tbd

TARIM BİLİMLERİ DERGİSİ
JOURNAL OF AGRICULTURAL SCIENCES
ANKARA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ
ANKARA UNIVERSITY FACULTY OF AGRICULTURE

p-ISSN:1300-7580 e-ISSN: 2148-9297

Yıl/Year 16 Sayı/Number 4
Cilt / Volume 22

KAPAK FOTOĞRAFI (COVER PHOTO)

General view of tubuler photobioreactor
Photo: Sena ÖZDEMİR et al

Tübüler fotobiyoreaktörün genel görünümü
Fotograf: Sena ÖZDEMİR et al

Ankara Üniversitesi
ZİRAAT FAKÜLTESİ

Ankara University
Faculty of Agriculture

TARIM BİLİMLERİ DERGİSİ

Journal of Agricultural Sciences

ISSN **1300-7580** (Print)
ISSN **2148-9297** (Online)

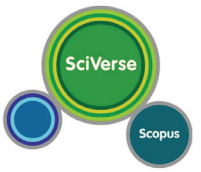
Cilt Volume	22	Sayı Number	4	2016

Tarım Bilimleri Dergisi aşağıdaki
veri tabanları tarafından taranmaktadır

Tarım Bilimleri Dergisi - Journal of Agricultural Science is
abstracted/indexed by the databases below

SCIENCE CITATION INDEX EXPANDED

SCOPUS



TÜBİTAK ULAKBİM

CAB INTERNATIONAL

FAO AGRIS /CARIS

DIRECTORY of OPEN ACCESS JOURNALS

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Amaç ve Kapsam

Tarım Bilimleri Dergisi, tarım bilimleri alanında yapılan özgün araştırma ve İngilizce makaleleri yayınlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar derginin kapsamı dışındadır.

Aims and Scope

Tarım Bilimleri Dergisi – Journal of Agricultural Sciences accepts original research articles in English in the field of agricultural sciences. Local, taxonomy and case related studies, ordinary and out-of-date research articles are out of scope of journal.

Cilt Volume **22** Sayı Number **4** **2016**

Ürün Bilgisi (Product Information)

Yayıncı Publisher	Ankara Üniversitesi Ziraat Fakültesi Ankara University Faculty of Agriculture
Sahibi (AÜZF Adına) Owner (On Behalf of AUZF)	Prof. Dr. Gökhan SÖYLEMEZOĞLU, Dekan (Dean)
Sorumlu Yazı İşleri Müdürü Editor-in-Chief	Prof. Dr. Ali İNAL
Yayın Ünitesi Sorumlusu In Charge of Publication Unit	Özlem ÇOĞALA
Dergi Yönetimi Journal Administrator	Uzm. Mücahit ŞAR
Web Sayfası Koordinatörü Web Page Coordinator	Uzm. Murat KÖSEÇAVUŞ
Grafik Tasarım Graphic Design	Zeynep ASLAN (m.zeynepaslan@gmail.com)
Basımevi Adresi Printing House	Pozitif Matbaacılık Çamlıca Mah. 12. Sok. No: 10/19 Yenimahalle/Ankara-TÜRKİYE Tel: +90 (312) 397 00 31
Basım Tarihi Date of Publication	12/12/2016
Yayın Dili Language	İngilizce English
Yayım Aralığı Frequency	Yılda dört kez yayınlanır Published four times a year
Yayın Türü Type of Publication	Hakemli yaygın süreli yayım Double-blind peer-reviewed, widely distributed periodical
Tarandığı İndeksler Indexed and Abstracted in	Science Citation Index Expanded (SCI-E) ELSEVIER-Scobus TÜBİTAK-ULAKBİM CAB International FAO AGRIS/CARIS Directory of Open Access Journals (DOAJ)

Yönetim Yeri Adresi
Tarım Bilimleri Dergisi
Ankara Üniversitesi Ziraat Fakültesi
Yayın Ünitesi
06110 Dışkapı/Ankara-TÜRKiYE

Telefon : +90 (312) 596 14 24
Belge geçer: +90 (312) 317 67 24
E-posta: tbdeditor@ankara.edu.tr

http://www.agri.ankara.edu.tr/dergi
adresinden dergiye ilişkin bilgilere ve
makalelerin tam metnine ulaşılabilir

Management Address
Journal of Agricultural Sciences
Ankara University Faculty of Agriculture
Publication Department
06110 Dışkapı/Ankara-TURKEY

Telephone : +90 (312) 596 14 24
Fax : +90 (312) 317 67 24
E-mail: tbdeditor@ankara.edu.tr

Instructions for authors and all articles in
this journal can be reached at
http://www.agri.ankara.edu.tr/journal

İÇİNDEKİLER - CONTENTS

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

A Study on the Nutritional Value of Hurma Olives (*Erkence cv.*) that Lose the Bitterness on the Tree

Erkan SUSAMCI[a], Ferişte ÖZTÜRK GÜNGÖR[a], Şahnur IRMAK[a], Handan ATAOL ÖLMEZ[a], Gönül TUSU[a]

[a]*Olive Research Institute, Bornova, İzmir, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Erkan SUSAMCI, E-mail: esusamci@hotmail.com, Tel: +90 (232) 462 70 73
Received: 19 July 2014, Received in Revised Form: 09 March 2015, Accepted: 09 March 2015

ABSTRACT

Hurma olive (*Olea europea* L.) is known to be a product that is formed as a result of debittering that occurs in the fruits of Erkence olive cultivars leading the removal of the bitter taste in the olive when it is still on the tree and thus making the olive edible. "*Debittering*" is the term expressed as the maturation period occurring in the olive fruit while it is still on the tree. In this study, the aim was to harvest Hurma olives from different locations of the Karaburun peninsula in order to determine the nutritional value. For this purpose, measurements were carried out on samples in order to determine their oil (%), protein (%), total sugars (%), reduced sugar (%), starch (%), energy (kcal 100 g^{-1}), pH, total phenolic compound (mg cafeic acid equivalent (CAE) 100 g^{-1}), mineral element (N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, B) contents. Besides, new harvested Hurma olives and dry salted ones stored for 1 year, that had been collected from three different locations of the peninsula, were compared in terms of some chemical properties. Hurma olives were determined to have 38.63% oil, 1.2% protein, 0.52% total sugar, 1.24% starch and the pH value of 5.52. They are regarded as a good source of energy due to the considerably higher oil (359.8 kcal 100 g^{-1}), phenolic compounds (288.71 mg CAE 100 g^{-1}) and mineral element content. It was found out that Hurma olives had high values in terms of mineral element content (N 0.57%, P 0.12%, K 1.42%, Ca 0.09%, Mg 0.04%, Fe 61.44 mg kg^{-1}, Mn 5.23 mg kg^{-1}, Zn 6.40 mg kg^{-1}, Cu 5.53 mg kg^{-1}, B 21.27 mg kg^{-1}) as well. The effects of the salt applications on phenolic compound and reduced sugar content of the olive samples was found statistically insignificant ($P>0.05$). According to the results obtained, the consumption of Hurma olive might be considered to be beneficial for human health due to its salt-free composition, nutritive compounds, total phenolic compound content and the amount of energy it provides.

Keywords: Hurma olive; Erkence; Total phenolic compound; Nutrient composition; Dry salted; Hypertension

Ağaç Üzerindeyken Acılığı Uzaklaşan Hurma Zeytinlerin (*Erkence cv.*) Besin Değeri Üzerine Araştırma

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Erkan SUSAMCI, E-posta: esusamci@hotmail.com, Tel: +90 (232) 462 70 73
Geliş Tarihi: 19 Temmuz 2014, Düzeltmelerin Gelişi: 09 Mart 2015, Kabul: 09 Mart 2015

ÖZET

Hurma zeytin (*Olea europaea* L.), Ege Bölgesi'nde Karaburun Yarımadası'nda Erkence zeytin çeşidinin meyvelerinin ağaç üzerindeyken acılıklarının kaybolarak yenebilir hale gelmeleri sonucu oluşan ürün olarak bilinmektedir. Ağaç üzerindeki bu süreç "Hurmalaşma" olarak ifade edilir. Bu çalışmada, tuzsuz olması nedeniyle sağlık sorunları yaşayan kişiler için önemli bir besin kaynağı olan Hurma zeytinin, yarımadanın farklı bölgelerinden hasat edilerek besin değerinin ortaya konması amaçlanmıştır. Bu amaçla yağ (%), protein (%), toplam şeker (%), indirgen şeker (%), nişasta (%), enerji (kcal 100 g^{-1}), pH, toplam fenolik madde miktarı (mg kafeik asit eşdeğeri (CAE) 100 g^{-1}), mineral madde (N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, B) analizleri gerçekleştirilmiştir. Ayrıca aynı bölgedeki üç noktadan alınan örneklerde sele tipi bir yıl muhafaza edilmiş ve yeni hasat edilmiş Hurma zeytinlerin bazı kimyasal özellikleri bakımından karşılaştırılması yapılmıştır. Hurma zeytinlerin % 38.63 yağ, % 1.2 protein, % 0.52 toplam şeker, % 1.24 nişasta içerdiği, 5.52 pH değerine sahip olduğu belirlenmiştir. Yüksek yağ içeriğinden dolayı iyi bir enerji kaynağı (359.8 kcal 100 g^{-1}) ve aynı zamanda toplam fenolik madde içeriği (288.71 mg CAE 100 g^{-1}) yüksek bir besin olduğu tespit edilmiştir. Hurma zeytinlerin mineral madde içeriği (N % 0.57, P % 0.12, K % 1.42, Ca % 0.09, Mg % 0.04, Fe 61.44 mg kg^{-1}, Mn 5.23 mg kg^{-1}, Zn 6.40 mg kg^{-1}, Cu 5.53 mg kg^{-1}, B 21.27 mg kg^{-1}) bakımından da yüksek değerlere sahip olduğu belirlenmiştir. Hurma zeytinlerin toplam fenol ve indirgen şeker içeriği üzerinde tuz uygulamalarının etkisi önemsiz bulunmuştur ($P>0.05$). Bu bilgiler ışığında, içermiş olduğu temel besin maddeleri, toplam fenolik madde ve enerji miktarı ile birlikte özellikle tuzsuz olarak tüketilmesi açısından Hurma zeytin tüketiminin sağlık için önemli olduğu söylenebilir.

Anahtar Kelimeler: Hurma zeytin; Erkence; Toplam fenolik madde; Besin kompozisyonu; Sele; Yüksek tansiyon

1. Introduction

Olive fruit does not differ from the other stone fruits morphologically. However, it differs from the other fruits due its low sugar content, high oil content and bitter taste that is formed by oleuropein (Balatsouras 1997). Olive fruit contains high amount of phenolic compounds. Oleuropein is the main phenolic compound existing in the pulp fraction of the olive fruit (Ryan et al 1999; Omar 2010). The bitter taste, that oleuropein is responsible for, is a factor inhibiting the olive fruit to be consumed directly. For this reason, oleuropein needs to be removed from the fruit with the application of several processes. Salt is commonly used in the processing of table olives or in the preservation of the processed olives. This constitutes a significant importance for the individuals that need to regulate salt intake in their diets. The World Health Organization, that carries out comprehensive studies intended for reducing the salt consumption, declares that the salt intake per day should not be more than 5 g (2 g sodium) in order to prevent chronic diseases (WHO 2006). There are some olive varieties in Karaburun Peninsula, Turkey that turn into edible form losing its bitter taste spontaneously while they are on the tree. The phenomenon occuring in the fruit on the tree is called "Debittering" and the olive obtained this way is called "Hurma Olive" (Susamcı 2011). Since Hurma olive is not a processed product and thus it does not contain salt, it has turned out to be a suitable option for the individuals seeking for salt-free olives. Hurma olives, which have all the mentioned properties, is the main source for the people living around Karaburun Peninsula to make money for living. Debittering is reported to occur commercially only in Izmir/Karaburun Peninsula and only for the olive cultivar *Erkence* in the world (Tutar 2010). Besides, some olive cultivars in Tunisia and Greece have been reported to have similar characteristics (debittering) to Erkence (Panagou 2006; Jemai et al 2009). Erkence is one of the most important olive cultivar for oil and table olive production around Izmir. This cultivar represents a medium rate of productivity, strong alternate bearing and early harvest characteristics (Mete & Cetin 2006). Debittering is reported to be depending on climate conditions (Buzcu 1969; Pamuk 1993) and soil characteristics (Tutar

2010). Besides, it is stated that a fungus named *Phoma oleae* is effective on the hydrolysis of oleuropein (Panagou 2006). The formation of Hurma olive on tree is reported to be related to the change in the phenolic compound composition and to the decrease in the phenolic compound content. Especially, the differences in the phenolic compound composition observed in the late phase of the maturation period of Hurma olives, non-debittered Erkence and Gemlik olive cultivars constitute a significant effect on debittering (Aktas et al 2013). If Hurma olives cannot be sold in a short period of time right after harvesting, the producers begin to store the olives after treating them with salt and this treatment causes Hurma olives to lose their salt-free characteristic. It is stated that Hurma olives might be preserved in modified atmosphere conditions at 1 ºC up to 90 days without representing any flavor change (Susamcı 2011).

In this study, nutritive contents of Hurma olive samples that had been collected from different locations of Karaburun Peninsula were determined. Besides, new harvested Hurma olives and the ones harvested a year ago and then preserved as dry salted Hurma olives were compared in terms of chemical properties.

2. Material and Methods

In this study, Hurma olive (salt-free) samples were freshly harvested from different locations of Karaburun Peninsula in November, 2010 (T_0) for the evaluation of nutritional composition and mineral element content. Besides, from only three locations, dry salted Hurma olive samples stored in closed plastic containers for 1 year were also used as materials (T_1). The samples were collected in duplicates and immediately transferred to the laboratory.

