as above), a dispenser without the floral attractants, a group of 10 fresh apple flowers and the fourth remained as a control. Flask contents were assigned randomly. One hundred hairy rose beetle adults were placed in the central chamber. The apparatus was placed in full darkness and number of beetles in each flask was recorded after 1 h. The experiment was repeated five times, each time cleaned apparatus with a new set of dispensers, apple flowers and beetles were used.

Evaluation of mass trapping

To evaluate the efficiency of mass trapping as a management tactic to reduce damage caused by the hairy rose beetles, traps at densities of 0, 1, 3 and 5 traps per 1000 m^2 were tested in 2009 and 2010 growing seasons. The experiment was arranged in a randomized complete block design with two blocks. Each trapping density was assigned randomly to a 1000 m^2 plot (25 x 40 m) of apple trees within each block. The plots were separated by a distance of 80 m within a row and a distance of about 150 m between rows (Figure 1). The traps used were white and placed on the ground as above. Each trap was supplied with one dispenser. The traps were placed two weeks before flowering and continued until the end of flowering (5 weeks). New dispensers were supplied to the traps every two weeks. Numbers of hairy rose beetles captured in the traps were recorded and the corpses removed weekly.

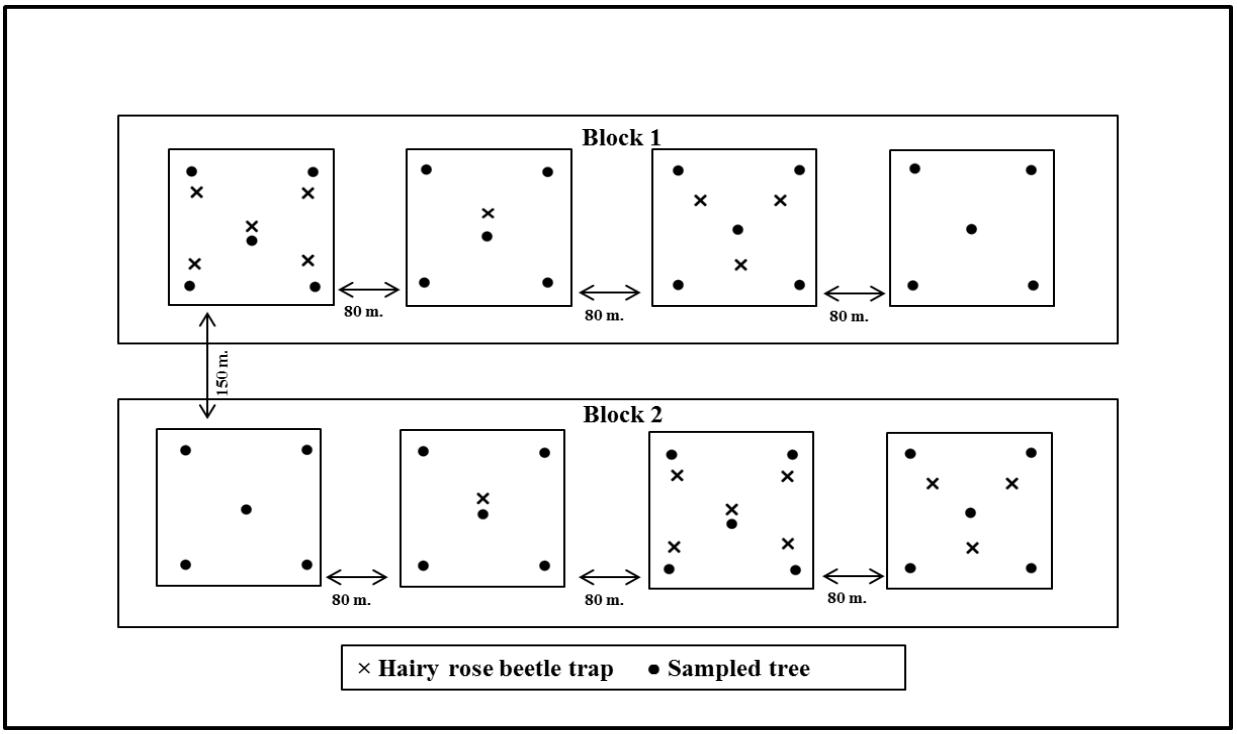


Fig. 1: Experimental setup of mass trapping field trials for the hairy rose beetle in apple orchard showing trap and sampled trees locations.

To assess apple flower damage in the plots supplied with different densities of traps, five trees from the corners and the center of each plot were selected during peak flowering (figure 1). From each selected tree, four apple branches from the four sides of the tree were randomly selected. Numbers of open, healthy apple flowers and numbers of apple flowers damaged by the hairy rose beetle were recorded on each selected branch.

Statistical analysis

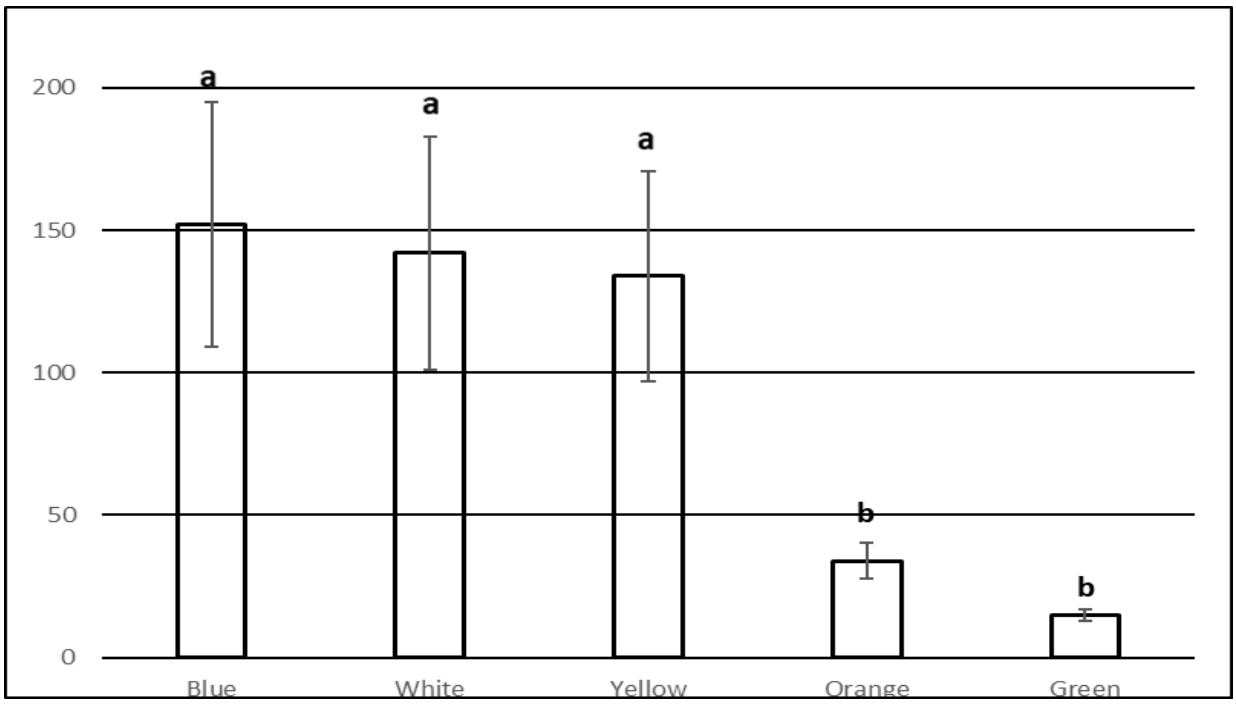
Results from trap colors, number of dispensers and attractiveness of dispensers compared to apple flowers were subjected to one way ANOVA and followed by mean separation using LSD-test if the F-value was significant. Trap placement data were analyzed by one way ANOVA. Trap capture and flower damage data from the mass trapping trials were analyzed by two-way ANOVA. When no significant effects were found between the 2009 and 2010 flowering seasons, data were pooled over the seasons and means were separated by LSD-test. When the tests for normality failed, trap captures were square root transformed while percentages data were subjected to arcsine square root transformation. Type I error (α) was set at 0.05 level for all the tests. Data were analyzed using SAS software version 9 (SAS Institute 2002).

Results

Factors affecting trapping efficiency

Trap color had a significant effect on numbers hairy rose beetle adults captured by the trap ($F_{4,25} = 4.37$; $P < 0.008$). Mean number of hairy rose beetles captured in blue (152 beetles/trap), white (142 beetles/trap) and yellow (134 beetles/trap) traps were significantly higher than mean number of beetles captured in orange (34 beetles/trap) and green (15 beetles/trap) traps. However, no significant differences were detected among mean numbers of beetles captured in blue, white and yellow traps (Figure 2). White traps were selected for further testing of mass trapping.

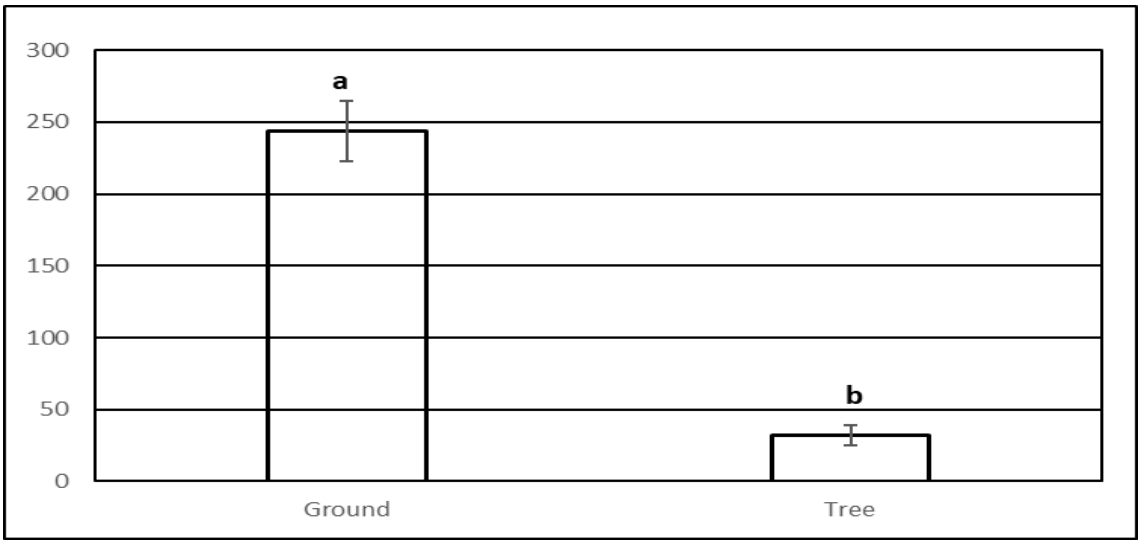
Fig. 2: Effect of trap color on the attractiveness of the hairy rose beetle to traps with different colors supplied with dispensers containing 100 mg of (E)-cinnamyl alcohol and (E)-anethol.



Bars with the same letters are not significantly different at 0.05 level using least significant difference (LSD) test.

Trap placement significantly affected trap efficiency ($F_{1,10} = 25.5$; $P < 0.0005$). Mean numbers of caught beetles was significantly lower in traps placed on the ground (32 beetles/trap) compared to traps placed on the trees (244 beetles/trap)

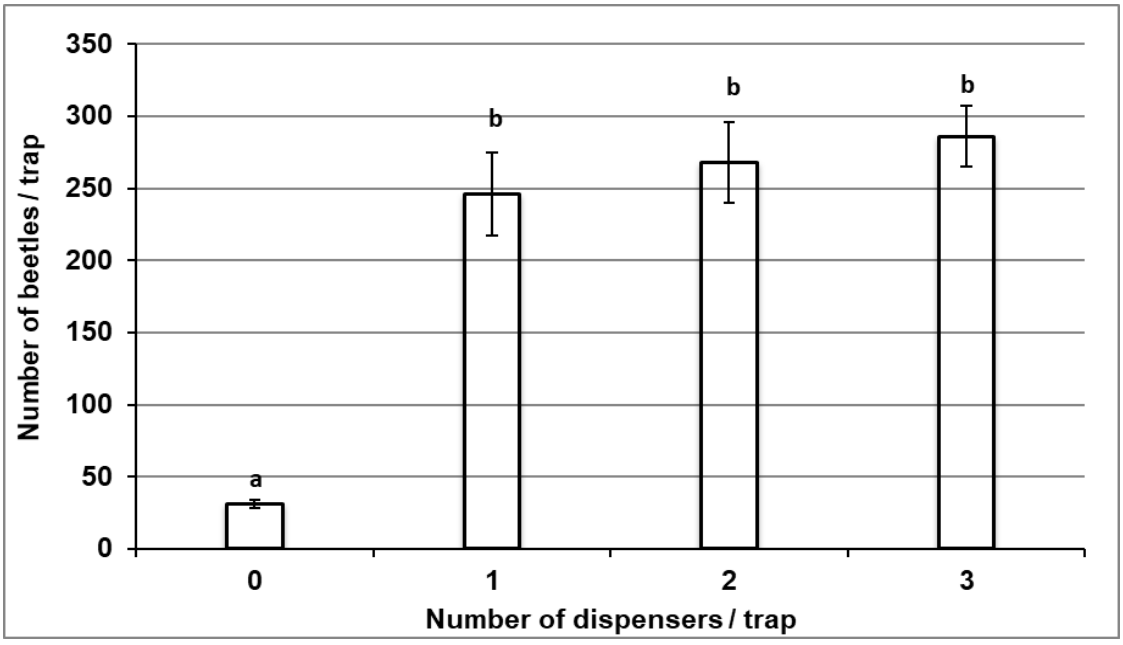
Fig. 3: Effect of trap position on attractiveness of hairy rose beetles to white traps supplied with dispensers containing 100 mg of (E)-cinnamyl alcohol and (E)-anethol and placed on the ground or hanged on apple trees.



Bars with the same letters are not significantly different at 0.05 level using least significant difference (LSD) test.

Similarly, the number of dispensers placed in the traps significantly affected trapping efficiency ($F_{3,20} = 6.2$; $P < 0.004$). Traps with no attractant dispenser significantly captured fewer hairy rose beetles compared to traps supplied with dispensers. Mean numbers of captured insects increased with increasing number of supplied dispensers per trap. However, no significant differences were found among mean numbers of beetles captured in traps supplied with 1 (246 beetles/trap), 2 (268 beetles/trap) and 3 (286 beetles/trap) dispensers (Figure 3).

Fig. 4: Effect of the number of dispensers on attractiveness of hairy rose beetles to traps supplied with different numbers of dispensers containing 100 mg of (E)-cinnamyl alcohol and (E)-anethol



Bars with the same letters are not significantly different at 0.05 level using least significant difference (LSD) test

Effectiveness of the attractive dispenser compared to the natural host

Results from the olfactometer, choice chamber, experiment showed that significant differences were found in the attractiveness of apple flowers as natural host compared to dispensers supplied with the artificial floral attractants or the control ($F_{3,16} = 144.5$; $P < 0.0001$). Mean percentage of beetles attracted to all treatments was 73.2%. From the number of attracted beetles, 58% were attracted to dispensers with floral attractants while 27% were attracted apple flowers. The percentage of beetles attracted to dispensers with floral attractants was significantly the highest among the treatments (Table 1).

Table 1: Mean percentage (±S.E.) of hairy rose beetles placed in an olfactometer and attracted to dispensers with floral attractants, apple flowers, dispensers without floral attractants and a control treatment.

Treatment	Percentage of hairy rose beetles (±S.E.)
Dispenser with floral attractants	58.0%a* (±2.0)
Apple flowers	27.0%b (±2.6)
Dispenser without floral attractants	6.8%c (±1.5)
Control	8.2%c (±1.6)

*Percentage of hairy rose beetles was calculated as: number of beetles in a treatment / (100 – number of beetles in all treatments). Means with the same letters are not significantly different at 0.05 level using least significant difference (LSD) test.

Evaluation of mass trapping

Statistical analysis of hairy rose beetle numbers captured during the mass trapping field trials showed no significant differences between 2009 and 2010 seasons in terms of average numbers of beetles caught per trap ($F_{1,6} = 1.30$; $P > 0.30$) as well as total numbers of beetles for all traps for each tested trap density ($F_{1,6} = 0.44$; $P > 0.53$). Thus, the data for the two seasons were combined.

The effects of trap density on average number of hairy rose beetles captured per trap and on total number of captured beetles are presented in Table 2. Increasing trap density had no significant effect on number of beetles captured per trap ($F_{2,9} = 1.45$; $P > 0.28$). Further mean separation showed no significant differences in beetle number per trap with increasing trap density (Table 2). On the contrary, increasing trap density significantly increased total number of beetles captured per all traps within each trap density ($F_{2,9} = 18.3$; $P < 0.003$). Trap density of 5 traps per 1000 m^2 resulted in significantly the higher number of captured insect (4730 beetles/all traps) compared to 3 traps (2689 beetles/all traps) and 1 trap (1253 beetles/all traps) (Table 2).

Table 2. Mean (±S.E.) of hairy rose beetles per trap and mean (±S.E.) of beetles for all traps per tested trap density captured in traps placed at different densities in apple orchards.

Trap density (trap^{-1} 1000 m^2)	Mean of captured hairy rose beetles (±S.E.)	
	Per trap	Per all traps
1	1253a (± 226.5)	1253a (± 226.5)
3	897a (± 130.2)	2689b (± 390.9)
5	946a (± 93.5)	4730 c (± 467.3)

Means within columns with the same letters are not significantly different at 0.05 level using least significant difference (LSD) test.

In terms of the effect of mass trapping on flower damage, statistical analysis showed significant differences in plant damage between the trials in 2009 and 2010 seasons. Results showed that increasing trap density significantly

reduced the percentage of damaged apple flowers by the hairy rose beetle in the treated plots in the two growing seasons. Percentage of damaged flowers per 50 cm apple branch was significantly reduced from 12.8% for no traps to 10.2, 7.9 and 4.1% in plots supplied with 0, 1, 3 and 5 traps, respectively in the first growing season. No significant differences were found in flower damage in plots supplied with either 1 or 3 traps, while flower damage was significantly lower in plots supplied with 5 traps compared to 1 and 3 traps. Similarly, Percentage of damaged flowers by the beetles was significantly reduced to 3.5% in the plots supplied with 5 traps / h compared to10.3, 13.8 and 15.3% in the plots supplied with 3, 1, 0 traps / h respectively, in the second growing season.

Discussion

The main objective of our study was to evaluate the efficiency of mass trapping in reducing damage caused by *T. squalida* to apple trees using the hairy rose beetle trap. To realize this objective, factors affecting the trap efficiency in capture including trap color, trap placement and amount of attractant were evaluated.

Adults of the hairy rose beetle responded differently to traps with different colors. Blue, white and yellow colored traps were highly attractive to the hairy rose beetle compared to the rest of the tested colors. Responses of the hairy rose beetle to different colors with no chemical attractant was previously reported when water blue traps attracted more beetles compared to white, yellow and green (Ali 1993). More recently, the efficiency of white vs. blue traps baited with floral attractant mixture of (E)-cinnamyl alcohol and (E)-anethol was evaluated (Toth et al. 2009). Although white traps caught more beetles, Toth et al. (2009) concluded that both colors can be used because no significant differences were found among them. In our study, we tested five different colors with the floral attractant including the white and blue. Again, results demonstrate the preeminence of blue and white colors. White traps were chosen for further trials because we noticed that trap color faded in the field which might affect the trap efficiency and require their periodic replacement. Response of the hairy rose beetles to differently colored traps suggests that visual stimulus is important for host finding by the hairy rose beetles. The colors of flowers of most stone and pome trees are white which might explain the attraction of white to the hairy rose beetle.

Trap placement affected the efficiency of beetle capture. More beetles were captured in traps placed on the ground than in traps hung on the trees. This might be due to the effect of wind on the beetle flying. We observed more beetles in the traps when there was little to no wind than on windy days. Traps hung on the trees might be subjected to stronger winds compared to traps placed on the ground. Weather conditions, including wind speed, were found to affect capture of the hairy rose beetle by colored traps in Egypt (Ali 1993).

Baiting the traps with the floral attractants (E)-cinnamyl alcohol and (E)-anethol prominently increased the efficiency of the traps in beetle capture. Baiting traps with a mixture of these two floral attractant vs no baiting has not been previously studied for the hairy rose beetle. These floral attractants were previously optimized for a closely related species, *E. hirta* (Schmera et al. 2004; Toth et. al. 2004). For *E. hirta*, it was found that traps baited with a mixture of (E)-cinnamyl alcohol and (E)-anethol at a ratio of 1:1 increased capture efficiency in blue traps compared to either no baited traps or traps baited with either one of them (Toth et. al. 2004). Recently, Toth et. al. (2009) concluded that blue traps baited with 1:1 E)-cinnamyl alcohol and (E)-anethol can be used for monitoring of both *E. hirta* and *T. squalida*. However, baited traps vs no baited traps were not evaluated.

Increasing the amount of the floral attractants per trap by baiting the traps with more than one dispenser did not increase the trap efficiency. Therefore, only one dispenser is sufficient to increase the trapping efficiency which is reflected positively by reducing the cost of the trapping system and the effort required to replace drained dispensers. Both (E)-cinnamyl alcohol and (E)-anethol are known floral volatiles (Knudsen et al. 1993) and such chemical cues influence the attraction of flower visiting insects. Our olfactory experiment showed that these floral attractants are highly attractive to the hairy rose beetle adults compared to apple flowers as a natural host.

After optimization of trapping efficiency, effective trap density and the evaluation of mass trapping were studied over two years. We have shown that increasing trap density increased beetle capture. The trapping of more hairy rose beetles was reflected on reduced damage to apple flowers. Percentage of damaged flowers by the beetles was reduced to 3.5% when 50 traps/h were used in the treated field plots compared to 8.3% in the controls with no traps in the first growing season and to 4.7% in the treated plots compared to 15.3% in the control plots in the second growing season. Mass trapping has shown to be effective in reducing damage caused by many coleopteran pests such as the

corn rootworms, *Diabrotica virgifera virgifera* (LeConte) and *Diabrotica barberi* (Smith 1998) (Wilde et al. 1998), the bark beetle, *Ips typographus* L. (Faccoli and Stergulc 2008).

El-Sayed et al. (2006) listed the following requirements for successful mass trapping: 1) deployed traps release pheromone/attractant that is perceived by a high proportion of the target insects in the specified area; 2) lures are able to attract insects more effectively than natural sources of attraction 3) traps are efficient in catching and retaining attracted insects before they mate or oviposit; 4) lures and traps are effective during the entire period of adult emergence and mating; and 5) costs of trapping materials and labor are less than economic benefits from alternative treatments. We have demonstrated that most of these requirements for successful mass trapping of hairy rose beetle in Jordanian apple orchards were fulfilled. In Jordan adults of hairy rose beetle emerge from soils in early February shortly before apple bloom (Ateyyat and Al-alawi, 2017). Using the mass trapping technique might remove a large proportion of the adult population before they damage the flowering crop. The floral attractants used were more effective in attracting the beetles compared to the natural host flowers as shown by the olfactometer experiments. The traps were effective in capturing and retaining the beetles. Finally, the cost of the trap was nominal as the cost of the materials was very low and it can be assembled on the farm. Based on these finding, we recommend the use of mass trapping as a safe, ecofriendly and economically acceptable method for management of the hairy rose beetle in apple orchards.

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Araştırma Makalesi/*Research Article (Original Paper)*

A New Edible Macrofungus Record for Turkish Mycobiota

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Abstract: In this study *Morchella prava* Dewsbury, Moncalvo, J.D. Moore & M. Kuo was presented as a new record for the mycobiota of Turkey. This is an edible macrofungus species and has potential economic value. A short description, the images of macro and the micro morphologies of the species are provided.

Keywords: Macrofungus, new record, *Morchella*, Van

Türkiye Mikobiyotası için Yeni Bir Yenen Makromantar Kaydı

Özet: Bu çalışmada *Morchella prava* Dewsbury, Moncalvo, J.D. Moore & M. Kuo türü Türkiye mikobiyotası için yeni kayıt olarak sunulmuştur. Bu makrofungus yenebilen ve potansiyel olarak ekonomik değeri bulunan bir türdür. Türün kısa deskripsiyonu ile birlikte, makro ve mikro morfolojilerine ait görseller sunulmuştur.

Anahtar kelimeler: Makromantar, yeni kayıt, *Morchella*, Van

Introduction

The knowledge of higher fungi in Turkey has been increasing over the years. More than 2500 species has been identified so far in the country, and most of them have been published as checklists (Sesli and Denchev 2014; Solak et al. 2015). Ascomycota divisio including the genus *Morchella* is represented by about 300 species in our country (Kaya and Uzun 2018).

Edible mushrooms have low carbohydrates, fat and calories as well as high protein, vitamins, and fibrous and giving a feel of fullness due to the difficulty of digestion in the stomach could be a source of nutrients appropriate for a healthy metabolism (Feeney et al. 2014).

Because of much difference in terms of geographical features, our country has rich and diversity of fungi. This wealth is not sufficiently evaluated by the local people.

Morels, belonging to *Morchellaceae* family, is one of the significant mushrooms when it comes to its commercial value. Since it can be seen almost everywhere in the country, Turkey is regarded as one the most significant exporters (Taşkın and Büyükalaca 2014).

The aim of this work is to contribute to the knowledge of edible macrofungal biodiversity in Turkey.

Material and Methods

The study material is the mushroom sample collected in the border of Gürpınar (Van) district in 2016 (Figure 1). The collected morphological characteristics of this sample were recorded and photographed on natural habitats and substrates. Micrographs were taken using a Leica ICC50 HD digital camera compound photo microscope. The specimens were identified according to the literature (Kuo et al. 2012; Beug et al. 2014; Kuo and Methven 2014). The identified macrofungus samples have stored in the Mycology Research Laboratory (VANF) of the Department of Biology, Faculty of Science, Van YYU.

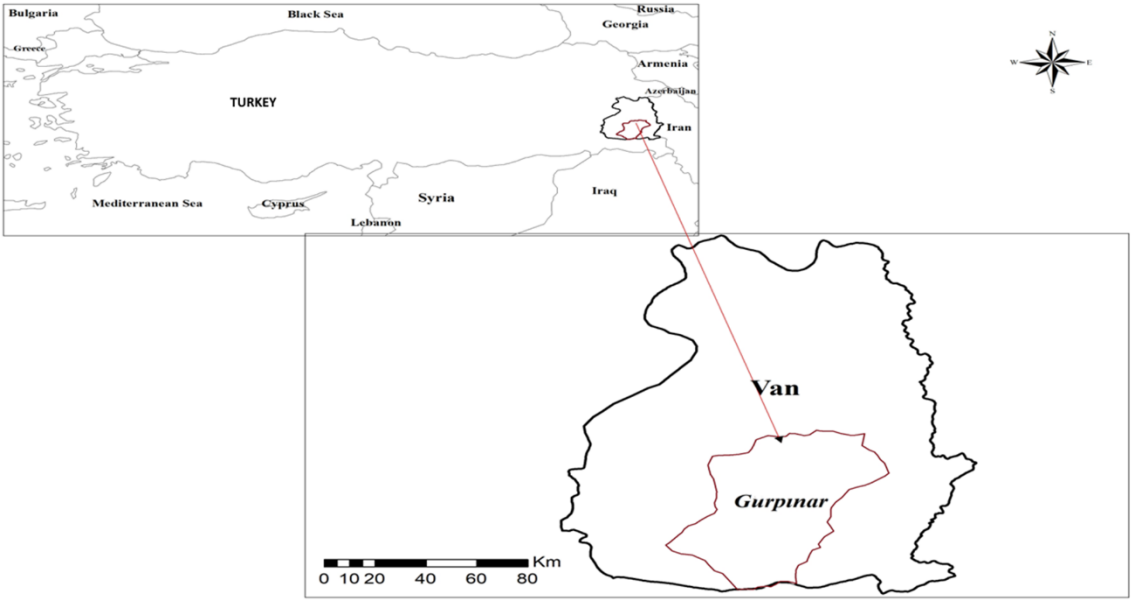

Figure 1. Research area.

Results and Discussion

The systematics of the species are given according to Index Fungorum (www.indexfungorum.org; accessed 31 July 2018). A short description, collection locality and date, ecological features, morphological and anatomical images of the species were presented.

Kingdom : *Fungi*
Divisio : *Ascomycota* Whittaker
Classis: *Pezizomycetes* O.E. Erikss. & Winka
Ordo: *Pezizales* J. Schröt.
Family: *Morchellaceae* Rchb.
Genus: *Morchella* Dill. ex Pers.
Morchella prava Dewsbury, Moncalvo, J.D. Moore & M. Kuo

Description

Ascomata, 3-6 cm high, 2-4 cm wide, variable in shape, but usually more or less egg-shaped or round, with a rounded apex, pitted and ringed. Stipe, 3-4.5 cm long, 1.5-2.5 cm wide, whitish or pale tan, usually developing indistinct ridges and folds near the base (Figure 2). Ascospores, 16-20 x 8.5-11 µm, smooth, ellipsoid, contents homogenous (Figure 3e). Asci, 250-350 x 15-25 µm, eight-spored, cylindrical, hyaline (Figure 3a-b). Paraphyses, 110-175 x 7.5-15 µm, cylindrical with variable apices, septate, hyaline (Figure 3c-d).

Ecological features

Possibly saprobic and mycorrhizal at different points in its life cycle; growing alone, scattered or gregariously under various hardwoods and conifers, often in sandy soil near bodies of water (lakes, rivers); April, May, and June; have widely distributed (Kuo at al. 2012).

Specimen examined

Exit of Gürpınar (Van/TURKEY), 14. km on Hakkari road, under *Populus* and *Salix* sp. trees, 38° 17.929' N, 43° 45.243' E, 2010 m, 03.06.2016, Selem. 94.

Figure 2. Ascocarp of *Morchella prava*.

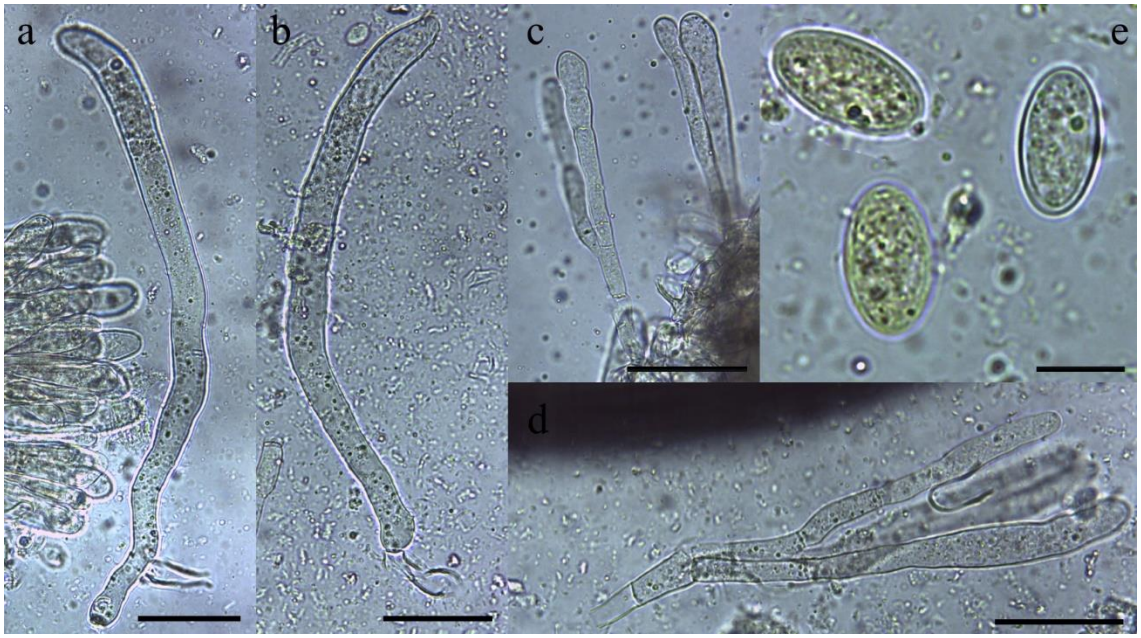

Figure 3. a-b = Asci, c-d = paraphyses, e = ascospores (with IKI) of *Morchella prava*. (Scale bars: a-d = 50 µm, e = 10 µm)

This species is one of the large yellowish morels. Although similar in appearance to *Morchella esculentoides* and *M. cryptica*, the hymeniophore of *M. prava* is more irregular and contorted compared to these two species. The pits and ridges of *M. prava* are asymmetrical and the mushroom has a crooked-looking in appearance. Although these three species have similar features in terms of micro-characters, the elements on sterile ridges are much less common in the *M. prava* than in the other two species (Kuo et al. 2012; Beug et al. 2014; Kuo and Methven 2014).

According to Sesli and Denchev 2014; Solak et al. 2015; Doğan et al. 2016, Taşkın et al. 2016; Acar and Uzun, 2017 so far, 27 *Morchella* species have been identified in Turkey. As a result of this study, the number of *Morchella* species increased to 28.

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Araştırma Makalesi/*Research Article (Original Paper)*

Freshness Assessment of Mullet (*Mugil cephalus*) Fillets Stored at 4˚C by Image Analysis Correlated to Chemical and Sensory Attributes

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Abstract: Fresh fish is described as fish being caught/harvested, chilled and stored for a short period before use. Color is one of the most important quality attributes of fish. Color measurements of fish samples have an acceptable potential for determination of freshness. The main objective was to assess freshness of mullet fillets based on color changes by machine vision and correlate this to chemical and sensory attributes.Mullet fillets in Ziploc bags were stored 8 days at 4˚C. Triplicate samples were analyzed at Days 0-1-2-4-6-8 by machine vision, sensory and chemical analyses (PV, TBARS, TVB-N and biogenic amines). Images of the same 6 fillets were also taken during storage using polarized-lighting. Color profiles were measured as the mean L*a*b* values from the entire area of the fillet surface.The a* and b* value for meat side of fillets were significantly different between Day-4 and Day-0, with a decrease in a* and an increase in b*. During storage, all chemical parameters analyzed increased. However, acceptable limits for human consumption were reached at Day-4 for TVB-N (34.30±1.62 mg N/100g fish) and biogenic amines (15.91±2.24 mg putrescine/1 kg fish; 9.04±0.49 mg cadaverine/1 kg fish). Chemical analysis revealed spoilage of fillets at Day-4. According to sensory evaluation, fillets were also rejected at Day-4 with an overall quality score of 6.770±3.945out of 15. Image analysis results were consistent with the chemical and sensory analysis. Therefore, machine vision system can be used as a rapid and non-destructive method to evaluate the freshness of mullet fillets.

Key words: Image analysis, freshness, sensory analysis, cold storage, mullet fillets

Soğukta Depolanan Kefal (*Mugil cephalus*) Filetolarının Tazeliğinin Duyusal ve Kimyasal Parametreler ile İlişkilendirilerek Bilgisayarlı Resim Analizi ile Belirlenmesi

Özet: Taze balık tüketiminden kısa bir süre önce avlanan, depolanan ve soğutulan balık olarak tanımlanmaktadır. Balığın kalitesinin belirlenmesinde kullanılanen önemli parametrelerden bir tanesi renktir. Balık örneklerinin tazeliğinin belirlenmesinde renk ölçümlerinin kullanımının kabul edilebilir potansiyeli bulunmaktadır. Bu çalışmanın temel amacı kefal filetolarının tazeliğinin bilgisayarlı resim analizi ile belirlenerek kimyasal ve duyusal analiz parametreleri ile ilişkilendirilmesidir. Ziploc poşetlerindeki kefal filetoları 4˚C'de 8 gün depolanmış vebalık örneklerine depolamanın 0-1-2-4-6-8. günlerinde sırasıyla bilgisayarlı resim analizi, duyusal ve kimyasal analizler uygulanmıştır. Depolama boyunca aynı 6 filetonun resimleri de polarize ışık ile çekilmiştir. Renk profilleri fileto yüzeyinin tüm alanının ortalama L*a*b* değerleri olarak ölçülmüş ve filetoların et yüzeyi için 0. ve 4. günler arasında a* ve b* değerlerinde önemli bir farklılık saptanmıştır. Bu farklılık a* değeri için azalma olarak, b* değerinde ise artış olarak gözlemlenmiştir. Depolama boyunca analizi yapılan tüm kimyasal parametreler artış gösterirken insan tüketimi için kabul edilebilir düzeylerin üzerinedepolamanın 4. günündesadece TVB-N (34.30±1.62 mg N/100g balık) ve biyojenik amin (15.91±2.24 mg putrescine/kg balık; 9.04±0.49 mg cadaverine/kg balık) değerleri ulaşmıştır. Kimyasal analiz sonuçları kefal filetolarının depolamanın 4. gününde bozulduğunu göstermektedir. Duyusal analiz sonuçlarına göre de kefal filetoları depolamanın 4. gününde 15 üzerinden 6.770±3.945toplam kalite parametresi skoru ile reddedilmiştir. Bilgisayarlı resim analizinden elde edilen sonuçlar ile kimyasal ve duyusal analiz sonuçları birbirleri ile tutarlı bulunmuştur. Bu sonuçlar doğrultusunda, kefal filetolarının tazeliğinin değerlendirilmesinde bilgisayarlı resim analizi sisteminin hızlı ve tahribatsız bir yöntem olarak etkili bir şekilde kullanılabileceği görülmektedir.

Anahtar kelimeler: Bilgisayarlı resim analizi, tazelik, duyusal analiz, soğuk depolama, kefal filetosu

Introduction

There is an increased awareness for the healthy and nutritive products of high quality such as regular consumption of fish and seafood(Smichi et al. 2017; Karouna-Renier et al. 2011). With increased expectations

for food products of high quality and safety standards, the need for accurate, fast and objective quality determination of these characteristics in food products continues to grow (Brosnan and Sun 2004). Freshness, nutritional value, sensory characteristics, physical attributes and safety are some of the quality parameters of fish products. Among these, freshness is a fundamental concept with a direct influence on fish quality (Dowlati et al. 2012). Freshness of fish and fish products can be determined using sensory, physical, chemical-biochemical and microbiological methods. However, the traditional microbiological and chemical methods cannot be employed at early stages of fish storage since these methods are sensitive in the latter phase of deterioration. Therefore, highly sensitive, nondestructive, inexpensive, precise, and rapid methods are required (Dowlati et al. 2013). Recently, automated systems mainly based on camera-computer technology are being used to invastigate the quality of food products. These systems (computer vision or machine vision) facilitates the objective and non-destructive assessment of visual (size, shape and color) quality attributes in food products (Brosnan and Sun 2004; Gümüş et al. 2011). Color is one of the most importantquality characteristic used to establish the acceptance of various foods especially fish products. Surface color has a direct effect on consumer's perception and can be used as a tool either to accept or reject fish products. Fish products are susceptible to color deterioration with decreasing freshness. Therefore, machine vision system can be used as an alternative inspection technique for freshness evaluation based on color changes (Dowlati et al. 2013; Quevedo et al. 2010).

Some of the studies related to machine vision applications of fish and fish products are;color, length and weight determination of Alaska Pollock roe (Balaban et al. 2012a; Balaban et al. 2012b), color determination of Atlantic salmon filletsand sorting of Atlantic salmon fillets according to their color (Quevedo et al. 2010; Misimi et al. 2007), effect of HPP (high pressure processing) and irradiation on quality parameters; such as color of different fish species like rainbow trout, mahi mahi, tilapia and Atlantic salmon (Yagizet al. 2007; Yagizet al. 2009a; Yagizet al. 2009b; Yagizet al. 2010). Machine vision system can also be used for sorting fish species based on their shape, size and color (Gümüş et al. 2011).Results of these studies indicated that the color measurements have an acceptable potential for fish freshness assessment.

Striped mullet (*Mugil cephalus*) iscommonly found in tropical, subtropical and temperate estuaries, where they play an important ecological and commercial roll supporting fisheries and also animportant food fish around the world. Striped mullet is one of the most commonly caught and consumed fish species inhabiting fresh and estuarine waters in Florida(Cardona 2000; Karouna-Renier et al. 2011; McDonough 2001).Many fish species have been consumed for quite some time in the form of fillets and the consumption of fillets will continue to increase in the future, due to increasing demand of consuming prepared products (Hernandez et al. 2009).
Striped mulletfillets were chosen as the material of this studydue to their abundance, popularity and high consumption in Florida and for being ready to cook in the form of fillets.There is also a lack of application of machine vision system for estimation of mullet freshness in the form of fillet.

The main objective of this study wastherefore to assess freshness ofmullet fillets stored at 4˚C based on color changes by machine vision systemand correlate this to chemical and sensory attributes.

Materials and Methods

Material

Fresh whole mullet (*Mugil cephalus*) (13 fish) were purchased from a local seafood supplier (Northwest Seafood Inc., Gainesville, Florida) within two days of harvest. Fish samples were filleted by the supplier. Each fillet (26 fillets in total) was labeled separately and put into Ziploc bags. Fish samples were transported into laboratory in ice.

Storage conditions
Fish samples were stored at 4˚C storage room. For triplicate analysis 18 fillets in Ziploc bags were put on the shelves in 4˚C storage room. According to the preliminary experiments storage time was determined as 8 days at 4˚C. Three fillets were taken at Days 0, 1, 2, 4, 6 and 8 for analysis. Images of each fillet was taken by machine vision(MV) system, followed by the sensory and chemical analysis, respectively. At the same time, 6 fillets were also stored and during the storage period only the images of these same fillets were taken by MV system.

Machine vision system
A machine vision (MV) system that was built as described by Luzuriaga et al. (1997) was used including polarized lighting.The MV system is consisted of an upper and lower light box and a digital camera (Nikon D200, Nikon Corp., Tokyo, Japan) connected to a computer. The MV system was calibrated with a reference material (Gretag Color Checker, X-Rite Inc., Grand Rapids, MI, USA) as described by Alçiçek and Balaban

(2012). The camera settings used were ISO 400 sensitivity, 1/1.3 s shutterspeed, f/13 aperture, 0 eV exposure compensation and direct sunlight whitebalance. LensEye software (ECS, Gainesville, FL, USA) was used to analyze images and obtain color profiles based on average $L*$ (lightness), $a*$(redness)and $b*$(yellowness) values of the whole fillet surface. Fish fillets were removed from the Ziploc bags and placed in the light box to acquire their images by using the 'two image' method (Alçiçek and Balaban 2012). The images of the fish samples were acquired for both the meat and skin side. All images were captured using polarized lighting. The Gretag Color Checker and a spoon was present in the light box for further color calibration and to check the condition of polarization (Balaban and Alçiçek 2015), respectively (Figure 1).

Chemical analysis
Triplicate fish fillets were homogenized with a homogenizer (Polytron, Kinematica, Switzerland) after removing the skin. Homogenized samples were stored at -80˚C for further chemical analysis. All chemical analysis except for TVB-N were performed in duplicate on three fish fillets (number of observations; n=3 fillets x2= 6).

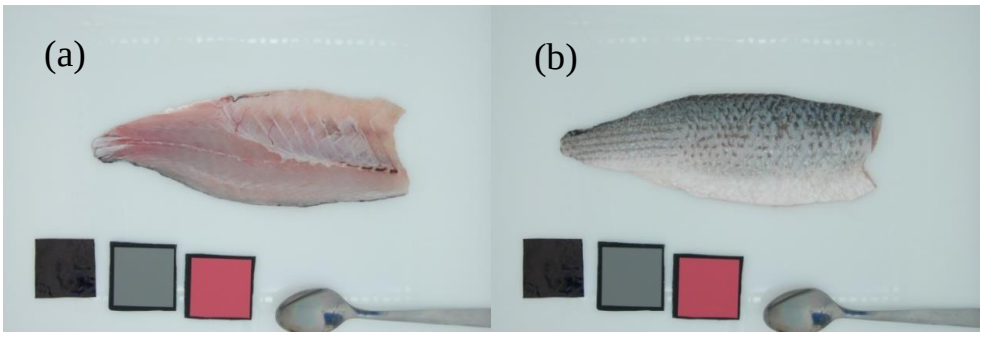

Figure 1. Polarized image of mullet fillet taken in the light box at Day 0 with the reference squares (black for size, medium grey for skin color, moderate red for meat color) and the spoon: a) meat side of mullet filet; b) skin side of mullet fillet.

Fat content
Fat content was determined only for Day 0 fish samples. Lipids were extracted from fish samples using a modified method of Bligh and Dyer (1959).

Moisture content
Moisture content was determined only for Day 0 fish samples. Moisture balance (CSC Scientific Company, Inc.) was used to determine the moisture content of fish samples.

pH measurements
One gram of fish sample was homogenized in 10 mLdistilled water in the ratio 1:10 (w/v) using a bio homogenizer (Bamix, Esge, Switzerland) and the pH was measured using a digital pH meter by inserting the electrodesinto the homogenates (Abelti 2013).

Total volatile basic nitrogen (TVB-N) analysis
The direct MgO method described by Woyewoda et al. (1986) was used forthe analysis of TVB-N. The method used steam distillation for extraction ofthe volatile bases from 10 g fish sample (n=3). TVB-N was expressed asmilligrams of nitrogen per 100 g fish (mg-N/100 g).

Thiobarbituric acid reactive substances (TBARS) analysis
Secondary lipid oxidation products were determined by TBARS method asdescribed by Yagiz et al.(2009b). Results were expressed as miligrams ofmalonaldehyde (MDA) per kg of fish sample.

Peroxide value (PV) analysis
Primary lipid oxidation products (lipid hydroperoxides) were determinedas described by Raghavan and Kristinsson (2008).Lipid hydroperoxides were measured as millimoles of cumene hydroperoxide per kilogram of fish sample.

Biogenic amines analysis
A rapid high-performance liquid chromatographic (HPLC) method for thedetermination of biogenic amines (putrescine, cadaverine and histamine) was used according to Özogul et al. (2002). HPLC analysis was carriedout

on a Perkin Elmer HPLC system consisting of a series 200autosampler, series 200 LC pump, and series 235C diode array detectorusing an Agilent Zorbax SB-C18 (5.0 μm, 4.6 × 250 mm). Elution wasperformed using mobile phase A (acetonitrile) and mobile phase B (water).

The following linear gradient was used: 0–1 min: 30% B, 1–6 min: 60% B,6-9 min: 70% B, 9-14 min: 80% B, 14–17 min: 80% B, 17-20 min: 30% B.The flow rate was 1.0 mL/min. Injection volume was 5 μLwith the UVdetector set to an absorbance wavelength of 254 nm. The retention timesfor each of the different components in the analyzed pool were comparedto the following standards: putrescine, cadaverine and histamine.

Sensory analysis

Generic descriptive analysis were selected as the sensory analysis method. This technique was selected since it was ideal for shelf-life testing and can be used to define sensory–instrumental relationships. A quantitative scale was used for intensity which allows the data to be statistically analyzed (Lawless and Heymann 2010). A 15 cm scale line anchored with descriptive words were used by the panelists for evaluation of the fish samples. Six parameters (flesh color, skin, texture, gaping, odor and overall quality) were evaluated by the trained panelists. Training sessions were performed twice in a month with 11 panelists from the Food and Environmental Toxicology Laboratory, University of Florida. Overall quality parameter was used to identify the acceptance and/or rejection of fish samples. Sensory evaluation of fish samples were performed inside the light box after the images of fish samples were taken by MV system.

Statistical analysis

Minitab 16 was used as the statistical software to analyze data. One-way ANOVA (Tukey's comparison) was used to determine the significant differencebetween storage days for each storage condition ($p<0.05$). All data werepresented as mean values ±standard error of means. Pearson correlation wasused to determine the correlation between chemical, sensory and coloranalysis results.

Results and Discussion

Color analysis

Triplicate mullet fillets were used for machine vision and followed by sensory and chemicals. Besidesthese triplicate samples, 6 mullet fillets were also stored at 4˚C. These 6 fillets were only used for machine vision system to acquirethe images of the same fillets during the storage period. The images of mullet fillets were acquired for both the meat and skin side. These images were analyzed by LensEye software to obtain their color profiles based on average $L*$(lightness), $a*$(redness) and $b*$(yellowness) values of the whole fillet surface. Color calibrated corrected images of the same mullet fillets are given in Figure 2 and color analysis results of these images are given in Table 1. Average L*a*and b* values were used to determine the color changes during storage.L* value for both meat and skin side of mullet fillets did not show a significant change during storage. There was a significant increase in a* and b* value for skin side of mullets during storage. This increase was significant between Day 6 and Day 0 for a* and between Day 4 and Day 0 for b*.The a* and b* value for meat side of fillets were significantly different between Day 4 and Day 0, with a decrease in a* and an increase in b*. Image analysis results showed that mullet fillets after 4 days of storage were significantly different from the fresh mullet fillets in terms of color parameters.

Table 1. Color analysis results of the same 6 mullet fillets stored at 4˚ C

	Skin Side			**Meat Side**		
	Polarized			**Polarized**		
DAYS	**L* value**	**a* value**	**b* value**	**L* value**	**a* value**	**b* value**
0	67.10±2.11^{a}	2.04±0.17^{ab}	3.56±0.13^{a}	62.64±1.25^{a}	10.40±1.38^{a}	8.72±0.39^{a}
1	65.61±1.89^{a}	2.12±0.31^{ab}	3.72±0.28^{a}	62.44±0.95^{ab}	8.17±0.76^{b}	9.87±0.45^{ab}
2	65.95±1.59^{a}	1.78±0.33^{a}	3.87±0.37^{ab}	62.21±0.75^{ab}	6.36±0.67^{c}	10.89±0.56^{bc}
4	65.90±1.78^{a}	2.39±0.25^{b}	4.39±0.34^{bc}	61.55±0.75^{ab}	6.18±0.48^{c}	11.86±0.77^{c}
6	64.54±2.03^{a}	3.73±0.37^{c}	4.48±0.35^{c}	61.74±1.25^{ab}	8.32±0.65^{b}	11.38±1.08^{c}
8	64.40±1.95^{a}	3.77±0.34^{c}	4.59±0.39^{c}	60.67±1.21^{b}	9.52±0.99^{ab}	12.19±1.18^{c}

*Mean values ± standard error of means, n=6.

Means with different letters in the same column are significantly different ($p<0.05$).

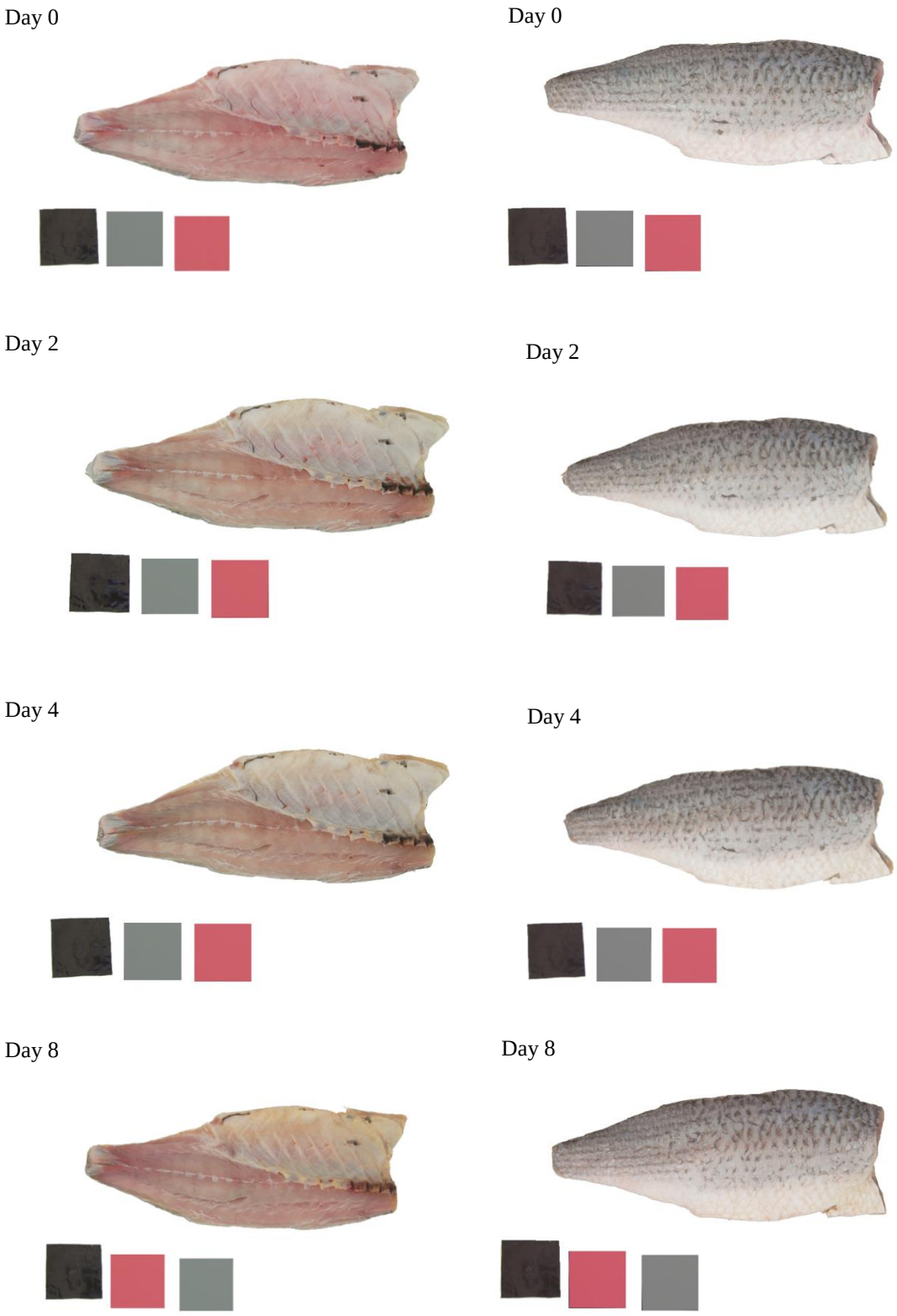

Figure 1. Corrected images ofthe same mullet filletsanalyzed by LensEye software at different days of storage at 4˚C.

Images of triplicate mullet samples were also analyzed by using LensEye software and color analysis results are given in Table 2. These results were used to correlate the color analysis results with sensory and chemical analysis results since the same three fillets were used for all these analysis.

Table 2. Color analysis of triplicate mullet fillets stored at 4˚C.