In order to determine the oil content in the olive samples, 10 g of sample was weighed on a coarse filter paper and was dried at 105 °C for 4.5 h. The dried samples were placed on a cartridge with the filter paper and the oil contents were determined through extracting the samples with n-hexane in the Soxhelet extraction device for 8 h according to the method given in IUPAC (1990). The oil contents were given as percentage (%). To determine the protein content of olive samples, crushed and homogenized olive sample of 0.25 g was weighed on aluminium folio, then it was kept for 3 minutes in nitrogen/protein analyzer (LECO FP-528, Michigan, USA) at 900 ºC. Protein content was calculated by multilying nitrogen value with 6.25 factor and results were expressed as % (AOAC 2005). Reduced sugar contents in the olive samples were determined according to Luff-Scroll method and given as percentage (%) (Uylaşer & Başoğlu 2000). Afterwards, supernatant obtained by Luff-Scroll method was used to determine total sugar content (TKB 1988). In the reduced sugar analysis, pitted and homogenized olive sample of 5 g was mixed with 5 mL potassium ferro cyanide (15%) and 5 mL zinc sulfate (30%) solutions. Afterwards, the mixture was made up to 100 mL with distilled water and set aside for a night in a closed lid sample container. The sample mixture was filtered on the next day and 25 mL Luff solution was added to the filtrated solution. The solution was boiled for 10 min in a heater condenser. Then, 10 mL potassium iodate, 25 mL sulphuric acid (25%) and a few drops of starch solution (5%) was added to the expeditiously cooled sample solution. The solution was titrated with 0.1 N sodium thiosulphate solution until the color of the solution turned into creamy yellow. Reduced sugar which consumed to sodium thiosulphate was read to related table in method. The results were calculated considering the volume of the titration solution consumed for the blank sample and the dilution carried out. In order to determine total sugar content, 50 mL of the clear supernatant (set aside for a night and filtered) obtained during reduced sugar analysis was taken into a 100 mL volumetric flask and 5 mL of hydrochloric acid was added. The solution was shaken in the water bath at around 70 °C for 5 min. Therefore, all sugars in solution were converted to reduced sugar by inversion treatment. Then it was cooled down to 20 °C and notralized with sodium hydroxide until the pH value reaches to 6.0. The solution was filled

to the volume with distilled water and mixed. 5 mL of this solution was taken and followed steps as it was performed in reduced sugar analysis after addition of Luff solution. Results were expressed as percentage (%). Starch content was measured with a polarimeter and expressed as percentage (%) (TKB 1988). For this purpose, crushed olive sample of 5 g was weighed on 100 mL flask and twice extracted with 25 mL of 1% HCl by shaking. This solution was kept on the boiling bath for 15 minutes and then, added 30 mL of distilled water and cooled. Compounds which comprise of nitrogen were precipitated with 10 mL of Wolfram acid and flask was filled to 100 mL with distilled water. After solution was filtered, supernatant was taken to polarimeter tube and measured its polarization. Starch content was calculated according to Equation 1. Energy value per 100 g was calculated according to Equation 2 and indicated as kcal.

$$\text{Starch content (\%)} = (100 \times a \times 100) / ((\alpha)\, D^{20} \times l \times 5) \quad (1)$$

Where; a, polarization degree read on polarimeter; (α) D^{20}, 182.7 (polarization degree of starch of wheat according to Ewers method); l, length of polarimeter tube (dm).

$$Energy(kcal) = (Oil\% \times 9) + ((Total\ sugar\% + Starch\%) \times 4) + (Protein\% \times 4) \quad (2)$$

pH values in the fruit pulp were measured with a pH-meter on 50 g of olive dough that had been prepared with pitted and homogenized in blender (TSE 2003).

Total phenolic compound content was determined with a spectrophotometer (UV2450, Shimadzu, Japan) utilizing the sample preparation method that Günç Ergönül (2006) had used. Calibration curve was obtained using the standard solutions in different concentrations prepared from cafeic acid stock solution. One g of homogenized olive sample mixed with 5 mL of methanol:water (60:40) solution for 2 min. Then, it was centrifuged at 3500 rpm for 10 min. The supernatant was taken into a 10 mL sample tube filtering through a coarse filter paper and the precipitate was washed with methanol-water solution, centrifuged and filtered. The filtered sample in 10 mL sample tube was made up to the volume with distilled water. Once the sample was well mixed, 0.1 mL was taken into 50 mL volumetric flask and 5 mL of distilled water and 0.5 mL Folin-Caiocalteu reagent were added. The solution was stood for 3 min and 1 mL of 36% Na_2CO_3 solution was added. Then, the sample solution was made up to the volume with distilled water. After the solution was kept in the dark for 2 h, the absorbance values were read at 725 nm wavelength using UV/VIS Spectrophotometer. The concentration values that correspond to the absorbance values were determined in the calibration curve for each sample and the results were calculated as mg CAE 100 g^{-1} considering the dilution factor.

Salt content in fruit pulp was determined with the titration of 10 g olive sample, which was pitted and homogenized, with 0.1 N silver nitrate and expressed as percentage (%) (TSE 2003).

Mineral element contents of the samples were also measured in the some locations. For this, 0.3 g of the sample was weighed into the digestion tube and, 5 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. Samples were digested in a laboratory microwave oven for 5 minutes at 200 psi at 180 ºC, for 10 minutes at 200 psi at 240 ºC. Solutions were made up to 15 mL with distilled water. After standards were read for calibration of every mineral, samples were read by using ICP-OES (inductively coupled plasma optical emission spectrometry). Results were calculated by concidering dilution factor and were expressed as percentage (%) for macro and mg kg^{-1} for micro nutrients (NMKL 2014).

The data from the newly harvested Hurma olives were subjected to one way ANOVA, whereas for the comparison of of newly harvested and salted hurma olives, a split plot design, where year was the main, and location was the sub plots, was used. A statistical package (JMP for Windows ver. 5.1) was used for both statistical analyses and the differences between the means were determined with Student's t test. Mineral element contents were not compared statistically.

3. Results and Discussion

Results of the oil, protein, total sugar, starch, energy, pH, and total phenolic compound contents for the harvested Hurma olives were given in Table 1. Oil content values varies between 33.83% and 42.26%. Although Hurma olive is generally consumed as table olive, it was determined that Hurma olive had higher amount of oil than olive oil cultivars. The amount of the Hurma olives processed for oil production is also quite much in the region. The oil content of Hurma olive, which is around 38.63%, enhances its nutritive value and makes it more valuable. Some of the important olive cultivars produced in Turkey such as Memecik, Ayvalık, Domat, and Gemlik contain oil in the amounts of 26%, 26%, 22%, and 28%, respectively. When compared to these cultivars, it is obviously seen that Hurma olive is a good source of oil (Günç Ergönül 2006). Another study has reported that there is no significant difference between the oil contents and the oil quality characteristics of the debittered fruits and non-debittered fruits of Erkence olive cultivar (Sevim et al 2013).

Protein contents were determined between 0.89% and 1.45%. Olives generally contain protein in the amount of 1-2% changing depending on the variety (Garrido Fernandez et al 1997), for instance unprocessed Memecik olives in the black mature form contain 1.31% protein (Ünal & Nergiz 2003). Although olives have low protein content, that low value is important due to the essential amino acid composition. It was observed that Hurma olives contain similar amount of protein that of the other olive cultivars.

Total sugar content differs between 0.18% and 1.05%. Reduced sugar content in the table olives before processing is reported be around 3-6% (Garrido Fernandez et al 1997). Total sugar content of the unprocessed Memecik olives in the black mature form is reported to be 2.20% and the reduced sugar content is reported as 1.90% (Ünal & Nergiz 2003). When compared to these information in literature, the results obtained shows that the sugar content of the olive decreases during debittering on tree. Tuna (2006) stated that total sugar content comprise of highly reduced sugars in olive. These sugars are indicated to be increasing the acidity level in the media as a result of being converted to lactic acid by homofermentative bacteria or to lactic acid, acetic acid, and some other metabolites by heterofermentative bacteria. It might be stated that the sugars in the olive are used by the microorganisms during debittering. Inconsistency between total sugar and reduced sugar values for Çamtepe (T_0) may be explained with differences among Hurma olive fruits due to naturally debittering (Susamcı 2011).

Table 1- Comparison of the some properties of the new harvested (salt-free) Hurma olives collected from different locations in Karaburun Peninsula (mean±standard deviation)

Çizelge 1- Karaburun Yarımadası'nda farklı noktalardan yeni hasat edilen (tuzsuz) Hurma zeytinlerin kimi özelliklerinin karşılaştırılması (ortalama±standart sapma)

Location	*Oil (%)*	*Protein (%)*	*Total sugar (%)*	*Starch (%)*	*Energy (kcal 100 g^{-1})*	*pH*	*Total phenolics (mg CAE 100 g^{-1})*
Ambarseki-1	33.83±0.33 c*	1.45±0.10 a	0.36±0.11 cd	1.20±0.07 b	316.5±2.8 c	5.41±0.11 c	288.93±119.11
Çamtepe	42.26±1.00 a	1.32±0.14 ab	0.45±0.06 c	2.19±0.06 a	396.2±9.6 a	5.70±0.03 b	282.40±76.01
Eğlenhoca	35.80±1.16 bc	1.24±0.06 ab	0.18±0.08 d	1.20±0.01 b	334.6±7.1 b	5.87±0.04 a	282.23±37.51
Gödence	41.80±0.94 a	1.18±0.20 ab	0.36±0.03 cd	0.44±0.03 c	384.1±7.9 a	5.67±0.04 b	299.35±48.65
Kösedere	41.40±0.82 a	1.13±0.04 bc	0.72±0.08 b	1.20±0.17 b	384.8±8.6 a	5.57±0.03 b	290.63±33.41
Urla	36.68±0.36 b	0.89±0.08 c	1.05±0.07 a	1.20±0.11 b	342.9±4.1 b	4.88±0.06 d	nm
Mean	38.63	1.20	0.52	1.24	359.8	5.52	288.71

*, different letters in the same column are significant (P<0.05); nm, not-measured

The starch content in the olive samples were determined between 0.44% and 2.19%. The energy values of the Hurma olive samples were also found high due to their high content of oil. Energy values differ between 316.5 kcal and 396.2 kcal for 100 g. Hurma olives were found to have higher values in terms of starch in Çamtepe and in terms of total sugar in Urla when compared to the values of the olive samples harvested from the other locations. Hurma olives might be classified in the group of foods having medium level of acidity and their pH values were determined between 4.88 and 5.87 in this study. Due to high pH and salt-free, Hurma olives have more sensitive to spoilage in short time after harvesting (Susamci 2011).

The phenolic compound contents of the Hurma olive samples were found to be between 282.23 and 299.35 mg CAE 100 g^{-1}. The phenolic compound content of the olives are affected by factors such as variety, maturation period, cultivation locations, seasonal climate changes, and agricultural applications (Patumi et al 2002; Marsilio et al 2005; Vinha et al 2005). In a study that Hurma olives were observed during 2 years, it was reported that the total phenolic compound content of the olives during debittering period in the first year was around 337.7-649.6 mg gallic acid equivalent (GAE) 100 g^{-1}, whereas it was found between the range of 29.2-344.3 mg GAE 100 g^{-1} in the second year. In the same study, it was stated that the natural debittering phenomenon of Hurma olive on the tree involves a decrease in phenolic content and a change in phenolic composition. Even though Hurma olive and Erkence belong to the same variety, the phenolic compound content of Hurma olives were found lower when compared to the phenolic compound content of Erkence (Aktas et al 2013). Zoidou et al (2010) reported that olives processed as dry salted contained significant amount of oleuropein (1.2 mg $fruit^{-1}$). Considering that a person can consume 20 olive fruit per day, approximately of 25 mg of oleuropein per day might be taken as safely for human use. Boskou et al (2006) determined between 82 and 145 mg CAE 100 g^{-1} the phenolic compound content of five different Greek olive cultivars purchased from the local market in Athens and they reported that 5-10 table olives might cover the daily intake of polyphenols. Similarly, it might be suggested to consume 5-6 Hurma olives, that are rich in phenolic compounds, per day as an antioxidant source. Phenolic compounds have significant benefits on human health and Hurma olives might be regarded as a rich source of phenolic compounds. Saija & Uccella (2001) pointed out that the risk of having chronic diseases are comparatively lower in the societies where based on the Mediterranean aliment culture. Total phenolic compound content, pH, and reduced sugar values of the new harvested Hurma olives and of the ones stored for 1 year were shown in Table 2. The salt contents of the dry salted Hurma olives were also listed in Table 2. The salt content of the Hurma olives that had been stored as dry salted for 1 year was determined as 6.98%. Total phenolic compound content of the salt-free Hurma olives (T_0) were determined as 290.79 mg CAE 100 g^{-1}, whereas the mean value of the total phenolic compound content for the Hurma olive samples that had been stored as dry salted olives (T_1) was 344.33 mg CAE 100 g^{-1}. 11.20% loss in the phenolic compound content of the Gemlik cultivar was reported after processed with trundle method (before processing 274.91 mg CAE 100 g^{-1}, after processing 244.10 mg CAE 100 g^{-1}) (Irmak et al 2010). The phenolic compound of the dry salted Hurma olives was comparatively higher when compared to the new harvested ones, even though they had been stored for 1 year. This might be a result of the difference in the climate between years. The mean pH value of the Hurma olives preserved as dry salted was determined as 4.71, whereas the mean pH value of the new harvested Hurma olives was found as 5.65. The effect of the salt application on pH values of the olive samples was found statistically significant ($P<0.05$). In the dry salt media, it might be stated that the acidity of the Hurma olives increased due to fermentation. The mean values for the reduced sugar contents were determined as 0.52% for both new harvested Hurma olives and the dry salted ones stored for 1 year. The effect of the location x salt application interactions on the reduced sugar contents of the olives were found significant

(P<0.05). Özay & Borcaklı (1996) reported in their study, that they had aimed at producing high quality black olive with natural fermentation, that reduced sugars had been efficiently consumed by microorganisms and the amount of the residues had been changing between 0.05-0.1 g 100 mL^{-1}. As reported by Garrido Fernandez et al (1997) that raw olives contain around 3-6% reduced sugars, it can be said that Hurma olives have reduced sugar content similar to olives processed with natural fermentation.

The mineral element contents of the Hurma olives are given in Table 3. The mineral element content (excluding nitrogen and iron) of the olive samples collected from Eğlenhoca were found higher than the mineral contents of the olive samples collected from the other locations. It was reported that the mineral element content of the olives (Ca, K, Fe, Zn) was affected by industrial processes and storage. Since the olives are treated with salt during processing, sodium content in the olive fruit was reported to increase after processing whereas calcium, potassium, zinc contents decreased and iron content value was flactuating. When the mineral contents of Hurma olives were compared to the values of Memecik cultivar olives when they are green, brownish and black in their mature period, it was found out that the Hurma olives contained higher amounts of potassium, calcium, iron and zinc (Ünal & Nergiz 2003). Average value of boron in Hurma olives was found as 21.27 mg kg^{-1}.

Table 2- Comparison of some chemical characterstics of the new harvested Hurma olives (T_0) and the dry salted Hurma olives (T_1) stored for 1 year (mean±standard deviation)

Çizelge 2- Yeni hasat edilmiş (T_0) ve sele tipi bir yıl muhafaza edilmiş (T_1) Hurma zeytinlerin bazı kimyasal özelliklerinin karşılaştırılması (ortalama±standart sapma)

Location		*Salt in fruit pulp (%)*	*Total phenolic compound (mg CAE 100 g^{-1})*	*pH*	*Reduced sugar (%)*
Çamtepe.	T_0		282.40±76.01	5.70±0.03	0.72±0.06 a*
	T_1	7.61	348.50±18.88	4.79±0.03	0.38±0.04 c
Gödence.	T_0		299.35±48.65	5.67±0.04	0.32±0.04 c
	T_1	7.37	368.85±24.96	4.80±0.03	0.67±0.03 a
Kösedere.	T_0		290.63±33.41	5.57±0.03	0.51±0.06 b
	T_1	5.97	315.63±19.83	4.54±0.06	0.50±0.06 b
Mean	T_0		290.79	5.65 a	0.52
	T_1	6.98	344.33	4.71 b	0.52

*, different letters in the same column are significant (P<0.05); T_0, new harvested Hurma olive; T_1, dry salted Hurma olive that had been stored for 1 year

Table 3- Mineral element contents of the new harvested (salt-free) Hurma olives from different locations of Karaburun Peninsula

Çizelge 3- Karaburun Yarımadası'nda farklı noktalardan yeni hasat edilen (tuzsuz) Hurma zeytinlerin mineral madde içerikleri

Location	*N (%)*	*P (%)*	*K (%)*	*Ca (%)*	*Mg (%)*	*Fe (mg kg^{-1})*	*Mn (mg kg^{-1})*	*Zn (mg kg^{-1})*	*Cu (mg kg^{-1})*	*B (mg kg^{-1})*
Ambarseki-1	0.43	0.09	1.00	0.06	0.03	19.68	2.99	4.84	3.65	12.28
Ambarseki-2	0.39	0.14	1.18	0.08	0.04	88.18	5.32	6.50	5.06	20.90
Tepe Bozköy	0.67	0.09	1.29	0.08	0.03	12.26	3.66	5.28	5.47	21.60
Eğlenhoca	0.48	0.18	2.64	0.15	0.07	60.53	8.20	9.04	10.93	37.04
Kösedere	0.77	0.08	1.15	0.09	0.03	56.82	4.58	5.15	3.67	20.00
Saip	0.65	0.11	1.26	0.09	0.04	131.14	6.65	7.56	4.41	15.78
Mean	0.57	0.12	1.42	0.09	0.04	61.44	5.23	6.40	5.53	21.27

4. Conclusions

The results showed that Hurma olives contained protein, sugar and starch in balanced amounts in addition to its high oil content. It represents similar characteristics with the other olive cultivars and provides high amount of energy due to its high oil content. It was determined that Hurma olives contained important amount of phenolic compound content, when it is considered as if it was the processed table olives due to naturally debittering. In this regard, Hurma olive is a good source of energy and phenolic compounds. The mineral element content of Hurma olives (K, Ca, Fe, Zn) are comparatively higher than the mineral element content values of Memecik cultivar olives both before and after processing (Ünal & Nergiz 2003). The possible consumption of Hurma olives right after harvested from the tree can compensate for the some of the natural nutrients needed by the human metabolism. It was concluded that the difference between the chemical properties of the new harvested and dry salted samples stored for 1 year was due to the changes in climate between years and the salt concentration in the media. Considering the olive processing methods decrease the phenolic compound content (Irmak et al 2010), dry salted Hurma olives would be expected to have a lower phenolic compound content. However, the results obtained showed an opposite trend. This trend might be originated from effect of factors like climate or differences between Hurma fruits. There is a little differences among Hurma olives (new harvested) in terms of texture, taste due to naturally debittering (Susamcı 2011). Besides having considerable amount of phenolic compounds, Hurma olives constitute an importance in terms of consumer health due to its salt-free characteristic.

Acknowledgements

This study was conducted with the support of Izmir Development Agency (IZKA), Izmir Olive Research Station and Karaburun Municipality.

References

AOAC (2005). AOAC Official Method 990.03. Protein (crude) in animal feed, combustion method. Official Methods of Analysis of AOAC International**.** 18th edition. AOAC International, Arlington, VA, USA; 2005 (pp. 30-31 (Chapter 4))

Aktas A B, Ozen B, Tokatli F & Sen I (2013). Phenolics profile of a naturally debittering olive in comparison to regular olive varieties. *Journal of The Science of Food and Agriculture* **94**(4): 691-698

Balatsouras G (1997). Table olive processing technology. (Ed: Plaza & Janes), *World live Encyclopedia*, International Olive Oil Council, Spain, pp. 297-344

Boskou G, Salta F N, Chrysostomou S, Mylona A, Chiou A & Andrikopoulos N K (2006). Antioxidant capacity and phenolic profile of table olives from Greek market. *Food Chemistry* **94**(4): 558-564

Buzcu N (1969). Karaburun Hurma zeytininin oluş sebeplerinin tespiti. Tarımsal Araştırmalar Genel Müdürlüğü, Ankara, Türkiye

Garrido Fernandez A, Fernandez Diez M J & Adams M R (1997). Table Olive, Production and Processing. Chapman and Hall, First Edition, London

Günç Ergönül P (2006). Zeytin meyvesinin olgunlaşması sırasında bileşimimdeki organik asit miktarındaki değişimler ve bu değişimlerin yağ birikimiyle olan ilişkisinin araştırılması. Yüksek lisans tezi, Celal Bayar Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Manisa

Irmak Ş, Öztürk Güngör F & Susamcı E (2010). Bazı sofralık zeytin çeşitlerimizin toplam fenolik madde miktarları ve işleme tekniklerinin bu bileşikler üzerine etkileri. *Zeytin Bilimi* **1**(2): 57-64

IUPAC (1990). International Union of Pure and Applied Chemistry. Standard methods for the analysis of the oils, fats and derivatives (6th ed.), Pergamon Pres, Oxford, UK

Jemai H, Bouaziz M & Sayadi S (2009). Phenolic composition, sugar contents and antioxidant activity of Tunisian Sweet olive cultivar with regard to fruit ripening. *Journal of Agricultural and Food Chemistry* **57**(7): 2961-2968

Marsilio V, Seghetti L, Lannucci E, Russi F, Lanza B & Felicioni M (2005). Use of lactic acid bacteria starter culture during green olive (*Olea europaea L cv Ascolana tenera*) Processing. *Journal of the Science of Food and Agriculture* **85**(7): 1084-1090

Mete N & Çetin Ö (2006). Zeytinin botanik sınıflandırılması ve bölgelere göre yerli zeytin çeşitlerimiz. *Zeytin Yetiştiriciliği*, Emre Basımevi, İzmir, s. 5-27

NMKL (2014). Metals. Determination by atomic absorption spectro-photometry after wet dig-estion in a microwave oven. Nordic committee on food analysis, No: 161, 1998, http://www.nmkl.org/ABONNEMENT/METPDF/161sk.PDF (Access date: 26.02.2014)

Omar S H (2010). Oleuropein in olive and its pharmacological effects. *Scientia Pharmaceutica* **78**(2): 133-154

Özay G & Borçaklı M (1996). Effect of brine replacement and salt concentration on the fermentation of naturally black olives. *Food Research International* **28**(6): 553-559

Pamuk H H (1993). Hurma zeytinin pomolojik özellikleri ve hurmalaşmaya etki eden faktörler üzerinde araştırmalar. Yüksek lisans tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), İzmir

Panagou E Z (2006). Greek dry-salted olives: Monitoring the dry-salting process and subsequent physico-chemical and microbiological profile during storage under different packing conditions at 4 and 20ºC. *LWT-Food Science and Technology* **39**(4): 323-330

Patumi M, d'Adria R, Marsilio V, Fontanazza G, Morelli G & Lanza B (2002). Olive and olive oil quality after intensive Monocone olive growing (*Olea europaea L., cv. Kalamata*) in different irrigation regimes. *Food Chemistry* **77**(1): 27-34

Ryan D, Robards K & Lavee S (1999). Changes in phenolic content of olive during maturation. *International Journal of Food Science and Technology* **34**(3): 265-274

Saija A & Uccella N (2001). Olive biophenols: Functional effects on human Wellbeing. *Trends in Food Science & Technology* **11**(9-10): 357-363

Sevim D, Köseoğlu O, Altunoğlu Y, Ataol Ölmez H & Burçin Büyükgök E (2013). Erkence zeytin çeşidinden elde edilen yağların (hurmalaşmış ve hurmalaşmamış) karşılaştırılması. *Gıda* **38**(3): 159-166

Susamcı E (2011). Farklı Depolama ve Sıcaklık Koşullarının Karaburun Hurma Zeytini'nin (Erkence) Muhafazası Üzerine Etkisi. Zeytincilik Araştırma İstasyonu Müdürlüğü, Genel Yayın No: 215, İzmir

TKB (1988). Gıda Maddeleri Muayene ve Analiz Metotları. T.C. Tarım ve Köyişleri Bakanlığı, Bursa, Türkiye

TSE (2003). Sofralık Zeytin Standardı. Türk Standartları Enstitüsü, Ankara, Türkiye

Tuna S (2006). Siyah sofralık zeytin fermantasyonunda alkali ve enzimatik yöntemlerin Fiziko-Kimyasal özellikler üzerine etkisi. Yüksek lisans tezi, Uludağ Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Bursa

Tutar M (2010). Erkence zeytin çeşidinde farklı tiplerin belirlenmesi. Doktora tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), İzmir

Uylaşer V & Başoğlu F (2000). Gıda Analizleri I-II Uygulama Kılavuzu. Uludağ Üniversitesi Ziraat Fakültesi Uygulama Kılavuzu No: 9, Bursa

Ünal K & Nergiz C (2003). The effect of table olive preparing methods and storage on the composition and nutritive value of olives. *Grasas y Aceites* **54**(1): 71-76

Vinha A F, Ferreres F, Silva B M, Valentao Gonçalves A, Pereira J A, Olivera M B, Seabra R M & Andrade P B (2005). Phenolic profiles of Portuguese olive fruits (*Olea europea L.*): Influences of cultivar and geographical origin. *Food Chemistry* **89**(4): 561-568

WHO (2006). Reducing Salt Intake In Populations. Report of a World Health Organization Forum and Technical Meeting, http://www.who.int/dietphysicalactivity/Salt_Report_VC_april07.pdf (Access date: 28.01.2014)

Zoidou E, Melliou E, Gikas E, Tsarbopoulos A, Magiatis P & Skaltsounis A L (2010). Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. *Journal of Agricultural and Food Chemistry* **58**(1): 46-50

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

TARIM BİLİMLERİ DERGİSİ — JOURNAL OF AGRICULTURAL SCIENCES 22 (2016) 480-491

Enginarın Basınçlı Havayla, Suyla ve Vakumla Ön Soğutulmasının Depolama Koşullarındaki Kalite Parametreleri Üzerindeki Etkisinin Belirlenmesi

İlknur ALİBAŞ[a], Rasim OKURSOY[a]

[a]*Uludag Universiesi, Ziraat Fakültesi, Biyosistem Mühendisliği Bölümü, 16059, Bursa, TÜRKİYE*

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: İlknur ALİBAŞ, E-posta: ialibas@uludag.edu.tr, Tel: +90 (224) 294 16 08
Geliş Tarihi: 28 Ocak 2015, Düzeltmelerin Gelişi: 27 Haziran 2015, Kabul: 29 Haziran 2015

ÖZET

Bu çalışmada, havayla, suyla ve vakumla ön soğutma yöntemleri kullanılarak 5±0.005 kg ağırlıkta tartılarak kasalara yerleştirilen enginarların tarla sıcaklığı olan 23.5±0.5 °C'den depolama sıcaklığı olan 1 °C'ye kadar soğutulması sağlanmıştır. Ön soğutma zamanı en kısa olan soğutma yöntemi 35 dakika ile vakumla ön soğutma yöntemidir. Bunu sırasıyla 58 dakika ile suyla ve 135 dakika ile havayla ön soğutma yöntemleri izlemiştir. Ön soğutma sistemleri enerji tüketimleri açısından incelendiğinde ise en az enerji tüketimi 0.38 kWh değeri ile vakumla ön soğutma sisteminde saptanmıştır. Bunu sırasıyla 0.65 kWh değeri ile suyla ve 0.84 kWh değeri ile de havayla ön soğutma sistemleri izlemiştir. Sistemlerin tükettikleri güç açısından yapılan değerlendirmeye göre en az gücün 0.37 kW değeri ile basınçlı havayla ön soğutma sistemi olduğu, bunu sırasıyla 0.48 kW değeri ile suyla ve 0.65 kW değeri ile vakumla ön soğutmanın izlediği belirlenmiştir. Ön soğutma işlemleri sonunda suyla ön soğutulmuş ürünlerde % 2.83 oranında bir ağırlık artışı gözlemlenmiştir. Buna karşın basınçlı havayla ve vakumla ön soğutulmuş enginarlarda ise soğutma işlemi sonrasında sırasıyla % 1.03 ve % 1.88 oranında ağırlık azalması meydana geldiği saptanmıştır. Basınçlı havayla, suyla ve vakumla ön soğutulmuş ve hiç ön soğutulmamış (kontrol) enginarlar kontrollü atmosfer odasında 30 gün, oda koşullarında ise 15 gün boyunca bekletilmiş ve meyve eti sertliği, ağırlık kaybı, bozulma oranı ve genel görünümleri açısından değerlendirilmiştir. Buna göre enginarlarda kalite parametreleri açısından en uygun ön soğutma yönteminin havayla ön soğutma olduğu; bunu sırasıyla vakumla, kontrol koşullarında ve suyla ön soğumanın izlediği saptanmıştır.

Anahtar Kelimeler: Basınçlı havayla ön soğutma; Enginar; Kalite parametreleri; Suyla ön soğutma; Vakumla ön soğutma

Determination of Quality Parameters during Air Blast, Vacuum and Hydro Pre-cooling of Artichoke under the Storage Conditions

ARTICLE INFO
Research Article
Corresponding Author: İlknur ALİBAŞ, E-mail: ialibas@uludag.edu.tr, Tel: +90 (224) 294 16 08
Received: 28 January 2015, Received in Revised Form: 27 June 2015, Accepted: 29 June 2015

ABSTRACT

The aim of the present study is to pre-cool artichokes which were placed in the cases after weighing 5±0.005 kg, from 23.5±0.5 °C which is field temperature to the storage temperature of 1 °C through using three different pre-cooling methods such as air blast, hydro and vacuum. The cooling method, which has the shortest pre-cooling time, is the vacuum pre-cooling method with 35 minutes. This was followed by hydro with 58 minutes and air pre-cooling method with 135 minutes. When pre-cooling systems were analyzed in terms of energy consumption, vacuum pre-cooling system was determined to have the lowest energy consumption with the value of 0.38 kWh, which was followed by hydro pre-cooling with 0.65 kWh and air pre-cooling system with 0.84 kWh. Based upon an evaluation carried out in terms of power consumption of the systems, air blast pre-cooling method has the minimum power with the value of 0.37 kW, which was followed by hydro and vacuum pre-cooling methods with the respective values of 0.48 kW and 0.65 kW. Following pre-cooling operations, it was observed that there is 2.83% weight gain in the products exposed to hydro pre-cooling. However, 1.03% and 1.88% weight loss was found to occur after pre-cooling operation in artichokes pre-cooled by means of air blast and vacuum. Artichokes pre-cooled through air blast, hydro and vacuum or those which are not pre-cooled were maintained for 30 days in modified atmosphere room while they were hold for 15 days in room conditions; moreover, they were analyzed related to weight loss, hardness, degradation rate and overall appearance. Accordingly, it was determined that the most suitable pre-cooling method is vacuum pre-cooling; this was followed by artichokes pre-cooled though vacuum and hydro or those not pre-cooled.