	Skin Side			Meat Side		
	Polarized			Polarized		
DAYS	**L* value**	**a* value**	**b* value**	**L* value**	**a* value**	**b* value**
0	63.09±1.28^{a}	2.73±0.44^{abc}	3.02±0.37^{a}	62.50±1.29^{ab}	11.80±0.28^{a}	10.03±0.43^{a}
1	65.38±1.14^{a}	2.36±0.22^{ab}	3.91±0.33^{ab}	65.22±1.90^{a}	10.12±0.56^{bc}	11.03±0.25^{ab}
2	63.67±1.20^{a}	1.87±0.14^{a}	3.34±0.40^{ab}	59.59±3.32^{ab}	11.62±0.28^{ab}	10.98±0.46^{ab}
4	63.45±2.84^{a}	2.44±0.60^{ab}	3.53±0.44^{ab}	58.13±2.71^{b}	9.83±0.18^{c}	11.55±0.15^{ab}
6	64.10±2.80^{a}	2.91±0.09^{bc}	4.12±0.27^{b}	59.98±2.82^{ab}	10.08±0.85^{bc}	11.87±0.46^{b}
8	61.20±2.12^{a}	3.37±0.07^{c}	3.90±0.41^{ab}	60.56±1.59^{ab}	10.85±0.87^{abc}	12.29±1.09^{b}

*Mean values ± standard error of means, n=3.
Means with different letters in the same column are significantly different ($p<0.05$).

Chemical assessment

The fat and moisture content of fresh striped mullet was determined as 4.93±0.57 g fat/ 100 g fish and %73.47±1.51, respectively. The changes in pH,TVB-N, TBARS, PV, putrescine and cadaverine values during storage at 4˚C are given in Table 3.Slight differencesin the pH of mullet fillets were observedbetween Day 0 and 8.TVB-N is the most common chemical method to measure fish spoilage. European Commission has been using TVB-N assessment as an indicator of fish spoilage since 1995. TVB-N values would be 'high quality' up to 25 mg/100 g, 'good quality' up to 30 mg/100 g, 'limit of acceptability' up to 35 mg/100 g, and 'spoilt' above 35 mg/100 g (Castro et al. 2012; Jinadasa 2014).TVB-N concentrationsof mullet fillets during cold storageare given in Table 3. TVB-N concentrations did not show a significant difference between Day 0, 1 and 2 with a value below 30 mg/100 g indicating good quality of fish. However, acceptable limits for human consumption were reached at Day 4 with a value of 34.30±1.62 mg N/100g and increased during storage.

The importance of estimating the concentration of biogenic amines in fish and fish products is related to their impact on human health and food quality. Histamine, putrescine and cadaverine are the most important biogenic amines found in fish and fish products (Özogul et al. 2002). No histamine was detected during storage of mullet fillets. For histidine-poor fish, putrescine and cadaverine are the main amines formed and the sum of them should be less than 20 mg/kg or putrescine concentration should be less than 10 mg/kg (Prester 2011). Concentrations of putrescine and cadaverine did not show a significant difference between Day 0- 1 and 2with very low values (Table 3). Acceptable limits for human consumption were reached at Day 4 with values of 15.91±2.24 mg putrescine/kg fish and 9.04±0.49 mg cadaverine/kg fish.

Lipid oxidation is one of the major quality deterioration for fish and fish products. Primary and secondary lipid oxidation was determined during cold storage by measuring PV and TBARS value, respectively. Both values increased significantly throughout the study reaching a final value of 0.95±0.07 mmole CPO/ kg fish for PV and 5.71±0.42 mg MDA/kg fish for TBARS value (Table 3). The upper limit of acceptability in terms of lipid oxidation was reached after 8 days of cold storage according to literature (Rizo et al. 2015; Shakhtour and Babji 2014).

Table 3.Chemical analysis results ofmullet fillets stored at 4˚C

DAYS	**pH**	**TVB-N mg N/100 g fish**	**TBARS mg MDA/kg fish**	**PV mmole CPO/kg fish**	**Putrescine mg/kg fish**	**Cadaverine mg/kg fish**
0	5.62±0.02^{a}	28.30±0.61^{a}	0.51±0.06^{a}	0.03±0.01^{a}	0.93±0.11^{a}	0.27±0.12^{a}
1	5.69±0.02^{b}	28.79±1.39^{a}	0.63±0.12^{a}	0.02±0.01^{a}	1.36±0.32^{a}	0.40±0.13^{a}
2	5.83±0.02^{cd}	28.95±1.21^{ab}	1.66±0.31^{b}	0.21±0.03^{b}	1.87±0.39^{a}	0.58±0.08^{a}
4	5.79±0.04^{c}	34.30±1.62^{bc}	3.26±0.41^{c}	0.17±0.04^{b}	15.91±2.24^{b}	9.04±0.79^{b}
6	5.98±0.05^{e}	39.96±5.10^{c}	3.48±0.48^{c}	0.16±0.08^{b}	46.93±8.42^{d}	114.25±8.28^{d}
8	5.86±0.02^{d}	39.55±1.25^{c}	5.71±0.42^{d}	0.95±0.07^{c}	35.99±6.05^{c}	83.86±10.87^{c}

*Mean values ± standard error of means.n=6 for pH, n=3 for TVB-N, n=18 for TBARS and PV, n=12 for putrescine and cadaverine.
Means with different letters in the same column are significantly different ($p<0.05$).

Sensory analysis

An evaluation sheet including a 15 cm scale line anchored with descriptive words were used to score six parameters (flesh color, skin, texture, gaping, odor and overall quality). The scale line was starting from 0 and low scores indicated best quality. Trained panelists made a mark on the scale line for each parameter of mullet fillets at Days 0, 1, 4, 6 and 8 of storage at 4˚C. The marks on the scale line was measured by a ruler from the left end of the line and these numerical values were recorded as the score for each parameter. This data was used to calculate the mean values of each parameter and for further statistical analysis. All sensory parameters significantly increased during storage (Table 4). Overall quality parameter was used to identify the acceptance and/or rejection of fish samples and it was determined as 5 during training sessions. According to sensory evaluation,mullet fillets were rejected at Day 4 with an overall quality score of 6.770±3.945out of 15. Sensory analysis results were consistent with chemical and color analysis results. However, compared to color and chemical analysis, standard error values were much higher for sensory analysis. This is due to the human subjective inspection which causes the inconsistent evaluation results. Together with the inconsistent results, being laborious, tedious, costly and variable emphasizes the need for rapid, accurate and objective measurement systems such as machine vision (Brosnan and Sun 2004; Du and Sun 2006).

Correlation results

Pearson correlation wasused to determine the linear correlation between chemical, sensory and color analysis results.Coefficients for Pearson correlation of fillet (meat side) color changes with chemical analysis are given in Table 5. All chemical analysis was performed after removing the skin of fish fillets. Since the results represent the deterioration of meat side, correlation was only investigated between chemical and meat side color analysis results. Although not significant, L* and a* value showed a moderate negative correlation with all chemical analysis, whereas b* value had a very strong positive correlation with all chemical analysis parameters being significant for TVB-N, TBARS and putrescine.

Table 4.Sensory analysis results of mullet fillets at 4˚C storage.

DAYS	Flesh color	Skin	Texture	Gaping	Odor	Overall Quality
0	1.539±1.297^{a*}	0.830±0.714^{a}	0.533±0.228^{a}	0.841±0.627^{a}	2.359±2.748^{a}	0.711±0.575^{a}
1	1.823±1.227^{a}	1.538±0.900^{a}	2.637±1.881^{b}	2.437±1.502^{a}	3.193±1.714^{a}	3.700±2.458^{b}
4	6.267±3.725^{b}	3.635±1.481^{b}	5.281±2.649^{c}	4.865±3.275^{b}	6.667±3.924^{b}	6.770±3.945^{c}
6	6.569±2.400^{b}	6.340±2.366^{c}	6.697±2.849^{cd}	6.354±2.599^{bc}	8.543±3.225^{ba}	8.777±3.088^{cd}
8	7.067±2.566^{b}	6.703±2.536^{c}	7.447±2.787^{d}	7.430±2.402^{c}	10.290±3.019^{a}	10.020±2.863^{d}

*Mean values ± standard error of means, n=30.
Means with different letters in the same column are significantly different ($p<0.05$).

Coefficients for Pearson correlationof fillet color changes with sensory analysis are given in Table 6. There was a significant and very strong positive correlation of meat side b* value versus flesh color, odor and overall quality scores. There was a not significant but strong negative correlation of meat side L* value versus flesh color, odor and overall quality scores. Although not significant there was also a strong positive correlation of skin side a* and b* value versus skin color, odor and overall quality scores. On the other hand, there was amoderate strong negative correlation of skin side L* value versus skin color, odor and overall quality scores and meat side a* value versus flesh color, odor and overall quality scores.

Table 5. Correlation of fillet (meat side) color changes with chemical analysis

	Pearson Correlation coefficients, r value		
Chemical Analysis	**L* value**	**a* value**	**b* value**
pH	-0.596	-0.379	0.797
TVB-N	-0.479	-0.477	**0.885***
TBARS	-0.568	-0.320	**0.911***
PV	-0.279	0.052	0.708
Putrescine	-0.417	-0.445	**0.821***
Cadaverine	-0.278	-0.322	0.749

*Significant correlation ($p<0.05$; df=4)

Table 6. Correlation of fillet color changes with sensory analysis

	Pearson Correlation coefficients, r value (p<0.05); df=3		
	Meat Side		
Sensory Analysis	**L* value**	**a* value**	**b* value**
Flesh color	-0.823	-0.460	**0.903***
Odor	-0.653	-0.343	**0.944***
Overall Quality	-0.596	-0.516	**0.988***
	Skin Side		
Skin Color	-0.498	0.737	0.691
Odor	-0.583	0.731	0.629
Overall Quality	-0.422	0.603	0.753

*Significant correlation (p<0.05; df=3)

Conclusion

The results illustrate that after 4 days of mullet fillets storage at 4°C, significant levels of TVB-N, putrescine and cadaverine were formed indicating spoilage. Color and sensory analysis results also revealed spoilage of mullet fillets at Day 4.Because of the very strong positive correlation ofmeat side b* value versus sensory and chemical analysis, it can be concluded that b* value for meat sideis a good colorindicator of the quality deterioration inmullet fillets stored at 4°C.Therefore, it was verified that machine vision system can be used as a rapid, economic, consistent, objective and non-destructive method to evaluate the freshness of mullet fillets stored at 4°C.

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Araştırma Makalesi/*Research Article (Original Paper)*

Determination of the Effects of Pumpkin Rootstock on Yield and Fruit Quality in Mini Watermelon Cultivation

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Abstract: The aim of this study was to determine the effects of the pumpkin rootstocks developed through hybridization breeding technique on the yield and fruit quality in grafted mini watermelon cultivation. In this research, seven rootstock candidates obtained from intraspecific pumpkin hybrids developed by rootstock breeding program and RS 841 commercial rootstock cultivar were used. These rootstocks were grafted with Bonanza mini watermelon cultivar. Non-grafted Bonanza seedlings were planted as the control. Total yield (kg/da), average fruit weight, fruit number/plant, fruit dimensions, fruit dry matter content, total soluble solids (TTS), fruit flesh color, fruit firmness and vitamin C values were determined to examine the effect of rootstocks on the yield and quality components of mini watermelon. Mean TTS value was found 11.1% in grafted mini watermelons. This value was 8.9% in non-grafted Bonanza fruits (control). Fruit flesh color, vitamin C and fruit dry matter contents were changed depending on the rootstock. At the end of this study; the 15/B, 26/B and 35/B rootstock/scion combinations had higher values for yield and quality compared to those of the non-grafted and other rootstock/scion combinations.

Keywords: Grafting, Mini watermelon, Pumpkin rootstock, Fruit, Quality, Yield

Introduction

Grafting is an ancient technique, especially with fruit crops. The production of grafted plants first began in Japan and Korea in the late 1920's with watermelon grafted onto gourd rootstock (Davis et al. 2008). The use of grafted seedlings in Cucurbits has increased greatly in recent years in many of the major vegetable producing regions of the world. The use of grafted seedlings is expected to increase rapidly throughout the world during the next few decades (Lee et al. 2010). In Turkey, by the year 2015, the number of farms producing grafted seedlings has increased to 31 and the number of grafted seedlings produced has reached to 175 million. Of the produced total grafted seedlings, 44.2% consist of grafted watermelon seedlings (Fidebirlik 2016).

Depending on their characteristics, rootstocks used for grafting in vegetable species have an effect on earliness, yield and fruit quality and biotic and abiotic stress conditions (Balkaya 2014). Application success in grafting technology depends on the stress factors and determination of the pathogen-resistant rootstocks and the rapid formation of the vascular bundles between the rootstocks and scions (Karaagaç 2013; Karaağaç and Balkaya 2013). Today, the most widely used commercial rootstocks in the production of grafted watermelons are *Cucurbita maxima* x *Cucurbita moschata* interspecific hybrid rootstocks. This is followed by the *Lageneria* rootstock. Also, *Citrullus lanatus* var. *citroides* is also preferred because of its resistance to nematodes (Yetişir et al. 2004; Balkaya 2013; Karaağaç 2013; Yıldız et al. 2013; Yıldız and Balkaya 2016, Kurum et al. 2017; Ceylan et al. 2018).

The use of miniature vegetables has started in the United States in the 1990's and rapidly spread in many different countries since the 2000 years. In Turkey, the first miniature vegetable growing has started in 1996 (Yanmaz 2009). Miniature vegetable production has become an attractive sector for producers and consumers especially in recent years due to people's different consumption demands and changing consumption habits. Today, it is possible to find many cultivars of miniature vegetables in many types of vegetables in the miniature vegetable market. Mini watermelons have later become widespread in other countries in Europe and Asia. In Turkey, mini watermelon cultivars with an average fruit weight of 2-3 kg

have been produced in recent years and they have been sold in markets (Güngör and Balkaya 2015). As a result of the transformation from traditional and crowded family structure to core family structure in Turkey, interest in midi and mini watermelons as well as traditional and large watermelon sizes is increasing day by day (Mert 2011; Güngör 2014). Therefore, in recent years, many researchers have started to carry out scientific studies in different regions in Turkey to increase mini watermelon cultivation techniques and yield parameters (Mert 2011; Karuserci 2011; Güngör 2014). High quality fruit in mini watermelon cultivation can be achieved successfully with good ecological conditions, cultural applications at desired level, determination of the ripening time correctly and harvesting on the appropriate time (Güngör and Balkaya 2016).

Most of the *Cucurbit* rootstock breeding studies have been carried out in China, Japan and Korea. These countries have a long history of rootstock breeding. These rootstock breeding studies for cucurbits are a new topic in Turkey. Up until now rootstock breeding and selection studies have been conducted on *Cucurbita* and *Lagenaria* populations in Turkey (Balkaya 2014; Karaağac et al. 2018). This study aimed to determine the effects of promising pumpkin (*Cucurbita moshata* Duch.) rootstock candidates on fruit quality and yield components in grafted mini watermelon cultivation.

Materials and Methods

In the study, the first local pumpkin rootstock candidates developed in Turkey by Göçmen et al. (2014) was used (Table 1). The RS841 cv. pumpkin rootstock was chosen as the control. In grafted mini watermelons, Bonanza mini watermelon cultivar was used as the scion.

Table 1. Pumpkin rootstock genotypes used in this study

No	Code	Hybrid Combinations
1	G7	HMO 2 × HMO 11
2	G15	HMO 11 × OMO 2
3	G16	HMO 11 ×HMO 8
4	G17	HMO 11 × MOE 5
5	G18	HMO 11 ×AMO 12
6	G26	OMO 5 × HMO 8
7	G35	AMO 12 × HMO 8
8	RS	RS-841

The field experiment of this study was carried out in the Samsun Province (Tekkeköy District, Çayleyik village) in 2014. Each genotype was planted at the 3–4 leaf stage with spacing of 2.5 m × 0.8 m. The fruit length and diameter, fruit dry matter, total soluble solids (TSS), fruit flesh color, fruit firmness, and vitamin C content were also determined to investigate the effect of rootstocks on the quality of mini watermelons. Taste analysis of the non-grafted watermelons and watermelons grafted on different pumpkin rootstocks were carried out according to Sarı et al. (2004). The experiments were carried out according to the randomized complete block experimental design in three replicates. A total of nine fruits of each genotype were used in the analysis. Ten individuals participated as panelists in the degustation analysis. The panelists used 1 (very poor) - 5 (very good) scales for the fruit taste. In addition, the panelists also made an evaluation regarding the presence of smell in pumpkin and its fiber content. The yield components examined are given below.

a. *Average fruit weight (g):* It was obtained by proportioning the total weight of all fruits harvested from each plant in the harvesting period to the total number of fruits.
b. *Fruit numbers (plant):* The number of total fruits harvested from each plant in the harvesting period.
c. *Total yield (kg/da)*

Statistical analyzes of quality and yield values in mini watermelons grafted on different rootstocks were analyzed using the JUMP 5.01 program.

Results and Discussion

It was found that there was a statistically significant difference between the average fruit length values of the grafted mini watermelons. The highest fruit length value was measured as 27.9 cm in the 17/B

combination (Figure 1). This combination was followed by 16/B (27.8 cm) and 15/B (27.4 cm) combinations, respectively. In all the rootstocks/scion combinations, fruit length was found to be higher than that of the non-grafted Bonanza cultivar.

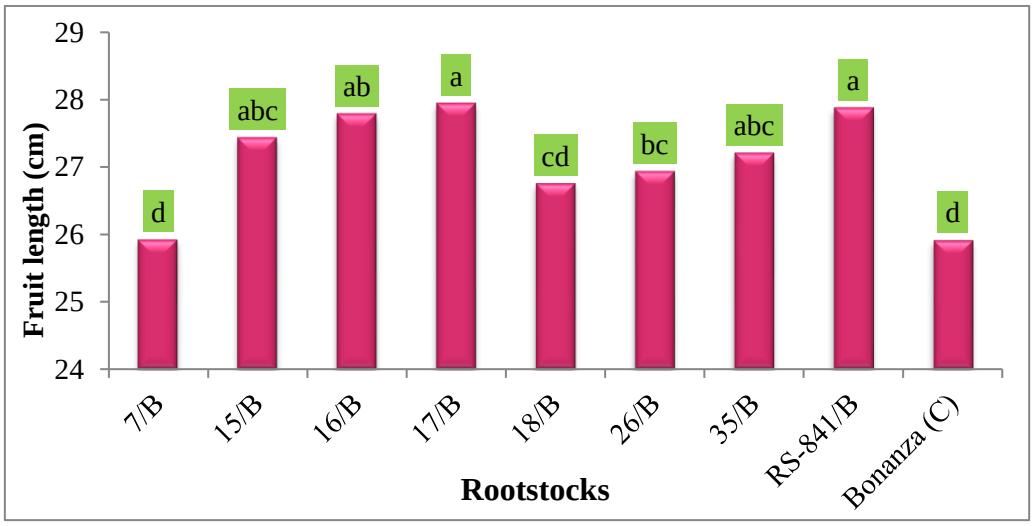

Figure 1. Fruit length in grafted and non-grafted mini watermelons (P<0.05, CV:1.86%).

The average fruit diameter values of grafted and non-grafted mini watermelon combinations are given in Figure 2. The highest fruit diameter value was measured in the 26/B combination (26.7 cm) (Figure 2). It was determined that all the selected rootstock combinations had a higher average fruit diameter than that of the non-grafted mini watermelon cultivar. In the study, it was determined that, depending on the rootstocks used, the grafting increased the diameter of the fruit by 12% in mini watermelons compared to that of the control cultivar. Karaagaç (2013) have reported that the diameter of the fruit changed between 20.7 cm and 27.9 cm in the grafted watermelons, depending on the rootstocks and grafting increased the diameter of the fruit by 15% in watermelon. Miles et al. (2004) mentioned that the fruit diameter values between 14.73 cm and 20.32 cm in mini watermelons. Mert (2011) found that the fruit diameter varied from 14.90 to 20.95 cm in mini watermelon cultivars. The average fruit diameter in the non-grafted Bonanza fruits was found to be 24.3 cm.

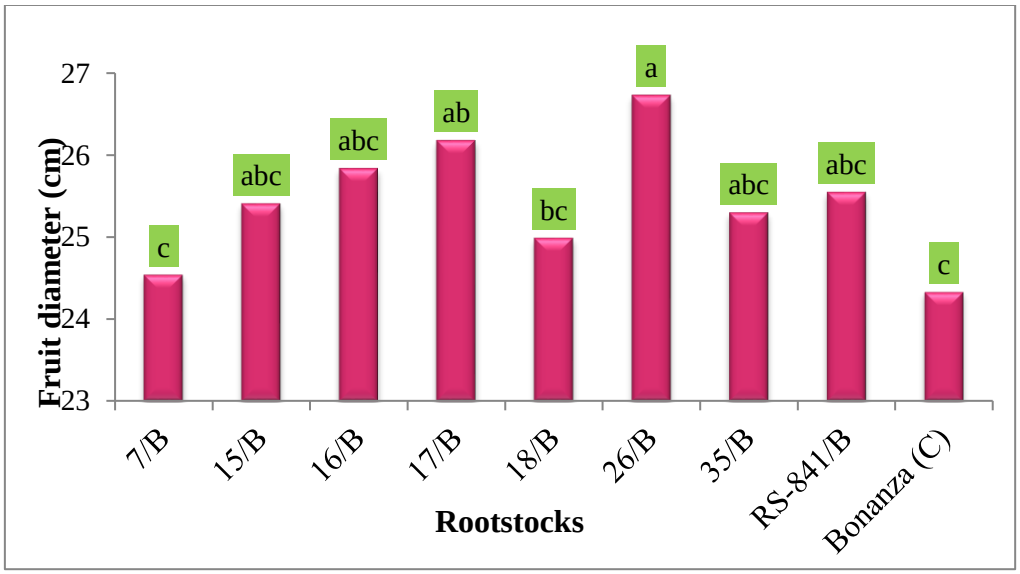

Figure 2. Fruit diameter values (cm) in grafted and non-grafted mini watermelons (P<0.05, CV:3.60%).

It was found that there was a statistically significant difference in the dry matter content of the grafted mini watermelon fruits grafted on different rootstock/scion combinations. As a result of the analysis, the fruit

dry matter contents in the grafted and non-grafted combinations varied between 7.4% and 11.1%. Fruit dry matter content was the lowest in the Bonanza mini watermelon cultivar (7.4%) (Figure 3). The highest fruit dry matter content in rootstock/scion combinations were found in 15/B combination (11.1%) and 17/B combination (10.0%), respectively (Figure 3). The average dry matter content of fruit was found to be 7.4% in non-grafted mini watermelons and 9.5% in grafted mini watermelons. Karaagaç (2013) found that the fruit dry matter content in grafted and non-grafted watermelons was 8.58% in grafted watermelons and 9.0% in non-grafted watermelons. In another study (Tokgöz et al. 2015), the total dry matter content of watermelon cultivars Crisby F_1 and Crimson Tide F_1watermelon cultivars grafted on three pumpkin rootstocks and the non-grafted watermelon variety used as the control group varied between 8.38% and 9.92%. The results of the research showed similarities with this findings.

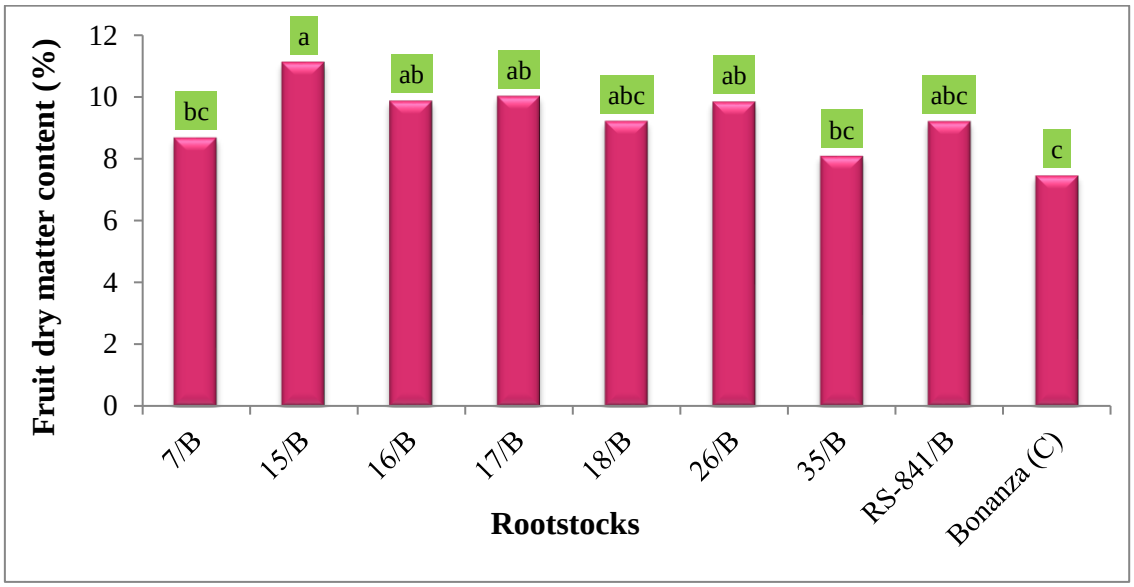

Figure 3. Fruit dry matter content in grafted and non-grafted mini watermelon fruits (%) (P<0.05, CV:13.84%).

In this study, it was determined that there was a statistically significant difference in the total soluble solid content of the mini watermelon fruits grafted on different rootstock/scion combinations. The average TSS content of fruit was found to be 8.9% in non-grafted mini watermelons and 11.1% in grafted mini watermelons. The highest TSS content in different rootstock/scion combinations were found in 17/B combination (11.5%), 35/B combination (11.5%), and 16/B combination (11.2%), respectively.

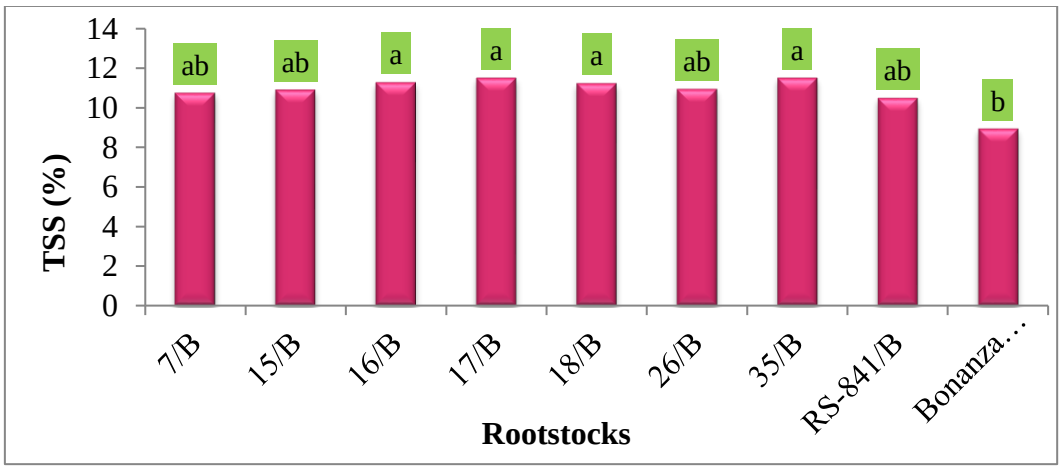

Figure 4. Total soluble solids (TSS) content in grafted and non-grafted mini watermelon fruits (%) (P<0.05, CV:11.28%).

One of the most important characteristics is the fruit color in watermelon quality. In the study, the effect of pumpkin rootstock candidates on the red color tone of mini watermelon was examined in detail. The highest fruit color brightness in rootstock/scion combinations were determined in 26/B (L: 36.75) and 15/B (L: 35.20) combinations (Table 2). In addition, it was determined that 18/B (L: 29.87) and RS-841/B (L: 29.92) combinations had the most mat colors in terms of fruit flesh color. In the study, a general evaluation of fruit flesh color in the grafted fruits, compared to non-grafted watermelons, five of the rootstocks were found to increase the brightness of fruit flesh, whereas three of them had a negative effect on the brightness of fruit flesh color. Examining the fruits in terms of the a* color value, it was determined that there were not statistically significant differences between the combinations according to the rootstocks used. As a result of the study, it has been reported that red color intensity positively correlated with the a* value (Karaağaç 2013). The highest a* color value was found to be 16.08, 15.88 and 15.82 for the combinations 35/B, 15/B and 26/B, respectively. Tokgöz et al. (2015) in Crisby F_1 and Crimson Tide F_1 watermelon cultivars grafted on three pumpkin rootstocks, have reported that a* color value varied between 23.35 and 29.15. Statistically significant differences were observed between the b* values of the examined rootstock/scion combinations. The b* values of the fruits varied between 10.58 and 14.43. The highest b* value was measured in 26/B rootstock/scion combination, whereas the lowest b* value was measured in 18/B rootstock/scion combination (Table 2). Non-grafted mini watermelon fruits had similar values to those of grafted mini watermelons. The results showed that grafting positively affected the a* and b* values in the majority of the rootstocks compared to those of the control.

Table 2. Fruit flesh color values of grafted and non-grafted mini watermelons

Combination	L	a	b
7/B	31.68 ab	14.66	11.01 ab
15/B	35.20 ab	15.88	13.99 ab
16/B	30.83 b	14.67	11.09 ab
17/B	35.03 ab	15.65	13.64 ab
18/B	29.87 b	14.72	10.58 b
26/B	36.75 a	15.82	14.43 a
35/B	34.13 ab	16.08	13.51 ab
RS-841/B	29.92 b	15.50	11.36 ab
Bonanza (C)	31.31 b	15.48	11.98 ab
	P<0.05	NS	P<0.05

The firmness of the fruit in watermelons has a positive effect on the preservation of the cultivars. The hollow heard disorder takes place in a prolonged period in watermelons with firm fruits; as a result it can be stored for longer periods (Arslan 2010). Fruit firmness values are shown in Figure 5.

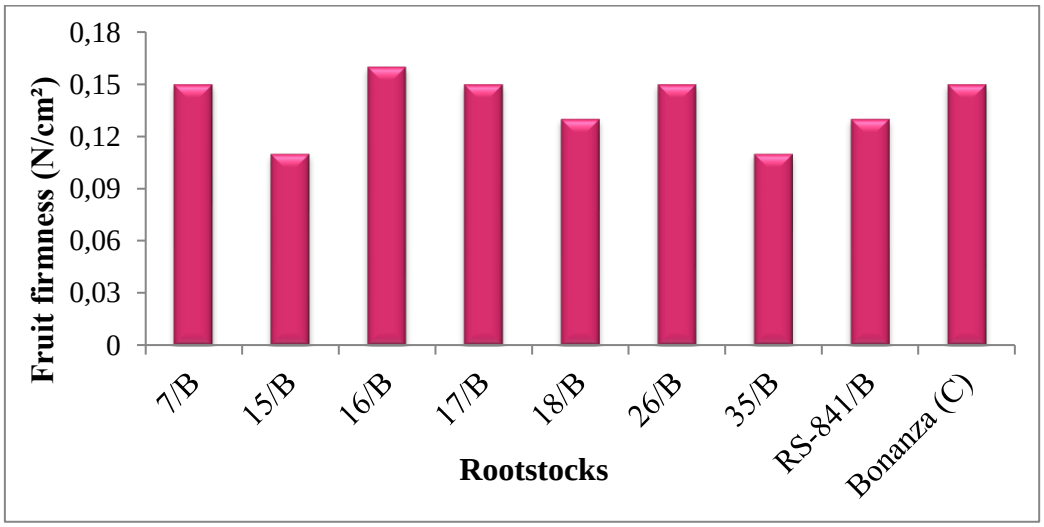

Figure 5. Fruit firmness (N/cm^2) values in grafted and non-grafted mini watermelons.

There were no statistically significant differences between the rootstock/scion combinations in terms of fruit firmness values. The highest values were determined in 16/B (0.16 N/cm^2) and 26/B (0.15 N/cm^2) combinations. Bonanza mini watermelon cultivar grafted on commercial pumpkin rootstock RS-841 and non-grafted Bonanza mini watermelon cultivars were similar to other selected rootstocks. The average fruit firmness values obtained in the study were 0.13 N/cm^2 in grafted mini watermelons and 0.15 N/cm^2 in non-grafted mini watermelons. Fruit firmness values were different in mini watermelons grafted with the selected rootstocks from the non-grafted mini watermelons.

In terms of the Vitamin C content of watermelon, it is one of the richest fruits after cabbages, pepper and tomatoes (Karaağaç 2013). It was determined that Vitamin C contents of grafted and non-grafted watermelons were statistically significant. In the study, Vitamin C content varied between 2.6 mg/100 g and 3.5 mg/100 g (Figure 6).

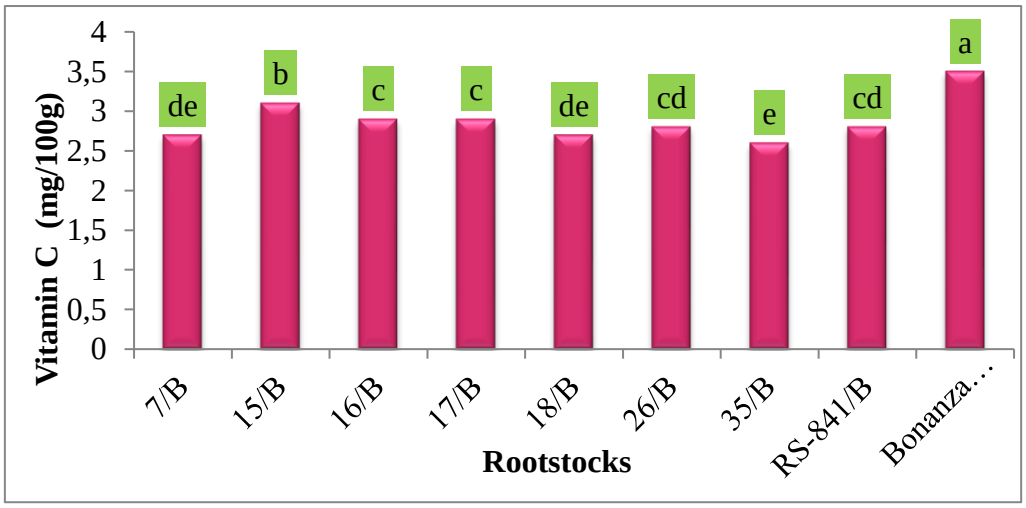

Figure 6. Vitamin C values in grafted and non-grafted mini watermelons (mg/100g) (P<0.05, CV: 3.60%).

Vitamin C level in Bonanza cultivar was 3.5 mg/100 g. It was found that Vitamin C of grafted mini watermelons decreased by 19.64% compared to the control (Figure 6). Among the pumpkin rootstocks, the highest vitamin C content was obtained as 3.1 mg/100 g from 16/B rootstock/scion combination. Accordingly, Leskovar et al. (2004) have reported 4.23-6.98 mg/100 g, Mélo et al. (2006) 57.62 mg/100 g, Proietti et al. (2008) 64-69 mg/100 g, Tlili et al. (2011) 10.5-23.9 mg/100 g and Karaağaç (2013) has reported 10.6-15.3 mg/100 g for the Vitamin C values. The significant differences between the results of the studies in the Vitamin C content of watermelon were associated with the genetic diversity and different ecological conditions.

In the study, sensory degustation analysis was carried out to determine the effects of rootstock candidates on the taste properties of mini watermelons. It was determined that there were no statistically significant differences between the taste scores of grafted and non-grafted mini watermelons (Table 3). The highest taste scores were obtained in 26/B (3.53), 17/B (3.50) and RS-841/B (3.50) rootstock/scion combinations. In studies on the effect of grafting on watermelon taste, it was determined that taste values varied depending on the rootstock (Yamasaki et al. 1994; Atasayar et al. 2005; Karaca et al. 2012; Karaağaç 2013). The results obtained in the study were similar with the literature given above. Panelists were requested to make an evaluation on the fiber formation in watermelon fruits. Fiber formation was not observed in 15/B rootstock/scion combination and non-grafted Bonanza cv. The fibrous structure formation was found in all of the other rootstock/scion combinations examined in the study. According to the sensory analysis results, 15/B and 26/B combinations found the best combinations in the study.

Table 3. Sensory analysis results of grafted and non-grafted mini watermelon fruits

Combinations	Taste points (1 very bad-5 very good)	The smelling	Fiber status
7/B	2.91	No	Little
15/B	3.33	No	No
16/B	3.00	Little	No
17/B	3.50	No	Little
18/B	3.00	No	Little
26/B	3.53	No	No
35/B	3.16	No	No
RS-841/B	3.50	No	Little
Bonanza (C)	3.21	No	No
	Non Significant		

It was found that there were statistically significant differences between the average fruit weights of the grafted mini watermelon fruits on different pumpkin rootstocks. The highest average fruit weight value was determined in 16/B combination (2923 g) (Figure 7).

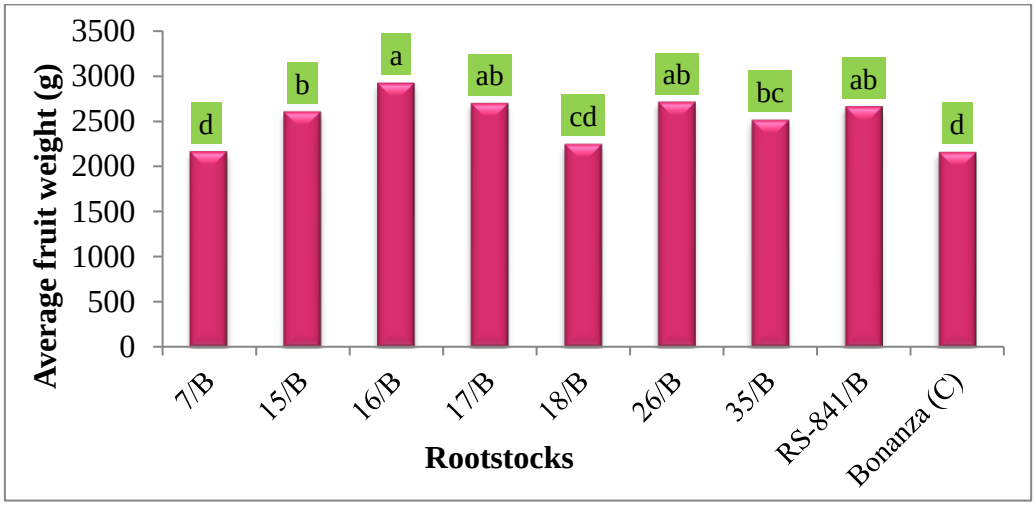

Figure 7. Average fruit weight values in grafted and non-grafted mini watermelons (P<0.05, CV:7.12%).

The majority of the rootstocks used in the grafted mini watermelons increased the average fruit weight compared to the control. Miles et al. (2004) have reported the average fruit weight in mini watermelons between 1.589 kg and 5.221 kg while Maynard (2003) has reported between 2.220 kg and 2.900 kg. Yield values in mini watermelons vary depending on the cultivars (seed, seedless, early, late) and ecology. Cattivello et al. (2007) conducted a study on the yield factors of 22 mini watermelon cultivars in Italy. The researchers have reported that the fruit weights in seedless cultivars varied between 2.2 kg and 5.8 kg. Karuserci (2011) have reported that fruit weights in non-grafted applications varied between 2194 g and 2356 g depending on the plant spacing and between 2622 g and 3268 g in grafted applications depending on the plant spacing.

The number of fruits in non-grafted and grafted mini watermelons on different pumpkin rootstocks changed between 2.21 and 4.76 fruits/plant (Figure 8). The highest number of fruits per plant was determined in 35/B combination (4.76 fruits/plant) whereas the lowest number of fruits per plant was determined in the non-grafted watermelon plants (2.21 fruits/plant). The average number of fruits in the combinations grafted with the selected pumpkin rootstocks increased by 88.6% compared to those in non-grafted mini watermelon. In many studies, it was determined that grafting had an increasing effect on the number of fruits (Colla et al. 2006; Rouphael et al. 2008; Huitrón-Ramírez et al. 2009; Karaağaç 2013).

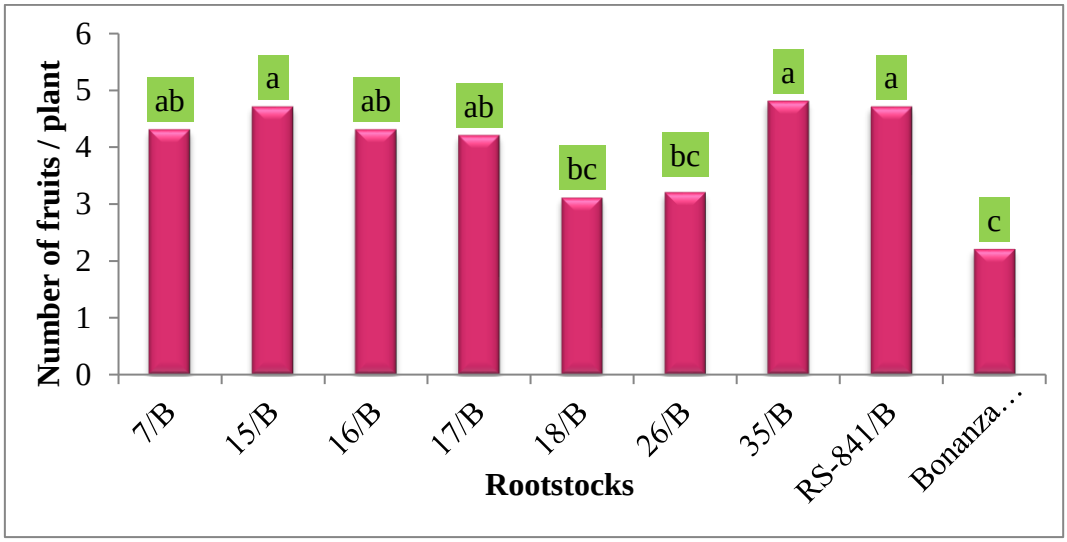

Figure 8. Number of fruits per plant in grafted and non-grafted mini watermelons (P<0.05, CV:16.75%).

Total yield values in mini watermelons vary depending on the cultivars and ecology. It was determined that there were statistically significant differences between the rootstock/scion combinations. The highest yield values were obtained in RS-841/B and 15/B rootstock/yield combinations to be 6086.1 kg/da and 6190.8 kg/da, respectively (Figure 9). The lowest yield was obtained in the Bonanza mini watermelon cultivar to be 2380 kg/da. It was determined that the total yields in rootstock/scion combinations were higher compared to that of the non-grafted watermelon. Maynard (2003) have reported that the yield values of nine different mini watermelon cultivars varied between 2.46 tons/da and 5.75 tons/da. In another study on the yield values of different mini watermelon cultivars under Adana ecological conditions, the highest yield values were obtained from WDL 7078 F_1, Crisby F_1, WDL 8051 F_1 and Bonanza F_1 (5.18 tons/da, 5.02 tons/da, 4.85 tons/da and 4.84 tons/da, respectively) cultivars (Mert 2011). In a general evaluation in terms of total yield values, depending on the rootstocks, in grafted mini watermelons yield values were 2-3-fold the non-grafted watermelon cultivar.

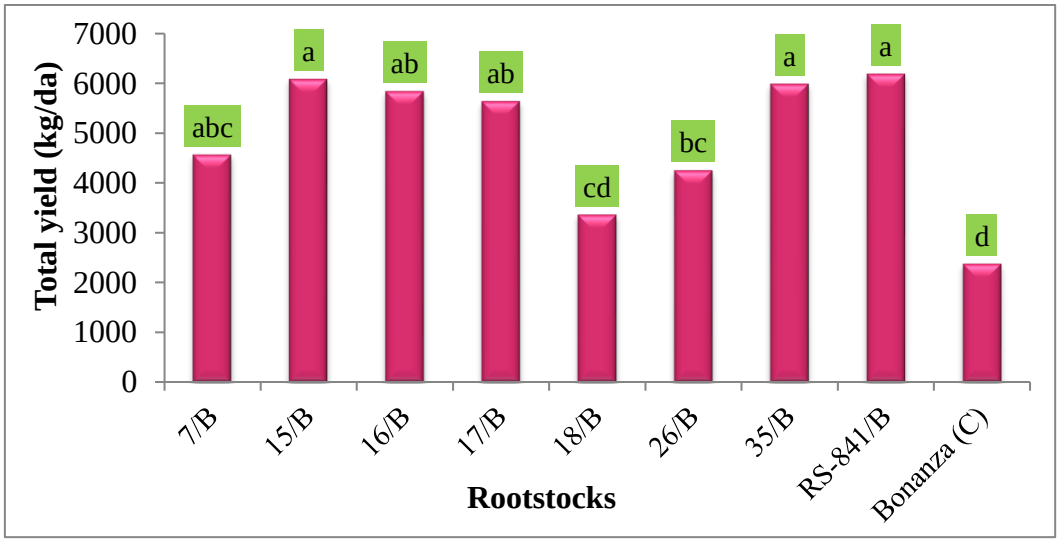

Figure 9. Total yield values in grafted and non-grafted mini watermelons (kg/da) (P<0.05, CV:19.24%).

Conclusion

The results showed that these selected pumpkin rootstock candidates can be used as commercial rootstock for the grafted mini watermelon seedling production. The rootstock/scion combinations of RS15/Bonanza, RS26/B, and RS35/B were found to be superior for yield, quality and some other characteristics than

ungrafted and other rootstock/scion combinations. Promising hybrid rootstocks will be registered according to the results. In addition, first certificated local rootstocks will be produced in Turkey.

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Araştırma Makalesi/*Research Article (Original Paper)*

Life table and some biological features of *Planococcus citri*, Risso (Hemiptera: Pseudococcidae) on 41-B grapevine variety (*Vitis vinifera* L.)

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Abstract: *Planococcus citri* (Hemiptera: Pseudococcidae), known as citrus mealybug, is a most crucial polyphgous pest on many plants around the world including the grapevine. In this study, information on development, fecundity, survival and population projection of citrus mealybug on grapevine has been collected in the climate chamber 25±1 ºC, 65-70 % RH, and 16:8 (L:D) h photoperiod. The preadult duration of females *Pl. citri* was 28.31 days, while the preadult duration for male individuals was 26.25 days. Total adult longevity and total longevity times for all cohort were found as 14.81 and 33.97 days, respectively. When reared on 41-B grapevine variety, the net reproductive rate (R_0), mean generation time (T), the finite rate of increase (λ), and the intrinsic rate of increase (r) were 32.07 eggs/individual, 41.43 days, 1.087 day^{-1}, and 0.084 day^{-1}, respectively. The population projection showed *Pl. citri* could increase to population of 357 individuals in 60 days with an initial population of 10 eggs. This study has provided the basic information about the general characteristics of the population of *Pl. citri* fed on the 41-B grapevine variety and can be useful for IPM studies.

Key words: *Planococcus citri*, *Vitis vinifera*, 41-B grapevine, Age-stage two-sex life table

Introduction

Planococcus citri Risso (Hemiptera: Pseudococcidae) is a highly polyphagous and most crucial pest around the world (Williams and Watson 1988). It is a common pest on citrus, grapevine, ornamental plants, cocoa, bananas, tobacco, coffee, passionfruits, pineapples, figs, taro, date palms, pomegranates, potatoes and greenhouse plants (Carter 1942; Strickland 1951a,b; Le Pelley 1968; Niyazov 1969; Entwistle 1972; Bivins and Deal 1973; Williams 1973; Gibson and Turner 1977; Murray 1978; Williams and Watson 1988).

In vineyards, mealybugs caused severe damage through direct feeding and virus transmission (Bigger 1972; Lockhart and Olszewski 1988; Brunt 1992; Cabaleiro and Segura 1997; Martelli and Boudon-Padieu 2006; Bertin et al. 2013). Numerous mealybug species are found in vineyards, e.g., *Ferrisia gilli* Gullan, *Pl. citri* (Risso), *Pl. ficus* (Signoret), *Pseudococcus calceolariae* (Maskell), *Ps. longispinus* (Targioni-Tozzetti), *Ps. maritimus* (Ehrhorn), *Ps. viburni* (Signoret), and *Maconellicoccus hirsutus* (Green). *Planococcus citri* and *Pl. ficus* is morphologically very similar and their separation is very difficult (Williams and G. Willink 1992). It was reported that *Pl. citri* is the most abundant in vineyards (Morandi Filho et al. 2009) than *Pl. ficus* (Foldi and Kozar 2006). The biology of *Pl. citri* is little known, although it is native to Eurasia, and has recently been regarded as an economically important pest (Daane et al., 2012).

The most comprehensive information on the biology of a species is obtain by using life tables (Yu et al. 2005; Atlihan et al 2017; Qayyum et al 2018; Özgökçe et al. 2018a, b). Chi and Liu (1985) developed a new method called the age-stage two-sex life table which is incorporated into life table theory. Unlike the traditional life table, this method can give very detailed information about the population characteristics, taking into account the population dynamics of both sexes and stage differentiation (Atlıhan and Chi 2008; Huang and Chi 2012; Tuan et al. 2015; Chang et al. 2016, Maleknia et al. 2016, Tuan et al. 2016, Bussaman et al. 2017). There are very few studies on the biology of *Pl. citri* on grapevine. To understand the growth potential and damage of *Pl. citri*, we collected the survival, development, and fecundity data reared on the 41-B grapevine variety, i.e., the rootstock in European vineyard plants against phylloxera damage. By using population projection based on 0.025 and 0.975 percentiles of the net reproductive rate as well, the variability on population growth was studied.

Materials and Methods

Pl. citri colonies were obtained from West Mediterranean Agricultural Research Institute (BATEM), Antalya in 2016 and reared potato tubers with shots at the laboratory. In the study, American grapevine (*Vitis vinifera* L., 41-B) leaves were used as host plants. Before the study, newborn nymphs were reared on grapevine leaves for a few generations.

Life table studies

Plastic Petri dishes with 3 cm in diameter containing 1.6%agar were used as experimental arenas for every individual. The grapevine leaves were placed upside down on agar solution and newly laid eggs were transfered on leafs. Thirty replicates were used at the study and they were kept in the climatic cabinet adjusted to 25 ± 1 ºC, 65-70% RH, and 16:8 (L:D) h photoperiod. All developmental stages of nymphs were observed and daily recorded. After the emergence of both sexes, they were paired in Petri dishes, and laid eggs were recorded daily until the end of lifetime of individuals.

Life table analysis

The TWOSEX-MSChart software (Chi 2018a) were used to analysis of the raw survival, development, and fecundity data of the *Pl. citri* on grapevine. Chi and Liu (1985) and Chi (1988) developed the theory of age-stage two-sex life table which gives stage differentiations and variations between sexes and among individuals in a cohort.

The life table parameters were estimated according to the Euler-Lotka formula (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

The bootstrap technique (200,000 resampling) was used to determine stable population parameters (Özgökçe et al. 2018 a,b).

Population Projection

The TIMING-MSChart software was used to determine the population size of *Pl. citri* using the life table data obtained on grapevine N-41 variety (Chi and Liu 1985, Chi 1990, Chi 2018b). The newly laid ten eggs as the initial population were used to population development for 60 days. We used the 0.025 and 0.975 percentiles of the net reproductive rate of 200,000 to include the uncertainty of population growth.

Results and Discussion

All newly laid *Pl. citri* eggs which were kept in leaf cells were hatched, and 70% of the nymphs emerging from these eggs could be adult (Table 1). Among them, 43.3% were female, and 26.7% were male. Females had three instars whereas males had four instars (Table 1) (Gullan 2000; Morandi Filho 2008; Goldasteh et al. 2009; Asiedu et al. 2014; da Silva et al. 2015; El-Aw et al. 2016). The egg durations of females, males and N-type (individuals that died at the preadult stage) were 4 days. The durations of the first instar were 6.92, 6.63 and 7.00 days, while the duration of the second instar were 8.62, 7.00 and 7.67 days, for females, males and N-type individuals respectively (Table 1). Total preadult duration were not statistically different between females (28.3 days) and males (26.25 days). The adult longevity of females (20.77 days) was statistically longer than males (5.12 days) (Table 1). Morandi Filho et al. (2008) reported that preadult durations of females of citrus mealybug were 4.00-4.25 days for egg, 8.13-8.65 days for the Nymph 2, and 8.27-8.33 days for the Nymph 3 in their study. These results are quite similar to our results, while other developmental periods are different from our studies. That is why the hosts are used in different varieties for their reasons.

Adult and total preoviposition periods (APOP and TPOP), and oviposition days of citrus mealybug on grapevine leaves were 10.0, 39.17 and 9.42 days, respectively (Table 2). The duration of oviposition was 10.7 days in the study of Ahmad and Abd-Rabou (2010) on grape plants at 24 °C conditions. This result is very close to our result. But they reported only preoviposition period (i.e., APOP) as 4.7 days. Gabre et al. (2005) stated that APOP ignores the preadult time so that the preadult time cannot show the regeneration effect and TPOP reveals the impact of the first reproductive age on the population parameter. Fecundity of citrus mealybug was 74.0 eggs/female. Morandi

Table 1. Development and longevity of *Planococcus citri* on *Vites vitifoli* (41-B variety) leaves (Mean ± standard error)

		n	Mean ± St. Error
Egg	Female	13	4.00 ± 0.00
	Male	8	4.00 ± 0.00
	N-type	9	4.00 ± 0.00
	P; F; df		-
Nymph 1	Female	13	6.92 ± 0.35
	Male	8	6.63 ± 0.53
	N-type	4	7.00 ± 0.41
	P; F; df		0.846; 0.17; 2
Nymph 2	Female	13	8.62 ± 0.0.50
	Male	8	7.00 ± 0.60
	N-type	3	7.67 ± 2.19
	P; F; df		0.229; 1.58; 2
Nymph 3 Prepupa+Pupa	Female	13	8.77 ± 0.77
	Male	8	8.63 ± 0.65
	P; F; df		0.898; 0.14; 19
Total preadult duration	Female	13	28.31 ± 1.17
	Male	8	26.25 ± 1.21
	P; t; df		0.261; 1.16; 19
Adult longevity	Female	13	20.77±2.11a
	Male	8	5.13±0.69b
	P; t; df		**0.000**; 7.05; 14.44
Total preadult duration (All)		21	27.52±0.87
Total adult longevity (All)		21	14.81±2.15
Total longevity (All)		30	33.97±3.12

* Tukey and t-student tests at the 5% significance level were used to comparisons

Table 2. The oviposition, fecundity and life table parameters of *Planococcus citri* on *Vites vitifoli* (41-B variety) leaves

	n	Means±SE
Adult preoviposition period (APOP) (d)	12	10.0±1.64
Total preoviposition period (TPOP) (d)	12	39.17±2.08
Oviposition days (d)	12	9.42±1.55
Fecundity (d)	13	74.00±14.24
The intrinsic rate of increase, r (d^{-1})	30	0.084±0.0094
The finite rate of increase, λ (d^{-1})	30	1.087±0.0102
The mean generation time, T (d)	30	41.43±2.85
The net reproductive rate, R_0 (eggs/individual)	30	32.07±8.93
Doubling Time, DT (d)	30	8.28

Filho et al. (2008) reported that fecundity of citrus mealybug on three different grape cultivars (Cabernet Sauvignon, Italia, Isabel) ranged from 53.33 to 67.27 eggs/female. These results are close to the fecundity value obtained on the 41-B variety in this study.

Age-stage specific survival rates (s_{xj}) of citrus mealybug on 41-B variety of grapevine are given in Fig 1. These curves depict the probabilities that a newly laid mealybug egg will survive to x age and j stages. Due to the different developmental rate of individuals, there is significant overlapping in the s_{xj} curves.

Fecundity (m_x), age-specific survival rates (l_x), and net maternity ($l_x.m_x$) curves of the citrus mealybug on the tested plant were plotted in Fig. 2. It shows that the citrus mealybugs started to produce eggs at the age of 29 d and has reached the peak at age 44 and 50 d on the tested plant. Due to the high mortality, the maternity curve ($l_x.m_x$) is rather low than the fecundity curve.

Population demographic parameters

The population demographic parameters of *Pl. citri* on grapevine 41-B variety estimated by using the bootstrap techniques were presented in Table 2. The finite rate of increase (λ), intrinsic rate of increase (r), the mean generation time (T), the net reproductive rate (R_0) and doubling time (DT) of citrus mealybug were estimated as

1.0873 d^{-1}, 0.0837 d^{-1}, 41.43 d, 32.07 eggs/individual and 8.28 d, respectively. A shorter mean generation time does not necessarily represent a faster population growth rate.

All values of R_0 and F are consistent with the proof of Chi (1988) that $R_0 = F \times (N_f / N)$, where N : the total number of individuals at the beginning of the study and N_f : the number of adult female emerged from N.

Karacaoğlu and Satar (2017) reported that the intrinsic rate of increase and the finite rate of increase of citrus mealybug at 25 °C on grapefruit leaves were 0.100 d^{-1} and 1.105 d^{-1}, respectively. These results are closer to our study results. Because it is based on female age-specific life table and due to the problems of applying female life table to two-sex populations (Huang and Chi 2012), it is not justified to compare the results of Karacaoğlu and Satar (2017) with the current study.

The net reproductive rates of 200,000 bootstrap samples were shown in Fig. 3. The bootstrap is a technique of resampling with replacement, hence the results fluctuated randomly (Fig. 3a). To reveal the distribution of 200,000 bootstrap samples, we sorted them in ascending order (Fig. 3b). The 0.025 and 0.975 percentiles of the net reproductive rates can be obtained from Figs. 3b and 3c. Because inviable cohorts gave a net reproductive rate R_0 = 0, no intrinsic rate of increase and finite rate of increase can be estimated. The 0.025 and 0.975 percentiles of the net reproductive rates can be used to reveal the variability of a population without the assumption of stable age-stage distribution. The age-stage specific life expectancy (e_{xj}) of citrus mealybug on grapevine 41-B variety leaves is the lifespan remaining for an individual age x and stage j, which is plotted in Fig 4. The life expectancy of a newborn egg of *Pl. citri* on the tested plant was 34 d, and this is precisely the mean longevity of all individuals on the host plants. The reproductive value (v_{xj}) of citrus mealybug on the tested plant is plotted in Fig. 5, and peak of v_{xj} occured at the age of 39 days as 38.53 d^{-1}. It is the contribution of an individual to the future population (Fisher 1930). As indicated by Gabre et al. (2005) and Liu et al. (2018), the ages of peak v_{xj} are found close to the TPOP (39.17 d).

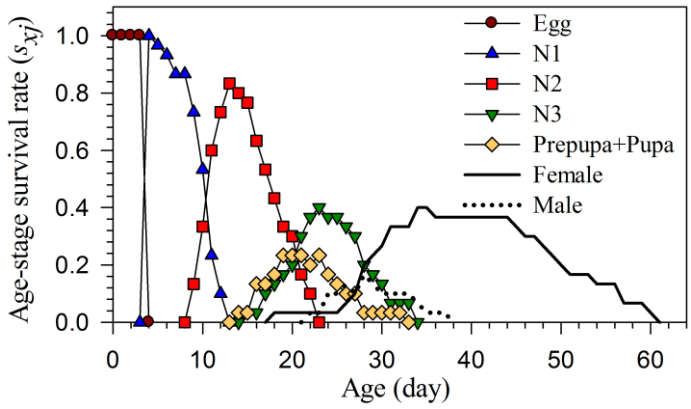

Fig 1. The age-stage specific survival rates (s_{xj}) of *Planococcus citri* reared on *Vites vitifoli* (41-B variety) leaves.

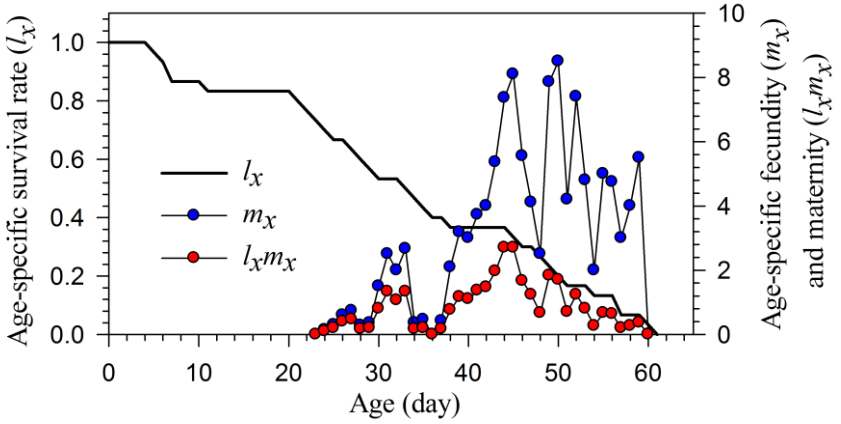

Fig 2. The age-specific survival rates (l_x), fecundity (m_x) and net maternity ($l_x.m_x$) curves of *Planococcus citri* reared on *Vites vitifoli* (41-B variety) leaves.

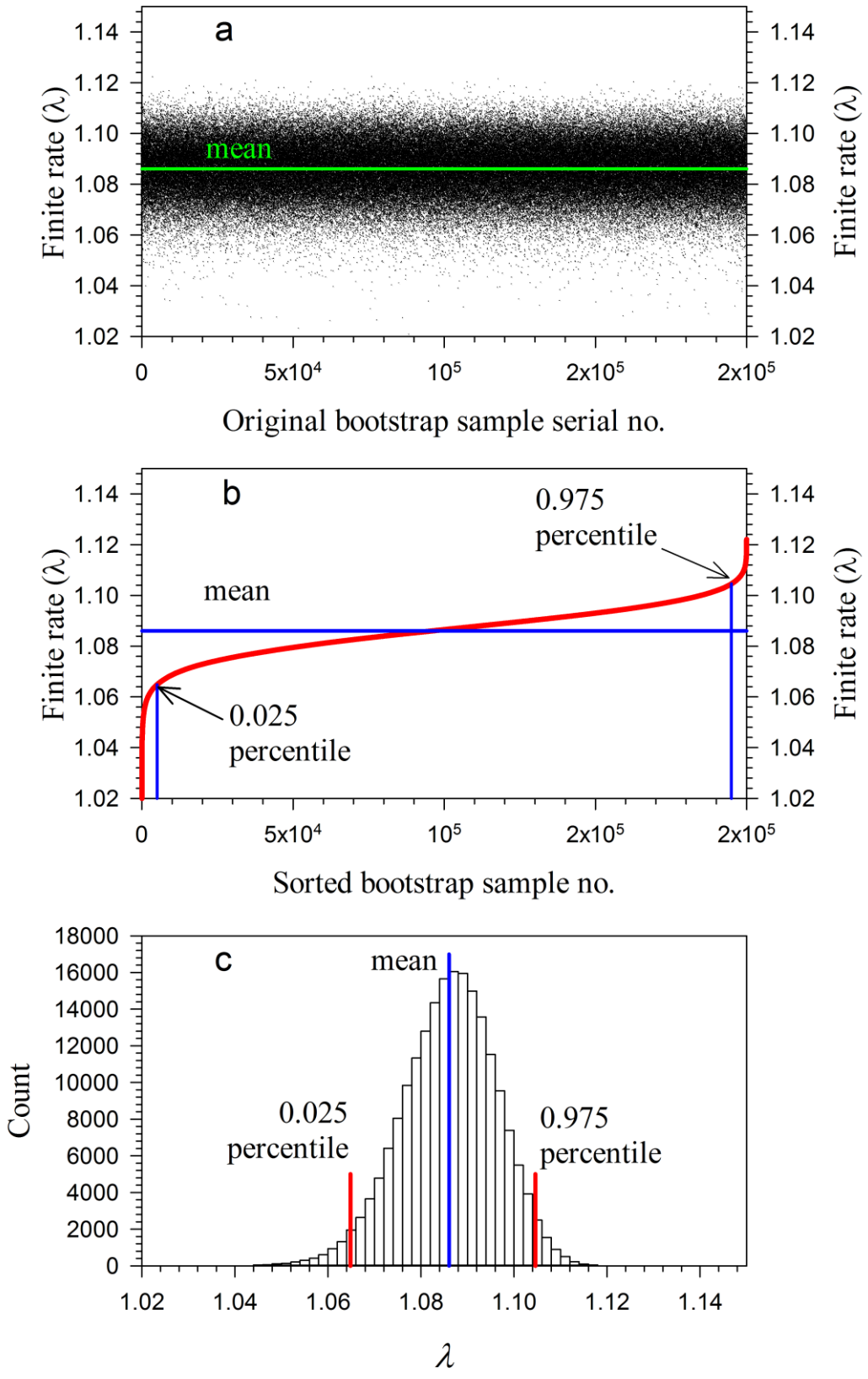

Fig. 3. The 200,000 finite rates ordered according to original bootstrap order (*x*-axis), fluctuated randomly around the mean. (b) The 200,000 finite rates sorted in ascending order. (c) The 200,000 finite rates shown as a frequency distribution. The 0.025 and 0.975 percentiles of finite rates can be obtained from the middle or bottom figures.

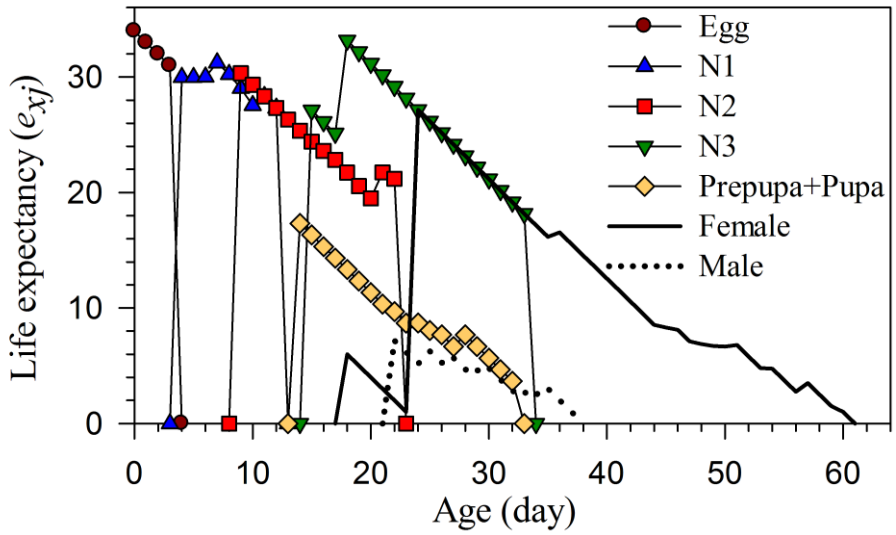

Fig. 4. The age-stage specific life expectancy (e_{xj}) of *Planococcus citri* reared on *Vites vitifoli* (41-B variety) leaves.

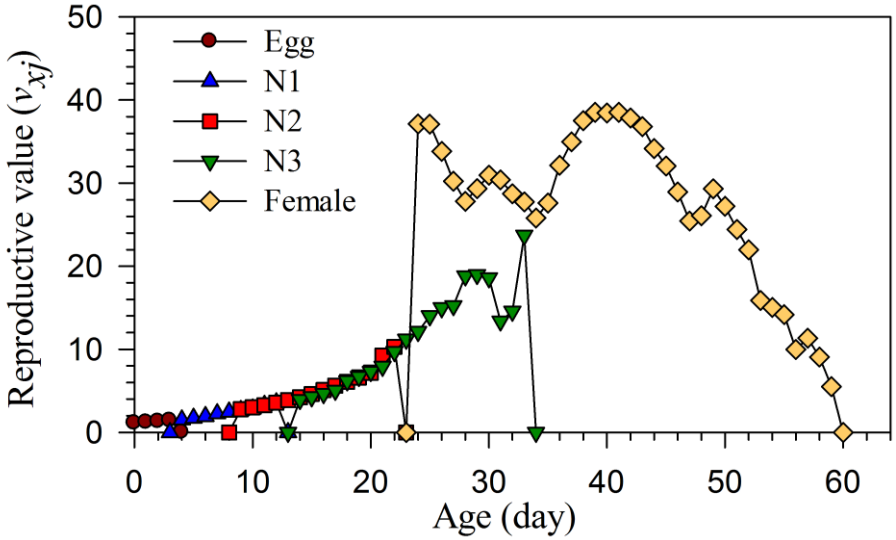

Fig. 5. The age-stage specific reproductive value (v_{xj}) curves of *Planococcus citri* reared on *Vites vitifoli* (41-B variety) leaves.

Population Projections

The population growth of citrus mealybug on the tested plant was simulated from an initial population of 10 eggs to revealed the stage structure of the egg, nymph, pupa, adult and total population size by using the life table data (Fig. 6, Fig. 7). The population projection demonstrated that the growth of the citrus mealybug was 357 individuals after 60 days, and there were 95 eggs, 76 Nymph 1, 113 Nymph 2, 51 Nymph 3 + Pupa, 14 females and 8 males. The stage curves reflect the emergence and change of each stage. The population projections can be used to depict the growth trends of a population without the assumption of approaching the stable age-stage distribution (Farhadi et al. 2011).

The life table data presents a summary of the entire biology of *Pl. citri* on host plant used in the study and on adjusted climatic conditions. The life table parameters, particularly the intrinsic rate of increase and the finite rate of increase, are powerful comprehensive tools for comparing the potential of different populations on the same host plants or the same population on different host plants.

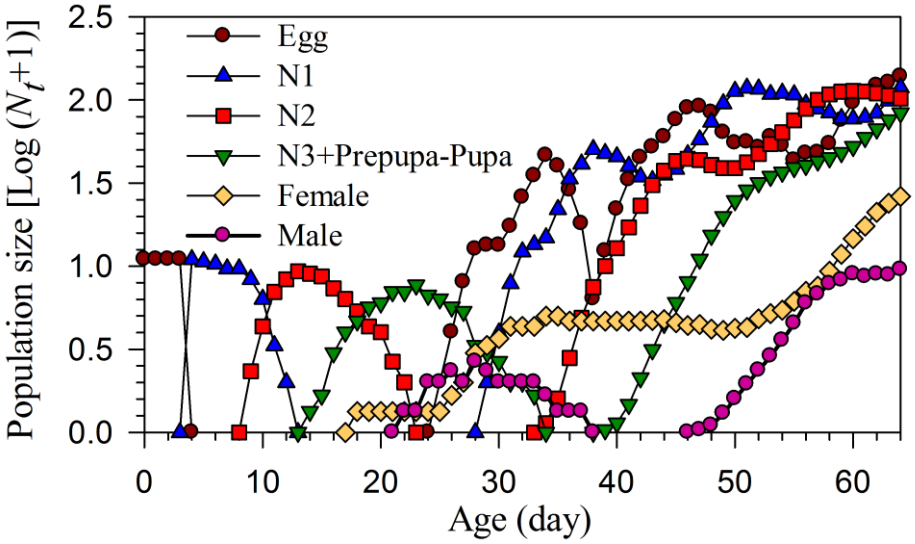

Fig. 6. The simulated population growth of *Planococcus citri* on *Vites vitifoli* (41-B variety) leaves.

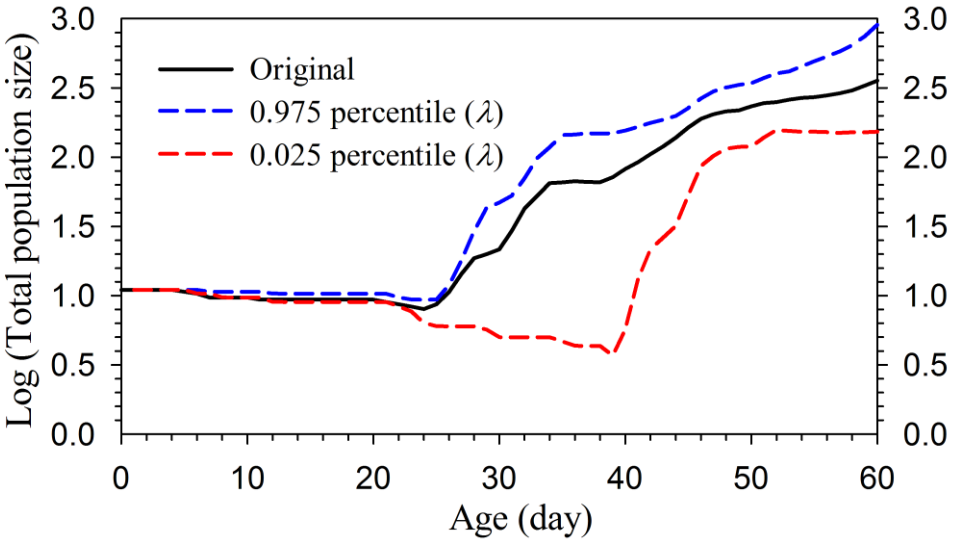

Fig. 7. The simulated total population size of *Planococcus citri* reared on *Vites vitifoli* (41-B variety) leaves.

There have been very few studies on the life table of the *P. citri* on the grapevines and most of them have been done according to female age-specific life tables. However, no investigation was based on the two-sex method. For this reason, both the life table parameters obtained in this study and the population size estimated by the population projection are the first reports. Besides, the graph of distribution of the net reproductive rates of bootstrap samples showing the uncertainty is an important new concept.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Effects of Trunk Height and Training Systems on The Some Physicochemical Properties of 'Karaerik' Berries

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Abstract: In this study, the effect of trunk heights and training systems on physicochemical properties of the 'Karerik' grape cultivar grown on different trunk heights (75-100-125 cm) and training systems (Wall, Y, Baran) were investigated. In the study, color (L*, a*, b*, C, h˚), cluster weight (g), berry weight, pH, TA%, TSS%, MI, sugar, organic acid, vitamin C and antioxidant activity (FRAP) as well as total and individual phenolic compounds were considered. As a result of the study, it was found that various training system and trunk height had no significant effect on the content of b*, h˚, cluster weight, berry weight, pH, TA%, TSS%, MI, sugar, organic acid and macro-micro nutrients and individual phenolic compound, however, effects on density of color (a*) and saturation (C), antioxidant activity and total phenolic content were found statistically significant. It can be concluded that 125 cm trunk height and double cordon training system supported by Y system are considerable due to some advantages for quality.

Keywords: *Vitis vinifera* L., quality characters, phenolics, antioxidant, vitamin C.

Karaerik Üzüm Çeşidinde Tanelerin Bazı Önemli Fizikokimyasal Özellikleri Üzerine Gövde Yükseklikleri ve Terbiye Sistemlerinin Etkileri

Özet: Bu çalışmada, farklı gövde yükseklikleri (75-100-125 cm) ve terbiye sistemleri (Duvar, Y, Baran) üzerinde yetiştirilen Karaerik üzüm çeşidinin bazı fizikokimyasal özellikleri üzerine gövde yükseklikleri ve terbiye sistemlerinin etkileri incelenmiştir. Çalışmada renk (L*, a*, b*, C, h˚), salkım ağırlığı (g), tane ağırlığı (g), pH, % TA, % SÇKM, Oİ, şeker, organik asit, C vitamini, antioksidan aktivite (FRAP) ile toplam ve bireysel fenolik bileşikler ele alınmıştır. Çalışma sonucunda, farklı terbiye sistemi ve gövde yüksekliğinin, b*, h˚, salkım ağırlığı, tane ağırlığı, pH, % TA, %SÇKM, Oİ, şeker, organik asit, makro-mikro besin elementleri ve bireysel fenolik bileşik içeriğine önemli bir etkisinin olmadığı, bununla birlikte rengin açıklık ve koyuluğu (L), yoğunluğu (a*), doygunluğu (C), antioksidan aktivite ve toplam fenolik içeriğini etkilediği görülmüştür. Elde edilen veriler doğrultusunda 125 cm gövde yüksekliği ve kalite açısından artıları nedeni ile Y destek sistemi ile desteklenen çift kollu sabit kordon terbiye şeklinin öne çıktığı söylenebilir.

Anahtar kelimeler: *Vitis vinifera* L., kalite özellikleri, fenolikler, antioksidan, C vitamini

Introduction

In modern viticulture, it is essential for control of the growth and development for grapevine. For providing to do this, vine are shaped properly by using various support materials. Training system in the viticulture specifies the combination of vine shape and organs that form this shape as well as support systems (Çelik et al., 1998a).

With a proper trunk height and training system, it aims to minimize the effects of diseases as well as harmful and negative climatic factors. Training systems in viticulture affect considerably the yield and especially berry composition by affecting utilization of berries from the sunlight (González-Neves et al., 2004; Reynolds et al., 2004; Pérez-Lamela et al., 2007; 2009; Reynolds and Heuvel 2009; Segade et al., 2009; Mota et al., 2011). Wireline training systems also play an importante role for determining exposure of the clusters to sunlight as well as affecting microclimate of vine canopy system. By depending on the number, array and volume of the leaves in the canopy system, microclimate changes with the environmental factors in the vineyard (Ağaoğlu, 2002). Therfore, changes in the microclimate of the canopy affect harvest time and berry quality (Smart, 1985). Low quality berries can be formed with improper training systems which cause overshadow for the clusters.

Shading increases potassium, pH and malic acid content in the berries, while decreases berries length, total soluble solid (TSS), phenols, anthocyanins and monoterpenes (Dokoozlian 1990; Peterlunger et al., 2002; Abd El-Razek et al., 2010; Palliotti et al., 2012; Cheng et al. 2015).

The previous studies that conducted to determine the effect of training system on berry content mostly focused on titratable acid (TA), TSS (Çelik et al., 1995; Çelik et al., 1998b; Çelik et al., 1999; Dardeniz et al., 2007; Kepenekçi, 2007; Karabat et al., 2015; Ünal et al., 2015) and the anthocyanin amount of colored cultivars (Wolf et al., 2003; Reynolds et al., 2004; González-Neves et al., 2004; Baeza et al. 2005; Kyraleou et al., 2015;Liu et al., 2015a; Marcon-Filho et al., 2017). Although the training and pruning systems affect the accumulation of phenolic compounds with related to light in leaves and berries (Crippen and Morrison, 1986; Marcon Filho et al., 2017), the studies about this subject are very limited.

Erzincan is the most important province in terms of having viticulture potential in the Northeast Agriculture Region in the eastern Anatolian region with continental climate. The vineyards area in Erzincan are 9200 decares and the production amount is 5607 tons (Anonymous, 2016). 90-95% of the cultivars in the province is only 'Karaerik' that is unique standard cultivar of the North Eastern Agricultural Region, and has distinctive flavor.

This study aims to determine effects of various trunk heights (75-100-125 cm) and trainining system (Wall, Y, Baran) on phytochemical, physical and chemical properties of 'Karaerik' cultivar.

Materials and Methods

This study was conducted at Van Yüzüncü Yıl University Agricultural Faculty Department of Horticulture and Erzincan Horticultural Research Institute Directorate in 2015 and 2016.

Materials

The grapes of 'Karaerik' were obtained from the Erzincan Horticultural Research Institute Directorate vineyard. The experiment vineyard is located in Erzincan Bahçeliköy with 1309 altitude and 39° 45'06.54 N, 39° 21'36.79E coordinates. According to the randomized block design with 4 replications, the vineyard was planted 3.0 x 2.0 m (vine x row) spacing with 6 vines in each replication. The height of the trunk was 75-100-125 cm and the double cordon was used as training system. 'Karaerik' grape cultivar is proper for short pruning, therefore short pruning was performed. By applying equal charge (24 buds) for each vine, pruning was carried out equally to all vines including the Baran training system.

Results of soil analysis and climatic data for the experimental vineyard are given in Table 1 and Table 2, respectively.

Table 1. Results of soil analysis for the experimental vineyard

Soil Analysis		
pH	7.65	Light alkaline
EC (dS m^{-1})	0.63	Low salinity
Texture	55	Clay loam
Organic matter	2:03	Medium
Lime (%)	6:48	Medium lime
Salt (%)	0.022176	Without salt
Phosphorus (kg da^{-1})	13.28	
Potassium (kg da^{-1})	56.2	

Drop irrigation system was used in the experimental vineyard. In the autumn, vineyard was fertilizied for nitrogen, phosphorus and potassium as well as vine manure with 2-3 tons/decares. In addition, fertilization was performed on leaves for the phosphorus and potassium.

According to the diseases and pests, spraying was carried out regularly. Mechanical control was preferred for the weed control. Axillary shoot removal, bottom shoots cleaned and topping were performed as summer pruning.

Table 2. Climate data for experimental vineyard

Months	2015 Annual Climate Data				2016 Annual Climate Data			
	Average temperature (°C)	Number of Frost Days	Precipitation (mm)	Relative Humidity (%)	Average temperature (°C)	Number of Frost Days	Precipitation (mm)	Relative Humidity (%)
J	-4.3	22	55	78.8	-3.8	21	35.2	71.5
F	1.2	11	35.2	71.5	-1	13	37.6	77.5
M	8.4	1	85	64.9	5.1	1	34.4	58.5
A	8.2		98.4	60.7	12.1		37.8	47.9
M	13.8		85.4	62.1	13.5		120	65.9
J	19.9		20.2	49.6	19.3		35.2	55.3
J	24.3		21.4	38.3	23		21	47.3
A	24.9		7.8	41.8	25		1.4	39.7
S	1.22		0	33.6	16.4		34.4	52.5
O	12.5		105.2	69.1	3.12		8.8	53.1
N	5.7		32.6	57.9	2.2	3	3.8	58.5
D	-2.3	30	6.8	67.7	-4.1	22	24.6	73.6
Total		64	553			60	394.2	

Methods

Collecting of grape samples

The stages of phenological development for 'Karaerik' cultivar are presented in Table 3. The maturation was determined by measuring the amount of dry matter with a digital refractometer. When total soluble solid value reached 17% to 18% the clusters were cut and the berries were collected by Amerine and Cruess (1960) method (taking of berries from 1/3 of the clusters). Then berries were stored at -20 °C until the analyses are performed.

Table 3. The stages of phenological development of 'Karaerik' grape cultivar

Training System	Trunk Height (cm)	Budburst	Blooming	Verasion	Harvest
Y system (Y)	75	10/05/2015	06/22/2015	08/17/2015	09/23/2015
		05/12/2016	06/21/2016	08/24/2016	05/10/2016
	100	05/11/2015	06/21/2015	08/16/2015	09/23/2015
		13/05/2016	06/21/2016	08/24/2016	05/10/2016
	125	10/05/2015	06/21/2015	08/15/2015	09/23/2015
		05/12/2016	06/20/2016	23/08/2016	05/10/2016
Wall system (W)	75	05/11/2015	06/21/2015	08/15/2015	09/23/2015
		13/05/2016	06/20/2016	23/08/2016	05/10/2016
	100	10/05/2015	06/21/2015	08/15/2015	09/23/2015
		05/12/2016	06/20/2016	23/08/2016	05/10/2016
	125	10/05/2015	06/21/2015	08/15/2015	09/23/2015
		05/12/2016	06/20/2016	23/08/2016	05/10/2016
Baran system (B)	-	08/05/2015	06/19/2016	08/15/2015	09/23/2015
		10/05/2015	06/18/2016	23/08/2016	05/10/2016

Physical properties

Color

For the analysis of grain skin color, three replications which include 10 berries were used and measurments were obtained from three parts of each berries by Minolta CR-400 colorimeter. In the samples, the colors were measured as L*, a*, b*, h° and C values according to the CIE Lab color system and the obtained values were transformed to the CIRG index that determined by Carreńo et al., (1996).

Cluster weight

Randomly sampled samples (10 cluster) were weighed on the sensitive scale and expressed as gram.

Berry weight

100 berries drawn randomly were weighed on a sensitive scale and expressed as gram.

Chemical properties

pH

100 berries were drawn randomly from the grape clusters and then squeezed for obtaining the grape juice. pH value of this juice was measured at the pH meter with glass electrode (Ough and Amerine, 1988).

Titratable acidity (TA)

100 berries were squeezed and must obtained. The solution was formed by adding 20 ml of purified water to 10 ml must. Until pH value reached 8.1, 0.1 N NaOH was added to the solution. Then the titratable acidity (%) of the must was computed from consumed NaOH (ml) amount.

Total soluble solid content (TSS)

100 berries were squeezed and must obtained. Then the total soluble solid content (%) of the must was measured by refractometer.

Maturity index (MI)

It was obtained by dividing of TSS (%) values to TA during the harvesting period.

Chromatographic analysis of sugars

For the determination of the sugar (glucose, fructose) content with chromatograpy, the method purposed by Melgarejo et al. (2000) was modified and used (Karadoğan and Keskin, 2017).

Chromatographic analysis of organic acids

For the determination and extractions of the organic acids with chromatograpy, the method purposed by Bevilacqua and Califano (1989) was modified and used (Karadoğan and Keskin, 2017).

Analysis of vitamin C (L-ascorbic acid)

Vitamin C amount was determined by considering the method purposed by Cemeroğlu (2007) .

Analysis of macro and micro nutrients

Analysis of macro and micro nutrient elements was performed in the whole berries which was pureed. Phosphorus was determined in the solution that obtained from wet burning according to the yellow color method with spectrophotometer (Kacar, 1984). Potassium (K), calcium (Ca) and magnesium (Mg) were determined in the solutions that obtained from wet burning with Atomic Absorption Spectroscopy (AAS) Kacar (1984). Simillarly, analyzes of iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were also performed with AAS in the solution that obtained from wet burning (Kacar, 1984).

Antioxidant activity (FRAP) analysis

Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1996) was used to determine antioxidant activity and the absorbance at 593 nm was recorded for the prepared solutions in the spectrophotometer, and then the antioxidant activity values were measured as μmol trolox equivalent (TE) mg^{-1}.

Phytochemicals characteristics

In the study, total and individual phenolic compounds (gallic acid, citric acid and quercetin) were analyzed as phytochemical characteristics. According to Swain and Hillis (1959), analysis of total phenolic compounds was

determined by Folin-Ciocaltaeu calorimetric method in the spectrophotometer (Karadoğan and Sharp 2017). Chromotographic analysis of individual phenolic compounds was carried out by Rodriguez-Delgado et al. (2001).

Statistical analysis

Descriptive statistics for the characteristics in the study were presented as mean and standard error. One-way ANOVA was used for comparison of the groups' mean. The statistical significance level was considered 5% and SPSS (ver: 13) statistical program was used for all statistical computations.

Results and Discussions

Results of Physical Analysis

Comparison results for the physical characteristics are presented in Table 4.

Table 4. Desciptive statistics and comparison results for the some physical characteristisc to trunk height and training systems

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
L *	24.00±0.20d	24.67±0.19cd	25.14±0.28cd	25.90±0.05c	27.65±0.94b	29.75±0.04a	20.55±0.20e
a *	3.42±0.01f	4.53±0.01d	4.33 ±0.04 e	5.35 ±0.01c	5.71±0.05b	5.84±0.05a	3.10±0.01g
b *	0.33±0.14	0.31±0.38	0.10±0.52	0.35±0.67	0.22±1.16	0.26±0.29	0.01±0.06
h	333.75±12.97	358.87±0.23	351.58±2.89	358.15±1.59	352.51±6.05	359.42±0.25	355.16±3.93
C	3.44±0.02 e	4.56±0.03c	4.35±0.03d	5.40±0.04b	5.83±0.10a	5.85±0.03a	3.10±0.01f
Cluster weight (g)	468.04±110.90	417.01±47.78	447.76±104.48	406.31±53.37	448.37±73.370	457.02±66.78	387.82±24.37
Berry weight (g)	5.62±0.28	5.29±0.31	5.46±0.23	6.13±0.41	6.10±0.43	5.43±0.55	5.15±0.39

a, b, c →: Different small letters in the same row indicate statistically significant differences ($p < 0.05$)

As seen in Table 4, the difference between the combinations in terms of the L* value expressing the lightness-darkness coordinates of the color was found statistically significant. The highest and lowest L* values are obtained from the combination of 125+Y (29.75) and Baran (20.55) training system, respectively. Although there is no visible difference in coloration between the two wire training systems, it was observed that the clusters were colored better than that of growing on the Baran training system.

The difference in the a* value was statistically significant ($p < 0.05$) and not significant in terms of b* value when the changes in the a* and b* values expressing the color intensity of different trunk heights and Training systems applied in the 'Karaerik' grape variety were found to be statistically significant.

The differences among the various trunk heights and and training systems were found statistically significant for a value, while not significant for b.

Similarly, the differences among the various trunk heights and and training systems were statistically significant for chroma (C) value indicating degree of saturation ($p < 0.05$) and the values varied between 5.85 (125+Y) and 3.10 (B).

Hue (hº) value that indicates the proportion of all colors were recorded as 333.75 (75+WD) and 359.42 (125+Y). However, there was no statisticaly significant differences among the treatments for this characteristic. When the obtained values were evaluated according to the CIE Lab color system, it was observed that the color of 'Karaerik' cultivar grown on different trunk heights and training systems was within the black color limits.

Dardeniz et al. (2007) stated that the effects of different training systems on berry color was statistically significant for 'Müşküle' grape cultivar. Light colored berry ratio was lower in double cordon and Guyot training systems, however the berries in vines with single cordon training system showed better coloring. Similarly, Falcão et al. (2008) reported that training systems are effective for the color tone and density of Cabernet Sauvignon grapes grown on two different training systems (Y and Vertical Shoot Position). On the other hand,

Marcon Filho et al., (2017) emphasized that there is no significant difference for color tone and density of berries Cabernet Sauvignon grape cultivar grown on Lir and Trellis training systems.

The cluster weight varied from 387.82 g (B) to 468.04 g (75+W) and effects of treatment were nonsignificant. In some previous studies (Çelik ve ark. 1998b; Ünal ve ark., 2009), it was stated that the effects of training system on the cluster weight were significant, however the findings of some other studies (Çelik ve ark., 1999) showed that cluster weight was unaffected from training system as in our findings.

For the beerry weight, the highest berry weight was observed as 6.13 g from the 75+Y combination while the lowest one recored as 5.15 g from the baran training system. However, differences among the treatments were nonsignificant. Our findings are supported by the previous studies (Çelik et al., 1995, Celik et al., 1999, Kepenekci, 2007, Zoecklein et al., 2008, Kim et al., 2014).

Chemical Analysis

The results of some chemical characteristics according to trunk heights (75-100-125 cm) and training systems (Wall, Y, Baran) are given in Table 5.

For the pH values, the highest one was obtained as 3.36 from the combination of 125+W, while the lowest one was 3.01 for 75+W combination. The difference among the treatment was found nonsignificant (Table 5). Dardeniz et al., (2007) reported that the effects of training system on pH were significant, however the findings of some studies (Auvray et al., 1999; Kepenekci, 2007) showed that pH was unaffected from training system as in our findings.

Table 5. Desciptive statistics and comparison results for the some chemical characteristisc to trunk height and training systems

	75 +W	**100 +W**	**125 +W**	**75 +Y**	**100 +Y**	**125 + Y**	**B**
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
pH	3.01±0.09	3.17±0.09	3.36±0.18	3.08±0.12	3.07±0.16	3.10±0.12	3.15±0.14
TA%	0.89±0.09	0.84±0.03	0.82±0.12	0.82±0.03	0.83±0.03	0.79±0.07	0.80±0.15
TSS%	16.85±0.25	17.10±0.40	17.60±0.40	16.90±0.40	16.90±0.50	18.10±0.50	18.00±0.20
MI	19.15±2.21	20.29±1.31	22.00±3.70	20.50±0.38	20.25±0.25	23.03±2.80	23.27±4.72

TA values ranged from 0.79% (125+Y) to 0.89% (75+W) and the differences were found nonsignificant (Table 5). In the previous studies, (Demirbüker et al., 1982; Özışık et al., 1986; Celik et al., 1998; Dardeniz et al., 2007; Zoecklein et al., 2008; Karabat et al., 2009a; Karabat et al., 2009b; Babalık, 2009; Marcon Filho et al., 2017) it was found that the trunk height and the training system were ineffective on TA% as in our study.

The highest TSS % was recorded as 18.10% in the 125+Y combination while the lowest one was 16.85% in 75+W combination. However these differences among the treatments were nonsignificant. Our findings are consistent with the results of previous studies (Çelik et al., 1998b; Çelik et al., 1999; Dardeniz et al., 2007; Kepenekci, 2007; Karabat et al., 2009a; et al., 2015, Kim et al., 2014, Marcon Filho et al., 2017).

The maturity index is an important criterion in the selection of table grapes. In this study, MI varied from 19.15 (75+W) to 23.27 (B) however, this differences were not statistically significant. This findings are consistent with the results of Dardeniz et al. (2007) and Karabat et al. (2015).

For the mineral content of the ‘Karaerik’ cultivar, comparison results to treatments are presented in Table 6.

Table 6. Desciptive statistics and comparison results for the mineral content (μg100g^{-1})

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
P	359.34±0.86	355.91±0.28	358.43±0.32	359.92±0.48	359.13±0.14	359.38±0.11	358.32±1.76
K	26.90±1.44	26.85±0.44	26.73±1.40	28.59±0.02	27.92±0.79	27.74±0.58	25.93±0.25
Ca	3274.17±52.23	3260.73±38.32	3261.35±37.21	3273.50±52.82	3279.74±55.09	3254.20±33.75	3267.11±38.14
Mg	3758.60±229.11	3990.39±9.22	3675.10±213.24	3604.25±63.13	3852.29±73.81	3788.73±96.57	3671.02±83.36
Fe	199.61±1.18	197.76±0.40	198.79±1.28	200.37±0.57	199.37±1.10	197.73±0.41	197.32±0.15
Mn	79.41±0.09	82.23±0.70	81.94±0.58	76.86±0.41	78.94±0.27	78.79±0.30	79.44±0.17
Zn	41.44±1.05	41.91±1.18	41.94±1.29	41.19±0.60	40.91±0.48	41.20±0.57	41.47±0.88
Cu	70.72±0.74	71.40±1.03	72.78±2.31	71.84±1.56	74.10±1.76	72.83±1.76	76.50±0.80

Effect of trunk height and training systems on the mineral content of the berry were found nonsignificant.

According to Winkler et al. (1974) 100 g flesh grape consists of 0-70 ppm boron, 100-250 ppm calcium, 0-3 ppm copper, 0-30 ppm iron, 100-250 ppm magnesium, 0-51 ppm manganese, 1500-2500 ppm potassium, 200-500 ppm phosphorus, and 0-200 ppm sodium. Minerals found in grapes are taken from the soil by the grapevine and transmitted to the plant, and indirectly to the berries. The amounts are within certain limits and vary according to the grape cultivar, maturity grades, type of soil, fertilization and climatic conditions (Martins et al., 2012). In general, the amount of mineral matter is lower in arid climatic conditions and dry conditions. The quantities of minerals are influenced by soil conditions, while some elements are affected by atmospheric conditions and some by drugs that are used against plant diseases and harmful effects.

The results of organic acid content (g100g^{-1}) are given in Table 7. As seen in Table 7, the effects of different trunk heights and training systems on the organic acid content were nonsignificant.

Table 7 . Desciptive statistics and comparison results for the organic acid content (g100g$^{-1)}$

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Tartaric acid	0.30±0.01	0.28±0.01	0.29±0.01	0.28±0.01	0.29±0.01	0.29±0.01	0.30±0.01
Malic acid	0.24±0.01	0.24±0.01	0.25±0.01	0.24±0.03	0.25±0.01	0.25±0.01	0.24±0.01

In the study, the highest tartaric acid content was obtained as 0.30 g100g^{-1} in the berries of the vines grown on Baran and 75+W. Although, there was no statistically significant difference in malic acid, the highest one was recorded as 0.25 g100g^{-1} in the berries of the vines grown on 100+Y, 125+Y and 125+W combinations.

Smart and Robinson (1991) pointed to the importance of crown system in terms of organic acids and reported that the sunlight caused deterioration of organic acids by pass through the clusters. Zoecklein et al. (2008) reported that differences between training sytems was nonsignificant for malic and tartaric acid of Viognier grape cultivar grown on VSP, SD, and GDC training systems. Similarly Mota et al. (2011) stated that although there is no statistically significant difference between VSP and GDC training systems for total organic acid of berries Syrah grape cultivar in the Brazil-Minas Gerais-Cerrado region, tartaric and malic acid values of GDC training system are higher than that of VSP.

For glucose and fructose contents, comparison results of different trunk heights and training systems are presented in Table 8.

Table 8. Desciptive statistics and comparison results for the glucose and fructose content (g100g$^{-1)}$

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Glucose	12.81±1.09	12.13±0.69	13.01±0.75	11.64±0.32	13.04±0.20	11.85±0.40	11.05±0.29
Fructose	12.11±1.09	11.70±0.61	12.55±0.78	11.22±0.13	12.25±0.18	11.37±0.27	10.49±0.27
Glucose / Fructose	0.94±0.01	0.96±0.01	0.96±0.01	0.96±0.02	0.94±0.01	0.96±0.01	0.95±0.02

As seen in Table 8, the effects of different trunk heights and training systems on the glucose and fructose content were not statistically significant. Both the highest glucose and fructose contents were 13.01 g100g^{-1} and 12.55

g100g^{-1} (125+W), respectively. The glucose/fructose ratio varied from 0.94 to 0.96. Liu et al., (2015b) stated that glucose/fructose ratio varied 1.14 to 3.07 for VSP and 1.08 to 3.26 for Y-shaped in 2011-2013, and two (VSP and Y) different training systems for Beibinghong *(Vitis amurensis* Rup.) were ineffective for sugar metabolism.

For vitamin C and the antioxidant content, comparison results of different trunk heights and training systems are presented in Table 9. The differences among the treatment were found statistically significant for both characteristics. In the literature, there was no study about the effects of different trunk heights and training systems on vitamin C content in grapes. However, Kretzschmar et al. (2014) reported that the highest vitamin C was obtained from the "X" of the four training system (free, vertical cages, X and V) for the Physalis *peruviana* L. fruit. On the other hand, Kyraleou et al. (2015), studied about the effect of 3 training systems (Lir, Guyot and Royat) on the phenolic compound of Xinomavro *(Vitis vinifera* L.) grape cultivar and they noted that the effect of training system on the antioxidant activity of the berry shell was found statistically significant. In their study the highest antioxidant activity was obtained as 0.134 mmol trolox g $^{-1}$ dry weight from the berry shell that of vine grown on Lir training system and this is followed by Guyot with 0.134 mmol trolox g $^{-1}$ and Royat with 0.119 mmol trolox g $^{-1}$.

Table 9 . Desciptive statistics and comparison results for Vitamin C (g100g$^{-1)}$ and antioxidant content

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Vitamin C	19.78±0.16a	16.44±0.09bc	19.38 0.28a	15.17±0.53c	19.41±0.08a	17.73±1.14ab	18.02±0.78ab
FRAP	153.85±0.53a	143.05±0.09bc	136.89±6.43cd	148.00±0.75ab	155.91±1.34a	133.78±1.92d	154.30±0.27a

a, b, c,: The difference between clone averages with different small letters in the same line is significant ($p < 0.05$)

Phytochemical Analysis

Comparison results for the trunk heights and training system of phytochemical characteristics presented in Table 10.

Table 10 . Desciptive statistics and comparison results for the phytochemical characteristics

	75 +W	100 +W	125 +W	75 + Y	100 + Y	125 + Y	B
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Gallic acid (µg 100 g^{-1})	59.12±0.020	59.38±0.05	61.00±0.05	59.34±0.05	61.92±0.38	59.48±0.38	60.14±0.05
Syringic acid (µg 100 g^{-1})	128.06±0.87	125.41±0.46	127.22±0.88	125.93±1.19	127.20±1.21	125.54±1.04	127.630±0.98
Quercetin (µg100g^{-1})	109.20±0.90	109.90±1.66	110.34±0.09	109.85±1.41	108.51±0.14	110.94±0.73	108.18±0.60
Total phenolic (mgGAEg^{-1})	1.17±0.06b	1.59±0.03b	1.18±0.09cd	1.28±0.05bc	1.56±0.05b	1.76±0.02a	1.07±0.03d

a, b, c →: Different small letters in the same row indicate statistically significant differences ($p < 0.05$)

For the individual phenolic compounds, gallic acid varied from 59.12 µg100g^{-1} (75+W) to 61.92 µg100g^{-1} (100+Y), citric acid 125.41 µg100g^{-1} (100+W) to 128.06 µg100g^{-1} (75+W) and quercetin 108.18 µg100g^{-1} (B) to 110.94 µg100 g^{-1} (125+Y).

Similarly, total phenolic content varied from 1.07 mgGAEg^{-1} (B) to 1.76 mgGAEg^{-1} (125+Y). Thus, according to these results, it can be stated that trunk heights and training systems were ineffective to individual phenolic compounds, while effective for total phenolic.

In some studies, (Cavollo et al., 2001; Falcão et al., 2008; Babalık et al., 2009) 2009; Bavougian et al., 2013), it was reported that the effects of different trunk heights and training systems on the phytochemical content of the berries were nonsignificant, while significant in some other studies (Segade et al., 2009; Bavougian et al., 2013). In viticulture, training systems and pruning both allow the clusters to be better exposed to the sunlight and provide good light transmission and ventilation. Therefore, training systems are effective on the phenolic compounds the period from maturation to harvest (Segade et al., 2009).

Both environmental and viticultural factors affect the phenolic composition of the grapes. At this point, it was reported that the effect of light was very important (Jackson and Lombard, 1993; Cheng et al., 2015). Some researchers expressed that light applications have not different effects, while others noted that the high light intensity decreases the anthocyanin content (Bergqvist et al., 2001). Spayd et al. (2002) reported that the high temperature in the clusters has reducing effect on total anthocyanin for the shell of fruits in the western location. Researchers also stressed that crown management, such as training systems, was still an important application in terms of taking enough light.

Conclusions

Today, in the viticulture, the studies about trunk height, training system and canopy management have been very common with the developing technology. It is a fact that the advantages of the wire training systems over conventional methods. It can be stated that local training systems (traditional system) are behind of modern systems in terms of many viticulture factors such as yield, quality, canopy management, cultural application.

In this study, it was to determine effects of various trunk heights (75-100-125 cm) and trainining system (Wall, Y, Baran) on phytochemical, physical and chemical properties of 'Karaerik' cultivar that is unique standard cultivar of the North Eastern Agricultural Region in Turkey gene potential. As a result of the study, it was found that various training system and trunk height had no significant effect on the content of b*, h°, cluster weight, berry weight, pH, TA%, TSS%, MI, sugar, organic acid and macro-micro nutrients and individual phenolic compound, however, effects on density of color (a*) and saturation (C), antioxidant activity and total phenolic content were found statistically significant.

In addition, this study also included the "Baran system" which is a traditional ground viticulture in Erzincan region. It can be concluded that 125 cm trunk height and double cordon training system supported by Y system are considerable due to some advantages for quality.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of Zinc Applications on Nutrient Contents of Up Ground Parts in Lentil (*Lens Culinaris*medic.) Varieties

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Abstract: Lentil is one of the oldest domesticated crops grown and used mostly in human diets in Turkey. The experiment was carried out in factorial randomized complete block design with three replications. Sazak-91, Yerli Kırmızı and Kışlık Kırmızı-51 lentil variety were used as lentil varieties. Five different zinc levels (0, 5, 10, 15 and 20 kg ha^{-1}) were applied in two years. In the study, the effects of zinc doses on the N, P, K, Cu, Fe, Mn, Ca, Zn and Mg content of up ground parts were investigated in lentil varieties. Zinc applications increased N, K, Fe, Zn and Mn contents of up ground parts in lentil whereas P, Cu, Ca and Mg decreased.

Keywords: *Lens culinaris*, zinc fertilization, nutrient content, up ground parts

Introduction

Legumes are an important source of protein, vitamins, minerals and complex carbohydrates in diets of a large number of people, especially in developing countries. Among legumes lentil is the second leading grain legume crop after chickpea in Turkey. The lentil, a legume crop, fixes atmospheric nitrogen in the root-nodules in a symbiotic relationship with *Rhizobium* bacteria. It is an important crop because of its high protein content of seed and straw for human and animal nutrition. The current productivity trends of lentil are decreased on decades. Poor fertilization area is considered as an important factor effecting this decreasing. The plant needs some macro- and micronutrients for its normal growth. Lentil seeds have a quite high protein content (18-30 %) varied according to environmental conditions, varieties and agronomic process.

Zn is an essential nutrient in plants, animal and humans. In human's Zn deficiency causes severe health complications, including impairments of physical growth, immune system and learning capacity, DNA damage and cancer development (Levenson and Morris, 2011). It was reported that more than 30% of the world's populations is Zn deficient, with Zn deficiency being the 11th most important factor causing disease or death in the world (WHO Micronutrient Deficiencies, 2011).

Zinc contents of plants plays an important role in plant reproduction. Its deficiency inhibits floral development, male and female gametogenesis, fertilization and seed development. Pandey et al. (2006) reported that zinc deficiency reduces plant growth, pollen viability, flowering and grain production in plants.

Studies show that 30% of the arable areas in the world and 50% of the agricultural soils in Turkey suffer from zinc deficiency (Kenbeay and Sade, 1987). The Van region is among the areas most severely affected by zinc deficiency (Eyüboğlu et.al. 1998, Çamaş et al. 1998).

This study was carried out to investigate the effects of zinc applications on nutrient contents of up ground parts of lentil varieties in the Van region in eastern Turkey.

Materials and Methods

Sazak-91, Yerli Kırmızı and Kışlık Kırmızı-51 lentil cu1tivars were well adapted lentil in Van, Turkey ecological conditions. The two years' field experiments on clay loam soils were conducted during the winter seasons in the Zeve Campus of Agricultural Faculty of Van Yuzuncu Yil University (Long. 43°172 E2, Lat. 38°332 N2, and 1655 m above msl). Sowing of Sazak-91 Yerli Kırmızı and Kışlık Kırmızı-51 varieties were done by hand with 20 cm row spacing in late October in both years. Plot size was 1 m x 5 m = 5 m². Sazak-91, Yerli Kırmızı and Kışlık Kırmızı-51 lentil varieties were applied at five different zinc doses (0, 5, 10, 15 and 20 kg ha^{-1}) in two

years. $ZnSO_4$ was used as Zn source. The seeding rate was 150 kg ha^{-1}. At sowing, 40 kg ha^{-1} P_2O_5 as a TSP and 20 kg ha^{-1} N as an ammonium sulfate were uniformly applied in the trial area as basic fertilization. Plots were hand-weeded twice each season. Plants were harvested in late June in both years. Some physical and chemical properties of research area soil are given in Table 1.

Some physical and chemical soil properties of the field were determined in soil sample taken from 0 – 20 cm depth as follows: particle size distribution by Bouyocous hydrometer method (Bauyocous, 1951); lime content by Scheibler Calcimeter, soil reaction (pH) in 1:1 (w:v) soil:water suspension by pH meter and soil salinity by EC meter in the same suspension (Black, 1965); organic matter content by Walkley-Black method, exchangeable cations by ammonium acetate extraction; available phosphorus by Olsen's method; total nitrogen by the Kjeldahl method (Kacar,1994) and DTPA extractable heavy metals (Fe, Mn, Zn, Cu) according to Lindsay and Norvel (1978).

Table 1. Some physical and chemical properties of the soil

Sand, %	19.90	NH_4OAc Extractable	
Silt,%	17.50	K, g kg^{-1}	0.29
Clay,%	62.60	Ca, g kg^{-1}	2.65
$EC_{25°C}$,mmhos cm^{-1}	0.77	Mg, g kg^{-1}	0.24
pH	8.54	DTPA Extractable	
Organic matter,%	0.44	Fe, mg kg^{-1}	6.80
$CaCO_3$,%	3.68	Cu, mg kg-1	1.20
Total N, g kg^{-1}	1.10	Zn, mg kg-1	0.37
Olsen P, mg kg^{-1}	31.20	Mn, mg kg-1	11.40

Some physical and chemical soil properties of the field were determined in soil sample taken from 0 – 20 cm depth in the experimental field are given in Table 1. The results can be summarized as; the textural class of soil is sandy loam, sufficient in a phosphorus and potassium contents, slightly alkaline in pH, low in organic matter, very slightly saline according to EC values. DTPA- extractable Zn concentration in soil was lower than the widely accepted critical Zn concentration of 0.5 mg kg^{-1}.

The nutrient contents of the harvested plant samples were analyzed in dried and grinded plant samples according to following methods reported by Kacar (1984). The N content was determined by the Kjeldahl method, the P level was analyzed by the spectrophotometric method, and K, Ca, Mg, Fe, Mn, Zn, and Cu levels were determined by using an atomic absorption spectrophotometer (Themo ICE 3000 series).

Statistical analyses were done using SAS package programs to show difference among the mean values of nutrient contents from the different applications.

Results and Discussion

The data on the effects of different zinc applications on nutrient contents of up ground parts in lentil varieties are given in Table 2 and Figure 1, 2, 3, 4, 5.