Keywords: Air precooling; Artichoke; Hydro precooling; Quality parameters; Vacuum precooling

1. Giriş

Anayurdu, Akdeniz ülkeleri olan enginar (*Cynara cardunculus* L.), toprağı zengin, iklimi yumuşak ve nemli olan pek çok ülkede yetiştirilmektedir (Romani et al 2006). Enginar içerdiği mineraller bakımından oldukça zengin bir sebzedir. Bol miktarda potasyum, kalsiyum ve mangan ile A, B1 ve C vitaminleri içermektedir. Enginar ayrıca, karaciğeri temizlemesi, kanı sulandırması, özellikle LDL-kolesterolü düşürücü etkiye sahip olması ve safranın kolay akışını sağlaması gibi özelliklere de sahiptir (Thompson Coon & Ernst 2003; Zhu et al 2004; Zhu et al 2005; Günhan et al 2014).

Tarımsal ürünler hasat edilmelerinin peşi sıra bünyelerindeki mikroorganizma faaliyetlerinin ve etilen gazı oluşumunun hızlanması, su kaybının oluşmaya başlaması ve solunumun yavaşlaması gibi nedenlerden dolayı bozulmaya başlarlar (Alibaş 2012). Hasat işlemini takip eden süreçteki bu bozulmaların önüne geçilebilmesi için hasat sonrası meyve ve sebzelerin tarla sıcaklığından depolama sıcaklığına kadar hızla düşürülmesi yani "ön soğutulması" gerekmektedir (Sankat & Mujaffar 1999; Wang & Sun 2001; Brosnan & Sun 2003; Sun & Wang 2004; Alibaş & Okursoy 2009).

Ön soğutma işlemleri soğutulacak ürünün özelliklerine ve uygulanacak teknolojiye göre genel olarak üç farklı yöntemle yapılmaktadır. Bu yöntemler; havayla, suyla ve vakumla ön soğutma yöntemleridir (Alibaş & Okursoy 2012). Havayla ön soğutma yöntemi bilinen en eski soğutma tekniğidir. Soğutucu akışkan olarak kullanılan havanın soğutulacak olan materyalin bulunduğu ortama gönderilmesi ile havayla ön soğutma işlemi gerçekleştirilmektedir. Hasat sıcaklığındaki ürün, soğuk havanın etkisi ile dış yüzeyden başlayarak iç yüzeye doğru konveksiyon yoluyla soğutulmaktadır (Alibas & Koksal 2014). Suyla ön soğutma yönteminde soğutulmuş su, soğutucu akışkan olarak sisteme verilir ve ürünlerin soğuması tıpkı havayla ön soğutma yöntemindeki gibi konveksiyon yoluyla sağlanmaktadır (Alibaş & İzli 2013). Bu yöntemin en büyük avantajı çeşitli ürün artıklarının, tarla tozu ve kirinin üründen yıkanıp temizlenmesine ve soğutma suyuna ilave edilen klor ve iyot çözeltileri gibi bazı kimyasal maddeler ile ürün yüzeyindeki zararlı bakterilerin yok edilmesine

olanak sağlanmasıdır (Alibaş & Okursoy 2009). Suyla soğutma yönteminde ürünün donmaması için bazı koruma önlemlerinin alınması gerekmektedir. Ayrıca suyla soğutma yöntemi ile soğutulacak ürünlerin ve kullanılan ambalaj malzemelerinin suya dayanıklı olması gerekmektedir (Alibaş & İzli 2013).

Vakumla soğutma tekniği ise ürünün bünyesinde bulunan suyun buharlaştırılması ile ürün sıcaklığının hızlı bir şekilde azalması prensibine dayanmaktadır (Brosnan & Sun 2003; Alibas & Koksal 2014). Soğutma sistemindeki basıncın sürekli olarak azalması ile materyalin bünyesindeki buharlaşmanın sürekli kalması sağlanmaktadır. Vakumla soğutma işlemi sırasında, materyalin bünyesinde serbest halde bulunan su, kaynama noktasına yakın bir sıcaklık değerinde buharlaşmaktadır (Dostal & Petera 2004). Ürün vakuma maruz bırakıldığında, ürünün bünyesinde bulunan suyun kaynama sıcaklığı düşmekte ve suyun bir kısmı yeni denge şartları oluşana dek kaynamaktadır (Wang & Sun 2004). Vakumla soğutma işleminin başlaması ile materyalin iç kısmında bulunan su, düşük basıncın etkisi ile kaynayarak materyalin dış kısımlarına çıkmakta ve dış kısmından da buharlaşmaktadır. Bu buharlaşma sırasında ortamdan ısı çekildiği için tarımsal ürünler soğumaktadır. Vakumla soğutma ile soğutma süresinde önemli ölçüde azalma sağlanmaktadır (McDonald & Sun 2000; McDonald et al 2002; Houska et al 2003; Sun & Wang 2004; Wang & Sun 2004).

Ön soğutma işlemlerinden sonra soğutulmuş ürünler, soğutma sıcaklığı korunarak ya satışa gönderilmekte ya da soğuk havada depolanmaktadır (Alibaş & Okursoy 2012). Ürünün satışa sunulmadan önce soğutulması, genellikle soğutma kapasitesi düşük olan taşıyıcı araçların soğutma yükünü azaltmaktadır. Depolanacak ürünlere daha önceden ön soğutma yapılması, soğuk depolama sırasında harcanan soğutma gücünden tasarruf sağlanmasına da olanak sağlamaktadır (Alibas & Koksal 2015).

Bu çalışmanın amacı; i) yeni hasat edilmiş enginar bitkisinin havayla, suyla ve vakumla ön soğutulmasını sağlamak, ii) ön soğutma süresi, ağırlık farkı, sistemlerin enerji ve güç tüketimlerini tespit etmek ve iii) ön soğutulmuş ürünlerin hem kontrollü atmosfer odasında hem de oda koşullarındaki kalite parametrelerinin belirlenmesiyle enginar bitkisi için en uygun ön soğutma yöntemini saptamaktır.

2. Materyal ve Yöntem

2.1. Örneklerin hazırlanması

Soğutulacak olan enginar (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori), Bursa ili Osmangazi İlçesi Nilüfer Köy'de üretim yapan bir üreticiden yeni hasat edilmiş olarak alınmıştır. Enginarlar hasat sıcaklığı korunarak hızlı bir şekilde laboratuvar ortamına taşınmış ve vakit kaybetmeden ön soğutma işlemlerine tabi tutulmuştur.

2.2. Ön soğutma sistemleri

2.2.1. Havayla ön soğutma sistemi

Genel görünümü Şekil 1(a)'da verilmiş olan basınçlı havayla ön soğutma sistemi; soğutma kabini, fan, kontrol panosu ve içinde buharlaştırıcı, yoğuşturucu, kompresör, genleşme valfi ve soğutucu akışkan bulunan soğutucu üniteden oluşmaktadır. Soğutma kabini 2 mm kalınlığındaki galvanizli sacdan boyutları 850 x 800 x 1100 mm, hacmi ise 0.748 m^3 olacak şekilde tasarlanmıştır. Soğutma kabininin çevresine, 12 mm kalınlığında 0.147 kJ m^{-2} h °C ısı geçiş katsayısına sahip cam yünü sarılmış olup soğutma kabininin arka duvarına buharlaştırıcı ve buharlaştırıcının hemen üzerine ise devir sayısı 1400 $dakika^{-1}$ olan 160 mm çark çapına sahip 5 kanatlı aksiyal bir fan yerleştirilmiştir. Sistem sıcaklığı dijital bir kontrol panosu aracılığıyla ayarlanmaktadır. Sistemde, 13.5 mm çapında, 8160 mm uzunluğunda, 0.3459 m^2 yüzey alanına sahip bakır borudan yapılı buharlaştırıcı ve 12 mm çapında, 16400 mm uzunluğunda, 0.6180 m^2 yüzey alanına sahip bakır borudan yapılı yoğuşturucu kullanılmıştır.

2.2.2. Suyla ön soğutma sistemi

Genel görünümü Şekil 2(b)'de verilmiş olan suyla ön soğutma sistemi genel olarak; soğutma odası,

su deposu, devir-daim su pompası, su püskürtme sistemi, kontrol panosu ve içinde buharlaştırıcı, yoğuşturucu, kompresör, genleşme valfi ve soğutucu akışkan bulunan soğutma ünitesinden oluşmaktadır. Soğutma kabininin ölçüleri, hem soğutma kabininin hem de su deposunun etrafını saran izolasyon malzemesi havayla ön soğutma sistemi ile aynı özelliklere sahiptir. Soğutma odasının tavanına, üzerinde 3 adet meme bulunan su püskürtme sistemi yerleştirilmiştir. Su deposu ana şasinin arka kısmında bulunmakta olup kalınlığı 2 mm olan paslanmaz çelik malzemeden, 980 x 780 x 700 mm boyutlarında ve 0.5351 m^3 hacminde imal edilmiştir. Su deposunun içerisine 15 mm çapındaki sarmal borulardan oluşan biri 10 sarımlı diğeri 9 sarımlı bakır borudan yapılı iki adet 450 mm çapında bir serpantin şeklinde buharlaştırıcı yerleştirilmiştir. Serpantin şeklindeki buharlaştırıcı 17.5 mm çapında, 19.1 m uzunluğunda ve 1.05 m^2 yüzey alanına sahiptir. Soğutucu kabinden tekrar sisteme dönen su, doğal akışıyla depoya geri dönmekte ve buradan tekrar sisteme 55 W gücünde bir devir-daim su pompası yardımıyla basılmaktadır. Sistemde; 10 mm çapında, 59.28 m uzunluğunda, 1.8623 m^2 yüzey alanına sahip bakır borudan yapılı hava soğutmalı yoğuşturucu bulunmaktadır.

2.2.3. Vakumla ön soğutma sistemi

Genel görünümü Şekil 1(c)'de verilmiş olan vakumla ön soğutma sistemi; vakum tankı, vakum pompası ve içinde buharlaştırıcı, yoğuşturucu, kompresör, genleşme valfi ve soğutucu akışkan bulunan soğutma ünitesinden oluşmaktadır. Vakum tankı 6 mm kalınlığında çelik sacdan, 0.6 m çapında ve 1 m uzunluğunda silindirik şekilde tasarlanmıştır. Soğutma tankının çevresine havayla soğutma sistemindeki ile aynı özelliklere sahip ısı yalıtım malzemesi kaplanmıştır. Vakumla ön soğutma sisteminde, soğutma tankının içine vakum pompasının etkinliğinin artırılması amacıyla, düşük basınçlarda ürün üzerinden buhar halinde uzaklaşan suyun tekrar sıvı hale gelerek ürüne dönmesini sağlayan 13.5 mm çapında 7350 mm uzunluğunda 0.3116 m^2 yüzey alanına sahip bakır borudan yapılı buharlaştırıcı kullanılmıştır (Alibaş & Okursoy 2009). Sistemde 11.8 mm çapında, 7.33 m uzunluğunda, 0.2716 m^2 yüzey alanına sahip bakır borudan yapılı yoğuşturucu kullanılmıştır.

(a) **(b)** **(c)**

Şekil 1- Ön soğutma sistemlemleri; a, basınçlı havayla ön soğutma sistemi; b, suyla ön soğutma sistemi; c, vakumla ön soğutma sistemi

Figure 1- Pre-cooling systems; a, air-blast pre-cooling system; b, hydro pre-cooling system; c, vacuum pre-cooling system

Vakumla soğutma sisteminde, döner hareketli ve yağlı tip bir vakum pompası kullanılmıştır. Vakum pompası (Carpanelli MMDE80B4, Italy) sistem basıncının 0.67 kPa basınca kadar düşürülmesi amacıyla kullanılmaktadır.

2.3. Ön soğutma yöntemleri

Soğutulacak olan enginarlar 0.001 kg hassasiyetli bir terazi kullanılarak 5±0.005 kg (Baster-plus, LCB-2, Türkiye) olacak şekilde tartılarak plastik kasalara yerleştirilmişlerdir. Sıcaklık ölçümleri, hassasiyeti 0.1 °C olan bir veri toplama cihazı (Datalogger-10, Türkiye) aracılığıyla, 1 saniyede bir ölçüm alacak şekilde, sıcaklık ölçüm uçlarından (prob) ikisi kasanın merkezi, dördü kasanın sağ, sol ön ve arka yüzeyleri, ikisi kasanın alt ve üstü, diğer ikisi de soğutma kabininin içine olacak şekilde on kanaldan ölçüm alınması sağlanarak gerçekleştirilmiştir. Tüm soğutma işlemleri kasanın merkezine konulan iki sıcaklık ölçüm ucunun okuduğu değer 1 °C oluncaya dek gerçekleştirilmiştir (Zhang & Sun 2006a; Zhang & Sun 2006b).

Havayla ön soğutma sisteminde kullanılan fanın hızı 1 m s^{-1} (Dincer 1995; Teruel et al 2001; Wang & Sun 2002a; Wang & Sun 2002b), suyla ön soğutma sistemindeki debi ise 2.5 L $dakika^{-1}$ olacak şekilde ayarlanmışlardır (Alibaş & İzli 2013; Alibas & Koksal 2014).

Vakumla ön soğutma sistemindeki basınç azalması analog (Viot 69044, US) ve cıvalı vakum metreler kullanılarak gerçekleştirilmiştir. Vakum tankının basıncı 0.67 kPa değerine kadar düşürülebilmektedir.

Havayla ve vakumla ön soğutma sistemlerinin enerji tüketimleri tek fazlı (Kaan, 001, Türkiye), suyla ön soğutma sisteminin enerji tüketimi ise üç fazlı elektrik sayacı (Kaan, 002, Türkiye) ile gerçekleştirilmiştir.

2.4. Kontrollü atmosfer odası (KAO) ve oda koşulları (OK)

Ön soğutulmuş ürünlerin soğuk depolama sırasındaki dayanım süresinin ölçülmesi amacıyla sıcaklığı 1 °C, oransal nemi % 90±5 ve atmosfer bileşimi ise 0:21 [(% CO_2:% O_2)] olan bir kontrollü atmosfer odası (KAO) kullanılmıştır (Alibas & Koksal 2015).