Table 2. Effects of Zn applications on nutrient contents of up ground parts in lentil varieties

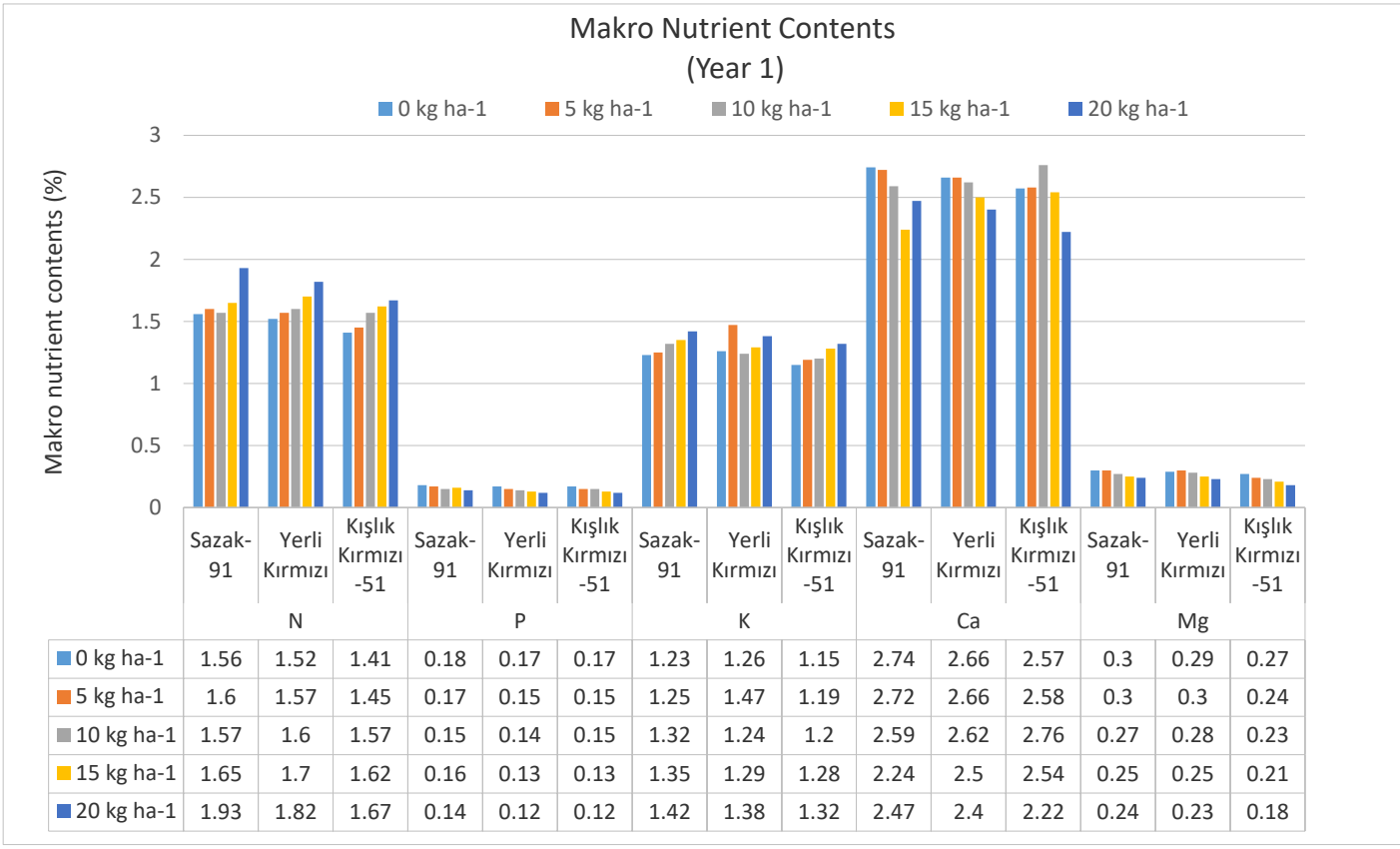
		N (%)				**P (%)**			
	Zinc levels (kg ha^{-1})	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	**Mean**	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	**Mean**
First year	0	1.56 gh	1.52 h	1.41 I	1.50 E	0.18	0.17	0.17	0.17 A
	5	1.60 e-g	1.57 fg	1.45 i	1.54 D	0.17	0.15	0.15	0.16 B
	10	1.57 g-h	1.60 e-g	1.57 fg	1.58 C	0.15	0.14	0.15	0.15 C
	15	1.65 cd	1.70 c	1.62 ef	1,.65 B	0.16	0.13	0.13	0.14 C
	20	1.93 a	1.82 b	1.67 cd	1.80 A	0.14	0.12	0.12	0.13 D
	Mean	1.66 A	1.64 B	1.54 C		0.16 A	0.14 B	0.14 B	
	0	1.63 g	1.60 gh	1.57 h	1.60 E	0.19	0.18	0.17	0.18 A
	5	1.71 ef	1.61 gh	1.60 gh	1.64 D	0.18	0.17	0.16	0.17 AB

Second Year	10	1.83 c	1.73 ef	1.71 f	1.76 C	0.18	0.17	0.15	0.16 B
	15	1.93 b	1.80 cd	1.76 de	1.83 B	0.17	0.15	0.13	0.15 C
	20	1.98 a	1.90 b	1.79 cd	1.89 A	0.15	0.13	0.12	0.13 D
	Mean	1.81 A	1.73 B	1.68 C		0.17 A	0.16 B	0.14 C	
		K (%)				**Ca (ppm)**			
	0	1.23	1.26	1.15	1.21 C	2.74	2.66	2.57	2.65 A
	5	1.25	1.47	1.19	1.25 BC	2.72	2.66	2.58	2.65 A
First year	10	1.32	1.24	1.20	1.30 AB	2.59	2.62	2.76	2.66 A
	15	1.35	1.29	1.28	1.30 AB	2.24	2.50	2.54	2.43 B
	20	1.42	1.38	1.32	1.37 A	2.47	2.40	2.22	2.36 B
	Mean	1.31 A	1.33 A	1.22 B		2.55	2.57	2.53	
	0	1.23 f	1.27 ef	1.26 ef	1.25 C	2.74 a	2.68 c-e	2.69 cd	2.70 A
	5	1.24 f	1.28 d-f	1.26 ef	1.26 C	2.76 a	2.65 de	2.65 e	2.69 A
Second Year	10	1.29 c-f	1.32 b-e	1.28 ef	1.29 B	2.73 ab	2.61 f	2.57 g	2.63 B
	15	1.34 bc	1.34 b-d	1.29 c-f	1.32 B	2.70 bc	2.56 g	2.50 h	2.62 B
	20	1.46 a	1.37 b	1.25 f	1.36 A	2.68 c-e	2.70 bc	2.47 i	2.59 C
	Mean	1.31 A	1.31 A	1.27 B		2.72 A	2.64 B	2.59 C	
		Mg (%)				**Fe (ppm)**			
	0	0.30	0.29	0.27	0.28 A	116.00 e	151.33 c-e	212.33ab	159.88 BC
	5	0.30	0.30	0.24	0.28 A	214.00 ab	149.00 c-e	178.66 b-e	180.55 AB
First year	10	0.27	0.28	0.23	0.26 B	184.00 b-d	205.33a-c	174.33b-e	187.88 AB
	15	0.25	0.25	0.21	0.24 C	192.66a-c	174.00b-e	248.33a	205.A
	20	0.24	0.23	0.18	0.21 D	167.33 b-e	149.00ce	129.33de	148.55 C
	Mean	0.27 A	0.27 A	0.23 B		174.76	165.73	188.59	
	0	0.29	0.27	0.27	0.28 A	151.4 de	150.1 e	152.2 c-e	151.2 C
	5	0.28	0.25	0.25	0.26 B	155.5 cd	150.7 e	150.1 c-e	152.1 C
Second Year	10	0.25	0.24	0.23	0.24 C	160.0 b	152.4 c-e	151.0 e	154.5 B
	15	0.24	0.21	0.19	0.21 D	164.7 a	156.2 bc	152.2c-e	157.7 A
	20	0.22	0.17	0.17	0.18 E	164.1 a	160.1b	151.9 c-e	158.7 A
	Mean	0.25 A	0.23 B	0.22 B		159.2 A	153.9 B	151.5 C	
		Cu (ppm)				**Zn (ppm)**			
	0	45.0b-d	68.6 a	68.3 a	60.6 A	5.66 hi	5.00 i	4.66 i	5.11 E
	5	49.3 b-d	42.0 d	52.6 a-d	48.0 B	6.66 gh	5.66 hi	5.00 I	5.77 D
First year	10	41.0 d	62.0ab	48.6 b-d	50.5 B	11.33 d	8.00 f	7.00 fg	8.77 C
	15	53.0 a-d	48.6 b-d	60.6 a-c	54.1 AB	14.66 b	11.66 d	9.33e	11.88 B
	20	56.0 a-d	43.3 cd	43.0cd	47.4 B	16.66 a	13.33 c	12.33 cd	14.11 A
	Mean	48.8 B	52.9A	54.6A		11.00 A	8.73 B	7.66 C	
	0	71.4 a	69.2 a-c	65.9 cd	68.8 A	5.98	5.62	5.36	5.66 D
	5	70.8 a	64.5 de	63.2 de	66.2 B	6.33	5.80	5.63	5.92 D
Second Year	10	69.5 ab	62.1 ef	57.6 g	63.1 C	7.36	7.00	7.03	7.13 C
	15	66.5 b-d	58.9 fg	53.4 h	59.6 D	10.30	8.40	8.70	9.13 B
	20	59.7 fg	56.3 gh	48.6 I	54.9 E	15.63	13.26	12.66	13.85 A
	Mean	67.7 A	62.2 B	57.7 C		9.12 A	8.01 B	7.88 B	

		Mn (ppm)			
	0	108.66a-d	112.00a-c	90.33d-f	103.66
	5	107.66a-d	101.00b-f	89.00d-f	99.22
First year	10	86.00ef	84.66ef	97.66b-f	89.44
	15	114.66ab	91.66c-f	97.33b-f	101.22
	20	123.66a	81.66f	104.00a-e	103.11
	Mean	108.13 A	94.20 B	95.66 B	
	0	90.6 b-e	88.8 de	88.1 b-d	89.2 D
	5	90.9 b-d	88.9 de	89.1 e	89.6 D
Second Year	10	92.7 a-c	90.0 c-e	89.3 de	90.7 C
	15	94.7 a	90.5 b-e	90.1 de	91.7 B
	20	95.1 a	91.0 b-d	91.1 b-d	92.4 A
	Mean	92.8 A	89.8 B	89.5 B	

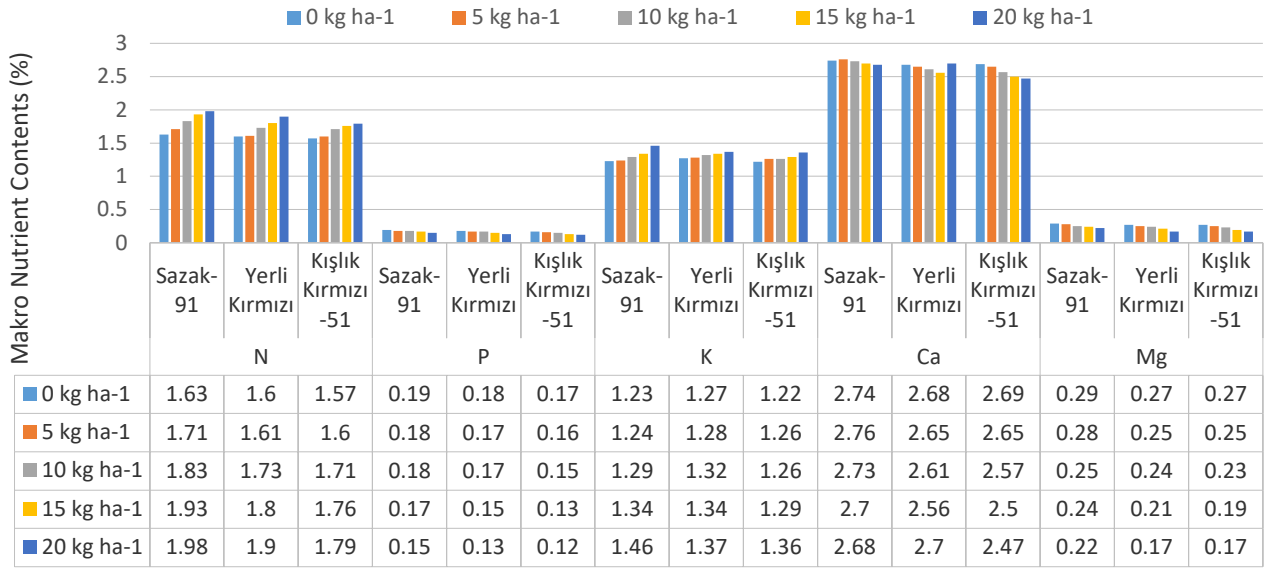
*There is no significant difference between the same letters in each column statistically at 5 % level.

The lowest nutrient contents were obtained in Kışlık Kırmızı-51 variety. It was reported that nutrient absorption by plants show differences among plant varieties (Aydemir and İnce, 1988). The nutrient contents of varieties did not show differences among years except Cu, Zn and Mg contents. Nitrogen, K, Fe, Mn and Zn contents significantly ($p<0.05$) increased by increasing Zn doses while P, Cu, Ca and Mg contents decreased both years.



	N			P			K			Ca			Mg		
	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51
0 kg ha-1	1.56	1.52	1.41	0.18	0.17	0.17	1.23	1.26	1.15	2.74	2.66	2.57	0.3	0.29	0.27
5 kg ha-1	1.6	1.57	1.45	0.17	0.15	0.15	1.25	1.47	1.19	2.72	2.66	2.58	0.3	0.3	0.24
10 kg ha-1	1.57	1.6	1.57	0.15	0.14	0.15	1.32	1.24	1.2	2.59	2.62	2.76	0.27	0.28	0.23
15 kg ha-1	1.65	1.7	1.62	0.16	0.13	0.13	1.35	1.29	1.28	2.24	2.5	2.54	0.25	0.25	0.21
20 kg ha-1	1.93	1.82	1.67	0.14	0.12	0.12	1.42	1.38	1.32	2.47	2.4	2.22	0.24	0.23	0.18

Figure1. Makro nutrients contents of different lentil varieties Lentil (First year)

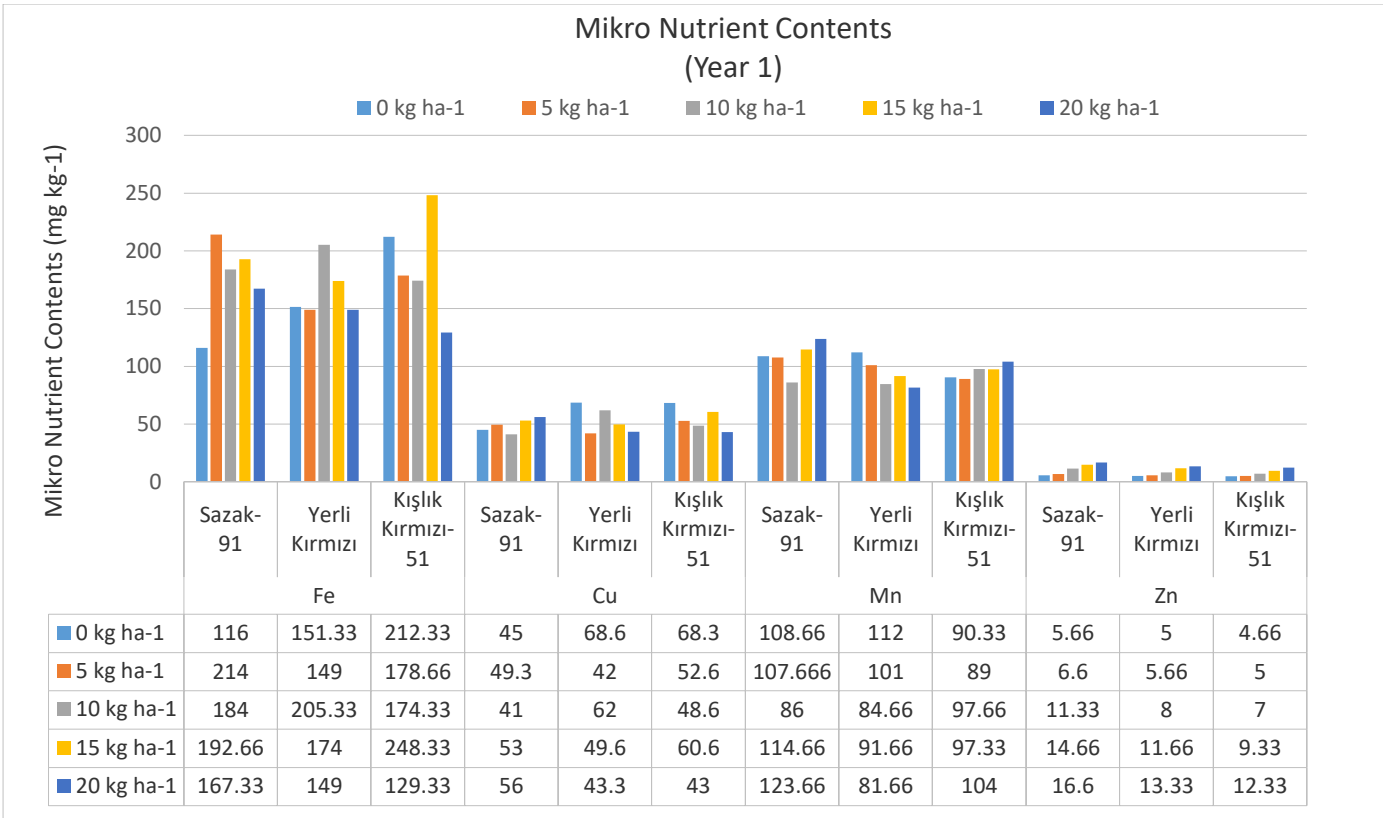


	N			P			K			Ca			Mg		
	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı -51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı -51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı -51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı -51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı -51
0 kg ha-1	1.63	1.6	1.57	0.19	0.18	0.17	1.23	1.27	1.22	2.74	2.68	2.69	0.29	0.27	0.27
5 kg ha-1	1.71	1.61	1.6	0.18	0.17	0.16	1.24	1.28	1.26	2.76	2.65	2.65	0.28	0.25	0.25
10 kg ha-1	1.83	1.73	1.71	0.18	0.17	0.15	1.29	1.32	1.26	2.73	2.61	2.57	0.25	0.24	0.23
15 kg ha-1	1.93	1.8	1.76	0.17	0.15	0.13	1.34	1.34	1.29	2.7	2.56	2.5	0.24	0.21	0.19
20 kg ha-1	1.98	1.9	1.79	0.15	0.13	0.12	1.46	1.37	1.36	2.68	2.7	2.47	0.22	0.17	0.17

Figure 2. Makro nutrients contents of different lentil varieties Lentil (Second year)

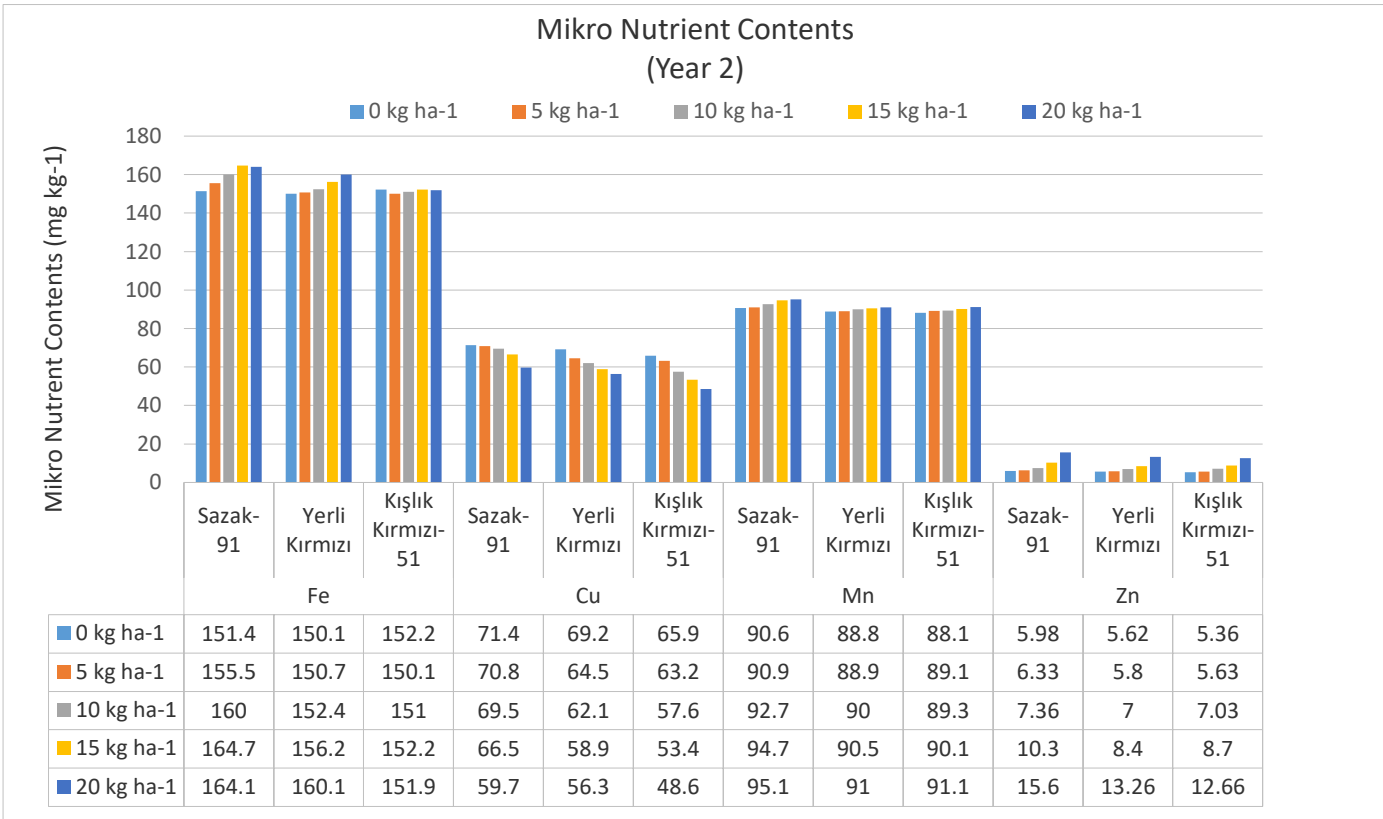
The lowest N, K and Zn contents were obtained as 1.41%, 1.15% and 5.11% mg kg^{-1} by 0 kg ha^{-1} Zinc application in Kışlık Kırmızı- 51 variety. The highest N, K and Zn contents were 1.98 %, 1.46% and 16.66 mg kg^{-1} in Sazak-91 variety with 0 kg ha^{-1} Zinc dose. There are many researchs about zinc fertilization in crop plants. But number of researchs related with effects of zinc fertilization on nutrient uptake in leguminosea are fewer and limited.

Kumar et al. (2016) determined that combined effect of biofertilizers and micronutrients (Fe+Zn) was significantly improved yield, yield parameters and nutrient uptake. Singh et al. (2017) reported that application of sulphur 40 kg ha^{-1} and zinc 20-30 kg ha^{-1} increased yield, quality and sulphur and zinc uptake in soybean. Abdul-Raziq et al. (2016) found that the highest zinc uptake obtained in 10 kg Zn ha^{-1} along with HA application and the lowest mean occured in control plants. Similarly they reported that alone zinc applications and zinc applications with humic acid increased yield and quality of french bean in zinc defficient soils.



	Fe			Cu			Mn			Zn		
	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51
0 kg ha-1	116	151.33	212.33	45	68.6	68.3	108.66	112	90.33	5.66	5	4.66
5 kg ha-1	214	149	178.66	49.3	42	52.6	107.666	101	89	6.6	5.66	5
10 kg ha-1	184	205.33	174.33	41	62	48.6	86	84.66	97.66	11.33	8	7
15 kg ha-1	192.66	174	248.33	53	49.6	60.6	114.66	91.66	97.33	14.66	11.66	9.33
20 kg ha-1	167.33	149	129.33	56	43.3	43	123.66	81.66	104	16.6	13.33	12.33

Figure 3. Mikro nutrients contents of different lentil varieties (First year)



	Fe			Cu			Mn			Zn		
	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51	Sazak-91	Yerli Kırmızı	Kışlık Kırmızı-51
0 kg ha-1	151.4	150.1	152.2	71.4	69.2	65.9	90.6	88.8	88.1	5.98	5.62	5.36
5 kg ha-1	155.5	150.7	150.1	70.8	64.5	63.2	90.9	88.9	89.1	6.33	5.8	5.63
10 kg ha-1	160	152.4	151	69.5	62.1	57.6	92.7	90	89.3	7.36	7	7.03
15 kg ha-1	164.7	156.2	152.2	66.5	58.9	53.4	94.7	90.5	90.1	10.3	8.4	8.7
20 kg ha-1	164.1	160.1	151.9	59.7	56.3	48.6	95.1	91	91.1	15.6	13.26	12.66

Figure 4. Mikro nutrients contents of different lentil varieties (Second year)

Iron and Mn contents also significantly ($p<0.05$) increased by zinc applications in only second year.

Access to these, the lowest P contents were obtained as 0.12% and 0.13% by 20 kg ha-1 Zn applications in all varieties and first year. Similarly, the lowest Cu, Ca and Mg contents were also in 10 kg ha-1 Zn applications in the first year. It was thought that the decreases of P, Ca, Mg and Cu contents in high zinc doses caused by interactions between Zn and P, Ca, Mg and Cu (Marschner,1995). Tather (2008) reported that among the various levels of zinc, application of 40 kg ha-1 gave highest pod yield and application of 20 kg ha-1 significantly increased uptake of N, P, K, Ca and Mg exhibiting synergistic effect of lower level and antagonistic effects at higher level. Awlad et al. (2003) investigated that effects of increasing zinc doses (0-2.5-5.0-10.0 and 20.0 kg Zn ha-1) and sulphur doses on nodulation, dry matter yield and nutrient content of soy bean. They determined that the highest zinc content was obtained in S30Zn20 application. Application of different levels of Zn accelerated nodulation, dry matter yield and nutrient content of soybean. Our results were corresponding with results of referred researches.

In this study, Zn applications increased N, K, Fe, Zn and Mn contents of up ground parts in lentil whereas P, Cu, Ca and Mg decreased.

Conclusion

Zinc applications increased the N, K, Fe, Zn, Mn contents of lentil up ground parts. As a result, in the soils of this region, which have poor zinc content and are highly alkaline, 20 kg ha^{-1} zinc fertilization would be beneficial and thus could be suggested in order to have efficient lentil farming. It was thought that investigations of effects of higher doses than 20 kg ha^{-1} zinc dose on nutrient contents can be useful for amelioration of crop amount and quality in lentil.

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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of EDDS application on phytoextraction of cadmium, lead and zinc contaminated soil with *Brassica napus*

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Abstract: Cadmium (Cd), lead (Pb) and zinc (Zn) contaminations of soils are a serious worldwide problem that affects human health and environmental quality. Phytoremediation, use of green plants to remove, sequester or detoxify contaminants, offers an environmentally friendly alternative solution for soil remediation. The effect of canola (*Brassica napus* L.) and a biodegradable chelate, ethylenediamine dissuccinate (EDDS), were tested on remediation of multi metal (Cd, Pb, and Zn) contaminated soil. In the pot experiment, plants were grown for two months on five soil mixtures obtained by mixing an uncontaminated soil sample with 0, 25, 50, 75, and 100% of multiple metal contaminated soil. The biomass of the plants were weighed, and the uptakes of Cd, Pb and Zn in the shoot were determined using ICP-MS. The addition of multi metals to the soil led to the increase of multi metal contents in plants. Consequently, the dry weights of the plants were increased with EDDS treatments in 25 and 50 % multi-metal doses compared to the control treatment. EDDS has significantly increased the uptake of metals (Cd, Pb, and Zn) from the soil and their accumulations in shoots of the plants. The Cd concentration of plant was higher than the hyperaccumulation limit of Cd (>100 mg kg^{-1}) with EDDS treatment in 25 and 50% multi-metal doses (141 and 174 mg Cd kg^{-1}, respectively) except for Zn and Pb concentrations.

Key words: *Brassica napus*, EDDS, phytoremediation, multiple metal, contaminated soil, decontamination

Kadmiyum, Kurşun ve Çinko ile Kirlenmiş Toprağın *Brassica Napus* ile Fitoksraksiyonuna EDDS Uygulamasının Etkisi

Özet: Toprakların kadmiyum (Cd), kurşun (Pb) ve çinko (Zn) ile kirlenmesi insan sağlığını ve çevre kalitesini etkileyen dünya çapında ciddi bir sorunudur. Fitoremediasyon, bitki kullanılarak kirleticilerin uzaklaştırılması, giderilmesi veya detoksifiye edilmesi yöntemi, toprakların ıslah edilmesinde çevre dostu alternatif bir çözüm sunar. Bu çalışmada, biyo-bozunabilir bir şelat olan etilendiamin disüksinatın (EDDS) kanola (*Brassica napus* L.) ile çoklu-metal (Cd, Pb ve Zn) ile kirlenmiş bir toprağın fitoremediasyonu üzerindeki etkisi araştırılmıştır. Saksı denemesinde, kanola bitkileri çoklu metallerle kirlenmiş toprağın temiz toprakla karıştırılmasıyla elde edilen beş toprak karışımı (%0, %25, %50, %75 ve %100) toprakta iki ay süreyle yetiştirilmiştir. Bitkilerin biyokütleleri tartıldı ve yeşil aksamın Cd, Pb ve Zn alımları ICP-MS kullanılarak belirlenmiştir. Toprağa çoklu metallerin eklenmesi, tüm bitkilerde çoklu metal içeriklerinin artmasına neden olmuştur. Sonuç olarak, kontrole kıyasla bitkilerin kuru ağırlıkları % 25 ve % 50 çoklu metal dozlarında EDDS uygulaması ile artmıştır. EDDS, topraktan metallerin (Cd, Pb ve Zn) alınmasını ve bitkinin yeşil aksamında birikmesini önemli ölçüde artırmıştır. Bitkinin, Zn ve Pb konsantrasyonları hariç EDDS uygulaması ile Cd konsantrasyonu % 25 ve % 50 multimetal dozlarında sırasıyla 141 ve 174 mg Cd kg^{-1}'e ulaşmıştır. Bu değer Cd hiperakümülasyon sınırından (<100 mg kg^{-1}) daha fazladır.

Anahtar kelimeler: *Brassica napus*, EDDS, fitoremediasyon, çoklu metal, kirlenmiş toprak, arıtma

Introduction

The increasing world population from the 20th century to the present day, the intensive agriculture, industrial, and mining, etc. activities cause soil pollution that became an important worldwide environmental problem (Dağhan 2007; Wuana and Okieimen 2011; Sumiahadi and Acar 2018). Contaminants cause an undesirable change in the physical, chemical and biological properties of the soil. Heavy metals are one of the most important kind of the contaminant in the soil. They are long-term persistence in the soil since they cannot be broken down to non-toxic forms. Heavy metal contamination is especially a problem of the industrial societies. The main source of heavy metal pollution is fossil fuel consumption. Other sources are industrial wastes, mining and

smelting wastes, fertilizers, pesticides, household wastes and sewage sludges (Wuana and Okieimen 2011; Sumiahadi and Acar 2018). These metals also naturally exist in the soil. Some of the heavy metals zinc (Zn), manganese (Mn), iron (Fe), copper (Cu) are essential for living organism in very low concentrations. Other heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), mercury (Hg), etc., are toxic to living organisms and they have a harmful effect on them even in very low concentrations. Heavy metals are highly toxic (mutagenic and/or carcinogenic) to human health. Plants grown in heavy metals contaminated soils exposed to heavy metals, which are consumed by the human can cause serious health problems such as damages on the lungs, kidneys, liver, and other vital organs (Evangelou et al. 2007; Dağhan 2011; Singh and Kalamdhad 2011; Wuana and Okieimen 2011; Sumiahadi and Acar 2018).

There are different soil remediation techniques such as isolation and immobilization, mechanical separation, pyrometallurgical isolation, biochemical, electro kinetic, soil washing, phytoremediation methods using to clean up heavy metal contaminated soils (Dağhan 2007; Angelova et al. 2015). Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost-effectiveness, aesthetic advantages and long-term applicability (Evangelou et al. 2007; Angelova et al. 2015; Sumiahadi and Acar 2018). Phytoremediation is the use of metal-accumulating plants to clean up toxic metals from soils. This technique is environmentally friendly, cheap, aesthetically pleasing, and long-term and wider range applicability than physical and chemical remediation technology (Tangahu et al. 2011; Angelova et al. 2015; Sumiahadi and Acar 2018). The main groups of this method are phytoextraction (using plant to uptake contaminants, particularly toxic metals from the contaminated soil by a plant), phytostabilization (the use of plants to limit the mobility and bioavailability of metals in soil) and rhizofiltration (removal of pollutants from groundwater via absorption by plant roots). Phytoextraction is widely utilized method to remediate heavy metal contaminated soils. This method is achieved by two ways: continuous (natural) phytoextraction and induced (assisted) phytoextraction.

Specially selected plants, known as hyperaccumulators, can extract and accumulate exceptionally high levels of contaminants from soil. There are approximately 450 types of natural hyperaccumulator plants exist in the world (Evangelou et al. 2007). Heavy metal accumulation limits of these plants are 100 times more than cultivated plant's concentration (accumulation). Hyperaccumulator plants can accumulate 10 000 mg kg^{-1} Zn and Mn; 1000 mg kg^{-1} As, Cr, Pb, cobalt (Co), Cu, nickel (Ni), selenium (Se) and 100 mg kg^{-1} Cd (Evangelou et al. 2007; Wuana and Okieimen 2011). On the other hand hyperaccumulator plants have low biomass and their growth period is long. *Brassica* species are commonly using in phytoextraction of heavy metal contaminated soil because of their high biomass production, deeply rooted and higher heavy metal accumulation ability (Kasiuliene et al. 2016). Different synthetic (such as ethylene diamine tetraacetic acid (EDTA), hydroxylethylene diamine tetraacetic acid (HEDTA), diethylene triaminee pentaacetic acid (DTPA), ethylenebis[oxyethylenetrinitrilo] tetraacetic acid (EGTA)) or natural chelates (such as EDDS, nitrilotriacetic acid (NTA)) are used to increase metal uptake capacities of plants (Evangelou et al. 2007). When the literature is examined, it is seen that EDTA is the most commonly used chelating agents in the removal of metals from the soil (Evangelou et al. 2007; Kasiuliene et al. 2016; Attinti et al. 2017). Similar to EDTA, EDDS increased the uptake of heavy metals, but as in the case of EDTA, only a fraction of the mobilized metals is effectively absorbed by the plant and subsequently translocated to the shoot, as much higher amounts of heavy metals were phytoavailable (Evangelou et al. 2007; Kasiuliene et al. 2016). However, EDTA is quite persistent in the environment due to its low-level biodegradability. The investigations reveal that EDTA has been destroyed of soil structure. Soil-applied EDTA can adversely impact soil enzymatic and microbial activities and at high concentrations, EDTA can negatively affect soil fungi and plants (Ullah et al. 2014; Attinti et al. 2017). For this reason, biodegradable chelating agents such as [S, S] -ethylenediamine succinic acid ([S, S]-EDDS) have begun to gain more attention as an environmentally friendly alternative to EDTA. Ethylenediamine disuccinic acid, $C_{10}H_{16}N_2O_8$, is an isomer of EDTA that produces either artificially or naturally by various microorganisms (Takahashi et al. 1999; Ullmann et al. 2013; Attinti et al. 2017).

The aim of this study is to investigate effects of EDDS treatment on phytoextraction of multi-metal (Cd, Pb, and Zn) contaminated soil using canola (*Brassica napus*) plant.

Material and Methods

The soil sample contaminated with multiple metals (Cd, Pb and Zn) was taken from Kayseri-İncesu (38 ° 42 '43 "N and 35 ° 15' 55" E) and the uncontaminated soil sample was taken from Çopurlu village on Mersin Gözne road (36 ° 87 '32 "N and 34 ° 56' 50" E) from 0-30 cm depth. Some physical and chemical properties of contaminated and uncontaminated soil samples were given in Table 1. The particle size distribution of uncontaminated soil was determined as 20.5%, 27.0%, 52.5% for sand, silt and clay, respectively. The soil texture was clay (C) according to the texture triangle. The particle size distribution of contaminated soil was

determined as 84.6%, 8.4%, 7.0% for sand, silt and clay, respectively. The soil texture was loamy sand (LS). The contaminated and uncontaminated soils demonstrated the following properties, respectively; pH 8.23 and 8.04, lime 28.34% and 7.26%, organic matter 3.04% and 5.27%. Both soils were alkaline, but uncontaminated soil has a high lime content than contaminated soil.

Soil samples were sieved from 4 mm sieve. Five soil mixtures (0, 25, 50, 75 and 100% of contaminated soil) were obtained by mixing the multiple metals (Cd, Pb and Zn) contaminated soil with a uncontaminated soil. The pot experiment was a randomized complete block design containing with and without EDDS treatments and three replications.

Table 1. Some physical and chemical properties of soil samples (from Çiftçi 2016)

Soil Properties	*Uncontaminated Soil*	*Contaminated Soil*	*References*
Sand (0.02-2 mm) (%)	20.5±1.1	84.6±2.3	Bouyoucos 1962
Silt (0,002-0,02 mm) (%)	27.0±0.9	8.4±0.5	
Clay (<0.002 mm) (%)	52.5±1.0	7.0±0.6	
Texture class	C (Clay)	LS (Loamy sand)	
pH (in the saturated soil	8.23±0.02	8.04±0.01	Soil Survey Staff 1951
Lime ($CaCO_3$) (%)	28.34±0.08	7.26±0.41	
Organic matter (%)	3.04±0.48	5.27±0.24	
Total Cd (mg kg^{-1})	1.92±0.10	483±32.9	USEPA 1995
Total Zn (mg kg^{-1})	92.4±3.35	13412±0.1	
Total Pb (mg kg^{-1})	19.1±0.01	25755±0.54	
DTPA extractable Cd (mg kg^{-1})	0.05±0.02	26.8±0.16	Lindsay and Norvell 1978
DTPA extractable Pb (mg kg^{-1})	1.03±0.21	31.1±1.72	
DTPA extractable Zn (mg kg^{-1})	0.75±0.33	128±11.6	

The seeds of canola (*Brassica napus* L.) were used as plant material in the study. Total 8 kg mixed soils (0, 25, 50, 75 and 100% of contaminated soil) filled into the pots and 10 of canola seeds were sown in pots. Those seedlings reduced to one seedling in each pot. Canola plant was grown for 2 months under controlled environmental conditions with 10 klux light intensity, 25ºC day and 20ºC night temperature cycle, 16 h light and 8 h dark period and 60% humidity.

At the beginning, canola seeds were planted in plastic pots in the absence or presence of 10 mg EDDS kg^{-1} soil, added in the form of EDDS ($C_{10}H_{13}N_2Na_3O_8$). A basal treatment of 200 mg kg^{-1} nitrogen (N) as calcium nitrate ($Ca(NO_3)_2$), and 100 mg kg^{-1} phosphorus (P) and 125 mg kg^{-1} potassium (K) as potassium dihydrogen phosphate (KH_2PO_4) were also applied to all pots. Before harvest, the chlorophyll (in old, middle and young leaves) content as SPAD (Soil, Plant Analysis Development) value was measured by using Konica Minolta SPAD-502 meter . The SPAD meter measures the optical density difference in 2 mm × 3 mm field, 2 wavelengths (650 nm and 942 nm) (Çiftci 2016). The numerical SPAD value refers to the proportional content of chlorophyll in the measured area in μg/cm^2 (Markwell et al. 1995). Accordingly, 1 SPAD unit is approximately 0.001 μg cm^2. After the SPAD measurement, plants were harvested. The samples were rinsed in distilled water and dried at the 65 ºC in oven for two days. After measurement of the dry weight (DW) of the plants, samples were ground in agat mill and then digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in microwave oven (MarsXpres CEM). Total multi metals (Cd, Pb, and Zn) concentrations of the samples were determined in ICP-MS (Inductively Coupled Plasma- Mass Spectrometry; Agillent 7500ce). All results were given based on dry plant matter at 65 °C. Certified reference materials (*SRM 1573A, SRM 1547*) were also analyzed in order to check the accuracy of the extraction technique used in this study. All data were presented as an average ±standard deviation (SD).

Results and Discussion

Chlorophyll Content

Chlorophyll content of canola was decreased with increasing multi metal (Cd, Pb, and Zn) concentration treatments (Figure 1). Plants did not grow in 75 and 100% multimetal doses. The lowest chlorophyll content (13.60 and 14.60 SPAD values) was measured in young leaves of the canola plants in 50% multimetal dose with

and without EDDS treatment, respectively. On the other hand, the highest chlorophyll content (28.77 and 29.77 SPAD value) was measured in the middle leaves of the plants in 25% multimetal dose with and without EDDS treatment, respectively.

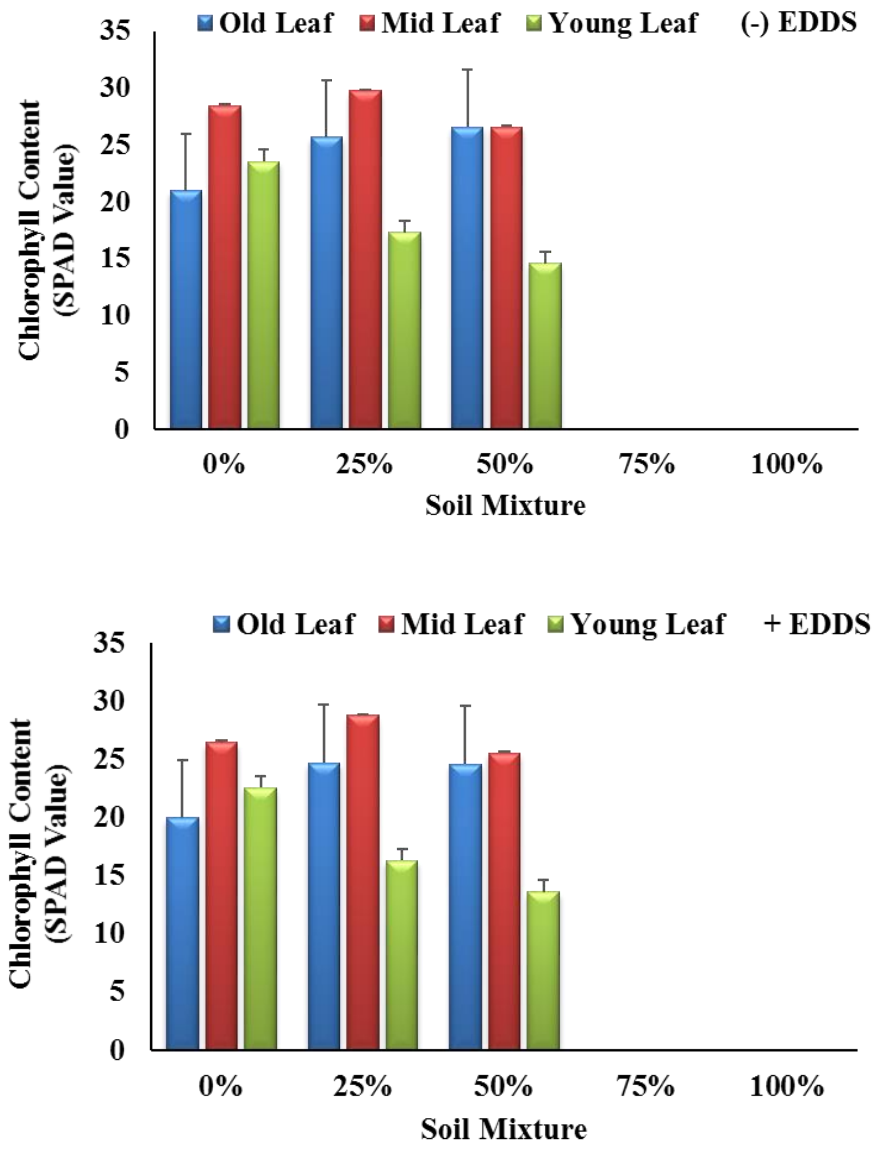


Figure 1. Effects of multi metal (Cd, Pb, and Zn) application on chlorophyll contents of canola plants without and with EDDS treatments (mean ±SD, *n*=3).

Chlorophyll content may be decreased because of the toxic effects of multi metals. Luo et al. (2005), reported that with the application of 5 mmol kg^{-1} EDDS to the soil, plants showed toxicity symptoms and biomass production decrease 14 days after treatment. Grčman et al. (2003) observed chlorosis and necrosis symptoms on *Brassica rapa* leaves grown in multi metal contaminated soil treated with 10 mmol kg^{-1} EDDS.

Dry Weights

EDDS treatments were increased dry weights of plants in the 25 and 50 % multi metal doses containing soils compared to the control treatment (Figure 2). On the other hand, dry weight of plants was lower with EDDS treatment than without EDDS treatment. This is due to the fact that the EDDS application reduces the dry weight of the plant by enhancing the heavy metal uptake by the plant. Similar results were reported by, Luo et al. (2005), who explained that the application of 5 mmol EDDS and EDTA to the 1 kg soil significantly inhibited plant

growth and 14 days after applications of EDDS and EDTA, the shoot dry weights decreased to 60% and 52% of the control plants for corn, and 76% and 61% of the control plants for bean, respectively.

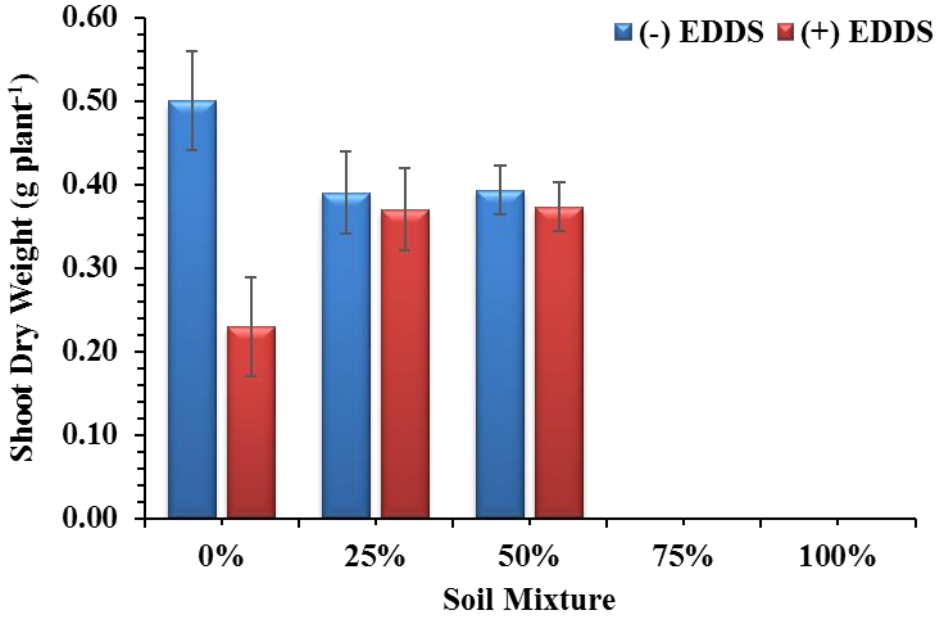
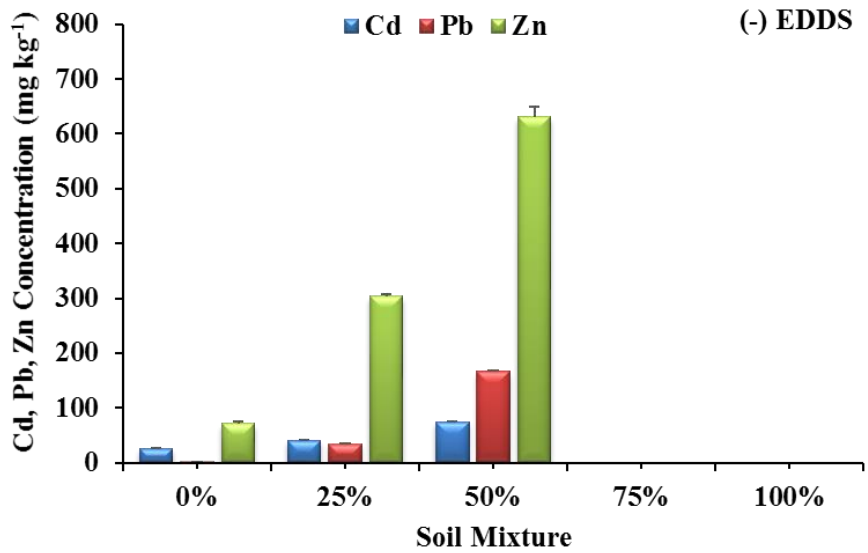


Figure 2. Effects of multi metal (Cd, Pb, and Zn) application on the dry weights of canola plants without and with EDDS (mean ±SD, *n*= 3).

However, the dry weight of canola was decreased due to the toxic heavy metal without EDDS treatments at 25 and 50% (0.39 g plant^{-1}) multi metal doses compare to the control (0.50 g plant^{-1}) treatments (Figure 2). Because of the toxic effects of heavy metals (Cd, Pb, and Zn) plants did not grow in the 75 and 100% multi metal doses.

Cd, Pb, and Zn Concentrations of Canola Plant

Plant multi metal concentrations were increased with increasing multi metal doses (Figure 3).



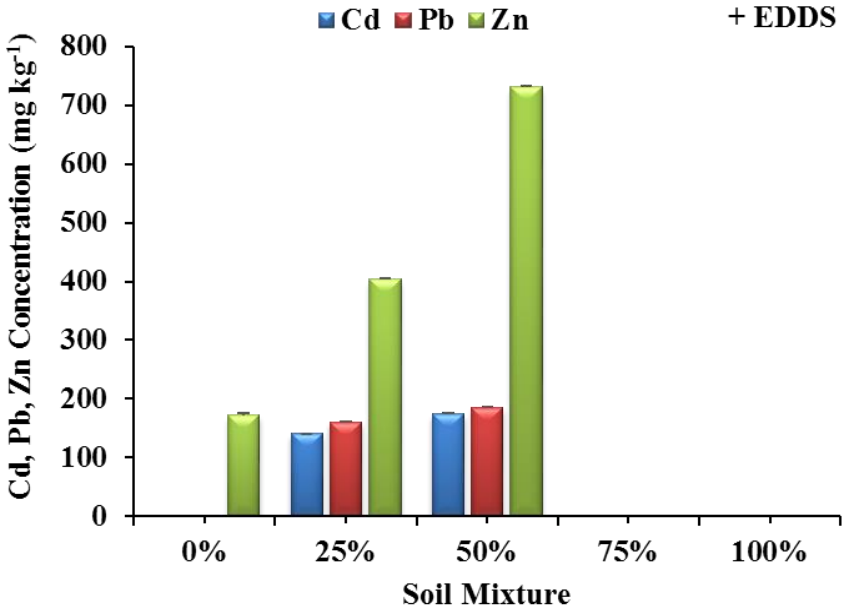


Figure 3. Effects of multi metal (Cd, Pb, and Zn) application on heavy metal concentrations in shoots of canola without and with EDDS (mean ±SD, *n*= 3).

The highest metal concentration of the plants were as follows Zn>Pb>Cd. The highest multimetal concentrations were obtained EDDS treatment at 50% multi metal doses as follows; 730 mg Zn kg^{-1}, 186 mg Pb kg^{-1} and 174 mg Cd kg^{-1}. Only Cd concentration was 1.4- and 1.7-fold higher than hyperaccumulation limit (>100 mg kg^{-1} DW, Baker 1981) with EDDS treatment in the 25 and 50% multimetal doses (141 and 174 mg Cd kg^{-1}, respectively). However, Zn and Pb concentrations in shoot of canola plants were lower than the critical toxicity level of hyperaccumulator (Pb: 1000 mg kg^{-1} and Zn: >10000 mg kg^{-1}). Grčman et al. (2003), obtained a 3-fold increase in Cd and Zn and 10.3-fold in Pb uptake by *Brassica rapa* with 4 times 10 mmol kg^{-1} EDDS treatment in multi metal contaminated soil. Luo et al. (2005) reported that 5 mmol kg^{-1} treatment of EDDS and EDTA were significantly enhanced heavy metal (Cd, Zn, Cu, and Pb) uptake of corn and white bean plants. Lee and Sung (2015) was reported similar results with us. They were investigated the effects of EDDS (5 mmol kg^{-1}) on the heavy metals (4 mg Cd kg^{-1}, 150 mg Cu kg^{-1}, 200 mg Pb kg^{-1}, 100 mg Ni kg^{-1}, and 300 mg Zn kg^{-1}) uptake of *Brassica campetris* and *Sorghum biocolor* plants. According to their results, EDDS could increase Pb, Cu, Ni, Cd, and Zn concentrations in the roots and shoots of *Brassica campetris* and *Sorghum biocolor* plants. *Cannabis sativa* was accumulated 105-fold Pb, 2.3-fold Zn and 31.7-fold Cd with 10 mmol kg^{-1} EDDS treatments than the control plant (Kos et al. 2003).

Conclusion

Although canola plant is a hyperaccumulator for a single heavy metal, but it is not suitable for phytoextraction of multi metal (Cd, Pb, and Zn) contaminations. EDDS treatment was enhanced multi metal uptake of the plants. Except for Zn and Pb, Cd concentration of *Brassica napus* was 1.4 and 1.7-fold higher than hyperaccumulation limit (>100 mg kg^{-1}) with EDDS treatment in the 25 and 50% multimetal doses. Nevertheless, it should be considered that elevated EDDS may be washed from the soil profile and contaminate the groundwater. According to Paracelsus, the father of toxicology, "The dose makes the poison" (Latin: sola dosis facit venenum) is indicating a basic principle of toxicology. It means harmless substances can be dangerous when consumed in large quantities. Due to this reason, suitable EDDS application in the soil and its movement in the soil profile should be monitored.

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Araştırma Makalesi/*Research Article (Original Paper)*

Antifeedant effect of two nano-capsuled essential oils against wheat weevil, *Sitophilus granarius* (L.) (Coleoptera: Curcurlionidae)

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Abstract: Novel application methods such as nano-encapsulation of essential oils enhanced effectiveness and improved the properties of natural insecticides. This study examined the effect of essential oils from *Cuminum cyminum* (L.) and *Lavandula angustifolia* (Mill.) and nanoparticles on nutritional parameters of *Sitophilus granarius* (L.). Nano-capsules were prepared using the emulsion solvent evaporation technique and the polymer was polyethersulfone. To evaluate nutritional indices alteration, wheat flour discs were prepared at 27 ± 1 ° C and 65 ± 5% R. H. Each flour disc was treated with 0, 1, 5, 10, 15, and 20 ppm concentrations of each essential oil and nano-capsules. The results showed that both essential oils and nanoparticles significantly reduced relative growth rate (*RGR*), relative consumption rate (*RCR*), and efficiency of conversion of ingested food (*ECI*) in treated adults. Conversely, the feeding deterrence index (*FDI*) percent increased significantly as the concentration increased. The lowest *RGR* and *ECI* at 20 ppm were observed in *C. cyminum* nanoparticles and the *RGR* reduced from 0.037 ± 0.003 mg/mg/day in control to -0.176 ± 0.01 mg/mg/day and ECI percent reduced from 16.8 ± 0.99 to -336.31 ± 6.95. The pure *C. cyminum* at 20 ppm caused the lowest *RCR* in comparison to *L. angustifolia.* The highest *FDI* was reached at 86.13 ± 0.89 % when *S. granarius* was exposed to *C. cyminum* oil. Therefore, both the essential oils and nano-capsules had a behavioural effect and the encapsulation technique improved the post-ingestive toxicity in treated adults.