Ön soğutulmuş ürünlerin satış koşullarındaki dayanımlarının ölçülebilmesi için sıcaklığı 22±1 °C ve oransal nemi % 55-60 olan bir laboratuvar oda koşullarını (OK) temsil amacıyla kullanılmıştır (Akbudak & Özer 2003; Alibas & Koksal 2015).

2.5. Kalite parametreleri

2.5.1. Sertlik ölçüm yöntemi

Enginarın sertliği bir penetrometrenin (FT 327) 5/16″'lik ucuyla "N" cinsinden ölçülmüştür. Penetrometre bir taşıyıcı sehpa (Bosch BS 45) üzerine yerleştirilmiştir. Penetrometrenin üzerine yerleştirildiği sehpanın ölçüm alanına yerleştirilen enginar taçlarının üst kısmı penetrometre ucu ile temas ettirilerek penetrometre kolu ve uç vidası yardımıyla sıkıştırılmıştır. Böylece sertlik değerleri penetrometrenin kadranından okunmuştur. Her bir yöntem için on ayrı ölçümün ortalaması alınarak sertlik değeri belirlenmiştir (Alibas & Koksal 2015). Ön soğutma yapılmamış enginarların 0. gündeki sertlik değerleri taze ürünün sertlik değerini vermiş ve bu değer kontrol değeri olarak kabul edilmiştir.

2.5.2. Genel görünüm testi

Tüm yöntemler için genel görünüm 0. günde "10" olarak kabul edilmiş ve bu değer kontrol değeri olarak alınmıştır. Genel görünüm testi, uzman bitki fizyologlarından oluşan bir jüri tarafından gerçekleştirilmiştir. Genel görünüm testinde biyolojik materyal bir puanlama sistemi kullanılarak değerlendirilmiştir. Bu puanlama; 10-9: çok iyi, 8-7: iyi, 6-5: satılabilir, 4-3: satılamaz, 2-1: kullanılamaz şeklindedir (Özer & Masatçı 2000; Özer 2002; Alibas & Koksal 2015).

2.5.3. Ağırlık kaybı ve bozulma oranı belirleme yöntemleri

Tüm yöntemler için hem KAO hem de OK ağırlık kaybı 0. günde % 0 olarak kabul edilmiş ve bu değer kontrol değeri olarak alınmıştır. Ağırlıkları 1 kg olarak belirlenen enginarların, depolama süresi

boyunca KAO'da her 15 günde bir ve OK'da her 5 günde bir ağırlık kaybı değerleri belirlenmiştir (Özer & Masatçı 2000; Alibaş & Okursoy 2009).

Tüm yöntemler için bozulma oranı 0. günde % 0 olarak kabul edilmiş ve bu değer kontrol değeri olarak alınmıştır. Bozulma oranının belirlenmesi genel görünüm, sertlik ve ağırlık kaybı değerlerinin hepsine birden bakılarak yapılmıştır (Alibaş & Okursoy 2012).

2.6. Verilerin analizi

Çalışma 3 tekerrürlü olarak kurulmuştur. Elde edilen verilerin ortalamaları ve diğer istatistik değerleri MINITAB 13 aracılığıyla saptanmış ve sonuçlar LSD testine ($P<0.01$) göre MSTATC istatistik programı kullanılarak harflendirilmiştir.

Veri toplama cihazı aracılığıyla ölçülen zamana bağlı sıcaklık değerleri doğrusal olmayan regresyon analizi yapan NLREG (NLREG 6.3) programı (Eşitlik 1) aracılığıyla tahminlenmiştir. Doğrusal olmayan regresyon analizinin sonuçları soğutma katsayısını (K_s), tahminin standart hatasını (SH) ve regresyon modeli karar katsayısını (R^2) vermektedir (Alibaş & Okursoy 2009; Alibaş & Okursoy 2012; Alibaş & İzli 2013; Alibas & Koksal 2014).

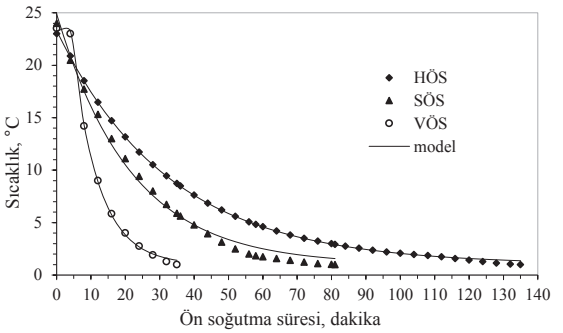
$$T = T_i + [T_m x \exp(K_s x t)] \quad (1)$$

Burada; T, veri toplama cihazı aracılığıyla ölçülen sıcaklık değeri (°C); T_i, ürünün ön soğutma işlemi sonunda ulaşması istenen sıcaklık değeri (°C); T_m, materyalin soğutulmadan önceki sıcaklığı (°C); K_s, soğutma katsayısı (birimsiz) ve t, soğutma süresi (s)'dir.

3. Bulgular ve Tartışma

3.1. Ön soğutma parametrelerinin belirlenmesi

Enginarın havayla, vakumla ve suyla ön soğutulmasına ilişkin zamana bağlı sıcaklık azalması Şekil 2'de verilmiştir. Şekil 2'ye göre, en kısa süreli soğutma işleminin 35 dakika ile vakumla ön soğutma yöntemi, en uzun yöntemin ise 135 dakika ile havayla ön soğutma yöntemi olduğu saptanmıştır. Buna göre vakumla ön soğutma yönteminin, havayla ön soğutma yöntemine göre 3.86 kat daha hızlı ön soğutma yapılabildiği belirlenmiştir. Enginarın suyla ön soğutulması ise 81 dakika sürmüştür. Suyla ön soğutma yöntemlerinde belirlenen soğutma süresinin vakumla ön soğutma yönteminde belirlenen soğutma süresine göre 2.31 kat arttığı; havayla ön soğutma yönteminde belirlenen soğutma süresine göre ise 1.67 kat azaldığı tespit edilmiştir. Benzer sonuçlar, çeşitli araştırıcılar tarafından da ifade edilmiştir (Sun & Wang 2004; Cheng 2006; Zhang & Sun 2006b; Jackman et al 2007; Alibaş & Okursoy 2009; Alibaş & Okursoy 2012; Alibas & Koksal 2014).



Şekil 2- Enginarın vakumla, havayla ve suyla soğutulmasındaki zamana bağlı sıcaklık eğrileri

Figure 2- The temperature curves depending on pre-cooling time during vacuum, air and hydro pre-cooling of artichokes

Enginarın havayla, vakumla ve suyla ön soğutulması sırasında elde edilen istatistiki veriler Çizelge 1'de verilmiştir. Ön soğutma denemelerinden elde edilen veriler ile soğutma modelinin belirlediği veriler arasındaki karar katsayısının (R^2) en yüksek olduğu ön soğutma yönteminin 0.9996 değeri ile havayla ön soğutma yöntemi olduğu tespit edilmiştir. Bunu sırasıyla 0.9991 değeri ile vakumla ve 0.9930 değeri ile suyla ön soğutma yöntemleri izlemiştir.

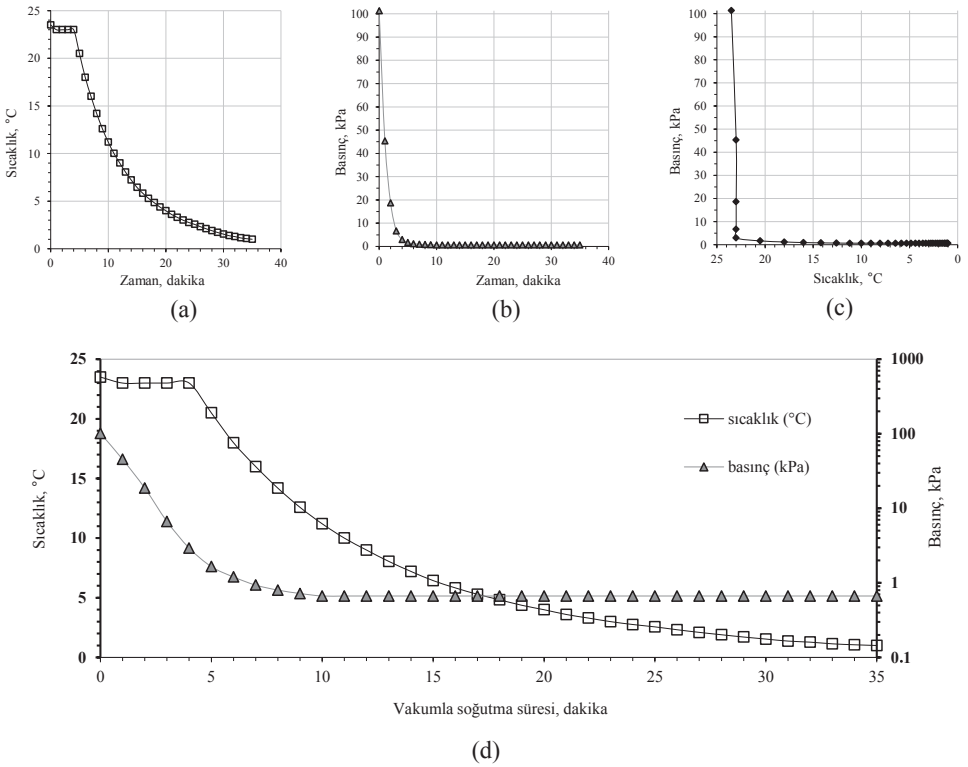
Enginarın vakumla ön soğutulması sırasında ölçülen sıcaklık, basınç ve zaman arasındaki ilişkiler Şekil 3'de verilmiştir. Toplam 35 dakika süren vakumla soğutma işleminin 4. dakikasında basınç 2.93 kPa değerine ulaşmış ve bu noktada üründe

Çizelge 1- Havayla, suyla ve vakumla ön soğutma yöntemlerinin ön soğutma verileri

Table 1- The statistical data of air, hydro and vacuum pre-cooling methods

Ön soğutma yöntemi	*Tahminin standart hatası (SH)***	*Regresyon modeli karar katsayısı (R^2)***	*Soğutma katsayısı ($K_ş$)***
HÖS	0.120816[b*]	0.9996[a]	0.0304295648[c]
VÖS	0.191241[b]	0.9991[a]	0.1288981560[a]
SÖS	0.563272[a]	0.9930[b]	0.0460402651[b]

*, aynı sütunda farklı harflerle gösterilen ortalamalar arası fark önemlidir; **, P<0.01



Şekil 3- Enginarın vakumla ön soğutulmasındaki a, sıcaklık-zaman; b, basınç-zaman; c, basınç-sıcaklık; d, basınç-sıcaklık-zaman grafikleri

Figure 3- The diagrams of vacuum precooling of artichoke a, temperature-precooling time; b, pressure-precooling time; c, pressure-temperature and d, pressure-temperature-precooling time

maksimum düzeyde buharlaşma meydana gelmiştir. Dördüncü dakikaya kadar sabit kalan sıcaklık, parlama noktası adı verilen bu andan sonra hızlı bir şekilde düşmeye başlamıştır. Sistem minimum basınç değeri olan 0.67 kPa basınca 10 dakikada ulaşmış olup bu basınç değeri vakumla ön soğutma işleminin sonuna kadar sabit kalmıştır.

Enginarın çeşitli yöntemlerle ön soğutulmasındaki enerji tüketimi, güç ve ağırlık farkı değerleri Çizelge 2'de verilmiştir. Enginarın ön soğutulmasındaki en

yüksek enerji tüketimi 0.84 kWh değeri ile havayla ön soğutma yönteminde ölçülmüştür. Bunu sırasıyla 0.65 kWh ve 0.38 kWh değerleri ile suyla ve vakumla ön soğutma yöntemleri takip etmiştir. Enerji tüketimi açısından en pahalı yöntem olan havayla ön soğutma yöntemindeki enerji tüketiminin, en ekonomik yöntem olan vakumla ön soğutma yöntemine göre 2.21 kat fazla olduğu belirlenmiştir. En fazla güç gereksinimi ise 0.6514 kW değeri ile vakumla ön soğutma yönteminde meydana gelmiştir. Bunu sırasıyla 0.4815 kW değeri ile suyla ve 0.3733 kW değeri ile havayla ön soğutma yöntemleri takip etmiştir. En fazla gücün harcandığı vakumla ön soğutma yöntemindeki güç gereksiniminin, havayla ön soğutma yöntemindeki güç gereksinimine göre 1.75 kat fazla olduğu saptanmıştır. Enerji ve güç tüketimi ile ilgili benzer sonuçlar literatür çalışmaları ile paralellik göstermektedir (Alibaş & Okursoy 2012; Alibas & Koksal 2014). Ön soğutma işlemlerinin sonunda suyla ön soğutulmuş ürünlerde % 2.83 değerinde bir ağırlık artışı, havayla ve vakumla ön soğutulmuş enginarlarda ise sırasıyla % 1.03 ve % 1.88 değerinde bir ağırlık kaybı saptanmıştır. Haas & Gur (1987) vakumla soğutma sırasında meydana gelen her 5-6 °C'lik sıcaklık düşüşünün marulun bünyesinde yaklaşık % 1'lik bir ağırlık kaybına neden olduğunu belirlemişlerdir. Ağırlık artış ve azalışlarına ilişkin benzer sonuçlar, çeşitli araştırıcılar tarafından ifade edilmiştir (Desmond et al 2000; McDonald et al 2000; Desmond et al 2002; McDonald et al 2002; Jackman et al 2007; Alibaş & Okursoy 2009; Alibaş & Okursoy 2012; Alibas & Koksal 2014).

3.2. Kalite parametrelerinin belirlenmesi

3.2.1. KAO muhafaza sırasındaki kalite parametrelerinin belirlenmesi

Havayla, vakumla ve suyla ön soğutulmuş enginarlar ile kontrol kabul edilen ön soğutulmamış enginarların kontrollü atmosfer odasında 0., 15. ve 30. günlerde belirlenmiş sertlik, genel görünüm, bozulma oranı ve ağırlık kaybı değerleri Çizelge 3'de verilmiştir.

Tüm soğutma yöntemleri içinde 30. günde en az bozulma % 25 değeri ile havayla ön soğutma yönteminde meydana gelmiştir. Bunu sırasıyla % 30 değeri ile vakumla ön soğutma, % 38 değeri ile kontrol koşulları ve % 55 değeri ile suyla ön soğutma yöntemi izlemiştir. Kontrol koşullarında depolanan enginarlarda oluşan bozulma oranının, havayla ön soğutulan enginarlarda oluşan bozulma oranına göre 130 kg t^{-1} ürün kaybına; suyla ön soğutulan enginarlara göre ise 170 kg t^{-1} ürün kazancına neden olduğu tespit edilmiştir.