Key words: *Cuminum cyminum*, *Lavandula angustifolia*, nutritional indices, nano-encapsulation, stored product pests

Introduction

Sitophilus granarius (L.), wheat weevil, is known as a common pest of barley and wheat all over the world. It can cause considerable damage to harvested stored grains and may intensively decrease crop yields. The females lay 150-300 eggs and the larvae eat the inside of the grain kernels (Haff and Slaughter, 2004). The life cycle average is about 40 days (Hill, 2002). Continuous application of synthetic insecticides has resulted in an increase resistance in pest species and caused several environmental damages. Regarding these problems, researchers focused on other alternatives for the management of stored-grain insect pests. Botanical insecticides represent feasible biological actions against stored pests as an eco-chemical agent (Isman, 2006; Isman *et al.*, 2011; Kordali *et al.*, 2006). Based on the considerable amount of published work, essential oils are known to be toxic to stored product beetles while their fumigant, contact, repellent and antifeedant activity due to the terpenoides and the phenols. Essential oils have negligible vertebrate toxicity and are safe for the environment (Pascual-Villalobos and Ballesta-Acosta, 2003; Rajendran and Sriranjini, 2008). Toxic, repellent and antifeedant effects of different essential oils against stored product pests have been investigated (Zapata and Smagghe, 2010; Abbasipour *et al.*, 2011; Stefanazzi *et al.*, 2011; Saeidi and Hassanpour, 2014; Ahmadi *et al*., 2015). Essential oils are unstable volatile organic compounds and break down simply by oxidation, heat and light. Therefore, they should be protected from external factors (Lai *et al.*, 2006; Pillmoor *et al.*, 1993). Essential oils stability could be increased by encapsulation (El Asbahani *et al.*, 2015). Encapsulation techniques enable loading of the essential oils in the polymeric shell which protects the bioactive compound against degradation and increases durability and stability (Kah and Hofmann, 2014; Perlatti *et al.*, 2013). The use of plant derivatives insecticides associated with nano-technology suggests a

remarkable potential for improving disadvantages of essential oils and enhances their persistence and also nanoparticles facilitate the production of new pesticides and insect repellents (Owolade *et al.*, 2008). In the present study, we synthesized nano-capsules composed of polyethersulfone as a capsule shell and *Cuminum cyminum* (L.) and *Lavandula angustifolia* (Mill.) essential oils as core materials by the emulsion solvent evaporation technique.

Two plants with insecticidal properties including *C. cyminum* from Apiaceae and *L. angustifolia* from Lamiaceae family and their nano-capsulated formulation have been studied in nutritional indices of *S. granarius* in the current research. *Cuminum cyminum* is an annual plant of the family of Apiaceae that is used as a spice and ancient medicine for many years in different countries (Jirovetz *et al*., 2005). *Lavandula angustifolia* is a perennial strongly aromatic shrub of the family Lamiaceae. *Lavandula angustifolia* oil is known for its great aroma and flavor and it is widely used in food, perfume and cosmetic industries (Cavanagh and Wilkinson, 2002). González *et al*. (2014) have studied nutritional and feeding deterrence indices of two essential oils and their nanoparticles on adults of *Tribolium castaneum* (Herbst) and *Rhizopertha dominica* (Fab). They showed that essential oils and their nanoparticles, significantly affected the relative growth rate, relative consumption rate, and efficiency of conversion of ingested food in studied insects.

Negahban and Moharrammipour (2007) also investigated the antifeedant property of Artemisia *spp*. extracts on the *T. castaneum*. Results showed that *Artemisia sieberi* (Besser) oil was highly effective compared to *A. scoparia* (Waldst et Kit) and significantly decreased the RGR and RCR. Moreover, in higher concentration (2 mL/disk), the ECI (9.81%) was significantly low. The *A. sieberi* oil was more effective on FDI than *A. scoparia*. Different studies examined the antifeedant toxicity of essential oils on stored pests, but little research has been performed to investigate the toxicity effects of either pure essential oils or their nano-capsulated form on nutritional and feeding deterrence indices on *S. granarius*.

The aim of this study was to test the antifeedant effects of *C. cyminum* and *L. angustifolia* essential oils and their synthesized nano-capsules on *S. granarius.* The results illustrated that essential oils and nanoparticles can be used as environmentally effective and safe control tools due to their visible changes in the common food consumption and digestion process by decreasing relative growth rate, relative consumption rate, and the efficiency of conversion of ingested food.

Materials and Methods

Insects rearing

Sitophilus granarius was mass reared in the Entomology Department Laboratory of Urmia University, Urmia, Iran. The insect rearing medium included wheat grain. The cultures were reared at 25 ± 2 ºC and 60 ± 5 % relative humidity (R. H.) in darkness. Adults with 1-3 days old of mixed-sex were used in the assays.

Materials

Essential oils of *C. cyminum* and *L. angustifolia* were provided from Eadeh Ara Pishgaam, the company for natural and herbal products, Tehran, Iran. For nano-capsules preparation, the following materials were applied: Polyethersulfone (PES Ultrason E6020P; Mw=58,000 $g.mol^{-1}$) was used as the polymer. Dichloromethane (DCM 99%; as a solvent for polyethersulfone) was supplied by Merck, Poly-ethylene glycol (PEG; Mw=600 $g.mol^{-1}$; as a surfactant and porosity agent) and Polyvinyl alcohol (PVA; as a surfactant) was purchased from Merck.

Nano-capsule preparation and nanoparticle size

Nano-capsules were prepared using emulsion solvent evaporation technique described by Pal *et al* (2011) and the polyethersulfone was used as the capsules shell. The preparation and characterization of nano-capsulated essential oils have been confirmed by scanning electron microscope (SEM) in the previous study (Bayramzadeh *et al*., 2016). Distribution size and the mean size of nano-capsules were estimated by dynamic light scattering (DLS) (Brookhaven, Instruments) before being used in the experiments.

Nutritional indices and antifeeding activity

The antifeeding activity and the changes in nutritional physiology were assessed according to Huang *et al.* (2000). Discs of wheat flour were prepared by suspension (200 µL) of wheat flour in distilled water (10 g in 50 ml). After 4 hours, the disks were stored in the hood for 12 hours to dry. The flour discs maintained for 24 hours at 27 ± 1 °C and 65 ± 5% relative humidity. Flour disks were treated with different concentrations (obtained by a preliminary test) 0, 1, 5, 10, 15, and 20 ppm of essential oils and nano-capsulated form. Control insects were kept under the identical conditions which included two groups: water, tween 3% and nano-capsules without oil. In each experimental unit, two flour discs and 10 starved (48 hours) insects were placed. At the beginning of the experiment, the weight of the disks and insects were calculated. After 72 h, the weight of insects alive and the discs was recorded. All bioassays were replicated four times at 27 ± 1°C and 65 ± 5% relative humidity. The nutritional indices were calculated as previously described by Huang *et al.* (1997):

- Relative growth rate (*RGR*) = (A - B)/B × day^{-1}, where A = weight of live insects on the third day (mg)/number of insects on the third day and B = initial weight of insects (mg)/initial number of insects.
- Relative consumption rate (*RCR*) = D/B × day^{-1}, where D= biomass ingested (mg)/number of live insects on the third day.
- Efficiency of conversion of ingested food (*ECI*) (%) = (*RGR*)/(*RCR*) × 100
- The percentage of feeding deterrence index (*FDI*) was calculated: *FDI* (%) = (C - T)/C ×100 where C = consumption of control discs and T = consumption of treated discs.

Data analysis

Before the statistical analysis, the data related to *ECI* and *FDI* were normalized. The data were analysed with SPSS software package (Ver. 20, 2011) using one-way analysis of variance (ANOVA) and the Tukey's multiple range tests. The independent sample T-test (significance at $P<0.05$) has been used to compare the effectiveness of each essential oils with their nano-forms.

Results and Discussion

Nanoparticles characterization

The distribution size and the mean size of two essential oils and coating polymer (polyethersulfone) is given in Fig. 1. The dynamic light scattering (DLS) was used as a measure for the size distribution of nanoparticles. The obtained average particle size distribution showed that the particles have a normal distribution. The mean size of particles, according to Fig. 1 was estimated about 823.4 nm for *L. angustifolia* nano-capsulated oil and 1266.4 nm for *C. cyminum* nano-capsulated. The lowest mean particle size distribution was obtained for *L. angustifolia* essential oil. Choupanian and Dzolkhifli (2018) reported that particle size range for nano-emulsion of four formulation of neem oil was about 208-507 nm. In addition, Louni *et al.,* (2018) estimated that the average nanoparticle size of nano-emulsion of *Mentha longifolia* (L.) oil was about 234 nm. Natrajan *et al*., (2015) prepared essential oil-loaded nano-capsules using the ionic gelatin method. Results of characterization particle size studied showed that in different heating condition and concentration of alginate and chitosan particle size range was about 256.6-895.5 nm. Comparison of particle size revealed that the used polymer, the applied method, synthesis condition and active ingredient type play a fundamental role in nanoparticle size (Vishwakarma *et al.,* 2016). Numerous studies have illustrated that applying nanoparticles loaded with essential oil improved the stability of essential oils bioactive compounds effectively (Yang *et al.,* 2009; González*et al.,* 2014; Ziaee *et al.,* 2014). There are several techniques that can be utilized to prepare nano-capsules for insecticides application (John *et al.,* 2011). In this study, nano-capsules were prepared using an emulsion solvent evaporation technique described by Pal *et al* (2011). This technique is one of the popular methods for the encapsulation within the water-insoluble polymer. The emulsion evaporation technique was developed at the end of the 1970s and has been used successfully in the preparation of microspheres made from several biocompatible polymers (Hoa *et al*., 2009).

Nutritional and feeding deterrence indices study

Cuminum cyminum and *L. angustifolia* oils and nanoparticles significantly ($P<0.05$) reduced relative growth rate (*RGR*), relative consumption rate (*RCR*), and the efficiency of conversion of ingested food (*ECI*) at all concentrations. In contrast, feeding deterrence indices increased by increasing concentration level. Results in Fig. 2 and 3 indicate that the tested plant oils and their nano-form showed considerable antifeedant activity against *S. granarius.*

Comparison between essential oils and nano-capsulated oils verify that the *RGR*, *ECI* and *FDI* of pure oils were higher than nano-forms. In the case of *RCR* values, *RCR* was higher in nano-form than pure oils (Table 1 and 2). The data illustrated that the increased rate of the *FDI* was higher in plant oils than nanoparticles.

Table 1. Comparing effects of *Cuminum cyminum* essential oils and nano-capsulated oils on nutritional indices of *Sitophilus granarius.*

Nutritional indices	Concentrations (ppm)	Mean ± SE		*t* (df=6)	*P*-value*
		Essential oil	Nanoparticle		
RGR (mg/mg/day)	0	0.038 ± 0.003	0.041	1	0.356
	1	-0.020 ± 0.004	-0.062 ± 0.008	4.71	0.003
	5	-0.048 ± 0.004	-0.090 ± 0.008	4.16	0.006
	10	-0.062 ± 0.004	-0.118 ± 0.008	6.37	0.001
	15	-0.090 ± 0.004	-0.145 ± 0.008	5.74	0.001
	20	-0.118 ± 0.004	-0.177 ± 0.010	5.37	0.002
RCR (mg/mg/day)	0	0.144	0.143 ± 0.001	1	0.356
	1	0.097 ± 0.001	0.122 ± 0.002	5.89	0.001
	5	0.087 ± 0.001	0.111 ± 0.002	11	0.000
	10	0.075 ± 0.001	0.095 ± 0.002	4.7	0.003
	15	0.062 ± 0.002	0.080 ± 0.003	4.58	0.004
	20	0.041 ± 0.001	0.051 ±0.004	3	0.024
ECI (%)	0	26.44 ± 2.40	28.84	1	0.356
	1	-21.44 ± 4.14	-50.93 ± 6.66	3.75	0.009
	5	-55.46 ± 4.10	-81.04 ± 7.02	3.14	0.02
	10	-83.44 ± 5.64	-123.36 ± 9.16	3.7	0.01
	15	-145.54 ± 10.7	-180.78 ±7.16	2.73	0.034
	20	-284.59 ± 14.62	-336.31 ± 6.95	3.21	0.018
FDI (%)	1	54.55 ± 0.7	34 ± 0.57	22.47	0.000
	5	56.74 ± 0.91	39.22 ± 0.94	13.31	0.000
	10	75.14 ± 1.51	55.55 ± 1.08	10.52	0.000
	15	82.47 ± 0.89	63.12 ± 3.26	5.71	0.001
	20	86.13 ± 0.89	69.77 ± 2.32	6.52	0.001

∗values $P \leq 0.05$ indicate a significant difference based on the independent *t*-student test to compare the effect of pure essential oil and nano-capsules.

Tukey comparison at highest concentration revealed that both essential oils were effective on nutritional indices of *S. granarius. Cuminum cyminum* oil reduced the amounts of *RCR* and *ECI* more than *L. angustifolia* oil (Table 3). The adults which were exposed to *C. cyminum* oil (at 20 ppm) had the highest *FDI* (86.13 ± 0.89 %). However, the lowest *RCR* was recorded for *C. cyminum* essential oils. The *RGR*, *RCR*, *ECI* and *FDI* values of the tested adults at 20 ppm concentration of pure and nano-essential oils which were fed on flour disks for 72 h are presented in Table 3. At concentration of 20 ppm, *RGR* of *C. cyminum* essential oil reduced from 0.037± 0.003 (mg/mg/day) to -0.118

± 0.004 (mg/mg/day) while in the case of *L. angustifolia* oil *RGR* value reduced to -0.093±0.006 (mg/mg/day) in comparison to the control but this decrease is not significant (Table 3).

Table 2 Comparing effects of *Lavandula angustifolia* essential oils and nano-capsulated oils on nutritional indices of *Sitophilus granarius*.

Nutritional indices	Concentrations (ppm)	Mean ± SE		*t* (df=6)	*P*-value*
		Essential oil	Nanoparticle		
RGR (mg/mg/day)	0	0.038 ± 0.003	0.041	1	0.356
	1	0.015 ± 0.002	0.000 ± 0.004	3	0.024
	5	-0.005 ± 0.002	-0.027 ± 0.007	2.8	0.031
	10	-0.030 ± 0.005	-0.062 ± 0.006	3.8	0.032
	15	-0.06 ± 0.007	-0.102 ± 0.007	4.123	0.006
	20	-0.090 ± 0.007	-0.150 ± 0.017	3.133	0.020
RCR (mg/mg/day)	0	0.361	0.361	0	1
	1	0.241 ± 0.005	0.305 ± 0.002	10.9	0.000
	5	0.215 ± 0.005	0.280 ± 0.005	8.51	0.000
	10	0.187 ± 0.004	0.249 ± 0.005	9.18	0.000
	15	0.155 ± 0.006	0.211 ± 0.006	5.9	0.001
	20	0.107 ± 0.003	0.145 ± 0.014	2.5	0.046
ECI (%)	0	10.576 ± 0.961	11.538	1	0.356
	1	8.575 ± 1.657	0.05 ± 1.812	3.47	0.013
	5	-3.227 ± 1.865	-10.890 ± 2.207	2.651	0.038
	10	-18.537 ± 2.178	-27.732 ± 2.063	3.064	0.022
	15	-42.195 ± 3.593	-50.950 ±3.374	1.776	0.126
	20	-87.052 ± 5.122	-110.19 ± 15.66	1.404	0.210
FDI (%)	1	36.777 ± 0.871	27.98 ± 0.283	9.602	0.000
	5	40.75 ± 0.605	31.7 ± 0.416	12.31	0.000
	10	54.83 ± 1.311	44.52 ± 0.255	7.714	0.000
	15	59.15 ± 0.973	48.34 ± 0.293	10.632	0.000
	20	63.73 ± 0.691	52.93 ± 0.590	11.88	0.000

∗values $P \leq 0.05$ indicate a significant difference based on the independent *t*-student test to compare the effect of pure essential oil and nanocapsules.

Table 3 Comparing effects of essential oils and nano-capsulated formulation on nutritional indices at 20 ppm of *Sitophilus granariu*

Treatment	Mean ± SE			
	RGR (mg/mg/day)	**RCR (mg/mg/day)**	**%ECI**	**%FDI**
Control: Nano capsules without oil	0.037 ± 0.003[a]	0.361 ± 0.000[a]	16.80 ± 0.99[a]	0 ± 0.000[e]
Control: Water+Tween 3%	0.035 ± 0.001[a]	0.360 ± 0.001[a]	16.77 ± 0.51[a]	0 ± 0.000[e]
Nano-capsulated *C. cyminum*	-0.176 ± 0.01[d]	0.052 ± 0.004[d]	-336.31 ± 6.95[d]	69.77 ± 2.32[b]
C. cyminum	-0.118 ± 0.004[bc]	0.041 ± 0.001[d]	-284.59 ± 14.62[c]	86.13 ± 0.89[a]
Nano-capsulated *L. angustifolia*	-0.155 ± 0.018[cd]	0.145 ± 0.014[b]	-110.19 ± 15.66[b]	52.93 ± 0.59[d]
L. angustifolia	-0.093 ± 0.006[b]	0.107 ± 0.003[c]	-87.05 ± 5.122[b]	63.73 ± 0.691[c]

*Means in the same column followed by same letters do not differ significantly in the Tukey's test at 5% level

In this study, we synthesized the nano-capsules of essential oils by polyethersulfone copolymer. Also, the bioactivity effects of essential oils in comparison to nanoparticles have been investigated on nutritional indices of *S. granarius*. It was found that the essential oils and nano-capsules significantly (P<0.05) affect the nutritional indices in a concentration-dependent manner. In all experiments, there was a higher reduction in the *RGR* and *ECI* of the nanoparticles and also the *FDI* values were lower than pure essential oils. Moreover, *C. cyminum* nanoparticles produced a significant reduction of *ECI* compared to *L. angustifolia* nanoparticles (P<0.05) at 20 ppm. Also, there was a significant difference (P<0.05) in *FDI* values at 20 ppm. So that the highest *FDI* was recorded for *C. cyminum* oil. The growth rate, food consumption and food utilization of *Sitophilus zeamais* (Motsch.) adversely affected when exposed to media treated with Eugenol, Isoeugenol and Methyleugenol (Huang *et al.*, 2002). The results showed that eugenol reduced *RCR* in the adults of *S. zeamais* at a concentration of 13.2 mg/g food. Isoeugenol reduced the *RGR* and *RCR* in a concentration-dependent manner and methyleugenol reduced the *RGR*, *RCR* and *ECI*. Our findings in reducing nutritional indices by botanical compounds were similar to Huang *et al.*, 2002 results. Stefanazzi *et al* (2011) studied post-ingestive and alteration of nutritional index of three essential oils (*Tagetes terniflora* Kunth, *Cymbopogon citratus* Stapf. and *Elyonurus muticus* Spreng Kuntz) on *Sitophilus oryzae* (L.) adults and indicated that all essential oils significantly reduced relative growth rate at both concentrations (1 and 4 mg/disc^{-1}). Feeding deterrence indices showed that the essential oils of *T. terniflora* and *C. citratus* at the highest concentration (4 mg/disc^{-1}) and *E. muticus* at both concentrations had strong feeding deterrent action.

According to the obtained results, either pure or nano-form of essential oils can modify and reduce nutritional indices of *S. granarius.* The antifeedant effect of some essential oils and their nanoparticles have been previously reported. The study of Abbasipour *et al* (2011) showed that *Datura stramonium* (L.) extract had significantly reduced the *RGR* and *RCR* of *T. castaneum* at higher concentrations. González*et al* (2014) indicated that at 1 and 2 mg/disc Geranium and Bergamot essential oils and nanoparticles, significantly affected the *RGR*, *RCR* and *ECI* (P<0.05) of *T. castaneum* and Geranium nanoparticles produced a significant reduction in *ECI* compared to Bergamot nanoparticles, while they reported that the essential oils alone did not alter the nutritional indices. This contradiction with our findings can be due to the differences in the type of essential oils composition and the concentration values which were used in bioassays.

In order to make practical use of essential oils, the compounds should be formulated and new technologies such as nano-pesticides should be applied. As we know botanical insecticides have a good potential for grain protection because of their antifeedant activity and we must effectively formulate and commercialize them when we want to use them in practice. So nano-encapsulation is an acceptable and practical solution for improving the disadvantages of essential oils. The findings of this study showed that nano-capsulated essential oils have considerable post-ingestive toxicity against *S. granarius.* The nano-capsulated essential oils caused more reduction in *RGR* and *ECI* and the *FDI* values were lower than pure essential oils, therefore, nanoparticles can increase the efficiency of post-ingestive toxicity of essential oils.

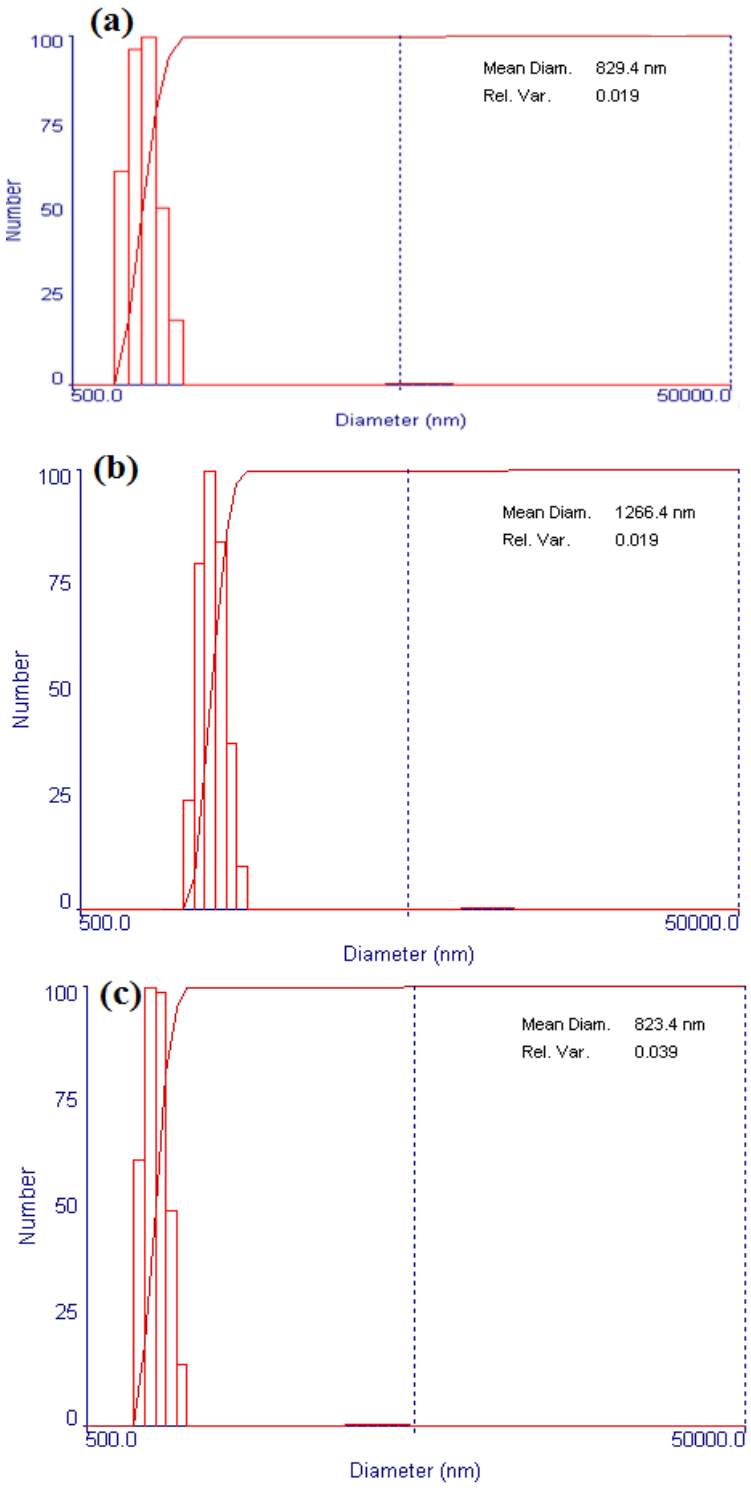

Figure 1 Particle size distribution of the polyethersulfone polymer (a), *Cuminum cyminum* nano-capsules (b) and *Lavandula angustifolia* nano-capsules (c).

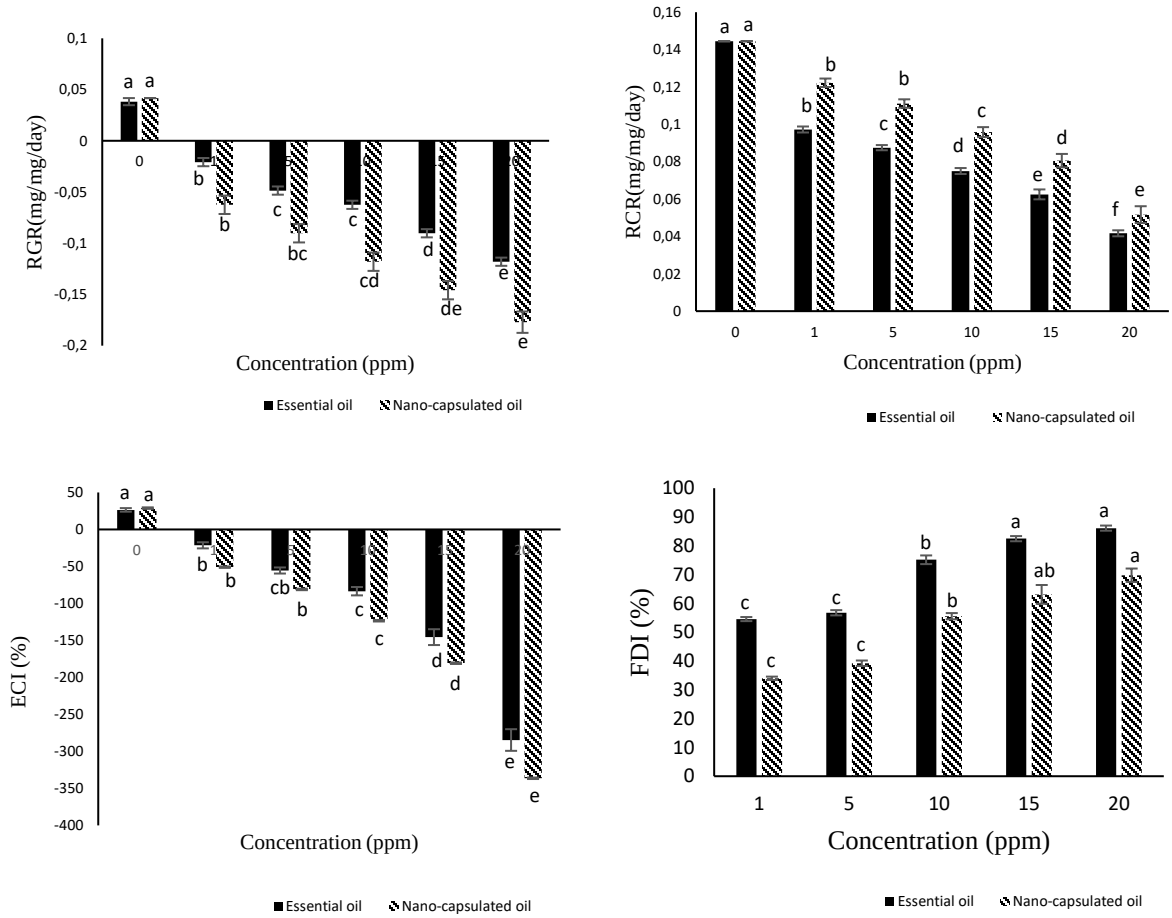

Figure 2 Effects of *Cuminum cyminum* essential oil and nano-capsulated oil on nutritional parameters of *Sitophilus granarius.*

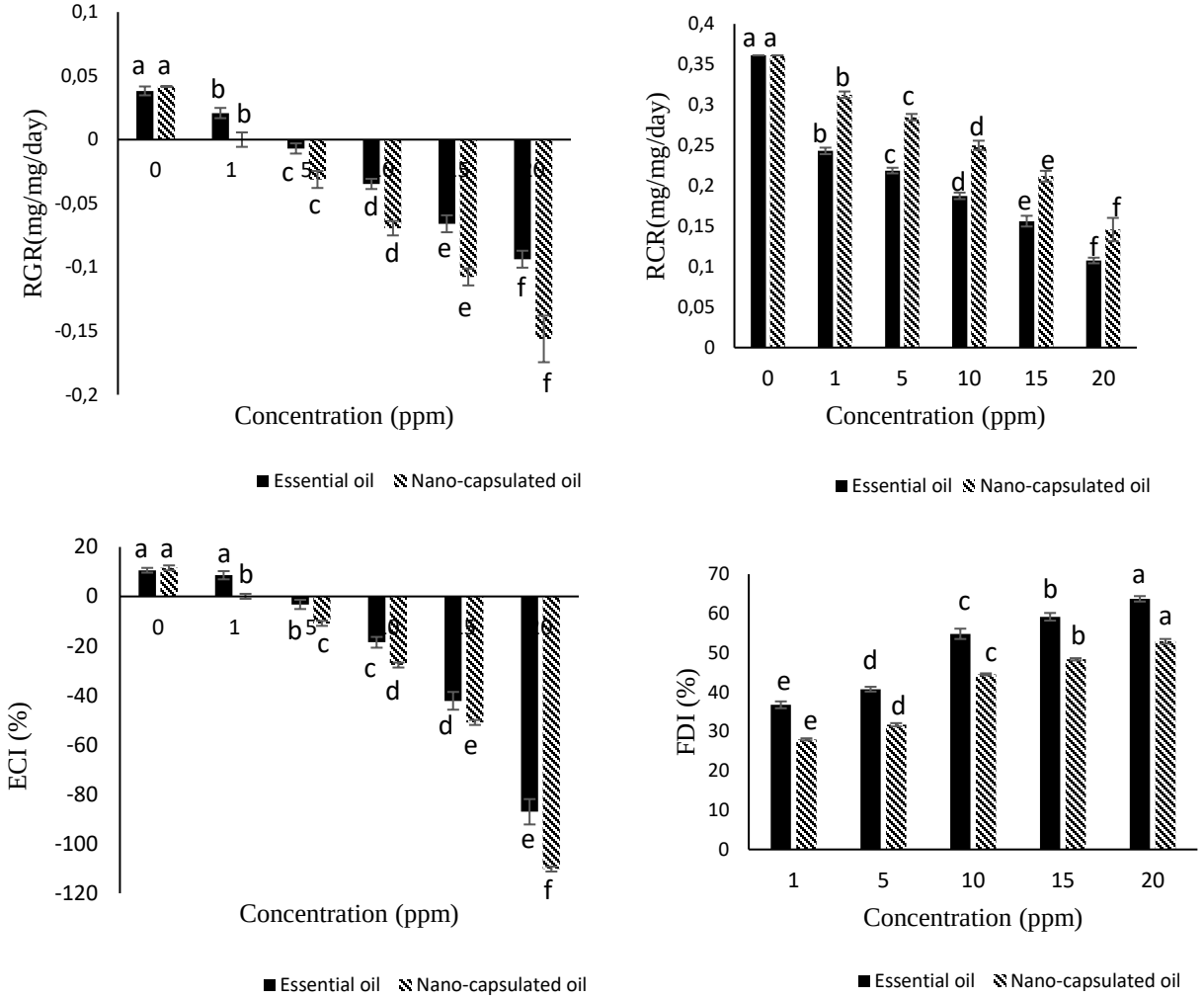

Figure 3 Effects of *Lavandula angustifolia* essential oil and nano-capsulated oil on nutritional parameters of *Sitophilus granarius.*

Increasing the stability, reducing the total concentration amount, sustaining release, and finally improving their efficacy are the reasons for nano-capsulation of essential oils. Therefore, the nano-capsulation of essential oils might provide a new method for the management of pests (Negahban *et al*., 2013). In conclusion, the lowest efficiency of conversion of ingested food was related to nano-capsulated *C. cyminum* and the highest feeding deterrence indices were obtained by *C. cyminum* essential oil. According to the results, *C. cyminum* had a significant effect on nutritional indices of *S. granarius* as compared to *L. angustifolia.*

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Araştırma Makalesi/*Research Article (Original Paper)*

Effect of Selenium Application on Selenium and Macro-Micro Nutrients Content of Grain Maize in Turkey

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Abstract: Selenium is essential for both humans and animals, and must be taken with nutrients in sufficient amount. Concentration of selenium in the food for both human and animal is desired between 100-1000 μg kg^{-1}. This study conducted field experiments on the Harran Plateau to investigate; the effect of soil applications in form of selenite on maize grain yield and Se content; the efficiency of soil application; the relationship between Se content in maize grain and Se application rate; and the effect of Se fertilisers on macronutrients and other micronutrients in grain maize. In this study, DKC 5783 F_1 which is grown intensively in the area as a grain maize variety, sodium selenite was used as selenium source with eight levels (0-5-10-15-25-50-75-100 g Se ha^{-1}). Sodium selenite was applied to the soil in liquid form before sowing. Sodium selenite application didn't affect the total Ca, Mg, S, Zn, B, Mo and Se content of grain, but it affected N, P, K, Fe, Cu and Mn content, statistically.

Keywords: Maize, selenium, soil application, sodium selenite,

Türkiye'de Selenyum Uygulamalarının Tane Mısırın Selenyum ve Makro-Mikro Besin Elementi İçeriğine Etkisi

Özet: Selenyum hem insanlar hem de hayvanlar için zorunlu bir besin maddesidir ve besinlerle yeterli miktarda alınmalıdır. İnsan ve hayvanlar için besin maddesindeki selenyum konsantrasyonu 100-1000 μg kg^{-1} arasında değişmektedir. Bu çalışma; Harran Ovası'nda tarla denemesi olarak selenit formunda toprak uygulamalarının tane mısır verimi ve selenyum içeriği üzerine etkisini; toprak uygulamasının etkinliğini; tane mısırın Se içeriği ile uygulama oranı arasındaki ilişkiyi ve selenyum gübrelemesinin tane mısırda makro besin elementleri ile diğer mikro besin elementleri üzerine etkisini saptamak amacıyla yürütülmüştür. Bu çalışmada mısır çeşidi olarak bölgede yoğun olarak yetiştirilen DKC 5783 F_1, selenyum kaynağı olarak da sodyum selenit sekiz farklı dozda (0-5-10-15-25-50-75-100 g Se ha^{-1}) kullanılmıştır. Sodyum selenit ekim öncesi toprağa sıvı formda uygulanmıştır. Sodyum selenit uygulamaları istatistiki olarak tanenin toplam Ca, Mg, S, Zn, B, Mo ve Se içeriğini etkilememiştir, ancak N, P, K, Fe, Cu ve Mn içeriğini etkilemiştir.

Introduction

In recent years, the importance of qualified food has been increasing steadily in the human and animal nutrution. In this context, selenium is one of the most studied micronutrients in the World, which is important for human and animal health. Selenium has been known for many years as a highly toxic, even carcinogenic element. It was first shown in 1957 as a useful element for biological systems. Although it is not considered an obligatory nutrient for most plant species, it is important for plants to become involved in the structure of amino acids and proteins after absorption (Erikson 2001).

Selenium is essential for both humans and animals, and must be taken with nutrients in sufficient amount (Cakmak et al. 2009; Surai 2000; David 1995). Habitual suboptimal dietary selenium intake leads to reduced selenium status, which is associated with a range of adverse health outcomes including cardiovascular disorders, impaired immune function, and some forms of cancer (Chilimbia et al. 2012). Food is the principal source of selenium intake. Meat and seefood contain the highest amounts of selenium,

with 0.4-1.5 µg per gram (Rayman 2008), but fruits and vegetables are also good food sources. According to Cakmak et al. (2009), Broadley et al. (2007), Adams et al. (2002), Allaway (1968), concentration of selenium in the food for both human and animal is desired between 100-1000 µg kg^{-1}. Selenium concentrations in food, including crops, depend not only on selenium concentrations in agricultural soils (which vary considerably between countries and regions) but also on selenium phytoaccessibility controlled by many abiotic and biotic factors such as soil pH, redox conditions, organic matter content, microbial activities, irrigation and compaction. In some countries or regions, low selenium levels in soil lead to low concentrations in food or forage, which in turn can result in selenium deficiency in livestock and humans. The rates of serum selenium in children alter between 50-70 µg L^{-1} and daily selenium intake alter between 30-40 µg day^{-1} in Turkey. This value is lower limit for children aged 9-13 and low for children aged 14-18 with regard to required daily value. Daily requirement of selenium is 40 µg for children between 9-13 ages and 55 µg for children between 14-18 ages. It was found that serum selenium levels were very low in the children with goiter and iron deficency anemia. In the same research, it was emphasized that taking higher rate of selenium can protect from several diseases including cancer. It was shown that wheat is the most important source of selenium in food for nutrition of humans (Cakmak et al. 2009). Major purchases of selenium for animals are with alimentation but the chemical selenium taken with the nutrition is thrown out by digestion. Organic selenium is taken with depending on feed, and stored in body proteins (Shamberger 1984). Selenium shows a synergistic effect with vitamin E in animals, it undertakes a powerful antioxidan task with glutathione peroxidase (Lawrence et al. 2003). Both selenium and vitamin-E are antioxidants, preventing oxidative damage in the cell and there is a link among these compounds; one can perform other's task (Surai 2000; Kaneko 1989).

Major source of selenium is soil in plant-based foods. Plants accumulate selenium in the body for functions but rather accumulate mainly in the seeds (Steven 1994). A lot of research have been done in this regard. For example, content of selenium increases by adding sodium selenite (Na_2SeO_3) to the freshwater for germination in chickpea plant (Zang et al. 2011) and by adding to the hydroponic irrigation water in maize plant (Longchamp et al. 2013). Chilimba et al. (2012) expressed that sodium selenate (Na_2SeO_4), NPK+Se and CAN+Se increased the content of selenium in grain maize, respectively to 19.7; 20.7 and 14.8 µg selenium kg^{-1}. Curtin et al. (2006) reported that they also obtained similar results in field trials and 0.03 mg kg^{-1} selenium concentration in the control sample increasing 0.39 mg kg^{-1} by the 20 g Se ha^{-1} soil application. Chilimbia et al. (2012a) reported that selenium content of grain maize increased to 21 mg kg^{-1} with each gram of Na_2SeO_3 application during the sowing time of the maize plant. They also indicate that selenium content of grain maize is 33% higher in late selenium application than early selenium application. Eurola et al. (2006) reported that soil application increases the contents of selenium in many plants. Selenium application in the soil is widely used in Finland to increase the content of selenium in the major nutrients, thus preventing selenium deficiency of humans. In contrast to humans, the role of selenium for plants is more ambiguous, although studies on young plants have led to a better understanding of selenium pathways in higher plants. Plant development and selenium metabolism are strongly dependent on the form of supplied selenium. The greater mobility of selenate compared to selenite results in differences in the absorption, translocation and metabolism of selenium within the plant. Indeed, when plants are exposed to selenite, selenium accumulation is less than selenate treatment, with a greater reduction in biomass production. After selenate treatment, selenium is almost entirely translocated to the leaves and weakly metabolized as selenoamino-acids, with a selenate concentration in shoots (i.e. stems and leaves) representing more than 90% of the total shoot selenium. In contrast, when supplied as selenite, selenium accumulates principally in roots with little translocation, although selenoamino-acid production (principally selenomethionine, selenocysteine and selenomethylselenocysteine) is greater and the selenium volatilization rate is about 2-fold higher from those plants (Longchamp et al. 2015). According to Deliboran and Nacar (2018), it is seen that the results of the low content of Se in the soil taken from the maize grown fields are overlapped with the findings of the researcher. In this research, Se contents of leaf samples ranged from <10-38.48 µg kg^{-1} in the Center to 0-27.53 µg kg^{-1} in Viransehir, depending on the provinces; in general ranges from 0 to 38.48 µg kg^{-1}. The samples taken from Ceylanpinar, Harran and Akcakale districts do not contain Se. Se contents are evaluated according to the Ozbek et al. (2001) with the qualification limit values; 100% soils of Akcakale, Ceylanpinar and Harran have a low Se content. In the Central district, 43% is low and 57% is adequate; in Viransehir, 80% is low and 20% is enough class. However, it can be said that leaf samples which appear in the sufficient group are insufficient in terms of Se feeding considering that they are in sufficient group with a very small difference when considering Se content. Considering that the available Se contents of soils are insufficient in all regions, it appears that there is a feeding problem in terms of Se feeding in maize grown areas.

This study was planned because of Se feeding problems in Sanlıurfa province. This study conducted field experiments on the Harran Plateau to investigate (1) the effect of soil applications in form of selenite on maize grain yield and Se content; the efficiency of soil application; (2) the relationship between Se content in maize grain and Se application rate; and (3) the effect of Se fertilisers on macronutrients and other micronutrients in grain maize. This study was conducted to determinine the proper Se fertilizer application rate for improving the Se content in maize grain and alleviating Se deficiency in humans and animals.

Material and Method

Experiment location

The research was carried out in 2013 and 2014 at the selenium deficiency area in Talat Demirören Research Station of GAP Agricultural Research Institute, located in Sanliurfa. Sanliurfa is located in the Southeastern Anatolia climatic region but it is under the influence of the Mediterranean climate. The climate is characterized by warm and dry summers and mild winters. The amount of precipitation increases from south to north and west to east. Monthly average temperatures are around 32 ^{0}C in July and Agust. The highest daily temperatures are observed in the same months, and the highest daily temperature recorded to date was 48 ^{0}C in July. The avarage of daily sunbathing time is over 12 hours in summer. Sanliurfa is on the lowest relative humidity line in Turkey and the relative humidity in summer is around 35%.

The soil in the experiment fields is clay loam. The main characteristics of the soil in each year of experiment are shown in Table 1.

Maize variety and selenium sources

In this study, DKC 5783 F_1 which is grown intensively in the area as a grain maize variety, Na_2SeO_3 were used as selenium source. Solid Na_2SeO_3 is a water-soluble compound (85 g/100 g water at 20 °C) and its molecular weight is 172.9 g. The selenium in Na_2SeO_3 is in the +4 oxidation state and is found naturally (Sangbom et al. 2005).

Table 1. Some of the physical and chemical properties of pre-test soil that belongs to the research area

Soil property	Year	
	2013	2014
Texture	clay	clay
Sand (%)	28.5	27.5
Silt (%)	19.6	19.3
Clay (%)	51.9	53.2
EC ds m^{-1}	0.98	1.06
pH	7.9	7.5
Lime (%)	31.2	29.2
Organic material (%)	1.57	1.92
Total N (mg kg^{-1})	600	700
Available P (mg kg^{-1})	30.4	16.8
Available K (mg kg^{-1})	552	604
Available S (mg kg^{-1})	18.5	16.6
Available B (mg kg^{-1})	0.32	1.27
Available Mg (mg kg^{-1})	1678	840
Available Mo (mg kg^{-1})	34.10	37.84
Available Se (µg kg^{-1})	3.90	3.50

Experimental design

Experiments were carried out in 3 replicates according to the design of random blocks as the grain maize test in 2013 and 2014. Eight selenium levels (0-5-10-15-25-50-75-100 g Se ha^{-1}) were used in the experiments. Na_2SeO_3 was applied to the soil in liquid form before sowing. Sowing operation was done with pneumatic seeder as 70 cm between rows and 16 cm above the rows on June 24 2013 in the first year of research, and on June 17 2014 in the second year.

The amount of fertilizer was determined after the analysis of soil samples taken before the experiment in the field in each two years of the research. The analysis results are given in Table 1. Amount of fertilizer given to maize plants during the vegetation period was completed with pure 25 kg da^{-1} nitrojen (N) and 10 kg da^{-1} phosphorous (P), potassium (K) was not applied since the amount of soil available K was sufficient. All of the P and some of the N were given to soil as a base fertilizer before the final tillage application. The remainder of the nitrogenous fertilizer was supplied as a top fertilizer with maize plants reached 30-40 cm in length.

Water was given immediately after sowing. Other irrigation was given equal water to the parcels by flood irrigation procedure. It was set between parcels to prevent surface flow. Anchor and once throat filling were done at appropriate times. Weed medicine were used against weeds after seed emergence, also drug fighting was done aganist harmful stalk and steed. The water sample was taken from the water source in the area where the pre-test experiment was analyzed. The analysis results are given in Table 2.

Table 2**.** Results of irrigation water analysis

Property		Year	
		2013	2014
Cations (me L^{-1})	EC (μSm^{-1})	354	356
	Na	0.08	0.08
	K	0.02	0.02
	Ca+Mg	3.2	3.3
	Soil cation	3.3	3.4
Anions (me L^{-1})	CO_3	-	-
	HCO_3	1.9	2.8
	Cl	1.1	1.2
	SO_4	0.3	0.4
	Soil annion	3.3	3.3
pH		7.45	7.49
SAR		0.06	0.06
B		-	-
Class		T_2A_1	T_2A_1

Sampling

The harvest was made on November 12 2013 in the first year of the research, and on November 6 2014 in the second year. Maize grain at harvest were measured by harvesting the entire plot through weighing the total straw and maize grain with the cob. After harvest, maize plants with cob were sampled, maize grains were threshed from the cob, and subsamples of mazie grain were washed twice with de-ionised water to remove the attached soil and other contaminants. Samples were then oven-dried at 60 ^{0}C to contant weight. The dried samples were ground into powder to pass through a sieve of 0.15 mm for nutrient analysis.

Chemical analysis

In soil samples, texture was determined by hydrometer method (Bouyoucos 1951); pH in 1:2,5 soil: water mixture, electrical conductivity (EC) with electirical conductivity instrument in the saturated soil paste, lime ($CaCO_3$) with Scheibler calcimetry (Tuzuner 1990); organic matter by modified Walkley-Black method (Black 1965); total N by modified Kjeldahl method; changeable K, calcium (Ca), magnesium (Mg) and sodium (Na) by 1 N ammonium acetate (pH=7) extraction (Kacar 1995); available P by $NaHCO_3$ extraction (Olsen and Sommers 1982); available iron (Fe), zinc (Zn), manganase (Mn) and copper (Cu) by di ethylene tri amine penta acetic acid-tri ethanol amine (DTPA-TEA) excraction (Lindsay and Norvell 1978); available boron (B) was determinated by exacting B from the soil with hot water based on the color intensity of azomethine-H complex (Kacar 1995), available Se was determined by KH_2PO_4 extraction method by reading the Atomic Absorption Spectrophotometer (ASS) connected to ETC-60 (Electrohermal Temperature Controller) and VGA-77 (Vapor Generator Aparatus) apparatus ontained (Cakmak et al. 2009).

In dried and ground grain samples; N analysis was determined by modified Kjeldahl method; total P with nitric acid (HNO_3)+perchloric acid ($HClO_4$) mixture by vanadomolibdophosphoric yellow color method in wetted burnt plants samples; total K, Ca, B, S, Mg, Fe, Cu, Zn and Mn contents were determined by ICP with the same solution (Kacar 1995). Grain samples were dried to a constant weight at 40 ºC in an air circulating dryer cabinet for selenium analysis. The dried and ground grain samples were prepared for selenium determination by wet digestion in microwave oven with 5 ml concentrared HNO_3 and 2 ml 30% hydrogen peroxide (H_2O_2) by using a digesting program, which have been developed for the grain samples. All selenium measurements in grain meterials were checked against certificated selenium values in different reference plant material (1547 Peach Leaves, NIST) obtained from the National Institute of Standarts and Technology (Gaithersburg USA). After digestion, the total volume was completed up to 20 ml, and Se concentration of the samples were measured by Atomic Absorption Spectroscopy (Varian) equipped with VGA 77 (vapor generation accessories) and ETC-60 (electrothermal temperature controller). First, Se (+ VI) in the samples was reduced to Se (+ IV) form by treatment with hydrochloric acid. After, Se was reacted with sodium tetraborate ($NaBH_4$) reductive in acidic medium and reduced to form volatile hydrogen selenide (SeH_2) in the hydride forming unit which a hydride generator module (VGA-77) mounted in front of the sample entry system of the AAS device, and measured by atomizing SeH_2 at high temperature (850-950 ºC) with ETC-60 instrument. The accuracy and reproducibility of the analysis values were controlled using standard reference materials in every 10 samples in the analyzes (Cakmak et al. 2009).

Statistical analyses

All data were anlysed using MSTAT-C software package. The data obtained from the experiments were evaluated with variance analysis every year, homogeneity tests were made and the differences between experiment subjects were cheched with LSD tests. The levels of significant was 0.05.

Results and discussion

In this study, the effects of Na_2SeO_3 on the grain yield and biomass, accumulation of selenium and other nutrients in grain maize have been investigated and the average values of 2013-2014 has been evaluated.

Grain yield and biomass of maize

There was no statistical difference between application doses, when the grain yield and biomass was examined with the Na_2SeO_3 applications. Grain yield and biomass ranged from 9.10-9.52 t ha^{-1} and 78.7-83.4 t ha^{-1} at different selenium doses, respectively, the highest values was obtained from 0 g Se ha^{-1} application in both (Table 3). This result is consistent with that selenium application didn't affect the plant yield and the other plant properties for example wheat (Cakmak et al. (2009); Broadley et al. (2007); Deliboran et al. (2018)), maize (Chilimbia et al. (2012); Longchamp et al. (2013)) and lettuce (Duma et al. 2011). For example, several studies showed that with selenium application, grain yield of maize ranged from 2764-7009 kg ha^{-1} (Chilimbia et al. 2012); grain yield of maize ranged from 5.41-9.13 t ha^{-1} in 2009, 7.93-12.25 t ha^{-1} in 2010 with soil application of Na_2SeO_3, 6.15-9.91 t ha^{-1} in 2009, 9.58-17.05 t ha^{-1} in 2010 with foliar application of Na_2SeO_3 (Wang et al. 2013). Howewer, several studies showed that selenium application posivitely affects the plant due to antioxidative activity of patato plants (Turakainen 2007) and maize plant (Sajedi et al. 2011); respiratory potential of young chicory (Germ et al. 2007) and brassica plants (Cakmak et al. (2009); Lyons et al. (2009)).

Selenium content in maize grain

There was no statistical difference between application doses, when the selenium content of the grain was examined with the Na_2SeO_3 applications. Total selenium levels of grain ranged from 19-22 µg kg^{-1} at different selenium doses, the highest value was obtained from 15-25-50-75-100 g Se ha^{-1} application (Table 4). When the selenium rate was increased from 0 to 100 g Se ha^{-1}, the grain selenium content increased by 1.16-fold (from 19 µg kg^{-1} to 22 µg kg^{-1}) and the selenium accumulation by 1.13 fold (from 181 mg ha^{-1} to 205 mg ha^{-1}) but both of them showed no significant change across the selenium rates. The selenium recovery in grain showed no significant change across the selenium rates with an average of 0.81%.

Table 3. Effect of different levels of sodium selenite application on maize grain yield and biomass

	2013		2014		Avarage 2013-2014	
Se rates (g Se ha^{-1})	Grain Yield (t ha^{-1})	Biomass (t ha^{-1})	Grain Yield (t ha^{-1})	Biomass (t ha^{-1})	Grain Yield (t ha^{-1})	Biomass (t ha^{-1})
0	7.51a	68.3	11.52	98.4	9.52	83.4
5	7.59	62.6	10.85	95.6	9.22	79.1
10	7.69	67.2	10.55	94.2	9.12	80.7
15	7.20	63.9	9.93	93.6	8.57	78.8
25	7.50	65.3	10.85	97.4	9.18	81.4
50	7.43	62.5	10.92	98.7	9.17	80.6
75	6.90	65.2	11.30	94.6	9.10	79.9
100	7.34	61.1	11.40	96.2	9.37	78.7

(Values in the same column followed by the same small letter are not significantly different at *P*=0.05.)

Table 4. Effect of different levels of sodium selenite application rates on Se content in maize grain and Se uptake and recovery by the grain.

Se rates (g Se ha^{-1})	Grain Se content (µg kg^{-1})			Grain Se uptake (mg ha^{-1})			Grain Se recovery (%)		
	2013	2014	Average 2013-2014	2013	2014	Average 2013-2014	2013	2014	Average 2013-2014
0	20	18	19	150	209	181			
5	21	21	21	159	228	193	1.87	3.77	2.45
10	21	21	21	165	226	195	1.47	1.71	1.41
15	22	22	22	155	214	184	0.33	0.34	0.22
25	21	22	22	161	244	202	0.45	1.41	0.82
50	22	22	22	161	236	198	0.22	0.55	0.35
75	22	21	22	150	243	196	0.00	0.45	0.20
100	22	22	22	161	248	205	0.11	0.39	0.24

(Grain Se accumulation (mg ha^{-1}) = grain Se concentration (µg kg^{-1}) x grain yield (g ha^{-1}). Grain Se recovery (%) = ((Grain Se uptake in the treatment (mg ha^{-1}) - (grain Se uptake in the control (mg ha^{-1})) / rate of applied Se (g ha^{-1}) x 1000. Values in the same column followed by the same small letter are not significantly different at *P*=0.05.)

According to Allaway (1968), Adams et al. (2002) and Broadley et al. (2007), it is desirable that the concentration of selenium in consumed foods is between 100-1000 µg kg^{-1} for adequate nutrition of both humans and animals. Miller et al. (1991) indicate that selenium levels should be at least 0.1-0.3 mg kg^{-1} for animal nutrition, 0.1-1 mg kg^{-1} selenium level is sufficient and >5 mg kg^{-1} is toxic effect. In our study, selenium levels of grain were increased with Na_2SeO_3 application from the control group, selenium accumulation was not sufficient in term of human and animal nutrition, it is thought that selenium may be absorbed from soil deu to the fact that soils of research is heavy. In addition, when compared to Wang et al. (2013), it is considered that the levels applied doses in our study are low to increase selenium content of grain for human and animal nutrition. According to Wang et al. (2013), selenium content of grain was increased from 3.7 µg kg^{-1} to 206 µg kg^{-1} at applications of Na_2SeO_3 from soil between 0 g Se ha^{-1} ile 600 g Se ha^{-1} doses. In term of increased selenium levels in Na_2SeO_3 applications, in our study the results obtained are in harmony with the researchers (Wang et al. 2013; Chilimbia et al. 2012; Chilimbia et al. 2012a; Zang et al. 2011; Curtin et al. 2006; Eurola et al. 2006). Otherwise, it is known that many factors increase Se activity in plants. For example crop spicies and variety in chickpea (Zang et al. 2011) and maize (Longchamp et al. 2013); application time and rate (Curtin et al. 2006; Chilimbia et al. 2012a; Wang et al. 2013); soil and climatic conditions (Cakmak et al. 2009; Kacar and Katkat, 1989; Zhao et al. 2007); selenium applications and selenium form (Wang et al. 2013; Chilimbia et al. 2012; Cartes et al. 2005). It is known that foliar selenium application caused than soil selenium application for increasing selenium content of maize grain. At the competence level with Na_2SeO_3 application to the soil, the selenium content of grain did not increase and this is the most important finding in our study as a results. Selenium levels

were higher than in the control group by Na_2SeO_3 application but the Se accumulation was not sufficient in term of human and animal nutrition.