Kontrollü atmosfer odasında 30. günün sonunda ağırlık kaybı değeri açısından en az kayba neden olan yöntemin % 12 değeri ile havayla ön soğutma yöntemi olduğu saptanmıştır. Bu değeri % 13 değeri ile vakumla, % 15 değeri ile kontrol koşulları ve % 18.5 değeri ile suyla ön soğutma yönteminin takip ettiği tespit edilmiştir. Kontrol şartlarında KAO'da muhafaza edilmiş enginarlarda 30. günde ölçülen ağırlık kaybı değerinin suyla ön soğutulan enginarlara göre % 23 oranında daha az olduğu belirlenmiştir.

Vakumla ve havayla ön soğutularak kontrollü atmosfer odasına konulan enginarların 30. günün sonundaki genel görünüm derecesi sırasıyla "7"

Çizelge 2- Enginarın ön soğutma parametreleri

Table 2- The pre-cooling parameters of artichokes

Ön soğutma yöntemi	*Soğutma süresi*** *(dakika)*	*Enerji tüketimi*** *(kWh)*	*Güç*** *(kW)*	*Ağırlık farkı*** *(%)*
HÖS	135±(5.030)^{a*}	0.84±(0.0252)^{a}	0.3733±(0.00299)^{c}	-1.03±(0.0321)^{c}
VÖS	35±(1.530)^{c}	0.38±(0.0208)^{c}	0.6514±(0.00218)^{a}	-1.88±(0.0451)^{b}
SÖS	81±(2.520)^{b}	0.65±(0.0265)^{b}	0.4815±(0.00414)^{b}	2.83±(0.0306)^{a}

*, aynı sütunda farklı harflerle gösterilen ortalamalar arası fark önemlidir; **, P<0.01

Çizelge 3- Enginarın kontrollü atmosferde (KA) soğuk depolanması sırasındaki kalite parametreleri

Table 3- The quality parameters of artichoke during cold storage in modified atmosphere room (MA)

Ön soğutma yöntemi	*Depolama süresi (gün)*	*Sertlik** (N)*	*Ağırlık kaybı** (%)*	*Bozulma oranı** (%)*	*Genel görünüm** (1-10)*
ÖS	0	48.66±(0.118)^{a*}	0.000±(0.000)^{d}	0.000±(0.000)^{g}	10.000±(0.000)^{a}
	15	37.80±(0.147)^{g}	6.000±(1.530)^{c}	10.000±(2.520)^{e}	9.000±(0.289)^{b}
	30	23.15±(0.206)^{k}	15.000±(1.730)^{b}	38.000±(1.530)^{b}	6.000±(0.577)^{e}
HÖS	0	48.07±(0.118)^{b}	0.000±(0.000)^{d}	0.000±(0.000)^{g}	10.000±(0.000)^{a}
	15	41.96±(0.147)^{d}	4.000±(0.577)^{c}	3.000±(1.000)^{fg}	10.000±(0.000)^{a}
	30	27.37±(0.206)^{i}	12.000±(1.530)^{b}	25.000±(3.610)^{c}	8.000±(0.577)^{c}
VÖS	0	46.99±(0.353)^{c}	0.000±(0.000)^{d}	0.000±(0.000)^{g}	10.000±(0.000)^{a}
	15	39.24±(0.265)^{f}	4.500±(1.040)^{c}	7.000±(1.530)^{ef}	10.000±(0.000)^{a}
	30	25.21±(0.353)^{j}	13.000±(1.730)^{b}	30.000±(3.210)^{c}	7.000±(0.289)^{d}
SÖS	0	40.19±(0.088)^{e}	0.000±(0.000)^{d}	0.000±(0.000)^{g}	10.000±(0.000)^{a}
	15	33.48±(0.196)^{h}	7.000±(1.530)^{c}	18.000±(1.530)^{d}	9.000±(0.289)^{b}
	30	19.94±(0.118)^{l}	18.500±(1.260)^{a}	55.000±(2.890)^{a}	5.000±(0.000)^{f}

*, aynı sütundaki farklı harflerle gösterilen ortalamalar arası fark önemlidir; **, P<0.01

ve "8" puan ile "iyi", kontrol şartlarında ve suyla ön soğutularak muhafaza edilen enginarların genel görünüm derecesi ise sırasıyla "6" ve "5" puan ile "satılabilir" olarak derecelendirilmiştir.

Taze enginarın sertlik değeri (ön soğutulmamış, 0. gün) 48.66 N olarak saptanmıştır. Havayla, suyla ve vakumla ön soğutulmuş enginarların KAO öncesi ölçülen sertlik değerleri ise sırasıyla 48.07, 40.19 ve 46.99 N olarak belirlenmiştir. KAO'da 30. gün sonundaki sertlik değerleri içinde taze ürüne en yakın sertlik değeri 27.37 N değeri ile havayla ön soğutma yönteminde bulunmuştur. Bunu sırasıyla 25.51 N değeri ile vakumla ön soğutma, 23.15 N değeri ile kontrol koşulları ve 19.94 N değeri ile suyla ön soğutma yöntemleri izlemiştir. Ön soğutma yapılmamış enginarlarda 30. gün sonunda ölçülen sertlik değerinin, suyla ön soğutma yöntemlerinde saptanan sertlik değerinden % 16.1 oranında daha fazla olduğu saptanmıştır. Suyla ön soğutma yöntemi enginarların soğuk depolanması sürecinde sertliklerinin azalmasına neden olmuştur.

3.2.2. OK muhafaza sırasındaki kalite parametrelerinin belirlenmesi

Havayla, suyla ve vakumla ön soğutma yöntemleri ile soğutulmuş enginarlar ile kontrol koşullarına sahip enginarların pazar koşullarında dayanımlarının belirlenmesi açısından nemi ve sıcaklığı sabit olan oda koşullarında (OK) bekletilmesi sonucu 0., 5. ve 10. günlerde ölçülen sertlik, genel görünüm, bozulma oranı ve ağırlık kaybı değerleri Çizelge 4'de verilmiştir.

Havayla, vakumla ve suyla ön soğutma yapılmış enginarlar ile kontrol koşullarındaki enginarların OK'da 10 gün boyunca bekletilmeleri sonucu saptanan bozulma oranı değerlerine göre en az bozulma % 7 değeri ile havayla ön soğutma yönteminde meydana gelmiştir. Havayla ön soğutma yöntemini sırasıyla % 13 değeri ile vakumla ön soğutma yöntemi, % 25 değeri ile kontrol koşulları ve % 31 değeri ile suyla ön soğutma yöntemi izlemiştir. Ön soğutma yapılmamış ürünlerde saptanan bozulma oranının suyla ön soğutulmuş enginarlarda gözlenen bozulma oranından % 24 daha az olduğu tespit edilmiştir. OK'da 10. günde ön soğutma yapılmamış ürünlerde saptanan bozulma oranının havayla ön soğutulmuş enginarlarda saptanan bozulma oranından 3.57 kat fazla olduğu saptanmıştır. Meydana gelen ağırlık kaybı değerlerinden yola çıkılarak bir ton (1000 kg) ürün başına bozulan ürün miktarı hesaplandığında, ön soğutulmadan satışa çıkarılmış ürünlerde 10. günün sonunda 250 kg ürünün bozularak atıldığı

Çizelge 4- Enginarın oda koşullarında (OK) depolanması sırasındaki kalite parametreleri

Table 4- The quality parameters of artichoke during storage in room conditions (RC)

Ön soğutma yöntemi	*Depolama süresi (gün)*	*Sertlik** (N)*	*Ağırlık kaybı** (%)*	*Bozulma oranı** (%)*	*Genel görünüm** (1-10)*
ÖS	0	48.66±(0.118)^{a*}	0.000±(0.000)^{g}	0.000±(0.000)^{h}	10.000±(0.000)^{a}
	15	37.25±(0.147)^{g}	23.500±(0.764)^{e}	7.000±(1.530)^{e}	7.000±(0.577)^{ef}
	30	20.41±(0.118)^{k}	39.000±(2.520)^{a}	25.000±(2.890)^{b}	4.333±(0.441)^{g}
HÖS	0	48.07±(0.118)^{b}	0.000±(0.000)^{g}	0.000±(0.000)^{h}	10.000±(0.000)^{a}
	15	42.02±(0.147)^{d}	15.500±(2.470)^{f}	2.000±(1.000)^{g}	9.000±(0.000)^{ab}
	30	36.07±(0.147)^{h}	31.500±(1.760)^{c}	7.000±(1.530)^{e}	7.000±(0.577)^{cd}
VÖS	0	46.99±(0.353)^{c}	0.000±(0.000)^{g}	0.000±(0.000)^{h}	10.000±(0.000)^{a}
	15	40.71±(0.167)^{e}	17.000±(2.000)^{f}	4.000±(1.000)^{f}	8.000±(0.577)^{bc}
	30	30.19±(0.147)^{j}	34.500±(2.470)^{b}	13.000±(2.520)^{d}	6.000 ±(0.289)^{de}
SÖS	0	40.19±(0.088)^{f}	0.000±(0.000)^{g}	0.000±(0.000)^{h}	10.000±(0.000)^{a}
	15	34.34±(0.118)^{i}	26.000±(1.000)^{d}	15.000±(1.000)^{c}	6.000±(0.289)^{cd}
	30	19.20±(0.088)^{l}	40.500±(2.750)^{a}	31.000±(3.060)^{a}	3.000±(0.577)^{f}

*, aynı sütundaki farklı harflerle gösterilen ortalamalar arası fark önemlidir; **, P<0.01

saptanmıştır. Ancak havayla ön soğutulduktan sonra pazara çıkarılan enginarlarda bu kaybın 70 kg olduğu belirlenmiştir. İşlem görmeden pazara çıkarılan enginarlara göre, havayla ön soğutulan enginarlarda 10. günde saptanan bozulma oranı ile bir ton üründe 180 kg ürün kazancı sağlandığı tespit edilmiştir. Suyla ön soğutularak OK'da 10 gün boyunca bekletilen bir ton üründe meydana gelen bozulma oranı ise 310 kg olarak belirlenmiştir. Suyla ön soğutularak pazar koşullarında satışa çıkarılmış bir ton enginarda 10. günde meydana gelen bozulmanın, hiç ön soğutma yapılmadan pazara çıkarılmış enginarlarda saptanan bozulma oranından 60 kg fazla olduğu saptanmıştır.

Çalışmada, en az ağırlık kaybına neden olan yöntemin % 31.5 değeri ile havayla ön soğutma yöntemi olduğu; bunu sırasıyla % 34.5, % 39 ve % 40.5 değerleri ile vakumla, kontrol şartları ve suyla ön soğutma yöntemlerinin izlediği belirlenmiştir.

Havayla ön soğutma yöntemi uygulanarak ön soğutulmuş enginarlar 10. günün sonunda "7" puan ile genel görünüm açısından "iyi"; vakumla ön soğutulmuş enginarlar ise "6" puan ile "satılabilir" olarak değerlendirilmiştir. Ön soğutma yapılmamış enginarlar ile suyla ön soğutulmuş enginarların 10 gün sonundaki genel görünüm derecesi ise sırasıyla "4" ve "3" puan ile "satılamaz" olarak değerlendirilmiştir.

Oda koşullarında 10. gün sonundaki sertlik değerleri içinde taze ürüne en yakın sertlik değerinin 36.07 N değeri ile havayla ön soğutma yöntemi olduğu belirlenmiştir. Havayla ön soğutma yöntemini sırasıyla 30.19 N değeri ile vakumla, 20.41 N değeri ile kontrol koşulları ve 19.20 N değeri ile suyla ön soğutma yöntemleri izlemiştir. Ön soğutma yapılmamış enginarlarda 10. gün sonunda ölçülen sertlik değerinin, suyla ön soğutma yöntemine göre % 6.3 oranında fazla olduğu tespit edilmiştir.

4. Sonuçlar

Bu çalışmada, enginar havayla, vakumla ve suyla ön soğutulmuştur. Soğutma süresinin en kısa, enerji tüketiminin ise en düşük olduğu soğutma yönteminin vakumla ön soğutma yöntemi olduğu saptanmıştır. Hem enerji tüketimi hem de soğutma süresi açısından vakumla ön soğutma yöntemini sırasıyla suyla ve havayla ön soğutma yöntemlerinin izlediği belirlenmiştir. Suyla ön soğutulan materyallerde soğutma işlemi sırasında ağırlık artışı, havayla ve vakumla ön soğutulmuş materyallerde ise ağırlık kaybı oluştuğu tespit edilmiştir. Ayrıca en fazla güç

gereksiniminin vakumla ön soğutma yönteminde meydana geldiği, bunu sırasıyla suyla ve havayla ön soğutma yöntemlerinin izlediği saptanmıştır.

Enginarın oda ve kontrollü atmosfer koşullarında muhafaza edilmesinde kalite parametreleri açısından en uygun soğutma yönteminin havayla ön soğutma yöntemi olduğu, bunu sırasıyla vakumla, kontrol şartları ve suyla ön soğutma yönteminin izlediği belirlenmiştir. Ön soğutulmaksızın oda ve kontrollü atmosfer koşullarında depolanan enginarın kalite parametrelerinin, suyla ön soğutulmuş enginarlara göre daha yüksek olduğu saptanmıştır. Buna bağlı olarak bu çalışmada, enginarı suyla ön soğutmanın önemli ölçüde kalite kaybına neden olduğu belirlenmiştir.