Macromineral content in maize grain

There was statistical difference between application doses, when the N, K and P content of the grain were examined with the Na_2SeO_3 applications, when years were evaluated collectively. At different Na_2SeO_3 doses, the total N values were varied between 10.25-11.64 g kg^{-1}, total K values between 4.25-5.51 g kg^{-1} and total P values between 2.92-3.37 g kg^{-1}, respectively. Se applications affected the values of N, K and P on grain. The highest values were obtained with 11.64 g N kg^{-1} from 10 g Se ha^{-1} application, with 5.51 g K kg^{-1} from 15 g Se ha^{-1} application and with 3.37 g P kg^{-1} from 10 g Se ha^{-1} application (Table 5, Figure 1).

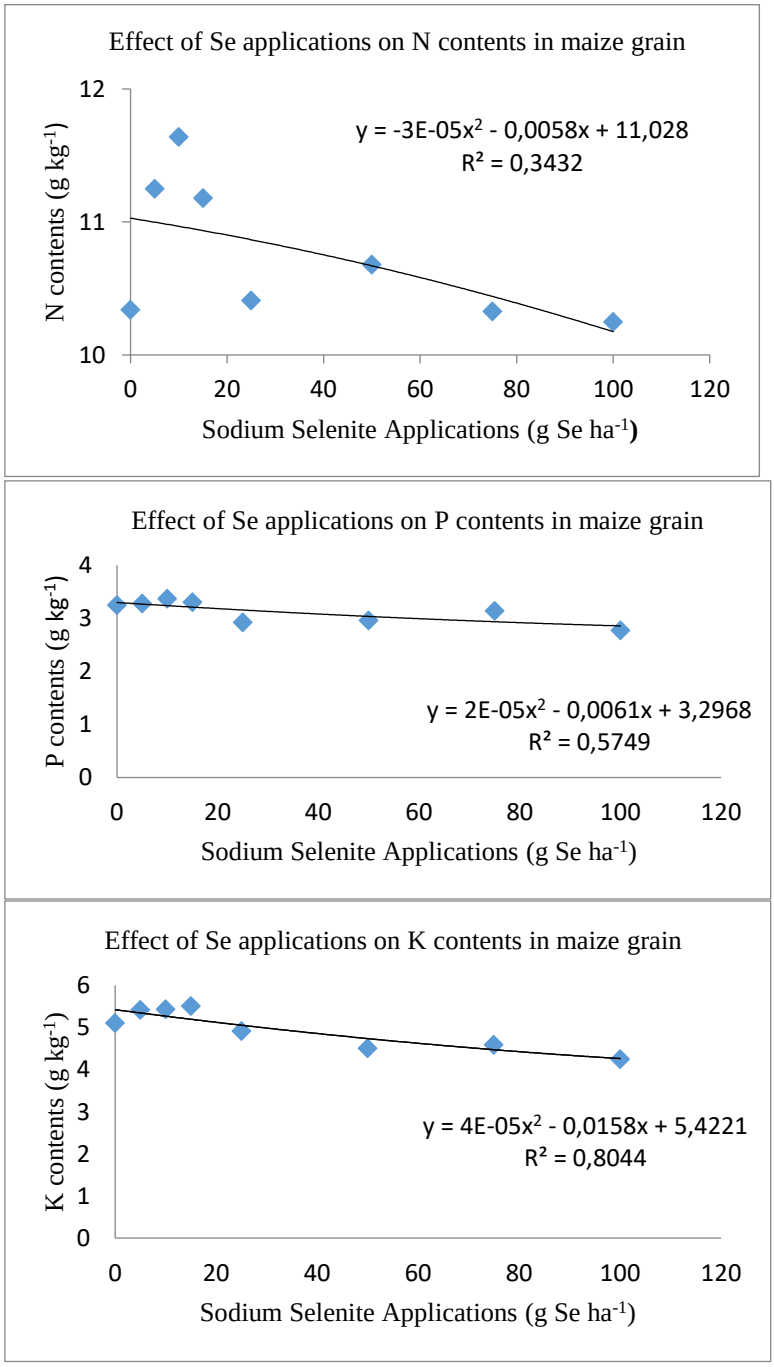

Figure 1. N, P and K content of maize grain with sodium selenite (Na_2SeO_3) application

There was not statistical difference between applications doses, when the Ca, Mg and S content of the grain were examined with Na_2SeO_3 applications when years were evaluated collectively. At different Na_2SeO_3 doses, the total Ca values were varied between 0.076-0.090 g kg^{-1}, total Mg values between 1.65-1.85 g kg^{-1} and total S values between 1.06-1.18 g kg^{-1}, respectively. Selenium applications did not affect as statistical the values of Ca, Mg and S in grain on Na_2SeO_3 applications (Tables 5). Howewer, when compered to the control group, the S content of the grain were decreased at increasing selenium levels. Wang et al. (2013) indicated that N, P, K, Ca and Mg content of grain can not be affected from soil and foliar application when compared with control groups. In term of N, K, P, Ca and Mg content of grain, our studies results obtained are in harmony with the researchers. In term of interaction between Se and N, K, P, the results obtained are in concordance with researchers for N, partly compatible for K and contradictory for P. The results obtained from our research are in contradiction for Ca and Mg. Hawrylak-Nowak (2008) found that Se application of 5, 25, 50, 100 μmol.dm^{-3} $Na_2SeO_3.5H_2O$ to maize plant in the hydroponic system increased the P content of the root and shoot of plant, espicially with 5 and 25 μmol.dm^{-3} doses, but the differences were not statistically significant, P content increased by 4-5 fold over the control group in 50 and 100 μmol.dm^{-3} Se applications. According to Huang et al. (2008); Cruz-Jimenez et al. (2005); White et al. (2004); Pezzarossa et al. (1999); Mikkelsen and Wan (1990), Barak and Goldman (1997) and Gissel-Nielsen (1973) there is significant interaction between S and Se. Our studies results obtained from Na_2SeO_3 are in concordance for S with researchers.

Micromineral content in maize grain

Statistical difference between selenium doses in Na_2SeO_3 application for Fe, Cu and Mn was found, when the Fe Cu, Zn, Mn, B and Mo content of grain were examined. Total Fe values of grain were changed between 27.10-38.59 mg kg^{-1}, total Cu values 4.89-7.14 mg kg^{-1}, total Mn values 2.63-5.20 mg kg^{-1}, total Zn 22.64-26.47 mg kg^{-1}, total B 27.14-34.45 mg kg^{-1} and total Mo 4.28-4.45 mg kg^{-1} with different Na_2SeO_3 doses. Selenium applications statistically affect the Fe, Cu and Mn values of grain maize, Fe, Cu and Mn content of grain were increased at the increasing selenium levels, the highest values were obtained with 38.59 mg Fe kg^{-1} from 10 g Se ha^{-1} application, with 7.14 mg Cu kg^{-1} from 15 g Se ha^{-1} application and with 5.20 mg Mn kg^{-1} from 10 g Se ha^{-1} (Table 6, Figure 2). Selenium applications did not affect Zn, B and Mo values of grain statistically. Krystyna et al. (2008) reported that applying 10^{-6} mol dm^{-3} sodium hydrogen selenite ($NaHSeO_3$), 10^{-4} mol dm^{-3} indole-3-acetic acid and IAA-$NaHSeO_3$ together (IAA) to maize plant in the hydroponic systems were effective on the root and leaf in term of Fe, Mg, Cu, Mn and Zn content. In addition, the researchers reported that interactions between these elements with selenium was found, the levels of these elements in the plant were increased and the accumulation organ changed according to the plant species. Wang et al. (2013) reported that soil and foliar applications of selenium did not affect Fe, Mn, Cu and Zn content of grain. In our study, the results obtained from selenite applications are consistent with Krystyna et al. (2008) in terms of the interaction between Se and Fe, Cu, Mn and their content.

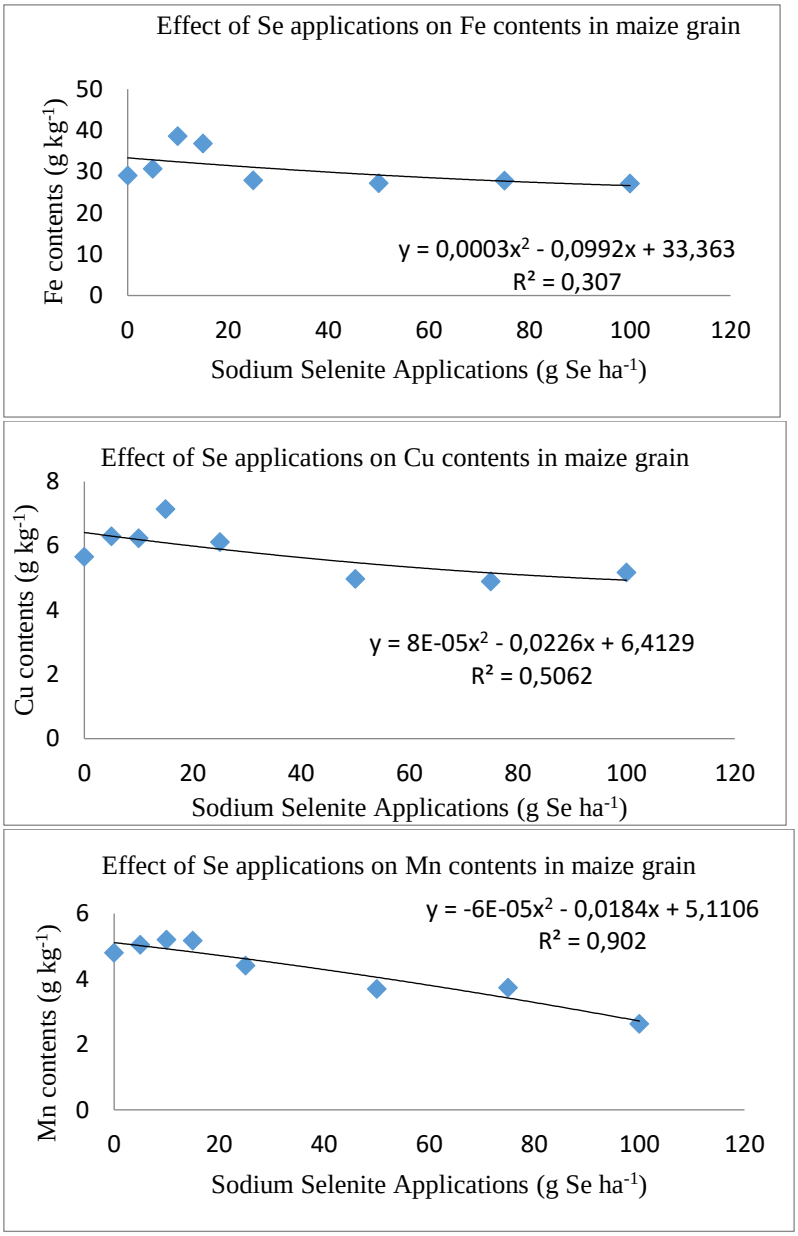

Figure 2. Fe, Cu and Mn contents of maize grain with sodium selenite (Na_2SeO_3) application

Table 5. Effect of Se applications on N, P, K, Ca, Mg and S contents in maize grain.

Se rates (g Se ha^{-1})	Nutrient contents (g kg^{-1})																	
	N			P			K			Ca			Mg			S		
	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Average 2013-2014
0	10.73	10.14	10.34 bc	3.64	2.86	3.25 ab	5.21	5.01	5.11 bc	0.095	0.066	0.081	1.68	2.02	1.85	1.80	0.55	1.18
5	10.75	11.74	11.25 ab	3.89	2.67	3.28 a	5.48	5.36	5.42 ab	0.103	0.067	0.085	1.63	1.89	1.76	1.69	0.52	1.11
10	12.29	10.99	11.64 a	3.93	2.81	3.37 a	5.58	5.43	5.44 ab	0.100	0.068	0.084	1.76	1.83	1.80	1.76	0.50	1.13
15	11.31	11.05	11.18 ab	3.96	2.64	3.30 a	5.61	5.48	5.51 a	0.109	0.071	0.090	1.74	1.84	1.79	1.75	0.50	1.13
25	9.57	11.25	10.41 bc	3.23	2.61	2.92 c	4.93	4.90	4.92 c	0.105	0.068	0.087	1.48	1.84	1.66	1.60	0.52	1.06
50	9.80	11.56	10.68 bc	3.19	2.73	2.96 bc	4.76	4.26	4.51 de	0.095	0.071	0.083	1.45	1.95	1.70	1.61	0.52	1.06
75	10.18	10.47	10.33 bc	3.71	2.56	3.14 ab	4.91	4.27	4.59 d	0.085	0.067	0.076	1.49	1.97	1.73	1.71	0.50	1.11
100	10.17	10.33	10.25 c	2.91	2.64	2.77 d	4.37	4.12	4.25 e	0.091	0.066	0.079	1.31	1.99	1.65	1.72	0.50	1.11

(Values in the same column followed by the same small letter are not significantly different at *P*=0.05.)

Table 6. Effect of Se applications on Fe, Cu, Mn, Zn, B and Mo contents in maize grain.

Se rates (g Se ha^{-1})	Nutrient contents (mg kg^{-1})																	
	Fe			Cu			Mn			Zn			B			Mo		
	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014	2013	2014	Avarege 2013-2014
0	37.08	21.13	29.10 c	5.53	5.79	5.66 bcd	5.10	4.50	4.80 ab	22.37	25.46	23.92	13.62	40.66	27.14	6.38	2.52	4.45
5	40.02	21.32	30.67 bc	5.53	7.06	6.29 ab	5.30	4.77	5.04 a	23.91	26.09	25.00	13.86	43.38	28.62	6.34	2.48	4.41
10	54.74	22.43	38.59 a	5.64	6.83	6.23 ab	5.48	4.93	5.20 a	25.12	27.81	26.47	14.20	40.17	27.19	6.33	2.46	4.40
15	48.16	25.49	36.82 ab	5.92	8.38	7.14 a	5.71	4.63	5.17 a	24.66	27.86	26.26	14.00	43.24	28.62	6.35	2.45	4.40
25	34.78	21.05	27.91 c	5.60	6.64	6.11 abc	3.21	5.61	4.41 ab	20.82	27.09	23.96	13.86	49.41	31.64	6.34	2.45	4.39
50	34.83	19.56	27.19 c	5.38	4.55	4.97 de	3.62	3.75	3.69 bc	20.75	26.48	23.62	13.58	50.83	32.21	6.32	2.38	4.35
75	35.68	20.03	27.85 c	5.38	4.40	4.89 e	4.28	3.19	3.73 bc	20.38	26.40	23.39	13.20	55.69	34.45	6.23	2.41	4.32
100	33.78	20.43	27.10 c	5.17	5.18	5.17 cde	3.19	2.07	2.63 c	18.87	26.41	22.64	13.55	48.18	30.87	6.23	2.34	4.28

(Values in the same column followed by the same small letter are not significantly different at *P*=0.05.)

Conclusions

When the effect of Na_2SeO_3 application to the soil in the grain maize investigated, it was seen that Na_2SeO_3 application did not affect the total Zn and Se content of grain, but it affected N, P, K, Fe, Cu and Mn content of grain statistically; N, P, K, Fe, Cu and Mn content of grain increased with selenium applications. It has been found that there was an interaction between the selenium applications and it is in accordance with the literature (Wang et al. 2013; Hawrylak-Nowak 2008; Krystyna et al. 2008). At the competence level with Na_2SeO_3 application to the soil, the selenium content of grain did not increase and this is the most important finding in this study as a results. According to Adams et al. (2002) and Allaway (1968) it is desirable that the concentration of selenium is between 100-1000 μg kg^{-1} in consumed foods. Miller et al. (1991) indicate that at least 0.1-0.3 mg kg^{-1} selenium must be present in feeds for animal nutrition and 0.1-0.3 mg kg^{-1} selenium is adequate and >5 mg kg^{-1} selenium is toxic. It was seen that selenium values of grain are not compatible when compared to the researchers' findings and the selenium values of grain are lower than the values required for human and animal nutrition. In this study selenium levels were higher than in the control group by Na_2SeO_3 application but the selenium accumulation was not sufficient in term of human and animal nutrition. It is thought that selenium clinges to soil due to the fact that soil of the research region is heavy, and also compared to Wang et al. (2013), the levels of selenium applied in our study are thought to be low to increase the selenium content of grain. According to Wang et al. (2013), with the application of Na_2SeO_3 to soil, the selenium content of grain maize was increased from 3.7 μg kg^{-1} to 206 μg kg^{-1} at doses between 0 g Se ha^{-1} and 600 g Se ha^{-1}.

It is clear that Na_2SeO_3 application is not more effective on the selenium contents of grain maize in this study which was carried out to determine the effect of Na_2SeO_3 applied to the soil. It is more effective on the N, P, K, Fe, Cu and Mn contents of grain maize.

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Araştırma Makalesi/*Research Article (Original Paper)*

Screening of High Temperature Tolerant Tomato Genotypes for Their Fruit Mineral Content

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Abstract: Agriculture is at the forefront of the sectors that will be most affected by climate change. It is inevitable that Turkey is exposed to the negative effects of climate change due to its geographical location. The development of new high temperature tolerant varieties is seen as an important economic measure in the adaptation to climate change. In this study, heat temparature tolerant tomato genotypes were investigated for their fruit mineral content. For this purpose, twenty tolerant tomatoes from the gene pool of the Çukurova University, Department of Horticulture the and two commerical cultivars were grown in the open field conditions during 2016 spring and summer periods in Adana, Turkey. Tomato fruits grown under control and high temperature stresses conditions were analyzed for phosphorus, potassium, calcium, magnesium, iron, manganese, copper and zinc. According to heat stress effects on the tomato fruit mineral content, the macro-nutrients were ordered P, K, Ca, Mg from the least affected to the most affected. Moreover, the micro-nutrients were ordered Cu, Fe and Zn from the least affected to the most affected. In the present study heat tolerant tomato genotypes showed better performance and their mineral content most cases were higher than mineral content of the control trade cultivars.

Key words: Calcium, copper, iron, magnesium, phosphorus, potassium, *Solanum lycopersicum* L., zinc

Yüksek Sıcaklık Stresine Tolerant Domates Genotiplerinin Meyvede Mineral İçeriği Bakımından Taranması

Özet: Tarım, iklim değişikliğinden en fazla etkilenen sektörlerin başında gelmektedir. Türkiye'nin coğrafi konumu nedeniyle iklim değişikliğinin olumsuz etkilerine maruz kalması kaçınılmaz görülmektedir. Yeni yüksek sıcaklık stresine toleranslı çeşitlerin geliştirilmesi, iklim değişikliğine uyumda önemli bir ekonomik önlem olarak görülmektedir. Bu çalışmada, meyve mineral içeriği açısından yüksek sıcaklık stresine toleranslı domates genotipleri araştırılmıştır. Bu amaçla, Çukurova Üniversitesi Bahçe Bitkileri Bölümü gen havuzunda bulunan yirmi adet toleranslı domates genotipi ve ticari iki çeşit kullanılarak Adana-Çukurova ekolojik koşullarında 2016 ilkbahar ve yaz aylarında açık alanda yetiştirilmiştir. Kontrol ve yüksek sıcaklık koşulları altında eld edilen domates meyvelerinde fosfor, potasyum, kalsiyum, magnezyum, demir, manganez, bakır ve çinko mineralleri analiz edilmiştir. Domates meyvesi mineral içeriği üzerindeki yüksek sıcaklık stresi etkisine göre, makro-minerallerde en az stresden etkilenenden en çok etkilenene göre P, K, Ca ve Mg olarak sıralanmıştır. Ve mikro-mineraller ise Cu, Fe, Zn olarak en az etkilenenden en çok etkilenene doğru sıralanmıştır. Bu çalışmada yüksek sıcaklık stresine toleranslı domates genotipleri meyvenin mineral içeriğini korumak için iyi performans göstermiş ve mineral içeriği çoğu durumda kontrol olarak kullanılan ticareti çeşitlerin mineral içeriğinden daha yüksek bulunmuştur.

Anahtar kelimeler: Kalsiyum, Bakır, Demir, Magnezyum, Fosfor, Potasyum, *Solanum lycopersicum* L., Çinko

Introductıon

Tomato (*Solanum lycopersicum* L.) fruit is the most popular as well as important vegetable in the world for consumption and also for production, the source of high nutritional contents with antioxidant compounds, minerals, vitamines and fibres. Tomato furit contain the largest quantities of potassium, although the quantities of calcium, magnesium and iron are also significant. Tomato also contains sodium, copper, zinc, manganese and numerous other minerals (Bjelić et al., 2005). Tomato crop has high commercial value since it is widely used not only as fresh fruit but also in processed forms in diets. Over 177 million and 12.6 million metric tons of tomatoes are produced each year on a world basis and Turkey, respectively (FAO, 2016). Tomato fruit provides

high nutrition in many forms such as raw in salads, cooked in meals, preserves, purees, ketchup, sauces, pickled and in other forms.

Tomato fruit quality is determined mainly by color, texture, and flavor. Demand and acceptance of fresh tomato fruit are based largely on these parameters. However, in recent years, antioxidant content such as phenols, flavanoids, carotenes, vitamin C, provitamin A and minerals of the tomato fruit have been prominent which are thought to protect and possibly prevent cancer. Nowadays, the increasing consumer fresh vegetables preference with high nutritional content is very important (Kowalczyk et al., 2011). Fruit composition and their desirability are affected by many factors such as growth environment, climatic factors, media, fertilizers, soil physical and chemical propeties and salinity sources (Haglund et al. 1997; Gundersen et al. 2001; Bjelić et al., 2005; Thybo et al. 2006; Kowalczyk et al., 2011).

In Turkey, fresh tomatoes are primarily grown in open field and greenhouses and the tomato fruits are produced all year round. In open field production in recent years drought threat has been emerged with low rains due to climate change symptoms; and low water storage for irrigation. Tomato is highly sensitive to environmental changes such as temperature, light, and water during growing period of plant (Murshed et al., 2013; Klunklin and Savage, 2017). Abiotic stress factors such as drought, heat, salinity, tropospheric ozone and excess UV radiation are causing agricultural yield and crop quality losses and will become even more prevalent in the coming decades due to the effects of global-climate change (Wang and Frei, 2011). The optimum temperatures for tomato cultivation are between 25 and 30ºC during day and 20ºC during night (Camejo et al., 2005). However, in tomato cultivation regions in sub-tropics and tropics where high temperatures are exceeded and often disturb plant growth and development, decrease yield and negatively affect fruit quality. Growth reduction, decrease in the photosynthetic rate and increase in respiration, assimilate partitioning towards the fruits, osmotic and oxidative damage, reduced water and ion uptake/movement, cellular dehydration are detrimental effectes of the heat stress.

The high temperature induces severe damage in the photosynthetic apparatus. Photosynthesis is one of the most heat-sensitive processes and it can be completely inhibited by high temperature before other symptoms of the stress are detected (Berry and Bjôrkman, 1980; Camejo et al., 2005). The electron transport chain is negatively affected in PSII (photosystem II). Moreover, chlorophyll fluorescence is decreased in PSII (Havaux and Tardy, 1996). Sensitivity of Calvin cycle activity and inactivation of Rubisco under high temperature stress are the other reason of the inhibition of photosynthesis (Camejo et al., 2005). Susceptibility of biomembranes to high temperature stress is other important reason of the inactivatipon of the photosynthesis. The membrane disintegration is a primary symptom of heat injury (Ristic et al., 1996). It is reporrted that the most affected plant growth stage is the reproductive growth and the affected process is pollen grain development and ultimately fruit set (Bita and Gerats, 2013). The retardation of carotenoid biosynthesis and red color (lycopene) development by high temperature have been reported (Buescher, 1979; Yakir et al., 1984; Chen et al., 1988). The firmness of tomatoes is negatively affected by high temperature (Chen et al., 1988). In the case of available leaf water status and stomatol opening under high temperature stress, photosynthesis and biomass production were not improved, probably due to non-stomatal limitation of photosynthesis mediated by nutrient deficiency. It is known that supraoptimal temperatures may develop multiple mineral deficiencies in roots and shoots, and this can adversely affect the nutrition of the fruit (Schwarz et al., 2010). There is a susceptibility of tomatoes to blotchy ripening and BER (Blossom end rod). These physiologycal disorders are related to deficiencies of K and Ca. Therefore, screening of tomato for efficiency of these nutrients under heat stress conditions can be a way of increasing tomato fruit K and Ca content (Adams and Ho, 1995).

The aims of this work were (1) to investigate the effect of heat stress on mineral contents of the fruits that are important from the point of view of fruit quality (2) to compare the mineral elements of tomato genotypes that are tolerant to high temperature stress.

Materials and Methods

Plant Material and Growing Conditions

Twenty-two different tomato genotypes including 20 genotypes heat tolerant and 2 commerical cultivars as control which grown commonly in the experiment region by the growers, were used in this study. The study was performed in open field under environmental conditions of Adana-Cukurova ((36°59'N, 35°18'E, 20 m above sea level) in 2016 spring-summer period. Two consecutive experiments were set up in the study; first one was control and second one was heat stress experiments. The heat stress experiment was the warmest time of the summer. Seed sowings were performed on February 27 and April 22, 2016 in control and heat stress experiments,

respectively. Seedling transplanting dates were April 14 and May 22, 2016 in successive experiments, respectively. Tomato fruit sampling dates were June 14 and July 22, 2016. Randomized complete block experimental design with 4 replications and 10 plants in each replicate were used in both experiments. Spacings were 120 cm between rows and 50 cm between plants were used. The nutrition of the tomato plants were done equally in the both experiments. For this purpose 140 kg N, 95 kg P_2O_5, 220 kg K_2O, 20 kg MgO and 40 kg CaO were used for ha area.

Table 1. Operation performed in control and heat stress experiments and dates

Operation performed	Dates in 2016
Seed sowing of the control experiment	February 27
Seedling transplantin of the control experiment	April 14
Tomato fruit sampling date in the control experiment	June 14
Seed sowing of the heat stress experiment	April 22
Seedling transplantin of the heat stress experiment	May 22
Tomato fruit sampling date in the heat stress experiment	July 22

Mineral elements analysis

The tomato fruits were washed once with tap water and then twice washed by deionized water. The fruit were sliced and dried in a forced-air oven at 65 °C for 96 hours and the dried material was ground in a laboratory mill for mineral element analysis. Ground samples were dry-ashed in a muffle furnace at 550 °C for 6 h. The ash was dissolved in 0.1 M HCl (hydrochloric acid) solution. The concentrations of K, Ca, Mg, Fe, Mn, Zn and Cu were determined using a Varian Spectra FS220 atomic absorption spectrometer (Jones, 2001). Phosphorus analysis was performed according to the colorimetric method Barton which uses vanadate–molybdate reagent in spectrophotometer.

Statistical analysis

Tomato fruit P, K, Ca, Mg, Fe, Mn, Zn and Cu concentrations were examined statistically by analysis of variance. Least significant difference (LSD) was calculated at 0.05 probability level for each parameter.

Results and Discussion

High-temperature stress reduces root growth which affects the growth of aboveground tissue by restricting the supply of water and mineral nutrients (Gri et al., 2017). Therefore it is inevitable that the fruit mineral content is also negatively affected. However, in the present study heat tolerant tomato genotypes showed better performance and their mineral content most cases were higher than mineral content of the control trade cultivars. The nutrient concentrations among the heat tolerant tomatoes and the control cultivars are presented in Table 2 and 3.

Phosphorus

The P content in tomato fruit was increased in the 12 heat tolerant genotypes and decreased in the 8 genotypes under heat stress (Table 2). The highest P increase in relative to control was 35.97% in Tom-230. In heat stress, the highest and lowest P concentrations were 29.9 and 13.2 mg 100 g^{-1} in Tom-201B and Tom-115, respectively. The mean P concentrations in control and heat stress were 18.67 and 20.79 mg 100 g^{-1}, respectively. Among the tolerant tomato genotypes 16 of them showed the higher P concentration than the mean of control cultivars (16.3 mg 100 g^{-1}) under heat stress. In this stdy, P concentration of tomatoes was generally increased under heat stress and mean increase of all genotypes in heat stress was 7.19 in relative to control (Table 2). Hernaéndez Suéarez et al., (2007) reported the P concentration of tomato fruit as 23.7, 25.0 and 29.8 mg100g^{-1} from conventional, organic and hydroponic grown tomato plants. Sainju et al., 2014 reported 27 mg100g^{-1} P in green and red tomato fruits. Our tomato fruit P concnetrations in both control and heat stress were lower than above mentioned literature. This can be due to genotype, variety, environmental conditions.

Potassium

The K content in tomato fruit was decreased under heat stress in 19 tolerant genotypes (Table 2). Tom-230 was only genotype that was not decreased its K concentration under heat stress. The K decreases of tolerant tomatoes in relative to control were changed between 3.97-55.93% under stress. The highest and lowest K concentrations

were under heat stress 77.9 and 55.3 mg 100 g^{-1} in Tom-114 and Tom-20, respectively. The mean K concentration in heat stress was recorded as 68.09 mg 100 g^{-1}. Among the tolerant tomato genotypes 14 of them showed the higher K concentration than the mean of control cultivars (65.10 mg100g^{-1}) under heat stress (Table 2). Hernaéndez Suéarez et al., (2007) reported the K concentration of tomato fruit as 247.6, 260.4 and 243.6 mg 100 g^{-1} from conventional, organic and hydroponic grown tomato plants. Sainju et al., 2014 reported 244 mg 100 g^{-1} K in green and red tomato fruits. The K concentrations in this study lower than above mentioned literatures. This may be due to growing and climatic conditions, fertilizers used and genotype characteristics. The K nutrient is quite important for tomato. Tomato fruits often express K deficiency as blotchy ripening, greenback or yellow shoulder. The fruit also lacks firmness and has low brix levels. Potassium has the greatest importance on the quality parameters determining the marketing of fruits, consumer preferences, and the concentration of vital phytonutrients for human health (Lester et al., 2010; Constán-Aguilar et a., 2014). K significantly affects fruit size, soluble solids, Vitamin C concentration and pigments (lycopene and beta-carotene) of tomato fruit (Kanai et al., 2007; Ramıérez et al., 2012).

Magnesium

The Mg content in tomato fruit was decreased under heat stress in all genotypes (Table 2). The Mg decreases of tomatoes in relative to control were changed between 32.50-70.94% under stress. The highest and lowest Mg concentrations under heat stress were 1.3 and 5.3 mg 100 g^{-1} in Tom-173 and Tom-14, respectively. The mean Mg concentration in heat stress was recorded as 2.80 mg100g^{-1}. Among the tolerant tomato genotypes 3 of them showed the higher Mg concentration than the mean of control cultivars (3.30 mg 100 g^{-1}) under heat stress. In the present study fruit Mg content was the most negatively affected nutrient under heat stress. The mean decrease of all genotypes in heat stress was 69.8% in relative to control. Mean Mg concentrations in control and heat stress were 11.4 and 2.8 mg100g^{-1}, repectively (Table 2). Hernaéndez Suéarez et al., (2007) reported the Mg concentration of tomato fruit as 11.6, 12.3 and 9.4 mg 100 g^{-1} from conventional, organic and hydroponic grown tomato plants. In the present experiment tomato plants were well fed with Mg fertilizer and the control fruits showed better Mg content; however, Mg concentration in the fruits were dramatically decreased under heat stress. Magnesium nutrition is particularly important in ensuring even ripening of well-formed tomato fruit. Fruits appear to ripen evenly but maturity is often delayed.

Calcium

The Ca content in tomato fruit was decreased under heat stress in all genotypes (Table 2). The Ca decreases of tolerant tomatoes in relative to control were changed between 48.68-75.85% under stress. The highest and lowest Ca concentrations under heat stress were 14.10 and 47.90 mg 100 g^{-1} in Tom-111 and Tom-115, respectively. The mean Ca concentration in heat stress was recorded as 26.42 mg 100 g^{-1}. Among the tolerant tomato genotypes 11 of them showed the higher Ca concentration than the mean of control cultivars (22.55 mg 100 g^{-1}) under heat stress (Table 2). Hernaéndez Suéarez et al., (2007) reported the Ca concentration of tomato fruit as 66.74, 65.01 and 87.4 mg 100 g^{-1} from conventional, organic and hydroponic grown tomato plants. In the present study fruit Ca content was second most negatively affected nutrient (after Mg) under heat stress. The mean decrease of all genotypes in heat stress was 62.9% in relative to control. Mean Ca concentrations in control and heat stress were 75.73 and 26.42 mg100g^{-1}, repectively. Exposure of cells to suboptimal temperature results in membrane injury. Calcium magnifies the heat tolerance of the membrane (Starck et al., 1995). Optimal Ca content and proper distribution in individual organs prevents the incidence and severity of physiological disorders that are caused in many cases by unfavorable external conditions (Poovaiab, 1993; Starck et al., 1995). In spite of their very low Ca content, fruits are sensitive to Ca decrease. Low calcium concentrations are observed with blossom-end rot in tomato fruits. The above physiological disease is often described under adequate Ca supply of the whole plant, indicating some perturbation in its distribution, especially its supply to the fruits (Adams and Ho, 1992; Starck et al., 1995)

Zinc

The Zn content in tomato fruit was decreased under heat stress in all genotypes (Table 3). The Zn decreases of tolerant tomatoes in relative to control were changed between 31.25-80.74% under stress. The highest and lowest Zn concentrations were 1.62 and 2.75 mg 100 g^{-1} in Tom-19 and Tom-119, respectively. The mean Zn concentration in heat stress was recorded as 2.30 mg 100 g^{-1}. Among the tolerant tomato genotypes 17 of them showed the higher Zn concentration than the mean of control cultivars (1.74 mg 100 g^{-1}) under heat stress. In the present study fruit Zn content was the most negatively affected micro-nutrient under heat stress. The mean decrease of all genotypes in heat stress was 62.69% in relative to control. Mean Zn concentrations in control and heat stress were 6.64 and 2.08 mg100g^{-1} repectively (Table 3). Bjelić et al., (2005) reported greenhouse grown

tomato fruits Zn concentration was 0.154 mg $100g^{-1}$ fruit and open field grown tomato fruits contained 0.123 mg Zn in 100g fruit. Bosiacki et al., (2009) reported that Cu concentration in tomato fruit was ranged between 0.49-3.19 mg $100g^{-1}$.

Iron

The Fe content in tomato fruit was decreased under heat stress in all genotypes (Table 3). The Fe decreases of tolerant tomatoes in relative to control were changed between 16.67-87.07% under stress. The highest and lowest Fe concentrations were 3.47 and 2.15 mg$100g^{-1}$ in Tom-19 and Tom-233, respectively. The mean Fe concentration in heat stress was recorded as 3.07 mg 100 g^{-1}. Among the tolerant tomato genotypes 18 of them showed the higher Fe concentration than the mean of control cultivars (2.21 mg 100 g^{-1}) under heat stress. In the present study fruit Fe content was second most negatively affected micro-nutrient (after Zn) under heat stress. The mean decrease of all genotypes in heat stress was 59.30% in relative to control. Mean Fe concentrations in control and heat stress were 7.86 and 2.74 mg 100 g^{-1}, repectively (Table 3). Bjelić et al., (2005) reported greenhouse grown tomato fruits Fe concentration was 0.223 mg $100g^{-1}$ fruit and open field grown tomato fruits contained 0.283 mg Fe in 100g fruit. Bosiacki et al., (2009) reported that Fe concentration in tomato fruit was ranged between 1.29-5.54 mg 100 g^{-1}. Iron is the most abundant microelement in the plant. It has significant influence on the quality of tomato. Iron plays a key role, since it is involved in metabolic processes, such as photosynthesis and respiration. It is also implied in many enzymatic systems like chlorophyll synthesis (Houimli et al., 2017). Immobility or slow transfer through the plant (the phloem) is characteristic for iron, so that it mostly remains in the root and in young leaves. This results in low and unstable content of this element in the fruits and in the seed (Bjelić et al., 2005).

Cupper

The Cu content in tomato fruit was decreased under heat stress in 18 tolerant genotypes (Table 3). Tom-20 and Tom-225 were two genotypes that were not decreased their Cu concentrations under heat stress. The Cu decreases of tolerant tomatoes in relative to control were changed between 14.81-80.00% under stress. The highest and lowest Cu concentrations were 2.17 and 0.47 mg 100 g^{-1} in Tom-20 and Tom-108, respectively. The mean Cu concentration in heat stress was recorded as 0.99 mg 100 g^{-1}. Among the tolerant tomato genotypes 19 of them showed the higher Cu concentration than the mean of control cultivars (0.53 mg 100 g^{-1}) under heat stress. In the present study fruit Cu content was the least negatively affected micro-nutrient under heat stress. The mean decrease of all genotypes in heat stress was 34.82% in relative to control. Mean Cu concentrations in control and heat stress were 1.78 and 0.99 mg$100g^{-1}$ repectively. Bjelić et al., (2005) reported that copper is very resistant to various influences (temperature, moisture, time of harvest, etc.) and greenhouse grown tomato fruits Cu concentration was 0.0499 mg $100g^{-1}$ fruit and open field grown tomato fruits contained 0.0488 mg Cu in 100 g fruit. So the authors concluded that the copper content in tomato was substantially stable. Bosiacki et al., (2009) reported that Cu concentration in tomato fruit was ranged between 0.12-0.76 mg $100g^{-1}$. The Cu concentrations in our study were higher than above mentioned two literatures. This is may be related soil, environment, genotype and fertilisers used in the study.

Conclusion

High-temperature stress reduces root growth which affects the growth of aboveground tissue by restricting the supply of water and mineral nutrients (Gri et al., 2017). Therefore, it is inevitable that the fruit mineral content is also negatively affected. However, in the present study heat tolerant tomato genotypes showed better performance and their mineral content most cases were higher than mineral content of the control trade cultivars. According to heat stress effects on the tomato fruit mineral content, the macro-nutrients were ordered P, K, Ca and Mg from the least affected to the most affected. And, the micro-nutrients were ordered Cu, Fe and Zn from the least affected to the most affected. It is recommended to use heat tolerant genotypes in tomato cultivation in hot regions. Thus, the mineral content of the fruit will be preserved from the stress negative effects.

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Table 2. Tomato fruit **macro-nutrient content** of the tolerant genotypes and control cultivars under heat stress and control conditions (mg $100g^{-1}$ of dry weight).

	P			K			Mg			Ca		
Tolerant Tom	Control	Stress	CRC*	Control	Stress	CRC*	Control	Stress	CRC*	Control	Stress	CRC*
Tom-12	28.5 a	27.2 ab	-4.56	130.4 a-c	75.1 a	-42.41	17.6 b	5.10 ab	-70.94	51.0 f-h	25.7 e-g	-49.61
Tom-14	27.2 ab	28.2 a	3.68	142.9 a	74.6 a	-48.15	19.2 ab	5.30 a	-72.35	50.4 f-ı	19.8 g-ı	-60.71
Tom-19	18.3 c-g	18.1 b-g	-1.09	110.8 de	68.2 a-e	-38.45	10.5 c-d	2.00 c-e	-80.71	48.8 f-ı	16.2 g-ı	-66.80
Tom-20	17.4 c-g	14.4 e-g	-17.24	107.7 e	55.3 f	-48.65	9.2 c-f	2.80 a-e	-69.63	44.1 f-ı	17.3 g-ı	-60.77
Tom-26	21.7 a-d	21.4 a-g	-1.38	67.0 ı	61.9 c-f	-7.61	7.0 d-f	4.60 a-c	-34.71	37.5 ı	16.6 g-ı	-55.73
Tom-40	21.7 a-d	21.4 a-g	-1.38	71.1 ı	67.3 a-e	-5.34	8.0 d-f	3.1 a-e	-61.01	42.5 g-ı	17.7 g-ı	-58.35
Tom-47	20.6 a-f	16.4 c-g	-20.39	71.9 hı	63.5 b-f	-11.68	10.4 c-e	3.10 a-e	-70.73	38.0 hı	19.5 g-ı	-48.68
Tom-108	17.0 c-g	13.6 fg	-20.00	73.0 hı	70.1 a-e	-3.97	9.8 c-e	2.20 c-e	-77.48	41.5 g-ı	15.3 hı	-63.13
Tom-111	19.68 b-g	21.4 a-g	8.74	135.3 a-c	72.7 a-c	-46.27	23.6 a	1.50 de	-93.64	43.4 f-ı	14.1 ı	-67.51
Tom-114	24.5 a-c	22.6 a-f	-7.76	141.8 a	77.9 a	-45.06	19.0 ab	3.3 a-e	-82.79	130.9 a	41.3 a-c	-68.45
Tom-115	13.0 f-g	13.2 fg	1.54	138.6 a	63.2 c-f	-54.40	19.3 ab	3.1 a-e	-84.16	130.9 a	47.9 a	-63.41
Tom-119	21.2 a-e	23.7 a-e	11.79	137.2 ab	60.6 d-f	-55.83	18.5 a-b	2.4 c-e	-87.01	125.4 ab	42.3 ab	-66.27
Tom-165	22.3 a-c	25.6 a-c	14.80	91.1 f	69.7 a-e	-23.49	7.1 d-f	2.7 b-e	-62.52	113.1 bc	36.0 b-d	-68.17
Tom-173	1.84 c-g	20.5 a-g	11.41	76.6 g-ı	67.9 a-e	-11.36	7.9 d-f	1.3 de	-83.48	110.5 cd	37.2 bc	-66.33
Tom-201-B	20.6 a-f	29.9 ab	35.87	76.2 g-ı	72.0 a-c	-5.51	6.5 d-f	2.6 b-e	-60.47	108.9 cd	26.3 d-g	-75.85
Tom-211	18.3 c-g	23.0 a-f	25.68	79.0 f-ı	68.9 a-e	-12.78	7.3 d-f	1.4 de	-81.38	106.1 cd	34.1 b-e	-67.86
Tom-225	13.1 e-g	16.8 c-g	28.24	75.8 g-ı	71.4 a-d	-5.80	4.9 e-f	3.0 a-e	-38.37	99.3 de	25.4 e-h	-74.42
Tom-230	13.9 d-g	18.9 a-g	35.97	69.1 ı	69.7 a-e	0.87	4.0 f	2.7 b-e	-32.50	92.2 e	31.9 c-f	-65.40
Tom-232	19.6 b-g	24.7 a-d	26.02	123.9 b-d	72.4 a-c	-41.57	9.8 c-e	1.6 de	-84.01	48.7 f-ı	24.4 e-h	-49.90
Tom-233	13.0 e-g	14.8 d-g	13.85	114.8 de	59.3 ef	-48.34	9.2 c-f	2.9 a-e	-68.26	51.3 f-h	19.4 g-ı	-62.18
Mean Tolerant	18.67	20.79	7.19	101.71	68.09	-27.79	11.4	2.8	-69.8	75.73	26.42	-62.98
Control Tom.												
Hazera 56 F_1	16.4 c-g	20.0 a-g	21.95	87.3 fg	71.2 a-d	-18.44	5.4 d-f	3.7 a-d	-31.11	37.2 ı	22.0 f-ı	-40.86
H2274	12.1 g	12.6 g	4.13	85.4 f-h	59.0 ef	-30.91	6.7 d-f	2.8 a-e	-58.96	41.7 g-ı	23.10 f-ı	-44.60
Mean Controls	14.25	16.30	13.04	86.35	65.10	-24.68	6.1	3.3	-45.0	39.45	22.55	-42.73

*: Changes in relative to control. Values with the same letter are not significantly different. Data represent means of five independent fruits.

Table 3. Tomato fruit **micro-nutrient content** of the tolerant genotypes and control cultivars under heat stress and control conditions (mg $100g^{-1}$ of dry weight).

	Fe			Zn			Cu		
Tolerant Tom.	Control	Stress	CRC (%)*	Control	Stress	CRC*	Control	Stress	CRC (%)*
Tom-12	13.17 a-b	2.72 b-g	-79.35	11.42 a	2.20 b-e	-80.74	1.57 c-e	1.20 bc	-23.57
Tom-14	7.27 e-h	2.55 d-ı	-64.92	6.02 c-e	2.05 c-h	-65.95	1.65 b-e	0.79 b-g	-52.12
Tom-19	14.40 a	2.15 h-ı	-85.07	9.62 a-b	1.62 h	-83.16	2.50 a-c	0.90 c-h	-64.00
Tom-20	5.40 h-	2.35 f-ı	-56.48	5.60 c-f	2.10 c-g	-62.50	1.20 c-e	2.17 a	80.83
Tom-26	5.57 g-j	2.55 d-ı	-54.22	5.52 c-f	2.50 a-c	-54.71	1.80 b-e	1.17 b-d	-35.00
Tom-40	6.57 f-ı	2.65 c-h	-59.67	5.87 c-e	2.00 d-h	-65.93	1.10 d-e	0.62 e-h	-43.64
Tom-47	6.10 g-ı	2.27 g-h	-62.79	5.17 d-f	1.75 e-h	-66.15	0.92 e	0.77 c-h	-16.30
Tom-108	8.77 c-f	2.77 b-g	-68.42	11.77 a	1.97 d-h	-83.26	2.35 a-d	0.47 g-h	-80.00
Tom-111	9.32 c-e	3.22 a-b	-65.45	12.20 a	2.12 b-d	-82.62	2.97 a-b	0.65 d-h	-78.11
Tom-114	9.85 c-d	2.52 d-ı	-74.42	11.02 a	2.00 d-h	-81.85	2.92 a-b	1.45 b	-50.34
Tom-115	10.62 c	3.20 a-b	-69.87	8.00 b-c	1.70 g-h	-78.75	2.95 a-b	1.15 b-e	-61.02
Tom-119	3.60 j-k	3.00 a-e	-16.67	4.00 e-f	2.75 a	-31.25	1.35 c-e	1.15 b-e	-14.81
Tom-165	10.70 b-c	2.82 b-f	-73.64	4.22 e-f	2.15 b-g	-49.05	1.35 c-e	0.72 c-h	-46.67
Tom-173	5.30 h-j	2.75 b-g	-48.11	4.20 e-f	2.47 a-c	-41.19	2.47 a-c	0.97 b-g	-60.73
Tom-201-B	4.37 ı-k	3.37 a	-22.88	3.77 e-f	2.07 c-h	-45.09	0.95 e	0.75 c-h	-21.05
Tom-211	4.60 ı-j	3.00 a-e	-34.78	4.12 e-f	2.17 b-f	-47.33	0.67 e	0.57 f-h	-14.93
Tom-225	4.25 ı-k	2.50 e-ı	-41.18	3.00 f	1.82 e-h	-39.33	0.70 e	0.75 c-h	7.14
Tom-230	7.97 d-g	2.77 b-g	-65.24	5.37 c-f	1.82 e-h	-66.11	1.00 e	0.65 d-h	-35.00
Tom-232	8.62 c-f	2.15 h-ı	-75.06	5.05 d-f	1.72 f-h	-65.94	1.67 b-e	0.95 b-g	-43.11
Tom-233	10.80 b-c	3.47 a	-67.87	6.92 c-d	2.57 a-b	-62.86	3.60 a	2.02 a	-43.89
Mean Tolerant's	7.86	2.74	-59.30	6.64	2.08	-62.69	1.78	0.99	-34.82
Control Tom.									
Hazera 56 F_1	9.17 c-e	2.12 ı	-76.88	5.65 c-f	1.75 f-h	-69.03	1.05 d-e	0.40 h	-61.90
H2274	2.60 k	2.30 g-ı	-11.54	3.05 f	1.72 f-h	-43.61	1.97 b-e	0.65 d-h	-67.01
Mean Control's	5.89	2.21	-44.21	4.35	1.74	-56.32	1.51	0.53	-64.46

*: Changes in relative to control. Values with the same letter are not significantly different. Data represent means of five independent fruits.

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Araştırma Makalesi/*Research Article (Original Paper)*

Direct *in vitro* Regeneration and Transient Gus Assay: Towards Stable Genetic Transformation in *Trifolium alexandrinum* L.

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Abstract: The process of genetic transformation allows insertion of transgene conferring trait(s) of interest to genetically modified plants. Here, we report direct *in vitro* regeneration and transient gus assay as a step forward for *Agrobacterium* mediated genetic transformation of Berseem (*Trifolium alexandrinum* L.). The cotyledons from *in vitro* grown plants of local berseem varieties "superlate Faisalabad" and "Anmol" were cultured on MS media supplemented with various concentrations of NAA and BAP, for direct shoot induction. Maximum regeneration was obtained on MS medium BAP (2.0 mg/L) and NAA (0.05 mg/L). Regenerated shoots were rooted on MS media augmented with IBA. For transformation, *in vitro* grown cotyledons were co-cultured with *Agrobacterium tumefaciens* strain *EHA*105 harboring plant transformation vector p7i-UG containing *uidA* gene. Histochemical GUS assay was performed for tracking transient expression of reporter gene in cotyledons. Direct shoot regeneration from cotyledons was peculiarity of this study. Hence, establishment of efficient direct shoot regeneration combined with transient *GUS* gene expression would pave the way towards efficient genetic transformation of *Trifolium alexandrinum* L for valuable traits in future.

Keywords: Direct regeneration, *Trifolium alexandrinum*, Transient GUS assay, Indole butyric acid, Benzyl amino purine

Introduction

Berseem (*Trifolium alexandrinum* L.) is also known as King of forages owing to its nutritive richness, wide-spread adaptability and multi-cut nature. Various members of genus *Trifolium* are widely consumed as fodder crop widely (Zayed et al. 2012). Moreover, it exhibits proficient nitrogen fixation ability as it can fix 297-400 kg of atmospheric N per hectare so, is an important choice for soil fertility improvement (Abd El-Hady 1993; Graves et al. 1996). Its cultivation is also helpful in soil conservation by preventing water and wind erosion. In recently reclaimed lands, berseem in known to enhance organic matter contents of soil, amend soil structure and improve chemical as well as physical characteristics of soil (Tilman et al. 2002; Peters et al. 2003).

Various methods are being employed by genetic engineers to incorporate transgenes into plant genome. As compared with traditional breeding, transgenic technology has proved more efficient to crop plants with desired valuable traits (Zayed 2013). *Agrobacterium tumefaciens* is gram negative soil bacterium that has natural ability to infect dicot plants causing crown gall disease. Various strains of *Agrobacterium* have been explored to transfer DNA to dicot as well as monocot plant species (Gelvin 2003; Nester 2014). One of the fundamental pre-requisites, to develop transgenic plants, is establishment of proficient in vitro regeneration protocol. For this purpose, a variety of explants, genotypes, media contents and culture conditions have been exploited (Joyia and Khan 2013).

Berseem is susceptible to numerous biotic and abiotic stresses but not limited to drought, salinity and fungal pathogens causing severe decline in plant growth and crop productivity. Rapidly increasing population demands increased dairy and meat production which in turn requires widespread dairy farming. To support huge animal population on shrinking arable land and scarce water resources, augmented productivity of fodder crops is instantaneously desirable (Hamdy *et al*. 2003; Vinocur and Altman 2005). Biotechnological tools can efficiently be employed to get improved fodder crop.

Materials and Methods

Plant material
Berseem seeds were obtained from Fodder Research Institute, Ayub Agricultural Research Institute (AARI) Faisalabad. Seeds were surface sterilized and were cultured on solid MS0 medium in growth incubator at 25 ± 2°C under 16/8 hrs light/dark regime.

Selection of explant and assessment of regeneration efficiency
For appraisal of regeneration efficacy, cotyledons from 3-4 days old plants were detached and cultured onto regeneration medium augmented with MS vitamins and plant growth regulators (PGRs).

Rooting
Individual shoots from *in vitro* regenerated shoot clusters were cut and treated with IBA before transferring to tubes/jars containing root induction medium (MS medium with 0.025 mg/L IBA).

Genetic transformation with Agrobacterium
Competent cells of *Agrobacterium tumefaciens* strain EHA105 were transformed with transformation vector P7i-UG using electroporation method and were confirmed through colony PCR.

Infection and co-cultivation
PCR positive colonies of *Agrobacterium* cells were grown in LB medium containing (Rifampicin and Spectinomycin 10 mg/L each) for 48 hours at 28ºC. Explants were dipped in bacterial suspension for 60 min., rinsed with autoclaved distilled water followed by washing with timentin solution. Then, explants were cultured on resting medium for 3-5 days.

Histochemical GUS assay
The inoculated explants were subjected to β-glucurodinase (GUS) assay after 5 days of infection following protocol given by Kanwal *et al*. (2017). Cotyledons were incubated at 37°C for 48 hrs in buffer containing X-Gluc (5-Bromo-4-chloro-3-indolyl-D-glucuronic acid), Potassium buffer (pH 7.0) and 0.5% Triton X-100 as a substrate. Gus expression was observed visually and photographed.

Statistical analysis
Statistical analyses were carried out employing analysis of variance (ANOVA) (Snedecor and Cochran 1989) and LSD (Least Significant Difference) test at 5% level of significance (Steel et al. 1997).

Results and Discussion

Direct in vitro regeneration in berseem (Trifolium alexandrinum L.)

The explants (cotyledons) were excised from 3-5 days old *in vitro* grown seedlings while taking care to avoid any shoot tip. Cotyledons with adaxial sides up (upper surface up wards) were cultured on MS medium having 2% sucrose, 2.66 g/L Gellan gum powder and various combinations of NAA and BAP. For initial 5 days, the petri plates were kept in dark followed by 16:8 hours light:dark regime. After 3-4 weeks, multiple shoots appeared on the surface of cotyledons showing direct regeneration (Fig 1 B-C). Among the different concentrations and combinations of growth regulator, maximum regeneration was observed on MS medium augmented with NAA (0.05mg/L) and BAP (2.0 mg/L). The results described here for Pakistani berseem varieties are in accordance with Abogadallah and Quick (2010) who reported similar results for Egyptian cultivars Sakha3 and Sakha4. However, mean number of shoots/explant (Table 2.0) reported here are higher than those reported by aforementioned authors. The genetic transformation is a tedious process and only few out of thousands of cells get transformed. Hence, it is solely dependent on the availability of enormous number of receptive cells responsive to regeneration. So, the reported direct regeneration protocol will prove a real milestone in the genetic transformation of this fodder crop. The multiple shoots regenerated from cotyledons were shifted to jars having MS0 medium for further proliferation (Fig 1 D). Then, individual shoots were separated and maintained on the same medium for elongation (Fig 1 E). Elongated shoots were shifted to rooting media (Fig 1 F). Then, plants with roots were shifted to pots for acclimatization (Fig 1 G).

Data analyses (ANOVA) revealed significant difference among two varieties used (Table 1.0). For the comparisons of number of shoots regenerated/explant of each variety, LSD was applied to the recorded mean values and a significant difference was observed. The variety "Superlate Faisalabad" was found better (41 shoots per explant) as compared to anmol (28 shoots per explant) (Table 2.0). The aim of this part of study was to establish a proficient direct *in vitro* regeneration system in berseem by eliminating callus induction step in order to get higher number of true to type regenerants within shortest possible time. Barakat (1990) reported callus induction from cotyledons and hypocotyls of two varieties Giza10 and Sakha4 and eventually employed those calli to produce cell suspension cultures. Bhowal *et al*. (2011) used MS or L2 media and concluded that shoot tip was best responsive explant. About 75% of explants produced shoots on L2 media augmented with IAA (0.1 mg/L) and BAP (1 mg/L).

Transient GUS expression in berseem (Trifolium alexandrinum L.) cotyledons

Agrobacterium tumefaciens has been exploited as a means of transgene delivery and induce transient expression of recombinant proteins including GUS in plants (Liu et al. 2018; Bendahmane et al. 2000). In this part of study, *Agrobacterium* mediated genetic transformation and transient GUS assay based early detection of transgene expression, in cotyledons of *Trifolium alexandrinum*, was carried out. Electro-competent cells of *Agrobacterium* were transformed using electroporation method. Transformed cells were selected on spectinomycin containing solid LB medium and were confirmed through colony PCR (Fig. 2). In order to infect seeds, secondary culture of *Agrobacterium* was established (Fig 3 B). Seeds, at different germination stages (Fig. 3 C) were transferred to bacterial culture and incubated for 60 minutes. After infection, seeds were cultured onto antibiotic free MSO medium (Fig. 3 D). After three days, seeds were rinsed with autoclaved double-distilled water containing timentin, so that all the *Agrobacterium* cells on the surface of explants become dead. This treatment proved very effective and bacterial contamination was reduced upto 99%. The explants were shifted to MSO medium containing timentin for 3-5 days. At this stage histochemical GUS assay was performed to track expression of the transgene (Fig. 3 E). Regions of cotyledons with successful transgene expression exhibited blue colour due to chromogenic cleavage of the substrate by X-Gluc (Fig. 3 E). Moreover, cotyledons were cultured onto regeneration medium supplemented with timentin for selection and subsequent regeneration of transgenic shoots. The GUS expression has been reported to serve as a proficient and reliable method for transient expression of the transgene in tobacco (Kanwal et al. 2017). In another study, β-glucuronidase (GUS) reporter gene has proved to be appropriate for the quantification of gene expression in many plants including *Arabidopsis*, tobacco and rice (Fior and Gerola, 2009). Hence, these studies prove transient integration of transgene in local berseem varieties so the retrieved results may be employed to engineer berseem for valuable traits.

Future directions:

The present study is a step forward towards stable genetic transformation in berseem. Though, GUS expression assay rendered cotyledons unusable for further tissue culture, however, the use of other reporter genes like green fluorescent protein (gfp), yellow fluorescent protein (yfp) and red fluorescent protein DsRed in future may help to further prove this concept.

Table 1.0 Analysis of variance (ANOVA) for direct shoot Regeneration from Cotyledons

S. O. V	DF	SS	MS	F	P
Variety	1	253.500	253.500	254	0.0001
Error	4	4.000	1.000		
Total	**5**	**257.500**			

Non-significant (P>0.05), Significant (P<0.05), highly significant (P< 0.01).
Grand Mean 34.500 CV 2.90

Table 2.0 LSD All-Pairwise comparisons test of direct shoot regeneration by varieties

Variety	Mean	Homogeneous Groups
Superlate Fsd	41.00	A
Anmol	28.00	B

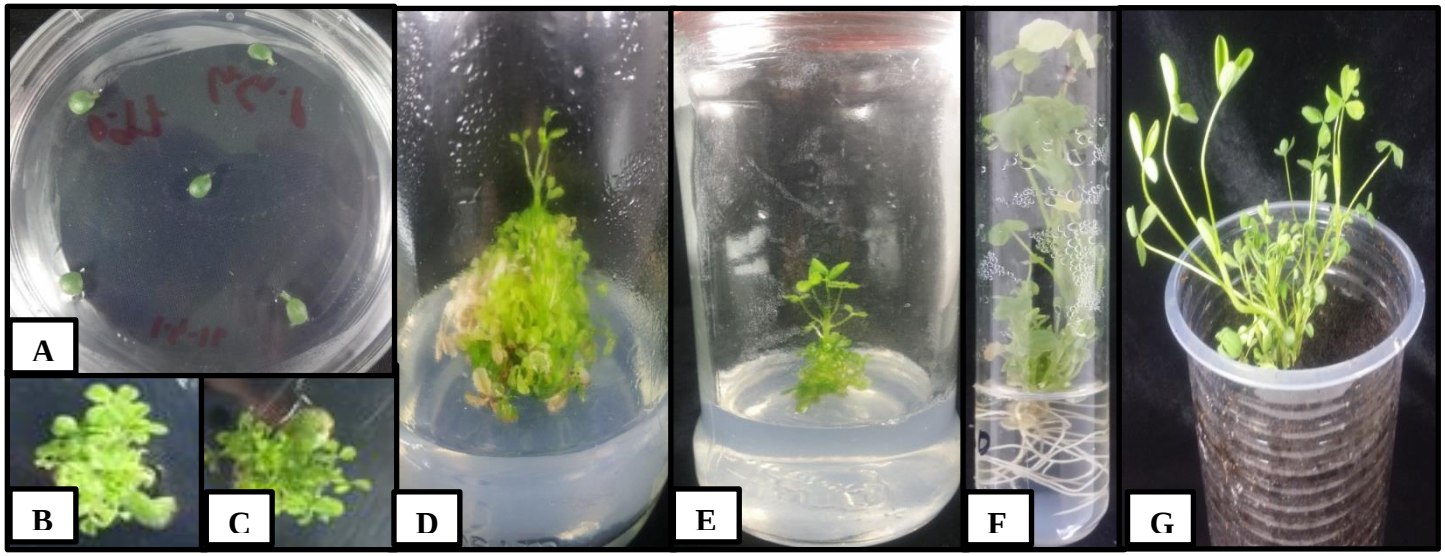

Figure 1. Direct *in vitro* regeneration in berseem (*Trifolium alexandrinum* L.) using cotyledons as explant (A) Cotyledons cultured on regeneration medium, (B-C) cotyledons showing regeneration of multiple shoots (D) individual cotyledons with multiple regenerated shoots shifted to jars for further proliferation (E) individual shoots shifted to jars for elongation (F) elongated shoots shifted to rooting media (G) acclimatization of rooted plants.

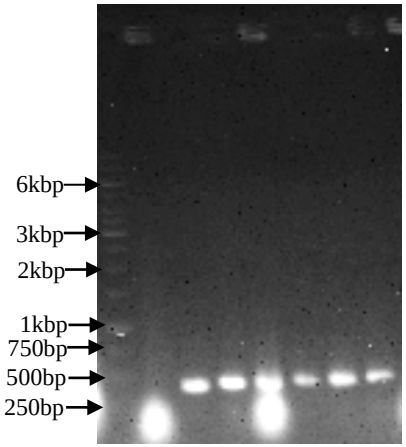

Figure 2. Colony PCR for confirmation of Agrobacterium transformation.

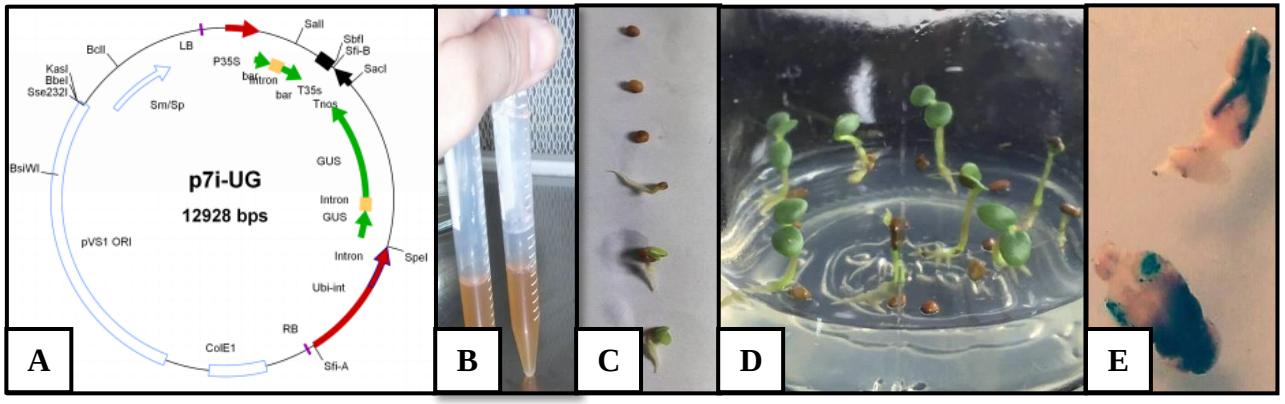

Figure 3. Transient GUS expression in berseem (*Trifolium alexandrinum* L.) cotyledons (A) Plant transformation vector *p7i-UG* vector having *ubi* 1 promotor, *uidA* gene and *nos* terminator (B) Agrobacterium cells transformed with *p7i-UG* through electroporation and grown in liquid culture (C) Explant treated with Agrobacterium (D) Treated explants growing *in vitro*, (E) Gus expression assay from *in vitro* grown cotyledons which were ready to be transferred to regeneration medium.

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Araştırma Makalesi/*Research Article (Original Paper)*

The Effects of Different Growing Media and Root Pruning Applications on Trakya Ilkeren Grape Variety Grown in Soilless Culture

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Abstract: In this research, the effects of shearing of 10 cm bottom part of the root zone of three-year-old "Trakya Ilkeren" grape variety grown in 32 L pots filled with cocopeat and perlite:peat (2:1, v:v) solid culture media on grape yield, cluster and berry weights, must characteristics and macro and micro nutrient levels of the leaves and stem diameter were investigated. Plants were under hail net. A modified Hoagland nutrient solution was applied to the plants once a week. The effect of the growing conditions on the stem diameter measured at bud burst and full bloom period was significant and the highest stem diameter values were determined in Perlite:Peat medium (17.18 mm and 17.65 mm, respectively). The effect of the applications on the yield, cluster, berry and must characteristics was not significant. The content of leaf nutrients in the full bloom and veraison period changed according to the growing media and root shearing application. Ca and Mg in plants grown in Perlite: Peat medium; P, K, Zn and Mn in Cocopeat; Zn and Mn in roots-pruned plants; Ca and Mg values in the unsheared (control) plants, were found to be higher. Considering N, Fe and Cu contents of the leaf samples taken in both periods, there was no significant difference between the applications.

Key Words: Grapevine, grape, soilless culture, growing medium, mineral element

Topraksız Kültürde Yetiştirilen Trakya Ilkeren Üzüm Çeşidinde Kök Budama Uygulaması ve Farklı Ortamların Etkisi

Özet: Bu araştırmada, dolu net altında ve 32 litrelik saksılarda, cocopeat ve perlit:torf (2:1) katı kültür ortamlarında yetiştirilen 3 yaşlı Trakya Ilkeren üzüm çeşidinde kökün alttan 10 cm lik kısmının kesilmesi uygulamasının üzüm verimi ve salkım ve tane ağırlıkları ile yaprakların makro ve mikro besin maddeleri düzeyi ve gövde çapı üzerine etkisi araştırılmıştır. Bitkilere haftada bir kez değiştirilmiş Hoagland besin çözeltisi uygulanmıştır. Araştırma sonucunda yetiştirme ortamlarının uyanma ve tam çiçeklenme döneminde ölçülen gövde çapı üzerine etkisi önemli bulunmuş ve bu bakımdan en yüksek değerler perlit:torf ortamında (sırasıyla 17.18 mm ve 17.65 mm) saptanmıştır. Uygulamaların verim, salkım ve tane ile şıra özellikleri üzerine etkisi önemsiz bulunmuştur. Yaprakların tam çiçeklenme ve ben düşme dönemindeki besin maddesi içerikleri yetiştirme ortamı ve kök kesme uygulamalarına göre değişmiştir. Perlit:torf ortamında yetişen bitkilerde Ca ve Mg; cocopeat ortamında P, K, Zn ve Mn; Kök budaması yapılanlarda Zn ve Mn; kök budaması yapılmayan (Kontrol) bitkilerde ise Ca ve Mg değerleri daha yüksek bulunmuştur. Her iki dönemde alınan yaprak örneklerinde N, Fe ve Cu içerikleri bakımından uygulamalar arasında önemli bir farklılık bulunmamıştır.

Anahtar kelimeler: Asma, üzüm, topraksız kültür, katı ortam, kök budama,

Introduction

Soilless cultivation is a technique generally used in the production of undercover. It is seen that the soilless culture technique which is widely used in the cultivation of vegetables and ornamental plants, nowadays is beginning to be used in grape cultivation as has been used in Italy since 2000 **(**Di Lorenzo and Mafrica 2000; Di Lorenzo et al. 2009, 2012 and 2013).

It is thought that this technique can be an important tool in controlling the yield and quality in table grape growing, especially in the problems caused by the rootstocks, more controlled feeding of grapevines and more efficiently applying of water. This technique is proven as one of the important production techniques that should be tried in grape growing in our country. Indeed, in Turkey several studies have been conducted on this issue.

Some of the works carried out on grape growing in soilless culture technique in Turkey are performed by Polat et al. (2003), Sabır et al. (2016 and 2017), Tangolar et al. (2016 and 2017) and Baştaş (2017) and Kaya et al. (2018).

In a soilless culture study carried out in Italy, 2.17 tons grape yield da^{-1} of "Cardinal" and "Victoria" and 2.94 tons grape yield da^{-1} of "Black Magic" and "Victoria" varieties were found (Buttaro et al. 2012). In a study conducted to determine the effect of crop load on soilless grape cultivation in our country, 4.2 tons da^{-1} of "Prima" grape variety and 3.8 tons da^{-1} of "Early Sweet" (Baştaş 2017) were obtained. In these studies, the cultivation technique applied in the first year is grown one cane and the product is obtained in this second year from it. In other words, two years have been spending for a product year.

An economically sufficient grape yield has been achieved by Tangolar et al. (2017) in the third year. In the second and third years of their study, consecutive 5.6 and 3.4 tons da^{-1} in "Trakya. İlkeren"; 6.7 and 4.2 tons da^{-1} in "Prima" variety, 4.7 and 3.7 tons da^{-1} in "Yalova incisi" variety could be obtained. However, according to the third year crop, the yield of grapes decreased by 39.3% in "Trakya Ilkeren", 37.3% in "Prima" and 21.3% in "Yalova incisi". It is thought that one of the important reasons for such decrease was probably due to the excessive increase and ultimate filling of the old roots in the pots, preventing the formation of new in roots. For this reason, it was decided to apply an idea for encouraging new root development by cutting a part from the bottom of the pot and thus avoiding loss of yield.

For this purpose, it was aimed to determine the effect of root cutting application on yield and some quality characteristics and mineral nutrient contents of the leaves of 3 years old "Trakya Ilkeren" (first year: one cane production, two and third year: obtaining product), which is grown in two different solid media Thus, it has been tried to find answers to the questions on the subject that the product can be obtained in sufficient quantities and quality at the age of 4 from the same vines in soilless culture conditions.

Materials and Methods

This study was carried out in Research and Experimental vineyard of Horticultural Department of Agricultural Faculty of Çukurova University, in 2017.

Materials

In the study, 3-year-old "Trakya Ilkeren" grape variety which was grown in different solid media in 32 liter pots, and applied a two-armed cordon shape was used. Here, authors may wish to provide certain explanatory information such as how many winter buds were left, or how many summer shoots per vine, were the shoots supported, etc.

For nutrient solution, we considered some researches which used Hoagland nutrient solution. (Hoagland and Arnon 1950; Ünlü Yılmaz and Tangolar 2007; Buttaro et al. 2012). Standard nutrient solution used in this study were prepared using 150 ppm N (as NH_4NO_3), 30 ppm P (as H_3PO_4) , 175 ppm K (as KSO_4 or KNO_3), 20 ppm Mg (as $MgSO_4$), 15 ppm (S) (in the form of the sulphate compounds), 5 ppm Fe (as Fe- EDDHA), 3ppm Mn (as $MnSO_4$), 0,4 ppm B (as H_3BO_3), Cu:0.2 ppm Cu (as $CuSO_4.5H_2O$), 1 ppm Zn (as $ZnSO_4.7H_2O$), 0,05 ppm Mo (as $NH_4Mo_7O_{24}.4H_2O$). The nutrient solution is applied to plants once a week between bud burst and veraison for 12 weeks and between veraison and maturity for six weeks.

In the study, the amounts of pure nutrients applied every week in the first and second periods, respectively, Nitrogen 1125 and 1500 mg, Phosphorus 226 and 300 mg, Potassium 1312 and 1750 mg, Magnesium 167 and 222 mg, Zinc 7.5 and 10.0 mg, Boron 3, and 4 mg, copper 0.15 and 0.20 mg, manganese 22.5 and 30 mg, molybdenum 0.37 and 0.50 mg and iron 41.67 and 55.56 mg.

The study area was 100 square meters and was covered with hail net. Drip irrigation system was used for irrigation, 1-3 L day^{-1} water was found to be sufficient during the growing period, less initially.

Methods

Growing Media Application

In the study, the effect of two different growing media, namely, mixture of perlite:peat (2:1) and cocopeat was tried.

Root Pruning Application

In this application, the control plants continued to be grown in their pots without any root pruning. After the other application plants were removed from the pots, the lower 10 cm sections were cut with a saw. In this application, vines with the remaining part of root system was planted again using the same media.

The white hail net was covered over the tunnels in which the pots were placed at a distance of 0.75 m and 1.5 m. For each application 3 replications were used, each replicate consists of 2 pots. After the berry set, every vine was thinned in a way to leave 10 clusters. The plant protection activities against to the diseases and disorders were done by using suitable fungicides and insecticides when necessary.

Investigations

Yield and Quality

In order to determine the effect of the applications, stem diameter (mm), yield (g vine^{-1}), cluster weight (g), berry weight (g 100 berry^{-1}), berry volume (mL 100 berry^{-1}), total soluble solids (TSS) (%), titratable acidity (TA) (g L^{-1} must) and pH were determined.

Plant Nutrition Analysis

In order to determine the effects of the applications on plant nutrition, leaf samples were taken in full bloom and veraison time. For each application, ten leaf samples taken from opposite of the clusters were washed twice in the laboratory with tap water and then twice with pure water. Wet leaves were removed from the water by coarse filter paper and then they were dried at 65 °C for 72 hours. Dried leaf samples were ground by agate mill and made ready for analysis.

N, P, K, Ca, Mg from macro elements; Fe, Zn and Mn contents of micro elements were determined:

Nitrogen (N): Nitrogen in the leaf samples was determined according to the Kjeldahl method as reported by Bremner (1965).

Phosphorus (P): Total phosphorus was determined using the Shimadzu model UV 1201 spectrophotometer according to vanadomolibdophosphoric yellow colour method (Kacar 1972).

Potassium (K): Total potassium was determined using an Eppendorf Elex 6361 fluorimeter.

Calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn) contents of the leaves were determined by Atomic Absorption Spectrophotometer.

Trial Design and Statistical Analysis

Variance analysis was performed according to the split plots experimental design with three replicates using JMP statistical programmer-based SAS, and least significant difference test (LSD) was used for separation of means of different treatments at 5% significance level. While root pruning subjects (Total 2) as a sub-plot, growth media (Total 2) as a main plot, were arranged in the blocks.

Results and Discussion

The effect of the application of "Trakya Ilkeren" grape variety on stem diameter development in different phenological periods is presented in Table 1. The effect of the media on the stem diameter measured during bud burst and full bloom period was found significant. The highest diameter values were in Perlite+Peat medium (17.18 mm and 17.65 mm, respectively) in both phenological periods. The effect of the media on the development of diameter measured during veraison and maturity time was not significant. It has also been found that the effect of root pruning applications on diameter was also insignificant. The effects of interactions were only significant during the full bloom period. Tangolar et al. (2017), who studied the effects of media on trunk diameter development, found the highest diameter value in Perlite+ Peat (2:1) medium.

Table 1. Effect of applications on stem diameter (mm) in different phenological periods[x]

Application	Bud burst	Blooming	Veraison	Maturity
Substrates				
Cocopeat	15.02 b	15.68 b	16.78	16.92
Perlite:Peat	17.18 a	17.65 a	17.52	18.38
LSD 5%	1.46	1.08	NS	NS
(Pr>F)	0.0147	0.0073	0.4121	0.0571
Root pruning				
Pruned	16.06	16.44	17.71	18.06
Non Pruned	16.14	16.90	16.60	17.24
LSD 5%	NS	NS	NS	NS
(Pr>F)	0.8880	0.3034	0.3076	0.2146
Interaction				
CocopeatxPruned	14.33	14.65 b	16.58	17.03
CocopeatxNon Pruned	15.71	16.72 a	16.99	16.82
Perlite:Peat x Pruned	17.94	18.22 a	18.48	19.09
Perlite:Peat xNon Pruned	16.57	17.07 a	16.35	17.67
LSD5%	NS	1.53	NS	NS
(Pr>F)	0.0673	0.0147	0.1770	0.3361

[x] Mean separation within columns by LSD mutiple test at 0.05 level, NS: Nonsignificant

As can be seen in Table 2, the effects of media and root pruning practices on yield and cluster and berry characteristics were statistically insignificant. Perlite+Peat medium yielded 4443 g vine^{-1}, cocopeat 4307 g vine^{-1} - 4485 g vine^{-1} was obtained from root cutting application, and 4266 g vine^{-1} was obtained from non-root cutting plants. It has been determined that the plants subjected to root pruning yielded approximately 200 kg da^{-1} more than their control plants. It is thought that the new roots formed by the cutting of the 10 cm old root zone at the bottom of the pot have increased the amount of the products by a little.

The highest bunch weight value was obtained from the Cocopeat medium (430.7 g) following Perlite+Peat (444.3 g) medium. The bunch weight, 100 weight and volume, were somewhat higher than those not made in root-pruned plants (448.5 g, 373.5 g and 353.3 mL, respectively). The cluster weights obtained in the study were found in medium sized cluster (Çelik 2011) group, in terms of 100 weight values, in media and root pruning applications, in large berry group, whereas those obtained from unroot-pruned were found to the medium-sized berry group (Çelik 2011). The effects of the media on the must characteristics were not significant. The effect of root pruning application on TSS, pH and maturity index was insignificant, while acidity was significantly affected. Acid values of root pruned vines were found higher than the untreated ones in the roots (Table 3). Furthermore Keskin et al. (2013) reported that pH ranges from 4 to 3 in mature grapes and minimum values of TSS should be 12 °Brix for the Alphonse Lavalleé and Cardinal while 13 °Brix for all other seeded table grapes cultivars and 14 °Brix for all seedless cultivars. The macro element levels examined in leaf samples taken during full bloom and veraison periods were given in Table 4.

Table 3. Effect of applications on must characteristics [x]

Application	TSS (%)	Acidity (g 100 mL^{-1})	pH	Maturity index
Substrate				
Cocopeat	15.20	0.396	3.92	38.87
Perlite:Peat	15.23	0.374	3.98	40.80
LSD5%	NS	NS	NS	NS
(Pr>F)	0.9434	0.0894	0.2828	0.3115
Root pruning				
Pruned	15.29	0.408 a	3.92	37.73
Non Pruned	15.14	0.362 b	3.98	41.93
LSD5%	NS	0.028	NS	NS
(Pr>F)	0.7845	0.0096	0.2595	0.0651
Interaction				
CocopeatxPruned	15.21	0.439 a	3.86	34.64
CocopeatxNon Pruned	15.18	0.353 b	3.98	43.10
Perlite:Peat xPruned	15.37	0.377 b	3.98	40.83
Perlite:Peat xNon Pruned	15.10	0.370 b	3.98	40.77
LSD5%	NS	0.039	NS	NS
(Pr>F)	0.8270	0.0163	0.2828	0.0624

[x] Mean separation within columns by LSD mutiple test at 0.05 level, Ö.D.: Önemli değil, N.S.: Nonsignificant

The effects of P, K, and Ca contents on leaf contents were significant in the growing media during both flowering and veraison period. N contents in both sample times and Mg contents during fall period were insignificant. The highest values of P (0.40% and 0.42%) and K (1.40% and 1.17%) were obtained from Cocopeat medium during both of the leaf sampling period. Ca (0.85% and 0.91%) values were obtained from samples taken from Perlite+Peat medium.

The effect of root pruning on the macro element content of leaves taken during the veraison was not significant. In the samples taken at full bloom, the effect on N, P and K content was insignificant and the effect on Ca and Mg contents was significant. Vines without root pruning gave higher values of Ca (0.69% and 0.54%) and Mg (0.52% and 0.41%, respectively) than root-pruned plants.

Jones et al. (1991) compared to the macro element limit values during full bloom and veraison, it was determined that the total amount of nitrogen in the tests performed during the full bloom and veraison in all applications was higher than in all the blooms during veraison period. Potassium and Calcium contents were found to be deficient in all applications and at two different sampling times. Magnesium content was found to be sufficient (0.37% - 0.56%) in all applications during full bloom period, and very high (0.67% -0.71%) in all applications during the veraison.

The microelement contents analysed in leaf samples taken during full bloom and veraison period were given in Table 5.

It was determined that growing medium and root pruning applications were not effective on Cu and Fe contents at the both of the time sampling and Zn content was not effective at full flowering. The effects of growing media and root pruning on Zn contents were found to be significant at the time of taking samples at both sampling times and during the veraison (Table 5).

It was found that Cu, Mn, Fe, Zn amount was sufficient in all applications during full flowering and veraison period when compared with micro element limit values during full flowering and veraison periods (Jones et al. 1991).

Conclusion

The effects of two growth media and root pruning on the most characteristics examined were found to be insignificant, in the case of soilless table grape production. For this reason, it has been determined that both growth media can be used for soilless grape cultivation. With root pruning, yield per plant increased by 219 g, but this value was not statistically significant.

It was seen that the values obtained were included in the standard quality values required for table grapes. Considering macro elements, the applications were found to be in excess of N and in the deficiency limit values of K and Ca when compared to the plant nutrition. For this reason, it may be suggested that different growth media and increasing levels of nutrients be tested for the elimination of K and Ca deficiency.

For example, Gül et al. (2005) noted that zeolite can contribute to sustainability in soilless agriculture by increasing fertilizer use efficiency. Buttaro et al. (2012) showed that as a result of the studies, the change in nutrient solution can be achieved without any adverse effect on yield and quality of grape. It was determined that the level of micro elements were sufficient in the leaf samples.

As a result, when a general evaluation is made, "Trakya Ilkeren" variety is not significantly affected by growth media and root pruning. From the overall findings of the present investigations, we can recommend to examine the effects of root pruning to cut the root not only from the bottom but also from all around because such application can anticipated affecting the yield and quality and the intake of plant nutrients by further increasing the number of roots.

Table 4. Effect of applications on macro (%) element contents of the leaves [x]

Application	N		P		K		Ca		Mg	
	B	V	B	V	B	V	B	V	B	V
Substrate										
Cocopeat	3.35	2.98	0.40 a	0.42 a	1.40 a	1.17 a	0.37 b	0.61 b	0.37 b	0.67
Perlite:Peat	3.30	2.78	0.35 b	0.24 b	1.05 b	0.80 b	0.85 a	0.91 a	0.56 a	0.71
LSD5%	NS	NS	0.04	0.11	0.28	0.24	0.05	0.14	0.05	NS
(Pr>F)	0.3684	0.3460	0.0403	0.013	0.0255	0.0120	<0.0001	0.0045	0.0004	0.1947
Root pruning										
Pruned	3.29	2.83	0.39	0.38	1.33	1.09	0.54 b	0.75	0.41 b	0.67
Non Pruned	3.36	2.93	0.35	0.29	1.12	0.89	0.69 a	0.77	0.52 a	0.71
LSD5%	NS	NS	NS	NS	NS	NS	0.05	NS	0.05	NS
(Pr>F)	0.2882	0.6279	0.0583	0.0869	0.1087	0.0792	0.0007	0.7324	0.0036	0.1248
Interaction										
CocopeatxPruned	3.36	3.00	0.41	0.50	1.54	1.40	0.31	0.54	0.32	0.61
CocopeatxNon Pruned	3.35	2.96	0.38	0.35	1.26	0.94	0.44	0.68	0.41	0.73
Perlite:Peat x Pruned	3.23	2.65	0.38	0.26	1.12	0.77	0.76	0.96	0.50	0.72
Perlite:Peat xNon Pruned	3.37	2.90	0.32	0.23	0.99	0.83	0.95	0.86	0.63	0.70
LSD5%	NS	NS	NS	NS	NS	0.33	NS	NS	NS	NS
(Pr>F)	0.2742	0.4861	0.3139	0.1900	0.5048	0.0371	0.1082	0.0739	0.4187	0.0542

[x] Mean separation within columns by LSD mutiple test at. 0.05 level. B: Full Blooming V: Veraison NS.: Nonsignificant

Table 5. Effect of applications on micro element (ppm) contents of the leaves [x]

Application	Cu		Mn		Fe		Zn	
	B	V	B	V	B	V	B	V
Substrate								
Cocopeat	10.63	17.87	90.22 a	137.9 a	181.9	166.3	34.07	54.16 a
Perlite:Peat	11.52	17.43	42.73 b	71.07 b	181.9	169.0	29.87	48.76 b
LSD5%	NS	NS	18.66	24.04	NS	NS	NS	1.24
(Pr>F)	0.3224	0.8146	0.0021	0.0015	0.9979	0.9039	0.2086	0.0003
Root pruning								
Pruned	10.73	19.89	84.94 a	123.58 a	191.2	169.75	32.96	56.35 a
Non Pruned	11.42	15.41	48.01 b	85.35 b	172.6	165.57	30.98	46.56 b
LSD5%	NS	NS	18.66	24.04	NS	NS	NS	1.24
(Pr>F)	0.4305	0.0627	0.0053	0.0116	0.5749	0.8527	0.5199	<0.0001
Interaction								
CocopeatxPruned	9.14 b	20.56	111.18	149.6	18.9	168.9	31.94	56.17 a
CocopeatxNon Pruned	12.11 ab	15.17	69.25	125.8	182.0	163.7	36.20	52.14 b
Perlite:Peat x Pruned	12.31 a	19.21	58.69	97.23	200.4	170.6	33.98	56.53 a
Perlite:Peat xNon Pruned	10.73 ab	15.64	26.76	44.91	163.3	167.4	25.77	40.98 c
LSD5%	3.12	NS	NS	NS	NS	NS	NS	1.77
(Pr>F)	0.0458	0.6324	0.4987	0.1788	0.5736	0.9663	0.0900	0.0002

[x] Mean separation within columns by LSD mutiple test at. 0.05 level. B: Full Blooming V: Veraison NS.: Nonsignificant

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Araştırma Makalesi/*Research Article (Original Paper)*

Use of Remote Sensing and Geographic Information Systems to Determine the Amount of Water Used for Agricultural Purposes at the Downstream Point in HEPP Projects

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Abstract: While Hydroelectric Power Plants are ideal sources for power generation, they may have various impacts on the environment as a result of diverting water from its bed and carrying it in the conveyance lines at river-type hydroelectric power plants in particular. In order to prevent or minimize these impacts, it is necessary to calculate the amount of water also called "prior water rights" and used for the natural habitats, people, and irrigation on the downstream side at the projecting stage of a power plant.
In this study, an integrated approach with remote sensing and geographic information systems which included the studies of data collection, data generation, mapping, and calculation was presented to calculate the water to be used for agricultural purposes at the downstream point that constituted part of the Environmental Impact Assessment report – the fundamental basis for the projecting of Hydroelectric Power Plants. In the field practices of this approach, great success was obtained in the generation and evaluation of the data about the topographical information, land characteristics, soil properties, and land use types of the HEPP areas and – considering this information – in the calculation of the water used for agricultural purposes and the approach was found feasible.

Keywords: Satellite images, digital elevation model, Blaney-Criddle, irrigation water

HES Projelerinde Mansaba Bırakılacak Tarımsal Amaçlı Kullanılan Su Miktarının Tespitinde Uzaktan Algılama ve Coğrafi Bilgi Sistemlerinin Kullanımı

Özet: Hidroelektrik Santralleri elektrik üretimi için ideal kaynaklar olmakla birlikte suyun yatağından saptırılarak iletim hatları içinde taşınması sonucu çevreye çeşitli etkileri olabilmektedir. Bu etkilerin engellenmesi yada en aza indirilmesi ise santralin projelendirme aşamasında mansaba bırakılacak doğal yaşam, insanlar ve tarımsal kullanım amaçlı suyun hesaplanması gerekmektedir. Bu çalışmada Hidroelektrik Santrallerinin projelendirilmesinde temel dayanak olan Çevre Etki Değerlendirme raporunun bir bölümünü oluşturan mansaba bırakılacak tarımsal amaçlı kullanılan suyun hesaplanmasında; veri toplama, veri üretimi, haritalama ve hesaplama çalışmalarını içeren uzaktan algılama ve coğrafi bilgi sistemleri ile bütünleşik bir yaklaşım ortaya konmuştur. Bu yaklaşımın arazide yapılan uygulamalarında, santrallerin kurulu olduğu ve etkilediği alanların topoğrafik bilgileri, arazi karakteristikleri, toprak özellikleri ve arazi kullanım türlerine ait verilerin üretilmesi, değerlendirilmesi ve bu bilgilerden hareketle tarımsal amaçlı kullanılan suyun hesaplanmasında büyük başarı elde edilmiştir.

Anahtar kelimeler: Uydu görüntüleri, sayısal yükseklik modeli, Blaney Criddle, sulama suyu

Introduction

One of the most heavily invested renewable energy sources is the hydroelectric power plants in Turkey. In order to open the way for privatization in the energy area, the law called "Establishment and Operation of Electricity Generation Production Facilities and Regulation of Energy Sales" which was no 4283 and dated 16/07/1997 were introduced and thus the "Build-Operate-and Transfer" model was applied. According to the provisions of the "Electricity Market Law" dated February 20, 2001, and numbered 4628, the construction of the power generation facilities was completely transferred to the private sector. HEPPs are the builds in which process the potential energy of the water is converted into the kinetic energy and the

electric energy is obtained. In the river type HEPPs, water is diverted from its bed and carrying it in the conveyance lines. After being used in electricity production, it is given to the bed again. In this process, the water is removed from the bed and the relationship with nature, air, and other living things are detached. In the case of the cascaded river-type power plants, the water comes out of the conveyance line of a company and goes into the conveyance line of the other company. It can reach the sea without relation to the habitat. Thus, the water is separated from the bed and passes through the conveyance lines at a distance of several kilometers. At these distances, water is cut off by nature, air and other living things, the oxygen content is reduced and the cultural elements in the surrounding natural environment are damaged.

Environmental Impact Assessment Regulation no 25318 has been developed in order to control the change in damage that may occur in the environment. In this regulation, it is necessary to determine the positive or negative effects of the planned projects on the environment. It is aimed to put forward the measures to be taken in order to prevent the negative effects or to reduce the minimum amount in order not to harm the environment. The regulation has vital importance in terms of the sustainable use of natural resources. The Regulation links area selection, implementation, monitoring and control of projects according to specific rules. At each stage of the HEPP projects, is encouraged/supported/promoted the use of technology alternatives to make the EIA works in a detailed and complete manner (Başayiğit and Uçar, 2012).

Different methods are used to determine the amount of environmental flow assessment. The most widely used hydrological flow evaluation method worldwide is the Tennant (1976) method (Tharme, 2003).

In a study evaluating various methods, it was emphasized that hydrological methods should be used for assessment at the level of discovery, then hydrological simulation or holistic methods should be used for comprehensive evaluation and calculation of water amount (Çimenci, 2011). In a study, Tennant-I, Tennant II, Q70, and Q95 methods were compared for at Koyunbaba Dam. The Tennant-II method was found to be more appropriate for the study area for the amount of environmental flow assessment (Köle, 2014). One of the most important elements encountered in reducing the environmental impacts of HEPPs is the necessity the separation of the water for the downstream point. This water is called such as living water, ecological water, ecological balance water. In addition, the domestic water of people and use of agricultural water should be determined and total water demand should be calculated. Three main types of water should be considered when determining the downstream point water. These are; habitat water, domestic water, and agricultural water.

In a study, irrigation water needs were calculated according to Blaney-Criddle and Penman-Monteith methods for 120 irrigation networks in our country. Then t-test was applied to two independent groups of results and the significant difference was found in 43% of the examined irrigation networks (Beyribey et al., 1997). To determine the amount of water used for agricultural purposes at the downstream point, it should be required the determination of basin boundaries, water resources, land use patterns and plant pattern, climate data and some soil characteristics. These data can be obtained from field surveys, maps and reports, and meteorological records. Nowadays, remote sensing and geographic information systems are used to calculate the amount of water to be separated for agriculture, and the result information can be generated in thematic maps as spatial data. In this study, Kürce HEPP was investigated as a sample. To determine the amount of water used for agricultural purposes at the downstream point, a new approach was developed. In this approach, Remote Sensing techniques and Geographical Information Systems have integrated the field applications. The results were presented in thematic digital maps and carried out spatial analyzes.

Material and Method

Material

The Kürce Regulator and HEPP were built on the Alakır Stream. Alakır Stream is located on the western Mediterranean region, within the Kumluca district of Antalya province. The Kürce Regulator is located approximately 600 m northeast of the Kürce quarter and at the 960 m south-west of İnlice, at an elevation of 643.53 m. The UTM coordinates of the regulator and power plant were 4063000 (K) / 261050 (D) and 4056000 (K) / 259900 (D) respectively. This study area is located in Alanya-O24-c1, Alanya-O2-c2 and

Alanya-O2-c4 areas in a standard topographic map with a scale of 1: 25.000. The coordinate of the basin that under the influence of Kürce HEPP is 254350 (K), 4064000 (D) -266000 (K), 4055000 (D).

Method

The approach was based on two basic concepts. The first of these was the production of data for the study area and the second was the calculation of the water need according to this data.

Data acquisition for the study area

In order to determine the characteristics of the study area, laboratory studies, field studies, and office studies were carried out. The laboratory work was consists of the processing of satellite data and the preparation of thematic map layers. For this reason, the studies were carried out in the Remote Sensing and Geographic Information Systems laboratory, the cartography laboratory and the data processing laboratory. These laboratories were required to have basic cartographic materials to be used in the field and software and hardware to produce the result maps. High-resolution digital satellite images are the most convenient tools for the production of maps. If this data is not available, new dated aerial photos can be used. If there are no aerial photos, archive satellite images obtained from DigitalGlobe can be used with Google Earth. In this study, archive satellite images were used. In addition, 1/25.000 scaled topographic maps produced by General Command of the Map and, Land Cover Provincial Soil Maps produced by the General Directorate of Rural Services and digital geological maps were used. These maps were digitized by using ArcGIS software. Photo interpretation of satellite images were done. And maps were overlaid on the interpreted image using the geographic information systems. Thus, draft maps were produced and these maps were printed on 1/5000 scale and made ready for field surveys. In addition, the result maps of the results obtained from the field studies and the data obtained from the office studies are produced in the GIS laboratories.

In the field surveys, ground controls were made using this cartographic material. For this purpose GPS was used for orienting on land. Arable land, major plant pattern, and other elements related to water were determined together with coordinate. Furthermore, in the field surveys, face-to-face interviews with local people and farmers were made to obtain more detailed information. In the office work, the reports about the study area and related literature were searched. The water requirement was calculated using the data obtained from the field. The final report was prepared. In order to understand the irrigation of area, the location of arable land, the catchment area and water resources map, slope, elevation classes, soil class map and land use map has been produced.

Crop Water Requirements

The amount of water use depends on the crop type, maturity, and atmospheric conditions. Seasonal water requirement is needed to match crops with available water supply and variation within season needed for irrigation scheduling. Blaney-Criddle method was used in the calculation of crop water requirements (Anonymous, 1982), below equation:

$U=k\times f$

$k=kc\times kt$

$kt=0.031\times t+0.24$

$f=((45.7\times t+813)\times P)/100$

Where; U is a seasonal crop water consumption or evapotranspiration (ET) ($mm.month^{-1}$), k is a seasonal ET coefficient and f is a monthly ET factors, kc is a monthly crop coefficient, t is the average monthly temperature (^oC) and kt is a climatic coefficient related to the mean monthly air temperature. P is a ratio of the monthly daylight hours to the annual daylight hours.

The irrigation water requirement is calculated by subtracting the effective rainfall values from the calculated plant water consumption.

I=U-re

I is monthly irrigation water requirement, $mm.month^{-1}$ U is crop water consumption, $mm.month^{-1}$, re is effective precipitation mm.

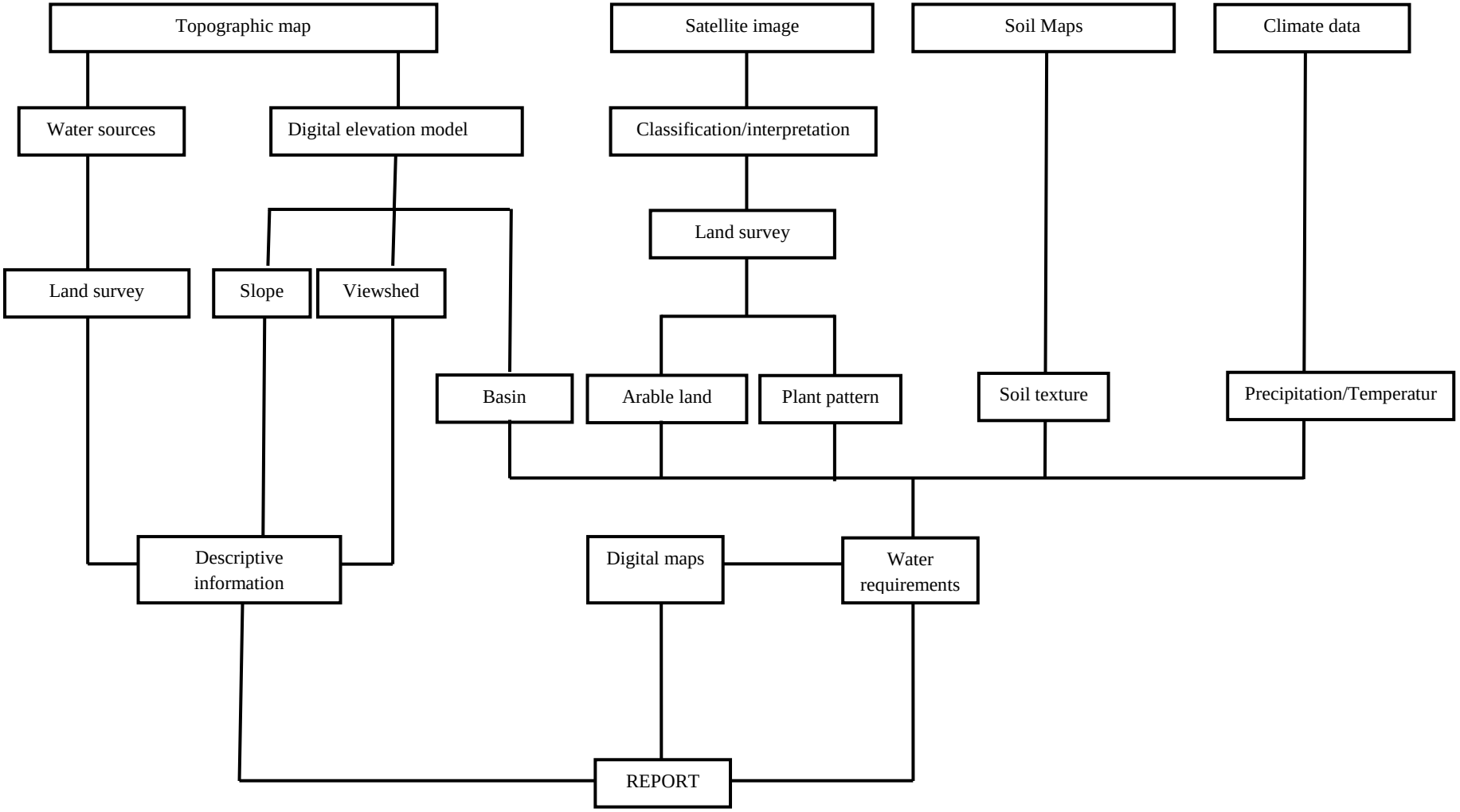

Figure 1. The flow chart of metodology

Results and Discussion

The evaluation of water basin and water resources

The study area was a basin that collects water on a conveyance line of approximately 6950 m which forms between the reservoir and the turbine gallery. The total area of the basin was covers 58.63 km^2. This basin consists of two separate parts, 25.35 km^2 to the east and 33.28 km^2 to the east of the Alakır stream.

Besides Alakır stream, there were four streams, ten intermittent stream and thirty springs in this basin (Figure 2).

Alakır Stream is the major water source of the hydroelectric power plant. According to the 5-year water flowing values of Alakır Stream, the annual average water flow is 110.56 hm^3 and the annual average flow is 3.52 $m^3.s^{-1}$. According to the 3-year water flowing values, the annual mean water is 316.86 hm^3, with an annual mean water flux of 10.05 $m^3.s^{-1}$ (Anonymous, 2010).

In the field surveys, the flow values of Koca Stream, Stream No1 and Dömenyeri Stream were measured to be 1-5-20 $l.s^{-1}$, 0.5-1 $l.s^{-1}$, 5-7 $l.s^{-1}$ respectively. There are also four intermittent streams (1, 2, 3 and 4 number) providing a short flow of range 1-10 $l.s^{-1}$ during intensive precipitation periods in the eastern part of the basin and six streams providing continuous flow in the western part of the basin.

Landform and elevation classes of basin

The study area was formed on "Mountainous Lands" and narrow "River Terraces". The mountainous lands were consists of extensions of Çalbeli Hill (2487 m) and Kızılarsivrisi Hill (2511 m) to the west of Alakır Stream. In addition, there are small pieces of land that are less inclined in place among these elevations (Figure 3). There was a hillside at the lower altitude (1300-1400 m) at the east of Alakır Stream at lower altitudes. On this hillside, terracing is a wide application. There are narrow river terraces on the right and left side of Alakır Stream with a slight slope of 100-200 m width. Accordingly, 71.04% of the study area is located on 600-1400 m. The total area of 6.4% is located on less than 600 m. 22.55% of the study area is higher than 1400 m altitude.

Slope

Due to the geomorphological formation of the basin, most of the study area is very steep (15-25%) and extremely steep (25-40%). The plain and slightly slopes (0-8%) land is located on the side of Alakır Stream as a narrow band and on the upper elevation of east and west parts. In most of the basin in west of Alakır Stream, the slope is between 25-40%. A large part of the basin in the east part is a steep slope of 15-20%. Extremely steep lands are mostly located on the high areas of the western part of the basin. Slight and medium slopes are mostly located on the high lands of the eastern part of the basin (Figure 4).

Viewshed

Alakır Stream is located in the north-south direction and the basin is a typical "V" profiled valley formed according to this direction. For this reason, the direction of major views in the basin was east and west. Change at frequent intervals of the slope values was caused by the complexity of the slope. Due to the formation of the hills, the terrain has different viewshed classes at south-east and north-west. Most of the eastern part of the study area is in the west and northwest viewshed. The west part is in the east and southeast viewshed. The most common type of viewshed in the study area is the north but also the southeast viewshed is the least (Figure 5).

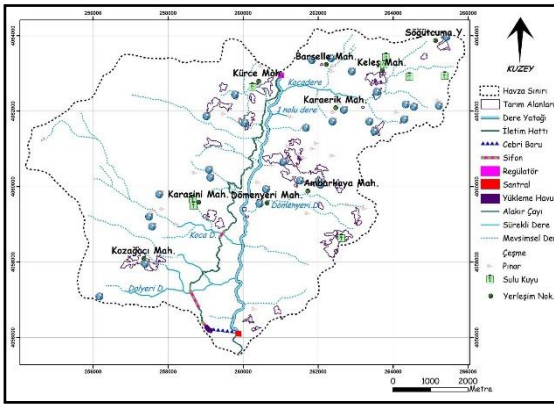

Figure 2. Water resources map

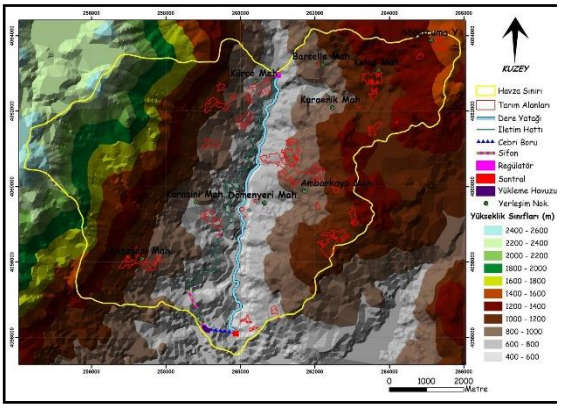

Figure 3. Elevation classes map

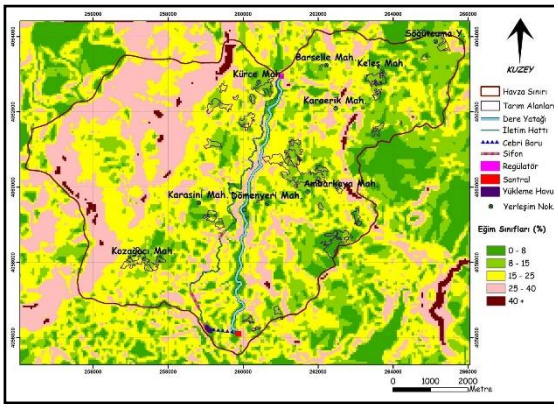

Figure 4. Slope classes map

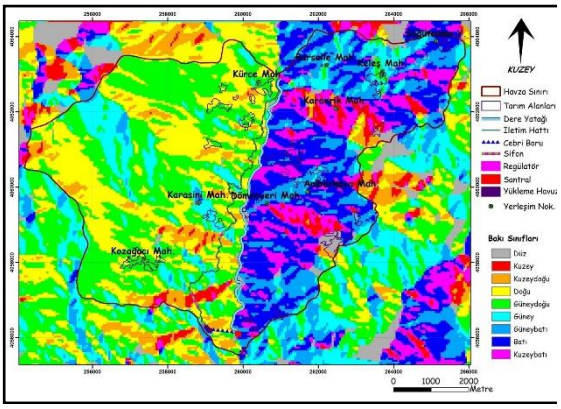

Figure 5. Viewshed classes map

Land use type and plant pattern

In terms of Floristic, the study area is defined as C3 in Davis (1965) 's quadratic system. Area is located on the Mediterranean region in terms of plant geography. The climate of the area is Mediterranean climate type, characterized by cool and rainy winters and hot and dry summers. The natural vegetation of the area is usually Calabrian pine (Pinus brutia Ten.) and oak in some places (Quercus L.). There are a lot of plane trees (Platanus orientalis), willow trees (Salix sp.), tamarisk (Tamarix sp.) and other hydrophilic flora on each side of Alakır Stream. Around of HEPP, there is dense maquis shrubland together with forestland. In the field observations, it was determination of other natural plants such as peppermint, pennyroyal (Mentha spicata L.), thorn apple (Phylleria latifolia), oleander (Nerium oleander L.), and tipton's weed (Hypericum oriéntale L.). Due to its topographical structure, agricultural areas are located on terraces in the study area. Soils are stony and gravelly. These lands have agricultural potential, but in the vast majority, it is not used for cultivation. In cultivation area, the major crop pattern is cereals. However, according to notify of farmers, it has been be convinced that various vegetables were produced in these terraces in the past. Seventy pieces of field that suitable for agriculture in the basin have been identified (Figure 6-8). Most of these lands are located near the settlement while others are located inside the forestlands and shrublands. The largest of these lands is 109,162 m^2 and the smallest is 715 m^2. The total area of arable land and the potential farmland area are calculated as 625599 m^2 (Table 1). The elevation of these lands ranges from 475 to 1460 m. In the study area, some orchards such as apples, cherries, apricots were observed. These trees are in the mixed garden in the village. In the study area, only olive cultivation was economically done as monoculture farming.

Soil properties

The study area is located in Alakırçay nappe. Alakırçay nappe generally consists of serpentine ultramafic rocks, massive lavas, red-brown pelagic limestone and radiolarite-chert and it is of ophiolitic melange type.

According to the soil map, there are brown forest soils (77.90%), reddish brown mediterranean soils

(1.21%), reddish mediterranean soils (0.30%), limestone brown forest soils, river bed and naked rock (20.47%) in the study area. Settlement is covered 0.09 % of total area. In the study area, very shallow (0-20 cm) and shallow (20-50cm) soils were cover 57,8 percent and 20,8 percent of total area. The 21.4 % of the study area was lithozolic surface that has a few soil depths (less than 10 cm) (Figure 7).

In the soil samples taken from the great soil groups (1: 2.5 soil-water), pH was found between 7.3-8.4 and EC was found between 50-150 μS.

Land use capability classes

In the basin, there are three different land use capability classes of VI (21.36%), VII (58.08%) and VIII (20.48%). The hazardous limitation in the classes of VI and VII is the risk of erosion and soil insufficiency (es). In the land of VIII class, it is not possible for growing cultural plants owing to land characteristics. These lands are usually suitable for natural habitat or recreation area. In this study, fragmented terrains, river banks, and bare rocks were included in this class.