Kısaltmalar ve Semboller	
HÖS	Havayla ön soğutma
KAO	Kontrollü atmosfer odası
K_s	Soğutma katsayısı
LSD	En küçük anlamlı fark
OK	Oda koşulları
R^2	Regresyon modeli karar katsayısı
SH	Standart hata
SÖS	Suyla ön soğutma
VÖS	Vakumla ön soğutma
T	Veri toplama cihazı aracılığıyla ölçülen sıcaklık
T_i	Ürünün ön soğutma işlemi sonunda ulaşması istenen sıcaklık
T_m	Materyalin hasat sıcaklığı
t	Ön soğutma süresi

Kaynaklar

Akbudak B & Özer M H (2003). Farklı sıcaklıklarda muhafaza edilen turşuluk hıyarda meydana gelen fiziksel ve kimyasal değişimler. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi* **17**(1): 33-46

Alibaş İ (2012). Asma yaprağının (*Vitis vinifera* L.) mikrodalga enerjisiyle kurutulması ve bazı kalite parametrelerinin belirlenmesi. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **18**(1): 43-53

Alibaş İ & Okursoy R (2009). Determination of operating and quality parameters during air blast, vacuum and hydro pre-cooling of spinach. *Tarım Makinaları Bilimi Dergisi* **5**(2): 149-160

Alibaş İ & Okursoy R (2012). A comparison study of some operating parameters during vaccum, air and hydro pre-cooling of faba beans, and determination of the quality parameters during storage period. *Tarım Makinaları Bilimi Dergisi* **8**(2): 185-197

Alibaş İ & İzli N (2013). Determination of the storage and quality parameters of nettle leaves hydro pre-cooleding different water temperatures. *Tarım Makinaları Bilimi Dergisi* **9**(1): 21-27

Alibas I & Koksal N (2014). Forced-air, vacuum and hydro precooling of cauliflower (*Brassica oleracea* L. var. *botrytis* cv. Freemont): Part I. Determination of precooling parameters. *Food Science and Technology* **34**(4): 730-737

Alibas I & Koksal N (2015). Forced-air, vacuum and hydro precooling of cauliflower (*Brassica oleracea* L. var. *botrytis* cv. Freemont): Part II. Determination of quality parameters during storage. *Food Science and Technology* **35**(1): 45-50

Brosnan T & Sun D W (2003). Influence of modulated vacuum cooling on the cooling rate, mass loss and vase life of cut lily flowers. *Biosystems Engineering* **86**(1): 45-49

Cheng H P (2006). Vacuum cooling combined with hydrocooling and vacuum drying on bamboo shoots. *Applied Thermal Engineering* **26**: 2168-2175

Desmond E M, Kenny T A & Ward P (2000). Effect of rapid and conventional cooling methods on the quality of cooked ham joints. *Meat Science* **56**: 271-277

Desmond E M, Kenny T A & Ward P (2002). The effect of ınjection level and cooling method on the quality of cooked ham joints. *Meat Science* **60**(3): 271-277

Dincer I (1995). Air flow precooling of individual grapes. *Journal of Food Engineering* **6**(2): 243-249

Dostal M & Petera K (2004). Vacuum cooling of liquids: Mathematical model. *Journal of Food Engineering* **61**(4): 533-539

Günhan T, Demir V & Yağcıoğlu A (2014). Mathematical modelling of convection drying characteristics of artichoke (*Cynara Scolymus* L.) leaves. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **20**(4): 415-426

Haas E & Gur G (1987). Factor affecting the cooling rate of lettuce in vacuum cooling installations**.** *International Journal of Refrigeration* **10**(2): 82-86

Houska M, Sun D-W, Landfeld A & Zhihang Z (2003). Experimental study of vacuum cooling of cooked beef in soup. *Journal of Food Engineering* **59**(2-3): 105-110

Jackman P, Sun D W & Zheng L (2007). Effect of combined vacuum cooling and air blast cooling on time and cooling loss of large cooked beef joints. *Journal of Food Engineering* **81**: 266-271

McDonald K & Sun D W (2000). Vacuum cooling technology for the food processing industry: A review. *Journal of Food Engineering* **45**(2): 55-65

McDonald K, Sun D W & Kenny T (2000). Comparison of the quality of cooked beef products cooled by vacuum cooling and by conventional cooling. *Lebensmittel-Wissenschaft Und-Technologie* **33**(1): 21-29

McDonald K, Sun D W & Lygn J G (2002). Effect of vacuum cooling on the thermophysical properties of a cooked beef product. *Journal of Food Engineering* **52**(2): 167-176

Özer M H (2002). Jonagold elma çeşidinin kontrollü atmosferde muhafazası. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi* **16**(2): 189-202

Özer M H & Masatçı F (2000). Domatesin kontrollü atmosferde (KA) muhafazası üzerine bir araştırma. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi* **14**: 45-57

Romani A, Pinelli P, Cantani C, Cimato A & Heimler D (2006). Characterization of violetto di toscana, a typical italian variety of artichoke (*Cynara scolymus* L.). *Food Chemistry* **95**(2): 221-225

Sankat C K & Mujaffar S (1999). Water balance in cut *Anthurium* flowers in storage and its effects on quality. *Acta Horticulturae* **368**: 723-732

Sun D W & Wang L (2004). Experimental ınvestigation of performance of vacuum cooling for commercial large cooked meat joints. *Journal of Food Engineering* **61**(4): 527-532

Teruel B, Cortez L & Fo L N (2001). A comparative study of the cooling of oranges using three cooling systems. *Revista Brasileira De Engenharia Agricola E Ambiental* **5**(3): 481-486

Thompson Coon J S & Ernst E (2003). Herbs for serum cholesterol reduction: A systematic view. *Journal of Family Practice* **52**: 468-478

Wang L J & Sun D W (2001). Rapid cooling of porous and moisture foods by using vacuum cooling technology. *Trends in Food Science & Technology* **12**(5-6): 174-184

Wang L J & Sun D W (2002a). Numerical analysis of the three-dimensional mass and heat transfer with inner moisture evaporation in porous cooked meat joints during vacuum cooling. *Transactions of the ASAE* **45**(6): 107-115

Wang L J & Sun D W (2002b). Evaluation of performance of slow air, air blast and water immersion cooling methods in the cooked meat industry by the finite element method. *Journal of Food Engineering* **51**(4): 329-340

Wang L J & Sun D W (2004). Effect of operating conditions of a vacuum cooler on cooling performance for large cooked meat joints. *Journal of Food Engineering* **61**(2): 231-240

Zhang Z & Sun D W (2006a). Effects of cooling methods on the cooling efficiency and quality of cooked rice. *Journal of Food Engineering* **77**: 269-274

Zhang Z & Sun D W (2006b). Effects of cooling methods on the cooling efficiency and qualities of cooked broccoli and carrot slices. *Journal of Food Engineering* **77**: 320-326

Zhu X F, Zhang H X & Lo R (2004). Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *Journal of Agricultural and Food Chemistry* **52**: 7272-7278

Zhu X F, Zhang H X & Lo R (2005). Antifungal activity of *Cynara Scolymus* L. extracts. *Fitoterapia* **76**(1): 108-111

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Effects of SNP within Exon 7 of the Insulin-like Growth Factor Receptor Type 1 (*IGF1R*) Gene on Growth Traits in Angus Cows

Małgorzata SZEWCZUK[a]

[a]*The West Pomeranian University of Technology, Department of Ruminant Science, Laboratory of Biostatistics, Judyma 10, 71-460, Szczecin, POLAND*

ARTICLE INFO
Research Article
Corresponding Author: Małgorzata SZEWCZUK, E-mail: malgorzata.szewczuk@zut.edu.pl, Tel: +48 (91) 449 68 07
Received: 12 March 2015, Received in Revised Form: 05 July 2015, Accepted: 06 July 2015

ABSTRACT

Insulin-like growth factor 1 receptor (IGF1R) signaling pathway plays a key role in the postnatal growth and development. The tyrosine kinase receptor IGF1R forms homodimers that is activated by IGF-I which trigger autophosphorylation of IGF1R and subsequent downstream signal transduction. The objectives of this study were to identify, characterize and the examination of the association between silent SNP (rs41961336; C→T) within exon 7 of bovine *IGF1R* gene and growth traits. A total of 672 cows of four breeds was genotyped using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A *Tai*I restriction endonuclease was used. Among all breeds under study (Polish Holstein Friesian, Angus, Hereford, Limousine), the presence of all three genotypes was observed only for the Angus breed. The *CC* genotype was the most frequent in all investigated breeds (0.7987-0.9904) followed by *CT* (0.0096-0.1946). Only Angus cows were selected for association analysis. At weaning weight adjusted to 210 days of age (WWT_{210}), statistically significant differences ($P \leq 0.05$) were shown-female calves with the *CC* genotype were heavier (+5.5 kg) than the individuals with the *CT* genotype. However, individuals carrying the *CT* genotype had significantly higher body weight at first calving (+10.62 kg; $P \leq 0.05$). The present work failed to show association between genotypes of the *IGF1R*/*Tai*I polymorphism and BWT, ADG or age at first calving.

Keywords: Beef; Genetic markers; Tyrosine kinase; Quantitative trait loci

İnsülin-benzeri Büyüme Faktörü Reseptör Tip 1 (IGF1R) Geninin Ekson 7 İçindeki Tek Nükleotid Polimorfizminin (SNP) Angus İneklerinin Büyüme Ölçütleri Üzerine Olan Etkileri

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Małgorzata SZEWCZUK, E-posta: malgorzata.szewczuk@zut.edu.pl, Tel: +48 (91) 449 68 07
Geliş Tarihi: 12 Mart 2015, Düzeltmelerin Gelişi: 05 Temmuz 2015, Kabul: 06 Temmuz 2015

ÖZET

İnsülin benzeri büyüme faktörü-1 reseptör (IGF1R) sinyal yolu, doğum sonrası büyüme ve gelişmede önemli bir rol oynar. IGF1R tirozin kinaz reseptörü, IGF1R'nin otofosforilasyonunu tetikleyen IGF-I tarafından aktive edilen homodimerleri ve sonrasında meydana gelen aşağı sinyal transdüksiyonu oluşturmaktadır. Bu çalışmanın amacı, sığır *IGF1R* geni içinde bulunan ekzon 7 içindeki sessiz SNP (rs41961336; C→T) ile büyüme özellikleri arasındaki ilişkinin tanımlanması, karakterize edilmesi ve incelenmesidir. Dört farklı ırktan toplam 672 adet inek polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi (PCR-RFLP) metodu kullanılarak genotiplenmiştir. Çalışılan tüm ırklar (Polonya Holstein Friesian, Angus, Hereford, Limousine) içinde, her üç genotip varlığı sadece Angus ırkında gözlenmiştir. *CC* genotipi araştırılan tüm ırklarda en sık gözlenen genotip olmuş (0.7987-0.9904) ve bunu *CT* genotipi takip etmiştir (0.0096-0.1946). Sadece Angus inekleri ilişkilendirme analizleri için seçilmiştir. 210 günlük yaşa (WWT_{210}) düzeltilmiş sütten kesim ağırlığında, *CC* genotipli dişi buzağıların, *CT* genotipli bireylerden istatistiki olarak daha ağır oldukları (+5.5 kg) bulunmuştur ($P \leq 0.05$). Buna karşılık, *CT* genotipini taşıyan bireyler, ilk buzağılamada daha yüksek canlı ağırlık kazanmıştır (+10.62 kg; $P \leq 0.05$). Bu çalışma, *IGF1R/TaiI* polimorfizminin genotipleri ile doğum ağırlığı, günlük canlı ağırlık kazancı veya ilk buzağılama yaşı arasındaki ilişkiyi göstermede başarısız olmuştur.

Anahtar Kelimeler: Sığır; Genetik markörler; Tirozin kinaz; Kantitatif özellik lokusu

1. Introduction

The insulin-like growth factor (IGF) signaling pathway has many important roles in normal cell growth and development. In mammals, insulin regulates cell metabolism, whereas insulin-like growth factor I (IGF-I) is an important regulator of cell growth (Humbel 1990). Remarkably, all of the components of this system (IGFs, receptors, and binding proteins) are also expressed in bovine mammary gland (Plath-Gabler et al 2001) and skeletal muscle (Blum et al 2007; Micke et al 2011) where development, nutrient uptake and utilization is largely coordinated by growth hormone (GH) and its main downstream effector, IGF-I. In laboratory animals, several studies have shown that GH treatment increases IGF-I mRNA in skeletal muscle through STAT5 molecules (Udy et al 1997; Teglund et al 1998; Woelfle et al 2003). IGF-I signals via the type 1 of IGF-I receptor (IGF-IR), which is a widely expressed cell surface $\alpha_2\beta_2$ heterotetramer held together by disulfide bridges, highly similar to the IR (Ullrich et al 1986). The importance of the IGF-IR in normal mammalian development is clear from studies in mice lacking functional receptors. Liu et al (1993) demonstrated that *igf1r* null mice were 45% of the size of wild-type individuals at birth, and died shortly due to severe organ hypoplasia while mouse embryonic fibroblasts cultured from *igf1r* null mice grew more slowly than wild-type fibroblasts, and were unable to proliferate under anchorage-independent conditions (Sell et al 1994).

The bovine IGF-IR, similar to human homologue, is synthesized as a single chain precursor composed of 1367 amino acids. The pre-proreceptor monomer of human IGF-IR includes a 30 residue signal peptide (residues 1 to 30) and an Arg-Lys-Arg-Arg furin protease cleavage site at residues 737-740, divided into one α-chain and one β-chain. The α-chain (residues 31-736) and 195 residues of β-chain comprise the extracellular portion of the IGF-IR and contain eleven and five potential N-linked glycosylation sites, respectively (Ullrich et al 1986). Subsequently, there is also a single transmembrane sequence (residues 936-959) and a 408 residue cytoplasmic domain (residues 960-1337) possessing intrinsic kinase activity (Favelyukis et al 2001). Free IGF-I molecule bind to the cysteine-rich domain of the α-subunits, leading to the transmission of a specific signal through the transmembrane domain to the β-subunit. Conformational change of the transmembrane domain causes stimulation of tyrosine kinase activity, followed by autophosphorylation of a cluster of tyrosine residues of the IGF-IR (Keyhanfar

et al 2007). It is known, that IGF-I induces skeletal muscle hypertrophy by activating the IGF-IR/IRS1/PI3K/Akt pathway (Shi et al 2011).

Human *IGF1R* gene is greater than 300 kbp in size and contain 21 exons, ten in the α-chain and eleven in the β-chain (Ullrich et al 1986). Bovine *IGF1R* homolog has been mapped to chromosome 21 (Moody et al 1996). More than 200 single nucleotide polymorphisms (SNPs) were identified in *Bos taurus* and submitted to the National Center for Biotechnology Information (NCBI) website (NCBI reference sequence: AC_000178.1; alternate assembly UMD 3.1 WGS) but most of them are located in non-coding regions. Several of these SNPs are silent mutation or localized within 3'UTR.

The objectives of this study were to identify, characterize and the examination of the association between SNP within exon 7 of bovine *IGF1R* and performance traits.

2. Material and Methods

The study involved a total of 672 female individuals including Angus (n= 298), Limousine (n= 141), Polish Holstein Friesian (n= 129) and Hereford (n= 104) cows kept on four different farms (one for each breed) belonging to the largest co-operative firm located in the West Pomeranian province, Poland.

Dairy cows were kept in an intensive system in free stalls with access to the feed. A complete Total Mixed Ration (TMR) feeding system was used and cows had continuous access to water from automatic drinkers. Milking took place twice a day in the milking parlor, "side by side", using the ALPRO system.

Beef cows were kept in a chamber system. Only calving cows were driven to the barn, where they stayed with their calves for one week after calving. After this time, dams with their offspring were moved to the outside run. Summer feeding was entirely based on the good pasture. In winter, the diet consisted of grass silage and maize, haylage and hay supplemented with minerals and vitamins. Calving cows were additionally fed the B-1 concentrate mixture. The animals had permanent access to water. Data on the beef performance were collected based on the breeding documentation ("heifer-beef cow" charts) and the information from the farm according to the guidelines of The Polish Association of Beef Cattle Breeders and Producers.