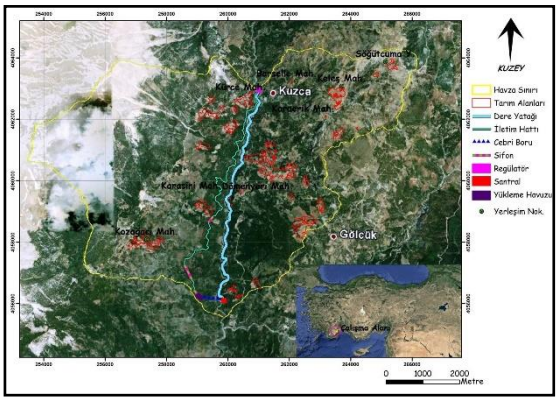

Figure 6. Distribution of potential agricultural areas

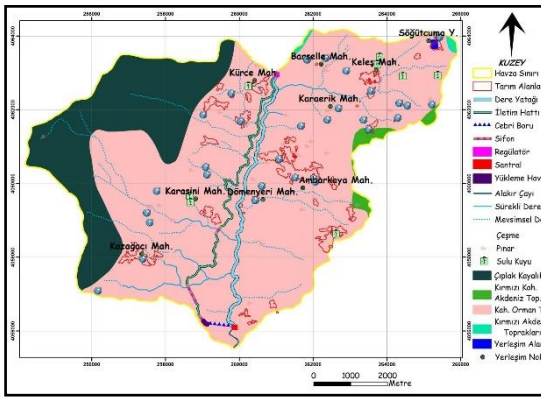

Figure 7. Soil map of study area

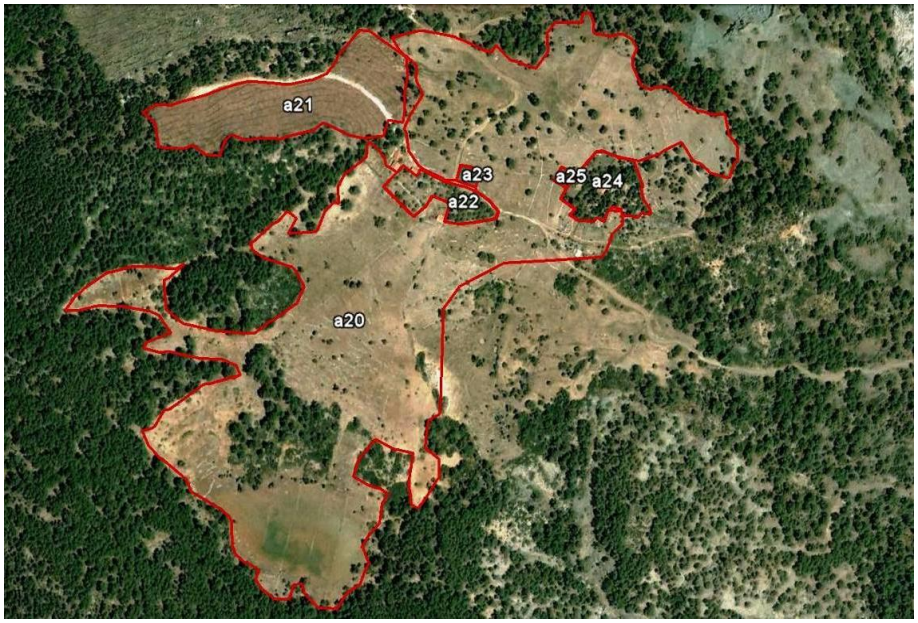

Figure 8. Examples of land use, agricultural areas and potential arable areas (Digital Globe, Google Earth 09.09.2009, 101001000A12650A image)

Table 1. The properties of the example areas

No	a20	a21	a22	a23	a24	a25
Area	45363 m^2	9465 m^2	4136 m^2	968 m^2	3658 m^2	834 m^2
Elevation	1110	1150	1120	1120	1130	1130
Distance from stream	2112 m	2155 m	2461 m	2528 m	2673 m	2661 m
Plant pattern	Cereal	Cereal	Orchards	Vegetable	Orchards	Vegetable

Water requirement for agriculture

As a result of the studies made with satellite images, and land surveys, a total of 625559 m^2 agricultural area was determined in which conveyance lines water flowing in through tunnel to plant. These areas do not have boundaries with each other but are scattered in the study area. The crop type was found as Cereal (78.2%), orchards (11.9%) and vegetables (9.9%) in these areas.
The parameters of the Blaney Criddle method are shown in Table 2. The ratio of the monthly daylight hours to the annual daylight hours (P) and the monthly crop coefficient (kc) was taken from Güngör et al. (2002), Kara (2005), and Kızılkaya (1983).

Table 2. Parameters of Blaney-Criddle

Latitude: 36º 39'	**Month**						
Parameters	**April**	**May**	**June**	**July**	**August**	**September**	**November**
t	12.7	16.7	21.2	24.6	24.9	21.1	16.1
kt	0.418	0.540	0.681	0.785	0.795	0.677	0.524
P	8.9	9.8	9.9	10.0	9.4	8.4	7.8
f	123.6	154.9	175.8	194.1	183.7	148.6	121.5
kc (Cereal)	1.38	1.45	1.05	0.36	-	-	-
kc (Orchards)	0.75	0.85	1.00	1.10	1.00	0.80	0.55
kc (Vegetable)	0.70	0.85	1.05	1.25	1.05	0.55	-

Approximately 76.4% of the areas defined as agricultural areas are located on more than 600 m elevation. Therefore, the pumped irrigation system could be used to irrigate these areas. However, no pumping system was observed in the field surveys.
It has been taken into consideration that surface irrigation methods can be used in case of these lands for irrigation. Therefore, surface irrigation parameters such as water application efficiency were used for calculating the water requirement. If drip or sprinkler irrigation methods are applied with a pumping system in these areas, less water will be needed than the calculated water requirement for surface irrigation. In other words, the water requirement calculated for surface irrigation was meant maximum water required for irrigation.

The mean crop water consumption for major plant pattern determined in field studies of vegetation period is presented in Table 3.

Table 3. Monthly crop water requirements for agricultural areas in downstream point according to Blaney-Criddle Method

Month	**Crop**	**Seasonal ET (mm.month-^{1})**	**Crop patern**	**Mean crop water consumption**	**Precipitation, mm**	**Effective rain, mm**	**Residual moisture from winter**	**Net irrigation requirement, mm**	**Total irrigation requirement, mm**	**Module, l/s/ha**
April	Grain Orchard 	71.4 38.8 	78.2 11.9 	55.8 4.6 **60.4**	27.9	26.7	80.5	0	-	-
May	Grain Orchard Vegetation	121.4 71.1 58.6	78.2 11.9 9.9	94.9 8.5 5.8 **109.1**	11.8	11.6	-	97.5	229.5	1.29
June	Grain Orchard Vegetation	125.7 119.7 101.8	78.2 11.9 9.9	98.3 14.2 10.1 **122.6**	0.9	9 0.	-	121.7	286.5	1.66
July	Grain Orchard Vegetation	54.8 167.6 160.0	78.2 11.9 9.9	42.9 19.9 15.9 **78.7**	5.9	5.8	-	72.9	5 171.	0.99
August	Grain Orchard 	146.0 182.5 	11.9 9.9 	17.4 18.1 **35.5**	0	0	-	35.5	83.5	0.47
September	Grain Orchard 	80.4 105.6 	11.9 9.9 	9.6 10.5 **20.1**	17.9	17.4	-	2.7	6.3	0.04
November	Grain Orchard 	35.0 35.0 	11.9 9.9 	4.2 3.5 **7.6**	69.8	62.0	-	0	-	-

*Residual moisture from winter (RMW): The amount of soil moisture available for plants at the beginning of the vegetation period. Residue from winter rainfall. This moisture can be used like ground rainfall. It is necessary to consider this moisture for crop water requirement. In this study, the mean value for Antalya was used 80.5 mm according to Kızılkaya (1983) in April.

The mean crop water consumption per month in the region was ranged from 7.6 to 122.6 mm. In this model, the water application efficiency is used as 50% for calculation of water amount requirement at the border of the field. For calculation of water amount at the resource, water conveyance efficiency was used as 85% (Kara, 2005). The daily irrigation time for the calculation of the irrigation module was used as 16 hours. Monthly irrigation water requirement that calculated according to the major crop water consumption was given in table 3. The total amount of irrigation water requirement was ranged from 6.3 to 286.5 mm.

It is expected that the highest irrigation module which calculated using the total irrigation water requirement will be one of the months of July or August. But the variation of the crop types can cause the highest module to appear in different months as it is here. In the study area, the most common crop is the Cereal with 78.2%. As the water consumption of Cereal is at its maximum level in June, the highest irrigation module has been determined in this month. With the Cereal harvest in July, crop water requirement was diminished likewise the irrigation module for the study area (Table 4).

Table 4. Monthly irrigation water requirement

Area:	625559 m^2							
Month	**Crop water requirement, mm**	**Field water requirement, mm (Efficiency:%50)**	**Irrigation water requirement, m^3/ha (Efficiency:%50)**	**Irrigation water requirement, mm (Efficiency: %85)**	**Irrigation water requirement, m^3/ha (Efficiency:%85)**	**Module, l/s/ha**	**Total water requirement, m^3**	**Total irrigation water, (l/s)**
April	-	-	-	-	-	-	-	-
May	97.5	195.1	1951	229.5	2295	1.29	143571	80.4
June	121.7	243.5	2435	286.5	2865	1.66	185165	103.7
July	72.9	145.8	1458	171.5	1715	0.99	110875	60.1
August	35.5	71.0	710	83.5	835	0.47	52261	29.3
September	2.7	5.3	53	6.3	63	0.04	4045	2.3
November	-	-	-	-	-	-	-	-

Conclusion

In this study, it was aimed to determine the amount of water used for agricultural purposes at the downstream point in HEPP Projects. As a tool, an integrated approach with remote sensing and geographic information systems was used.

The total area of arable land and the potential farmland area are calculated as 625599 m^2. In the area, Cereal was a major crop pattern. The highest irrigation water requirement was found in June with 185,165 m^3. This approach was a successful tool for this purpose.

When all evaluation criteria are considered, it may be proposed to use the Blaney-Criddle method to determine the irrigation water requirement for HEPP basin.

Penman-Monteith equation can also be used reliably to compute irrigation water requirement in the places which resemble the study area.

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Araştırma Makalesi/*Research Article (Original Paper)*

New Chromosome Counting of A Natural Hybrid (*P. Cerasifera*× *P.Spp.*) or a New Member in *Prunus* Genus and Chromosomal Similarities with Two Closely Related Species

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Abstract: Chromosome number and karyotype morphology including chromosome length, centromere position, arm ratio, secondary constriction and staining properties by Aceto-Iron-Hematoxylin were analyzed for *Prunus* species of Apricot (*Prunus armeniaca* L.), Plum (*P. cerasifera* Ehrh.) and a natural hybrid or new species known as "TANASGOL" (*P. cerasifera*× *P. spp.*). The obtained root tips from germinated seeds at 4-6 °C and young seedlings were pretreated in Hydroxyquinoline solution with 50 drops of DMSO. All species had 2n=2x=16 very small chromosomes and were uniformly stained. The ranges of chromosome length in the three species were as follows: Apricot 1.50 to 2.70 µm; Plum 1.5 to 2.77 µm; and "TANASGOL" 1.35 to 2.82 µm. Other features of the chromosomes, such as variation in the length of chromosomes within species, number of satellites and size, constrictions, centromere placements, and karyographs were applied to distinguish species. Chromosomal similarities between "TANASGOL" and Plum were more evident.

Keywords: Cytogenetics, Karyotype, Natural Hybrid, *Prunus*, TANASGOL

Introduction

Chromosome counts are an important biological character necessary to multiple fields of plant sciences, from plant breeding and genetics to systematics and taxonomy. A karyotype describes the phenotypic aspects of the chromosome complement of a species in terms of number, size, arm ratio (or centromere position), and other landmark features of its chromosomes(Levin 2002). Karyotypes are dynamic structures evolving through numerical and structural changes (for a comprehensive overview on chromosomal changes in plants, see (Levin 2002).

Prunus L. (Rosaceae) consists of over 200 species of deciduous and evergreen trees and shrubs with several members that are economically important fruit and nut crops. These include peach (*P. persica* (L.) Batsch), apricot (*P. armeniaca* L.), almond (*P. dulcis* D.A.Webb), and sweet cherry (*P. avium* L.) (Chin et al. 2014). Basic chromosome number in *Prunus* is x = 8, Polyploidy played an important role in the process of evolution of new species or strains. Natural interspecific hybridization is responsible for polyploidy of *Prunus* during phylogeny. This is the main cause for self-sterility and intersterility in this genus. Somatic chromosome numbers (Rehder 1947; Zohary 1992; Tropicos.org. 2011) of different *Prunus* species vary from diploid to hexaploid. Under plum, *P. salicina* is 2n = 16, 32, *P. simonii* is 2n = 16, *P. domestica* is 2n = 48, *P. spinosa* is 2n= 32, 24, 40, 48, *P. cerasifera* is 2n = 16, 17, 24, 32, 48, *P. insititia* is 2n = 24, 48, *P. x dasycarpa* is 2n= 16 and *P. americana* is 2n= 16. Most of the American species are 2n= 16. The European plum (*P. domestica* including *P. domestica spp. insititia*) is hexaploid (2n=2x=48), the sloe tetraploid (2n=4x=32), whereas the Japanese plum, as well as most of the other *Prunus* species belonging to the group of plums, are diploid (2n= 2x= 16). Therefore, the chromosome status has to be considered in interspecific hybrids.

Botanical classification of species within this genus is sometimes controversial, partly because of the easiness of interspecific hybridization (Casas et al. 1999), which creates numerous intermediate types, and fades the limits between species (Dosba et al. 1994). On the other hand, the somatic chromosome analysis

of the genus *Prunus* is very difficult due to poor stainability, stickiness and tendency to overlap at metaphase and diffuse appearance of primary and secondary constrictions of the chromosomes.

In this study, an efficient squash technique, developed by authors for determining precise somatic chromosome numbers and obtaining karyomorphological details in plants were applied to three stone fruit germplasm Apricot (*Prunus armeniaca* L.), Plum (*P. cerasifera* Ehrh.) and a natural hybrid known as "TANASGOL" (*P. cerasifera* × *P. spp.*) of *Prunus* genus.

Materials and Methods

Plant materials

Seeds and young seedlings of three species of stone fruit trees; Apricot (*Prunus armeniaca* L.), Plum (*P. cerasifera* Ehrh.) collected from the research station in Karaj, Iran and a natural hybrid (*P. cerasifera* × *P. spp*) known as "TANASGOL", cultivated in regions of, Tabriz, Mashhad, and Karaj (Mirabdulbaghi et al. 2011), were used for karyological studies.

Chromosome analysis

The important step in the cytogenetic study of plants is to obtain cells that are active in cell division and are available. For this purpose, Seeds of different species were removed from hard shells (pericarps) and were germinated (Zarifi 2013). Their root tips meristem were used in the chromosomal study (Zarifi 2013). One-year-old young seedlings were transferred from the ground to black pots (10×10 cm^3) (Zarifi and Güloğlu 2016) and were grown inside a greenhouse with a temperature between 20-26 °C. From each species 2 to 3 seedlings that had grown very well, were used for root sampling.

The obtained root tips from germinated seeds and young seedlings were pretreated in 2mM solution of 8-Hydroxyquinoline at about 4°C for 3-4 h (Agayev et al. 2010). In this study, 50 drops of dimethyl sulfoxide (DMSO) were added to 400 ml of pretreatment solution then roots were treated, and were fixed in Lewitsky solution (Zarifi and Güloğlu 2016) at a temperature of 4-10 °C for 30-36 (Agayev et al. 2010). Roots were rinsed in distilled water then stained by Aceto-Iron-Haematoxylin at 30-34°C for 15- 20 h (Agayev 2002; Zarifi et al. 2006; Agayev et al. 2010; Zarifi and Güloğlu 2016). Preparations were studied with research Microscope (Ni-U) made in Japan, and well-spread metaphase plates of chromosomes were photographed by a digital camera.

The chromosomes parameters were calculated on five metaphases plates, based on magnification by application of MicroMeasure 3.3 software (Reeves 2001). Arm rations, average lengths, relative lengths, and standard errors were calculated using Excel 2013 (Microsoft Excell) and SPSS v16. (http://www.ibm.com/analytics/us/en/technology/spss). The chromosomes were classified according to Levan et al. (1964) considering their centromere position (Levan et al. 1964).

Results

Chromosomal studies on three species of stone fruit trees; Apricot, Plum and a natural hybrid or new member of *Prunus* genus known as "TANASGOL", showed that the somatic chromosomes number of these species were 2n=2x=16, diploid and did not differ in ploidy, but species differed significantly in chromosome morphology and type. Differences occurred in chromosome size, the position of centromeres, secondary constrictions or satellites, tertiary constrictions, and staining ability of the chromosomes. Characteristics of chromosomes for each species are described below. Metaphase plates and quantitative data of characteristics of chromosomes are shown in Figure 3 and Table 1 and Table 2, respectively for each species.

Apricot (Prunus armeniaca L.)

Chromosome number in *Prunus armeniaca* was 2n=2x=16 (Figure 3). This is in agreement with previous reports (Zohary 1992; Doroftei et al. 2010; Tropicos.org. 2011; Parveen 2015). Eight pairs of homologues were identified in regard to the morphology of the chromosomes. Differences in size were significant and

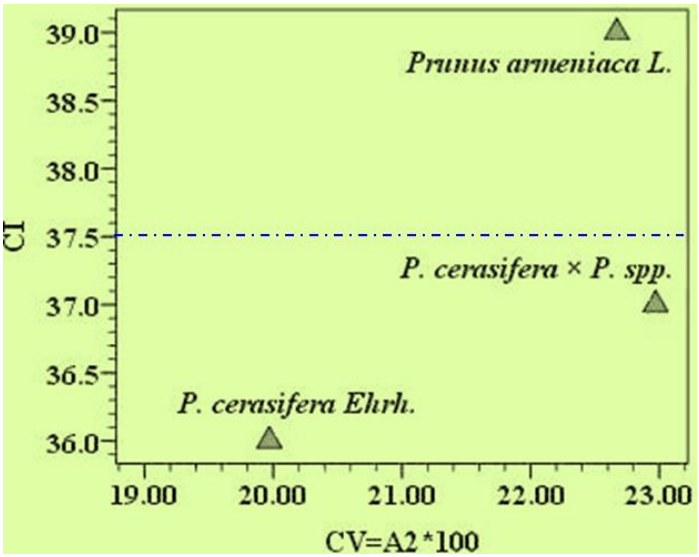

Figure 1. Scatter diagrams for Apricot, Cherry Plum and "TANASGOL"; the CV_{CL} parameter against the CV_{CI} parameter.

ranged from 1.5- 2.70 µm (Table 2). The karyotype of Apricot (*P. armeniaca*) included a pair of very small chromosomes and a pair of very large chromosomes than the rest, which was quite visible, and both pairs were metacentric. Two pairs of sattelited chromosomes were completely visible by staining Aceto-Iron-Hemaetoxyline (Zarifi and Güloğlu 2016), and their type was submetacentric (Figure 2 and Figure 3). Satellites were observed clearly at the end of the short arms (Pairs 2 and 3). The small size of chromosomes in this genus and the use of classical methods have not been shown satellite chromosomes yet. These chromosomes are cytogenetic markers of this species. The chromosomes types of Apricot were metacentric and submetacentric; number of metacentric chromosomes (10m) was further than submetacentric chromosomes (2sm + **4sm**^{sat}) (Table 1 and Table 2).

Plum (P. cerasifera Ehrh.)

The somatic chromosome number in *P. cerasifera* was 2n=2x=16 as diploid (Figure 3), which confirms previous reports(Eremin and Rassvetaeva 1992; Chen 1993, 2003; Salesses and Bonnet 1993; Yamamoto 2012). The chromosomes were mostly small with their length ranging from1.54- 2.77 µm (Table 2). In the plum karyotype, a pair of metacentric chromosomes, significantly longer (Pair 1) and a pair of submetacentric chromosomes shorter than others were found (Table 2 and Figure 3). Four pairs of chromosomes in the complement were metacentric (8m) and two pairs were submetacentric (4sm) type. Two pairs of the SAT chromosomes with different type were identified in the complement and these SAT located in different chromosomes that numbered 2 (sub-telocentric **2st**^{sat}) and 4 (submetacentric **2sm**^{sat}), respectively (Table 2, Figure 2 and Figure 3). The size of the Satellites was similar in both pairs.
The existence and number of satellite chromosomes in this species have not previously been reported, but this study clearly revealed these chromosomes.

"*TANASGOL" (P. cerasifera × P. spp) a natural hybrid of Prunus genus*

Cytogenetic studies and chromosomal counts on this natural hybrid tree between two different species (*P. cerasifera × P. spp*), which is endemic of Iran, were not performed before. In this study, for the first time, the chromosomal features of this hybrid have been studied. The somatic chromosome number observed in our study was 2n=2x=16 and diploid, it was similar to other studied species (Figure 3 and Table 2). The chromosomes were small with their length ranging from 1.35- 2.82 µm (Table 2). Two pairs of the SAT chromosomes were clearly identified in the complement with the same chromosome types (sub-telocentric (st)) and these SAT located in short arms of different chromosomes that numbered 2 and 4, that these chromosomes were very similar to plum species (*P. cerasifera*). But the SAT chromosome number 4 in plum was submetacentric (Figure 2 and Table 2). The most chromosome types in "TANASGOL" were metacentric (12m), but satellite chromosomes were subtelocentric (**4st**^{sat}).

Table 1. Chromosome number and karyotype formula of Apricot (*Prunus armeniaca* L.), Plum (*P. cerasifera* Ehrh.) and a natural hybrid between them (*P. cerasifera* × *P. spp*) "TANASGOL" from Iran.

No.	Species	Name	Location	Chromosome No.	Karyotype Formula
1	*Prunus armeniaca* L.	Apricot	Research Station Karaj,Iran	2n= 2x= 16	10 m+ 2sm + **4sm**^{sat}
2	*P. cerasifera* Ehrh.	Plum	//	2n= 2x= 16	8m+ 4sm + **2sm**^{sat} + **2st**^{sat}
3	*P. cerasifera* × *P. spp*	"TANASGOL"	Tabriz, Mashhad, Iran	2n= 2x= 16	12m+ **4st**^{sat}

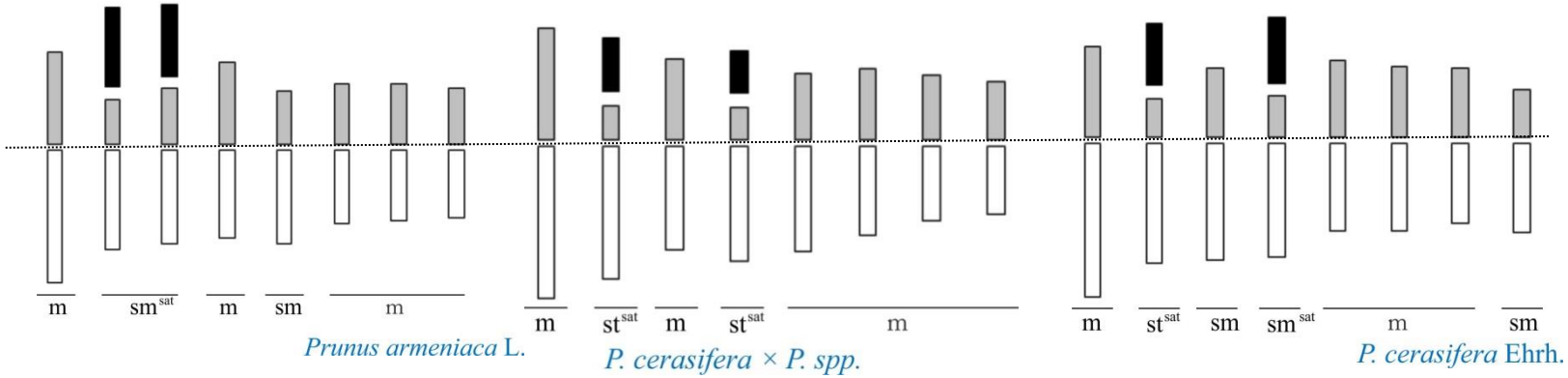

Figure 2. Haploid ideogram of Apricot (*Prunus armeniaca*), Cherry Plum (*P. cerasifera*) and the natural hybrid or new species "TANASGOL"(*P. cerasifera*× *P. spp.*) from Iran accordance to chromosomes of table 2and fig. 2.

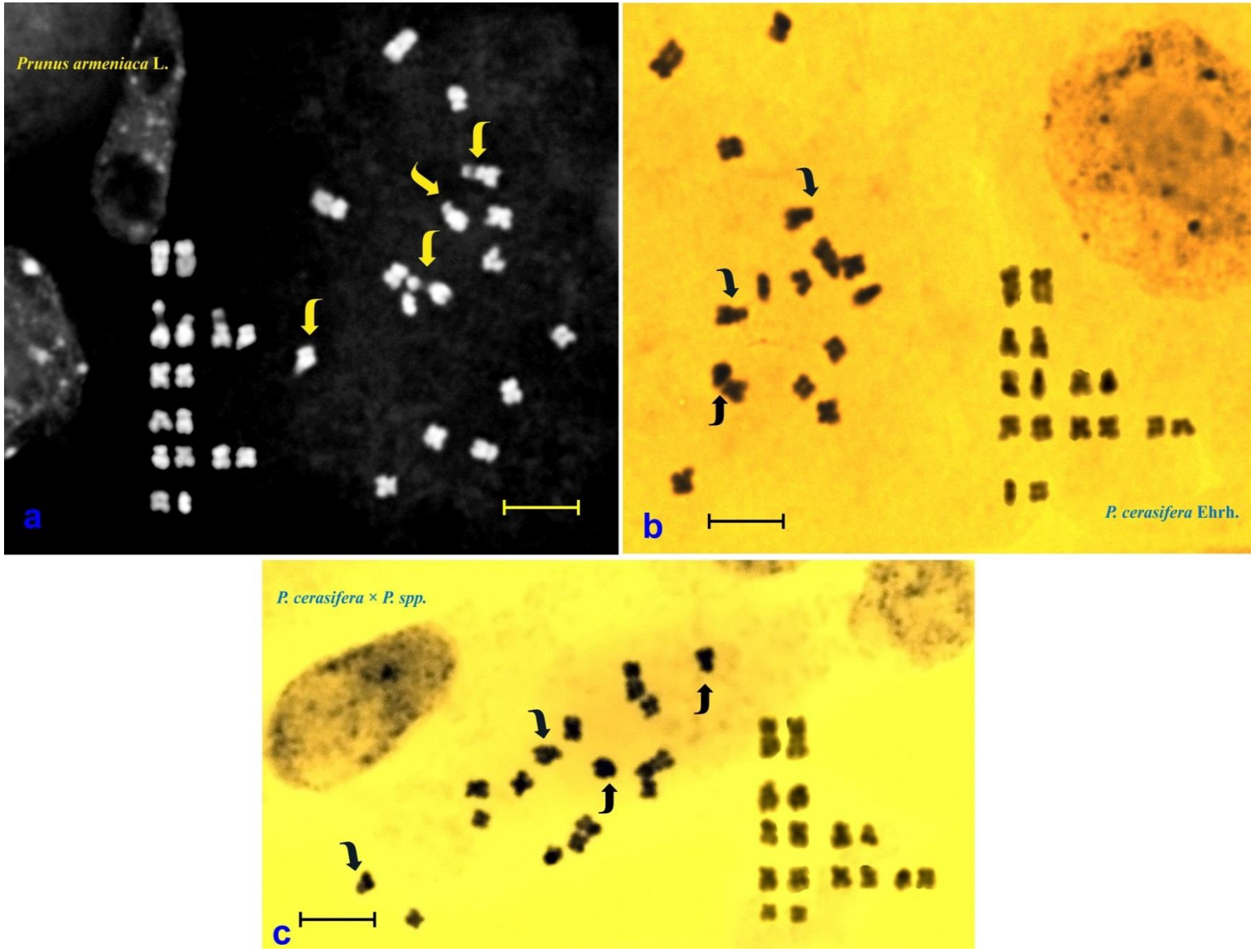

Figure 3. Somatic chromosomes of root tip meristem obtained from germinated seeds and young seedlings and karyogram of them. **a:** Apricot (*P. armeniaca*); **b:** Cherry Plum(*P. cerasifera*); **c:** TANASGOL; (2n=2x=16), Bar=5 µm

Table 2. Measurements (mμ) of somatic chromosomes for Apricot (*Prunus armeniaca* L.), Plum (*P. cerasifera* Ehrh.) and natural hybrid between them (*P. armeniaca* × *P. cerasifera*) "TANASGOL" from Iran; L: Length of the long arm of the chromosome (μm), S: Length of the short arm of the chromosome (μm), CI: Centromere index, (m: Metacentric, sm: Submetacentric, st: Submetacentric); Centromere type according to Levan et al. (1964), sat: Satellites, L% and S%: Indices that express the contribution of each arm of each chromosome to the total length of karyotype, RL%: Relative length of chromosomes.

1. *Prunus armeniaca* L.										
Pair No.	**Total (L+S) μm**	**Long arm (L) μm**	**Short arm (S) μm**	**Armratio (AR=L/S)**	**CI (S*100/(L+S))**	**Sat**	**Type**	**L%**	**S%**	**RL%**
1	2.71± 0.08	1.6± 0.08	1.11± 0.04	1.45± 0.10	41	-	m	9.91	6.88	16.79
2	**2.69± 0.13**	**1.2± 0.07**	**0.54± 0.02**	**2.23± 0.14**	**20**	**0.95**	sm	7.43	3.35	16.67
3	**2.24± 0.27**	**1.13± 0.08**	**0.67± 0.06**	**1.75± 0.20**	**32**	**0.87**	sm	7.00	4.15	13.88
4	2.05± 0.11	1.06± 0.05	0.99± 0.06	1.08± 0.03	48	-	m	6.57	6.13	12.70
5	1.77± 0.09	1.13± 0.06	0.64± 0.04	1.76± 0.05	36	-	sm	7.00	3.97	10.97
6	1.61± 0.04	0.89± 0.03	0.72± 0.04	1.27± 0.10	44	-	m	5.51	4.46	9.98
7	1.57± 0.06	0.84± 0.04	0.73± 0.02	1.16± 0.05	46	-	m	5.20	4.52	9.73
8	1.50± 0.05	0.82± 0.04	0.68± 0.02	1.21± 0.03	45	-	m	5.08	4.21	9.29
Mean	**2.02± 0.08**	**1.08± 0.04**	**0.76± 0.03**	**1.49± 0.06**	**39**		m			
Total (TC)= 16.14								**53.72**	**37.67**	
2. *P. cerasifera* Ehrh.										
1	2.77± 0.08	1.74± 0.06	1.03± 0.03	1.69± 0.07	37	-	m	10.81	6.40	17.22
2	**2.50± 0.11**	**1.36± 0.07**	**0.43± 0.03**	**3.23± 0.28**	**17**	**0.71**	st	8.45	2.67	15.54
3	2.09± 0.07	1.32± 0.05	0.77± 0.04	1.73± 0.08	37	-	sm	8.20	4.79	12.99
4	**1.88± 0.14**	**1.29± 0.06**	**0.46± 0.04**	**2.95± 0.4**	**25**	**0.74**	sm	8.02	2.86	11.68
5	1.86± 0.05	0.98± 0.02	0.87± 0.04	1.14± 0.05	47	-	m	6.09	5.41	11.56
6	1.77± 0.05	0.98± 0.05	0.79± 0.01	1.24± 0.06	45	-	m	6.09	4.91	11.00
7	1.68± 0.07	0.9± 0.05	0.78± 0.03	1.16± 0.06	46	-	m	5.59	4.85	10.44
8	1.54± 0.07	1± 0.05	0.54± 0.03	1.88± 0.08	35	-	sm	6.22	3.36	9.57
Mean	**2.01± 0.06**	**1.2± 0.04**	**0.71± 0.03**	**1.88± 0.12**	**36**		**sm**			
Total (TC)= 16.09								**59.48**	**35.24**	
3. *P. cerasifera*× *P. spp*										
1	2.82± 0.07	1.63± 0.07	1.19± 0.04	1.38± 0.08	42	-	m	10.56	7.71	18.26
2	**2.37± 0.16**	**1.43± 0.06**	**0.36± 0.04**	**4.24± 0.5**	**15**	**0.58**	st	9.26	2.33	15.35
3	1.97± 0.07	1.11± 0.04	0.86± 0.05	1.3± 0.09	44	-	m	7.19	5.57	12.76
4	**1.88± 0.12**	**1.23± 0.1**	**0.35± 0.03**	**3.72± 0.54**	**18**	**0.46**	st	7.97	2.27	12.18
5	1.84± 0.08	1.12± 0.08	0.71± 0.04	1.6± 0.18	39	-	m	7.25	4.60	11.92
6	1.72± 0.08	0.96± 0.05	0.76± 0.05	1.27± 0.1	44	-	m	6.22	4.92	11.14
7	1.49± 0.04	0.8± 0.03	0.69± 0.02	1.17± 0.05	46	-	m	5.18	4.47	9.65
8	1.35± 0.03	0.72± 0.02	0.63± 0.03	1.15± 0.05	47	-	m	4.66	4.08	8.74
Mean	**1.93± 0.07**	**1.13± 0.05**	**0.69± 0.04**	**1.98± 0.19**	**37**		sm			
Total (TC)= 15.44								**58.29**	**35.95**	

Conclusion

The chromosomal features in somatic cells are more constant morphologically comparing with meiotic cells. Karyotype marker structures, such as satellited chromosomes, constrictions, centromeres position/location, and arm length of the chromosomes are exposed more accurately in mitosis. Hence, the karyotype analysis of species is often done on somatic cells in mitosis stage.

Comparison of the results in this study with other studies (Parveen 2015; Yamamoto 2012; Biswajit Das 2011; Doroftei & Arcuş, Mariana, Trandafirescu, Marioara, Moldoveanu 2010; Salesses & Bonnet 1993; Zohary 1992; Eremin & Rassvetaeva 1992; Salesses & Bonnet 1993; Bradford & Bradford 1991; Salesses 1975; Tropicos.org. 2011), showed an agreement only in the chromosome count of apricot species (*Prunus armeniaca* L.), plum (*P. cerasifera* Ehrh.) and *P. cerasifera* × *P. spp.* as: 2n=2x=16, but, no reports were for the existence and number of satellites in the chromosomes of these species, location of satellites, length and types of chromosomes. So, the chromosomes features of *P. cerasifera* × *P. spp*., "TANASGOL", were analyzed in this study for the first time and were compared with the karyotypes of two species that are likely to be its ancestors.

In the chromosomal complex of studied species, all of which were diploid (2n=2x=16), two pairs satellited chromosomes (**4sm^{sat}**, (**2sm^{sat} +2st^{sat}**) and **4st^{sat})** were observed respectively in apricot, plum, and "TANASGOL", and the length of these satellites in apricot were greater than the rest. Satellites were located on the short arm of relatively large chromosomes of species. No report found for these marked chromosomes in previous works.

Differences of the results obtained in this study for satellite containing chromosomes and also other characteristics of the karyotyping shown up here, with other studies, are not due to the different plant materials and biological factors, but to newly improved techniques of cytogenetic studies and laboratory staining procedure with hematoxylin which applied by Zarifi et al. 2016, and also how to prepare microscopic specimens, the quantitative characteristics and morphology of the chromosomes are accurately and clearly expressed(Figure 2 and Figure 3).

The "TANASGOL (a naturally occurring hybrid) parents", is a controversial subject, so that in some sources it has been reported as a natural hybrid between apricot and plum (*P. armeniaca* × *P. domestica*), (Mirabdulbaghi et al. 2011). However, this claim cannot be correct, since the number of chromosomes of the species *P. domestica* L. is reported to be 2n=6x=48 (Rehder 1947; Tropicos.org. 2011; Zohary 1992). But the number of chromosomes of the two species used in this study, apricot (*P. armeniaca* L.) and plum (*P. cerasifera* Ehrh.) both diploid species is 2n=2x=16, And so it's very likely that the "TANASGOL" will be hybrid of them. In terms of the morphology of chromosomes, the similarity of this hybrid to *P. cerasifera* is also greater. In the analysis of the karyotypes symmetry and asymmetry parameters of these species, especially new parameters CV_{CL} (Coefficient of Variation of Chromosome Length) and CV_{CI} (Coefficient of Variation of Centromeric Index) and also their karyotype formula showed that the chromosomal similarity between "TANASGOL" and *P. cerasifera* Ehrh is more evident and their karyotype both are asymmetric and in their karyotypes formula they have sub-telocentric chromosomes and overlapping of their available karyographs could be seen (Figure 1). So it seems likely that the probability of considering apricot as one of the parents of "TANASGOL" is not strong, and another member of *Prunus* must be involved in this natural hybridization. This candidate is more similar to *P. cerasifera* with chromosome number; 2n=2x=48. On the other hand, it seems that "TANASGOL" might be a new species in *Prunus* genus or a sub-species with a high degree of similarity to *P. cerasifera*. It is more acceptable as sub-species, but needs more studies in depth at the molecular level by means of molecular markers and controlled inter-specific hybridization.

European Plums (*P. domestica* L.) are originated from a natural hybridization between a diploid *P. cerasifera* (2n=2x=16) and a tetraploid *P. spinosa* (2n=4x=32). The progeny is a triploid plant, of course, which due to the doubling of the number of its chromosomes in nature, the hexaploid plum (*P. domestica* L.; 2n=6x=48) is created (Salesses 1975; Zohary 1992; Faust et al. 1998; Rieger 2006).

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Araştırma Makalesi/*Research Article (Original Paper)*

The Difficulties Encountered During Establishment and Implementation Of Iso 22000:2005 Food Safety Management System in Olive Oil Plants

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Abstract: ISO 22000:2005 Food Safety Management System is one of the major elements that created added value to olive oil in the sector and all the organizations involved in the food chain could establish this system with the help of a consultancy firm or on their own. The aim of this study is to determine the difficulties encountered by companies that have established or are planning to establish the ISO 22000:2005 Food Safety Management System. In this study, the opinions of the olive oil companies that have operated as olive oil mills and bottling plants about the ISO 22000:2005 Food Safety Management System. Because the statements related to the difficulties were composed of data containing a large number of variables were reevaluated with the help of factor analysis. The results were analyzed using single sample t-test and independent sample t-test. The test results showed that all companies are not indecisive about the difficulties encountered in setting up the ISO 22000:2005 system and that the problems they encountered when installing the system were not different from each other, except for "the difficulty of adapting ISO 22000:2005".

Key words: ISO 22000:2005, food safety, olive oil, factor analysis

Zeytinyağı İşletmelerinde ISO 22000:2005 Gıda Güvenliği Yönetim Sisteminin Kurulması ve Uygulanmasında Karşılaşılan Zorluklar

Öz: Zeytinyağı sektöründe zeytinyağına katma değer yaratan önemli unsurlardan biri ISO 22000:2005 Gıda Güvenliği Yönetim Sistemi olup, gıda zincirinde yer alan tüm kuruluşlar bu sistemi bir danışmanlık firmasının yardımıyla veya kendi olanaklarıyla işletmelerinde kurabilmektedir. Bu çalışmanın amacı, ISO 22000:2005 Gıda Güvenliği Yönetim Sistemini işletmelerinde uygulayan ve uygulamayı düşünen firmaların karşılaştığı sorunları belirlemektir. Bu çalışmada, yağhaneler ve şişeleme tesisleri olarak faaliyet gösteren zeytinyağı firmalarının ISO 22000:2005 Gıda Güvenliği Yönetim Sistemi hakkındaki görüşleri alınmıştır. Zorluklarla ilgili ifadeler çok sayıda değişken içeren verilerden oluştuğu için faktör analizi yardımıyla tekrar değerlendirilmiştir. Analiz sonucu elde edilen ifadeler tek örneklem t-testi ve bağımsız örneklem t-testi ile analiz edilmiştir. Test sonuçları tüm işletmelerin ISO 22000:2005 sisteminin kurulmasında karşılaşılan zorluklar hakkında kararsız olmadıklarını, sistemi kurarken karşılaştıkları sorunların "ISO 22000:2005 adapte etme zorluğu" hariç birbirinden farklı olmadığını göstermiştir.

Anahtar kelimeler: ISO 22000:2005, gıda güvenliği, zeytinyağı, faktör analizi

Introduction

Quality and safety are important for the food industry. While quality is important for consumer satisfaction, safety is necessary to protect public health. Safe food can be defined as food material which is suitable for consumption and has not lost its nutritional value regarding its physical, chemical and microbiological properties (SPO, 2001). Therefore, food safety is related to the presence of food-borne hazards at the point of consumption since food safety hazards can occur at any stage in the food chain. Thus, a combined effort of all stages, in other words "a standart" is required. International Organization For Standardization (ISO) is an independent, non-governmental international organization with a membership of 161 countries and it is centered in Geneva/ Switzerland. ISO and its members bring together experts to share knowledge and develop voluntary, consensus-based, market relevant international standards that support innovation and provide solutions as global challenges. The ISO 22000:2005 Food Safety Management System was published in September 2005 and set as an international common standard which deal with food safety in food chain. It is a general derivative of ISO 9001 (Quality Management) and involves HACCP (Hazard Analysis Critical Control Point) principles whose implementations were published with the "Hygiene Regulation of 852/2004 / Ec Food Legislation" in 2004 and

have been accepted as the basic system of food safety in the European Union (Kocak, 2007). ISO 22000:2005 is more comprehensive than HACCP because of pre-requisite programs, documentation and traceability.

In Turkey, TS EN ISO 22000:2005 Food Safety Management System implements the standards for all establishments in the food chain with a decision taken at the TSE Technical Committee meeting dated April 24, 2006. Dated June 13, 2010, no:5996, The Republic of Turkey, The Ministry of Food, Agriculture and Livestock published a legislation and mentioned that "Food and feed plants are obliged to establish and implement a food and feed safety system based on hazard analysis and critical control points principles". Therefore, the establishment and application of HACCP principles became compulsory in food and feed plants by this government law.

Today, olive oil production has an important role in many countries and Turkey was in sixth place, with an average of 177 thousand tonnes of olive oil in the world production in 2016/2017 season (The Republic of Turkey Ministry of Customs and Trade, 2018). More than 50% of the total olive oil production of Turkey is in the Aegean region. The olive oil consumption of Turkey was approximately 140 thousand tonnes in this season and had an olive oil consumption with an average of 2 kilos per/person annually which was the least among Mediterranean countries. Exported olive oil with packing or bulk and total exports of Turkey was 44.5 thousand tonnes in 2016/2017 season and almost 35% of it was exported with package (Aegean Exporters Associations, 2018).

Packaged olive oil, especially extra virgin olive oil, with low acidity and high nutrition value provide added value to Turkish economy. In order to compete with strong domestic competition, as well as international markets and increase the production of packaged extra virgin olive oil, it is very important to establish ISO 22000:2005 system in their plants. Thus, the aim of the study is to determine the problems of the companies encountered in the olive oil sector which have established or considering establishing to ISO 22000:2005 Food Safety Management System and to find the solutions.

Some of the studies have shown that olive and olive oil sectors had difficulties about ISO 22000:2005 Food Safety Management System. For example, in a study conducted by Cukur et all. (2011), olive oil companies identified the outstanding problems of quality food safety system and it was found that the most important problem was "the lack of seminars and training studies". According to Bas et all. (2005), the most important problem was "the lack of prerequisite programs". The other key barriers were identified for all food businesses as "the lack of knowledge about HACCP, lack of time, staff turnover, lack of employee motivation, complicated terminology and lack of personnel training. In a study carried out by Tunalioglu et all. (2011), showed that food safety system documentation was too extensive and qualified staff was required in the certification process. Karaman et al. (2012) stated that the constraints that companies had namely difficulties imposed by the managerial level, technical aspects and even constraints arising from within the organization. According to Teixeira and Sampaio (2013), the ISO standard's procedures, constraints imposed by time insufficiency, as well as the employees skills and reluctance to change. Macheka et al. (2013) claimed that other difficulties triggered by the implementation of ISO 22000 were an inappropriate infrastructure that cannot support the application process and the absence of financial funds and of food safety procedures. Furlan and Morozini (2013) found three major constraints limiting the dissemination and usage of ISO 22000. It is not a well-known standard; many food companies were unaware of its potential and they also perceive high costs and hard time associated to the adoption. Similar findings were shared by Escanciano and Santos-Vijande (2014), unreasonable formality, the large amount of documents that were needed, the lack of standard recognition from the customers' part, communication issues at the company's level and the trouble with outside consultancy. Paunescu et all., (2018) said that the more prevalent difficulties were employees' qualification, costs associated with food safety management system implementation and legal requirements, followed closely by internal resistance to change. Hence, the review of past literature has indicated that the most important problems to changes in food safety were attributed to the employee and managerial commitment issues and the lack of education and training programs etc.

Material and Methods

There were 63 olive oil company members in Aegean Region Chamber of Industry in 2011. 45 of the companies accepted our request and answered the questionnaire form face-to-face. Therefore, data was obtained from authorized officer in the olive plants. The questionnaire consisted of demographics, multiple choice, open-ended, and likert scale questions. A total of 93 questions were asked to participants and the results of some questions were presented with simple descriptive statistics tables. Some of them were analyzed using some statistical techniques. One of them was factor analysis which was originally developed by Spearman at the beginning of

the 20th century, and which is a multivariate technique that is commonly used to reduce a large number of variables into fewer numbers of factors. Through factor analysis, it is possible to explain the relation between a large number of variable clusters invert to a small number of factors (Ozdamar, 1999; Tavsancıl, 2002). In the study it had been applied to decrease and group 25 likert scale questions to 6 factors about encountered difficulties dealing with ISO 22000:2005. In addition, the factor analysis results were analyzed by both one-sample t test and independent sample t test and were interpreted.

Results and Discussion

Nearly half companies (24 company) of the total 45 olive oil companies in Izmir Province that participated in the survey had no filling facility; they were operating as olive oil mills. Nine of them were just buying olive oil as bulk and making packed olive oil in bottling plant. Twelve companies were handling both as olive oil mills and as packaging facilities. Almost 40% of them had been operating in the sector for over 30 years (Table 1).

Table 1. Activity years of olive oil plants

Years	N	%
<5 years	9	20.0
5-15 years	7	15.6
15-30 years	11	22.4
30-50 years	9	21.0
>50 years	8	18.8
Total	44	97.8
Missing Value	1	2.2
General	45	100.0

More than third of companies (35.5%) had already established and was implementing ISO 22000 system in their plants while 13.3% of them were planning to establish the system soon. (Table 2). According to a similar study conducted in Aydın province in the same time, 39% of table olive companies had also ISO 22000 certification system (Tunalioglu et all., 2011).

Tablo 2. Current status of companies about ISO 22000:2005 in their plant

Current status	N	%
We have ISO 22000 documentation and we are fully implementing the system	15	33.3
We are implementing the system, we are in the documentation phase	1	2.2
We have HACCP document, not yet passed ISO 22000 system	1	2.2
We plan to implement the system soon	6	13.3
We do not implement the ISO 22000 system	14	31.1
Total	37	82.2
Missing Value	8	17.8
General	45	100.0

The difficulties encountered in establishing and implementing the ISO 22000:2005 Food Safety Management System was assessed on a 5-point Likert scale (Table 3). Number of answers (N) was 45. The difficulties were listed from strongly disagree (1) to strongly agree (5). According to the table, the most important difficulties were defined as “high workload and lack of trained personnel”. Similar problems had been reported by other authors in the past literature (Furlan and Morozini, 2013; Escanciano and Santos-Vijande, 2014; Teixeira and Sampaio, 2013; Bas et all., 2005; Tunalioglu et all.; 2011; Teixeira and Sampaio; 2013; Paunescu et all., 2018). While the statements “high cost or too expensive certification” were determined as the most important outstanding problems according to some authors (Tunalioglu et all., 2011; Macheka et al., 2013; Furlan and Morozini, 2013; Paunescu et all., 2018), “high cost” statement was found as one of the unimportant difficulty in our study (Table 3).

Before using factor analysis, Kaiser-Meyer-Olkin (KMO) and Bartlett's test were done whether the sample was big enough. According KMO test (0.699), the measure of sampling adequacy was acceptable. Because the sample is adequate if the value of KMO is greater than 0.5 (Field, 2000). The KMO index ranges from 0 to 1, with 0.6 suggested as the minimum value for a good factor analysis (Tabachnick & Fidell, 2001). This value showed that the sample with 25 difficulties was adequate for the level of representation. Also, Bartlett's test was significant with a value of 0.000. The Bartlett’s test of sphericity should be significant ($p<.05$) for the factor analysis to be considered appropriate (Pallant, 2005). Significance value is calculated 0.00 which is a greater than 0.05 ($p<.05$).

The value showed that the data come from a multivariate normal distribution and was suitable for factor analysis. The eigenvalue of a factor represents the amount of the total variance explained by that factor which is called the Kaiser's criterion. Only the factors with an eigenvalue of 1.0 or more are retained for further investigation (Pallant, 2005).

Table 3. Opinions of the participants about encountered difficulties about ISO 22000:2005

Difficulties*	Mean	Standart deviation
Having enough information when preparing documentation is an important difficulty	2.05	0.947
Analysis and documentation costs are high	2.08	0.957
Workplace management does not adopt food safety management system	2.14	1.160
Employees do not adopt food safety management system	2.26	1.149
The building infrastructure and the working environment are not suitable for this system	2.35	1.122
Working habit of staff with documentation system has not developed enough	2.54	1.002
Lack of qualified staff	2.55	1.041
Systematic certified supplier is hard to find	2.56	0.808
Finding accredited laboratories for analysis is difficult	2.64	1.226
Separation of requests by related personnel influences the maintenance of the system	2.71	1.255
It is difficult to provide traceability from the raw product to the final product	2.73	1.109
The workforce that can be allocated for work is sufficient	2.88	1.166
Customers prefer different certification firms	2.83	0.844
Lack of communication	2.85	1.276
It is difficult to test the withdrawal method	2.89	0.894
Timeless internal audit practices create problems	3.10	0.917
System installation and documentation costs too high	3.10	0.982
Advisory services are not provided at sufficient level	3.15	1.099
There is lack of training and inadequacy in personnel	3.24	1.067
Employees are not actively involved in this system	3.32	1.192
It is difficult to provide adequately trained personnel due to seasonal employee work	3.35	1.098
Workload is too high	3.44	0.940

*1= Strongly Disagree / 5= Strongly Agree

According to the factor analysis, statements were grouped and renamed into 6 factors and total variance explained was 72.79%. Factor analysis reliability test was found as 0.829 since Cronbach Alpha is more than 0.7, the reliability of the test is suitable to measure the scale of variables (Kurnaz and Yiğit, 2010). After determining the number of factors, since the indecisive of the companies wouldn't allow us to comment or suggest any solutions, new statements (factors) were analyzed with one-sample t-test. The test value was 3 (neither agreed nor disagreed). The null hypothesis (Ho) was that the companies answered indecisive response in the difficulties encountered. According to the test results in Table 4, it was determined that companies were not indecisive about all difficulties deal with ISO 22000:2005 (Ho-reject, p = 0.000).

Table 4. One-sample t test result of difficulties encountered towards ISO 22000:2005

Factors*	**Test Value = 3**					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Difficulty of adapting ISO 22000:2005	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004
Lack of education	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004
Lack of qualified staff	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004
Difficulties in the application	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004
No enough importance	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004
Highly cost	-20.357	44	0.000	-3.00000000	-3.2969996	-2.7030004

*1= Strongly Disagree / 5= Strongly Agree

New statements formed by factor analysis were analyzed for olive oil mills (bulk production) and bottling plants (packaged production) in Table 5 using independent sample t test. The null hypothesis was that there was no difference in the difficulties encountered among the companies. Only for the first statement (the difficulty of adapting ISO 22000:2005) Ho was rejected, which means olive oil mills and bottling plants had different views

for the first difficulty (Ho–reject, p = 0.00). “The lack of education, lack of qualified staff, difficulties in the application, no enough importance and highly costs” statements are not different from each other (Ho-acceptable, p> 0.05) (Table 6).

Table 5. Group statics for independent samples t test

Group Statistics

Factors*	Production Plant Type	N	Mean	Std. Deviation	Std. Error Mean
Difficulty of adapting ISO 22000:2005	bulk production	24	0.5649139	0.74888130	0.15286476
	packaged production	21	-0.6456159	0.82730838	0.18053349
Lack of education	bulk production	24	-0.1347902	0.84230819	0.17193544
	packaged production	21	0.1540459	1.13462580	0.24759565
Lack of qualified staff	bulk production	24	0.1300109	0.64563925	0.13179056
	packaged production	21	-0.1485838	1.27560106	0.27835897
Difficulties in the application	bulk production	24	-0.0388035	1.08913325	0.22231839
	packaged production	21	0.0443468	0.88429930	0.19296993
No enough importance	bulk production	24	0.0854627	0.76699909	0.15656303
	packaged production	21	-0.0976717	1.20610441	0.26319356
Highly cost	bulk production	24	-0.0375616	0.84162143	0.17179526
	packaged production	21	0.0429275	1.15403484	0.25183105

*1= Strongly Disagree / 5= Strongly Agree

Table 6. Independent t test result about comparison of difficulties between olive oil plants

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Difficulty of adapting ISO 22000	Equal variances assumed	0.690	0.411	5.152	43	0.000	1.21052981	0.23496181	0.73668416	1.68437546
	Equal variances not assumed			5.117	40.746	0.000	1.21052981	0.23655861	0.73269964	1.68835998
Lack of education	Equal variances assumed	3.195	0.081	-0.977	43	0.334	-0.28883614	0.29554284	-0.88485507	0.30718279
	Equal variances not assumed			-0.958	36.549	0.344	-0.28883614	0.30143888	-0.89986378	0.32219151
Lack of qualified staff	Equal variances assumed	4.015	0.051	0.942	43	0.351	0.27859470	0.29577139	-0.31788515	0.87507455
	Equal variances not assumed			0.905	28.717	0.373	0.27859470	0.30798128	-0.35156785	0.90875725
Difficulties in the application	Equal variances assumed	2.708	0.107	-0.279	43	0.782	-0.08315028	0.29853798	-0.68520949	0.51890893
	Equal variances not assumed			-0.282	42.784	0.779	-0.08315028	0.29438557	-0.67692202	0.51062146
No enough importance	Equal variances assumed	1.964	0.168	0.616	43	0.541	0.18313442	0.29749917	-0.41682984	0.78309867
	Equal variances not assumed			0.598	33.059	0.554	0.18313442	0.30623983	-0.43987294	0.80614177
Highly cost	Equal variances assumed	2.422	0.127	-0.270	43	0.789	-0.08048904	0.29855494	-0.68258246	0.52160438
	Equal variances not assumed			-0.264	36.140	0.793	-0.08048904	0.30484830	-0.69866669	0.53768860

Conclusions and Suggestions

The assurance of food safety is compulsory for the protection of public health. Implementation of the ISO 22000 food safety management system is a basic approach to supply the safety of the food supply, providing a systematic procedure for the identification, evaluation and control of hazards and risks in each process. The study has been determined the main problems grouped into 6 factors with factor analysis during ISO 22000:2005 implementation process in Izmir Province of Turkey. The prevalent difficulties were redefined as “the difficulty of adapting ISO 22000:2005, lack of education, lack of qualified staff, difficulties in the application, no enough importance and highly costs”.

The main result of the study reveals both olive oil mills and bottling plants had the same difficulties and they were not indecisive in the face of difficulties. Also they were not different from each other about encountered difficulties while establishing and application ISO 22000:2005 Food Safety Management System except for "the difficulty of adapting ISO 22000:2005". Selling in bulk to the final consumers is not allowed by the government in Turkey. However, the farmers and oil mills usually sell their olive oil directly to customers and it leads unfair consumption due to prices. Some oil mills are very old or uncared, so they produce low quality olive oil at low capacities in bulk. Therefore, government has to increase the control of the olive mills and also give them some technical, economical support and training programs in order to implement HACCP principles, to adapt ISO 22000:2005 system in their plants or to encourage the establishment the system. Certification and consultancy costs should be supported by the government to increase safe and quality olive oil production so that they can sell olive oil with their own brands and provide added value. If the packed olive oil production increases, the margin of market prices will decrease and also the safe and quality olive oil consumption will rise.

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