Peripheral blood was taken from animals from the external jugular vein into test tubes containing the EDTA anticoagulant. The DNA was isolated using MasterPure™ DNA Purification Kit (Epicentre Technologies) according to the manufacturer's instructions.

A silent transition at the third nucleotide of the codon encoding for aspartic acid (D^{491}; GA**C** → GA**T**) is localized within exon 7 of the *Bos taurus* *IGF1R* gene and submitted as rs41961336 (NCBI 2015).

Genotyping of the selected *IGF1R* SNP marker was carried out using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A *Tai*I restriction endonuclease (neoschizomer of *Mae*II) is able to recognize cytosine at this position. To genotype the *IGF1R*/e7/*Tai*I polymorphism, a pair of primers (IGF1Re7F 5' acagtgtttgggtccttagtgg 3' and IGF1Re7R 5' aggtgatgatgattcggttctt 3') were designed based on the sequence of the *IGF1R* gene (GenBank Accession No. JQ715681) and then used to amplify a 236-bp DNA fragment.

The PCR reaction volume of 20 µL contained approximately 30-50 ng of genomic DNA, 0.5 units of *Taq* DNA polymerase (FERMENTAS, Lithuania), 1×PCR buffer with $(NH_4)_2SO_4$ (FERMENTAS, Lithuania), 2 mM $MgCl_2$, 10 pmol of each primer (IBB PAS, Poland), 200 µM of each dNTP (FERMENTAS, Lithuania) and nuclease-free deionized water (Epicentre Technologies, Madison, USA). Thermal cycling conditions were as follows: 5 min at 94 °C, 30 cycles of 94 °C for 30 s, annealing temperature (60 °C) for 30 s, and 72 °C for 40 s, followed by a final step of 72 °C for 5 min in the Biometra thermal cycler. Amplified fragments were digested for 3 hours at 65 °C with 5 units of *Tai*I restriction enzyme (10 U μL^{-1}, A**C**GT↓;

FERMENTAS, Lithuania), and next subjected to electrophoretic separation in 2% ethidium bromide-stained Agarose gel (Basica LE GQT, Prona, Spain). The length of the obtained products was compared with the pUC19/*Msp*I molecular mass marker (FERMENTAS, Lithuania). To confirmation, selected samples for a particular genotypes were additionally sequenced (IBB PAS, Poland).

Association analyses between genotype and birth weight (BWT), weaning weight adjusted to 210 days of age (WWT_{210}) as well as average daily gains between birth and weaning (ADG) following by age and body weight at first calving was analyzed based on the data obtained from the official recordings.

The differences between particular genotypes were evaluated with Duncan's test and Bonferroni's test (STATISTICA 10.0 PL software package, Statsoft Inc. 2009). Statistical calculations were performed using a General Linear Model (GLM). As the statistical models Equation 1 and Equation 2 were used.

$$BWT, ADG, WWT_{210}, Y_{ijkl} = \mu + G_i + s_j + BYS_k + e_{ijkl} \quad (1)$$

Where; Y_{ijkl}, analyzed trait; μ, overall mean; G_i, fixed effect of *IGF1R* genotype (i= 1, 2); s_j, random effect of sire (j= 1,…,53); BYS_k, fixed effect of birth year/season (k= 1,…,20); e_{ijkl}, random error

Age and body weight at first calving,

$$Y_{ijklm} = \mu + G_i + s_j + CYS_k + b_l(cBW - cBW_l) + e_{ijklm} \quad (2)$$

Where; Y_{ijklm}, analyzed trait; μ, overall mean; G_i, fixed effect of *IGF1R* genotype (i= 1, 2); s_j, random effect of sire (j= 1,…,53); CYS_k, fixed effect of year/season of 1st calving (k= 1,…,16); b_l, regression coefficient on birth weight of calves; cBW, mean birth weight of calves; cBW_l, birth weight of a *l*-calf; e_{ijklm}, random error

The chi-square test was used to verify whether each population is in Hardy-Weinberg equilibrium.

3. Results and Discussion

Among all breeds under study, the presence of all three genotypes was observed only for the Angus breed (Table 1). Based on the chi-square test, it was found that genotype frequencies of *IGF1R*/*Tai*I polymorphism in all breed under study were under genetic equilibrium. The PCR products with cytosine at position 191 (GenBank acc. No JQ715681) were digested by *Tai*I into four fragments of 141, 45, 26 and 24 bp (allele C) while allele T gave a cleavage pattern of 167, 45 and 24 bp (Figure 1). The *CC* genotype was the most frequent in all investigated breeds (0.7987-0.9904) followed by *CT* (0.0096-0.1946). Due to the *TT* homozygote deficiencies, C allele frequency was very high-more than or equal to 0.9 in all population, whereas that of T allele was only equal or less than 0.1.

Table 1- Numbers and frequencies of genotypes and alleles of the *IGF1R*/e7/*Tai*I polymorphism

Çizelge 1- IGF1R/e7/TaiI polimorfizminin genotip ve allellerdeki sayı ve frekansları

Breed		*IGF1R/TaiI genotypes*			*Total*	*Allele*	
		CC	*CT*	*TT*		*C*	*T*
Polish Holstein Friesian	n	124	5	0	129	0.9806	0.0194
	f	0.9612	0.0388	0.0000	1.0000		
Angus	n	238	58	2	298	0.8960	0.1040
	f	0.7987	0.1946	0.0067	1.0000		
Hereford	n	103	1	0	104	0.9951	0.0049
	f	0.9904	0.0096	0.0000	1.0000		
Limousine	n	138	3	0	141	0.9894	0.0106
	f	0.9787	0.0213	0.0000	1.0000		
Total					672		

n, number of cows; f, frequency

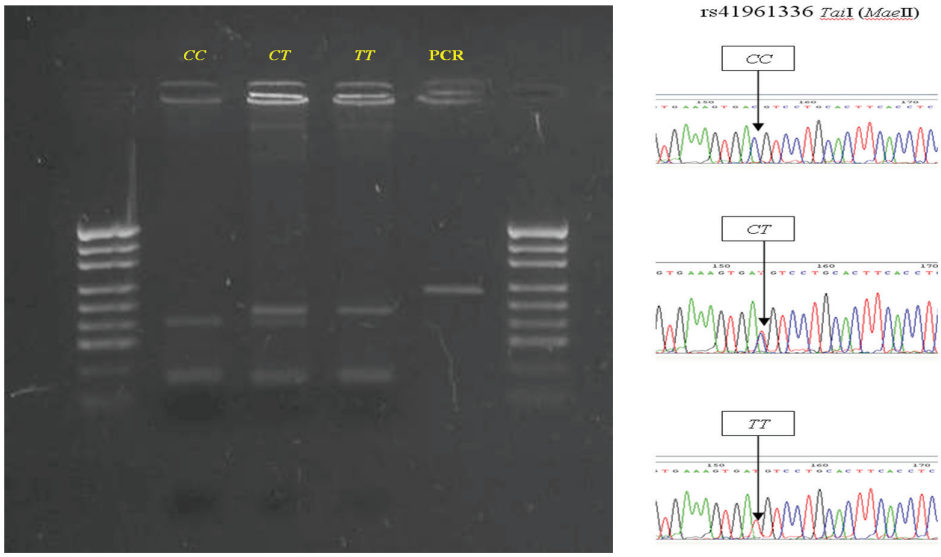


Figure 1- Agarose gel electrophoresis and fluorogram to distinguish *Tai*I restriction fragment length polymorphism in 236-bp fragment of the bovine insulin-like growth factor receptor type 1 gene, lanes 1 and 6 - pUC19/MspI DNA mass marker, lanes 2-4 genotypes, lane 5 PCR product without digestion

Şekil 1- 236 bp büyüklüğündeki sığır insülin-benzeri tip 1 büyüme faktörü reseptörü genindeki TaiI kesim parçasını ayırtetmek için agaroz jel elektroforezi ve fluorogramı, hat 1 ve 6- pUC19/MspI DNA belirteci, hat 2-4 genotipler, hat 5 kesimsiz PCR ürünü

Despite the fact that a total of 672 cows of three breeds raised for meat production and one breed used for milk production were genotyped, only Angus cows with two groups of genotypes (*CC* and *CT*) could be selected for further analysis. Due to the low number of individuals in the group (n= 2), the *TT* genotype group was excluded. Results for association analysis between *IGF1R*/*Tai*I genotypes and studied traits in Angus are presented in Table 2. At WWT_{210}, statistically significant differences (P≤0.05) between body weights of female calves with the *CC* genotype (+5.5 kg) and the female individuals with the *CT* genotype were shown. However, individuals carrying the *CT* genotype had significantly higher body weight at first calving (+10.62 kg; P≤0.05).

Table 2- Frequencies and mean values of the analyzed performance traits of Angus cows with the different *IGF1R* gene variants (standard errors in parentheses)

Çizelge 2- Farklı IGF1R gen değişkenliğine sahip Angus sığırlarında analiz edilen performans özelliklerinin ortalama değerleri ve frekansları (standart hatalar parantez içerisinde verilmiştir)

Polymorphism	*Genotype*	*n*	*f*	*BWT (kg)*	*ADG (g)*	*WWT_{210} (kg)*	*Age at first calving (days)*	*Body weight at first calving (kg)*
IGF1R/*Tai*I	CC	238	0.7987	36.61 (0.20)	992.97 (4.40)	246.91[a*] (1.27)	1067.71 (13.55)	563.86[a] (2.06)
	CT	58	0.1946	36.81 (0.42)	978.43 (10.48)	241.41[a] (2.72)	1060.72 (26.44)	574.48[a] (3.57)

*, means within columns bearing the same letters differ significantly at P≤0.05; f, frequency

In the present manuscript, there was no association between genotypes of the *IGF1R*/*Tai*I polymorphism and BWT, ADG or age at first calving.

Multiple genes potentially influencing meat and milk production have been investigated in a lot of breeds in order to find interbreed and intrabreed polymorphism and association with production traits. However, without clearly defined conclusions so far. The insulin-like growth factor I receptor (IGF1R) has an important effect on growth, carcass, and meat quality traits in many species. In this study, we have designed, optimized and validated protocol capable of detecting rs41961336 SNP within exon 7 of bovine *IGF1R*. In spite of the *IGF1R*/*Tai*I being a SNP in a coding region as a silent mutation, it could be only linked or in linkage disequilibrium with causative polymorphisms associated to phenotype differences. However, in addition to the rs41961336 only few SNPs within bovine exons are known and only one of them is potentially *missense*-rs209595810. Two polymorphic sites are recently described and validated (Szewczuk et al 2013). According to our research carried out in the years 2009-2011 (data not shown), a significant part of submitted SNPs (including unique–*missense* and majority of silent mutations) was not present in breeds under study. This can be explained by the fact that the NCBI database contains submission from whole world often taking into account rare native breeds or frequencies of genotypes. Furthermore, extreme low frequency of minor allele (MAF) of rs41961336 SNP was demonstrated. Only Angus breed (0.010) may be comparable to Brahman cattle (Fortes et al 2013) in this respect. Therefore, low MAF strongly limits the usability of this mutation in the association studies.

Analysis of evolutionary conservation of IGF-I and their receptors had provided insights into essential regions, in which the tyrosine kinase domain is highly conserved among vertebrate species (residues 999-1274) (Le Roith et al 1993) including an activation loop (tyrosines: Y^{1161}, Y^{1165} and Y^{1166}) (Favelyukis et al 2001). Studies performed on humans indicated that there were important associations of the IGF1R gene with growth and development (Inagaki et al 2007). As is shown by Blum et al (2007), the reduced presence of *IGF1R* may have been the underlying cause of dwarfism in calves. The association between SNP within exon 7 and growth traits in beef cattle has not been reported so far. For this reason, it was also the aim of this study to validate this SNP by analyzing the effect of the *IGF1R*/*Tai*I polymorphism on birth weight followed by daily gaining and body weight at different stages of live. It has been limited to Angus breed. Cows with the *CT* genotype with initially lowest WWT_{210} were then heavier at first calving in comparison to *CC* female individuals, despite the fact that first calving was an average of 7 days earlier. In the present work, rs41961336 failed to show association between genotypes and age at first calving. Recent studies show only that rs41961336 did not revealed associations with age of puberty in Brahman cattle (Fortes et al 2013). However, other polymorphism-*IGF1R*/*Msp*I within exon 12-had a significant association with WWT_{210} in Angus cows (Szewczuk et al 2013). No significant differences in growth traits were observed between the genotypes of intronic *IGF1R*/*Taq*I polymorphism in Nanyang cattle (Zhang & Li 2010). However, evidences suggest the presence of a QTL for growth traits on chromosome 21. Davis et al (1998), Casas et al (2003) and Morris et al (2003) reported a significant *Quantitative Trait Loci* (QTL) for birth weight in the centromeric region of BTA21 with support interval 1-10 cM, where almost exactly in the middle the *Bos taurus IGF1R* gene is localized. On the other hand, Kim et al (2003) and Casas et al (2004) found a QTL for birth weight with support interval 50-63 cM and relative positions at 62 or 56 centimorgans from the beginning of the linkage group (telomeric), respectively. As it can be seen, two QTL for the same trait reside on the same chromosome. This inconsistency across above mentioned studies can partly be explained among other things by the density and kind of the markers and the different breeds used in the studies.

4. Conclusions

Fine mapping around the marker site may eventually lead to the confirmation of some of the underlying genes associated with BW and other growth traits, and this may provide important insights into the biology of growth and development. For this reason, it is reasonable to evaluate the impact of other SNPs (more than 200) within bovine *IGF1R* gene. Once an objective has been defined and appropriate genetic relationships determined, marker-assisted selection may be a cost-effective option in breeding work in a beef herds.

References

Blum J W, Elsasser T H, Greger D L, Wittenberg S, de Vries F & Distl O (2007). Insulin-like growth factor type-1 receptor down-regulation associated with dwarfism in Holstein calves. *Domestic Animal Endocrinology* **33**: 245-268

Casas E, Shackelford S D, Keele J W, Koohmaraie M, Smith T P L & Stone R T (2003). Detection of quantitative trait loci for growth and carcass composition in cattle. *Journal of Animal Science* **81**: 2976-2983

Casas E, Keele J W, Shackelford S D, Koohmaraie M T & Koohmaraie R T (2004). Stone identification of quantitative trait loci for growth and carcass composition in cattle. *Animal Genetics* **35**: 2-6

Davis G P, Hetzel D J S, Corbet N J, Scacheri S, Lowden S, Renaud J, Mayne C, Stevenson R, Moore S S & Byrne K (1998). The mapping of QTL for birth weight in a tropical beef herd. *Proceeding 6th World Congress on Genetics Applied to Livestock*. Armidale NSW. Australia 26. pp. 441-444

Favelyukis S, Till J H, Hubbard S R & Miller W T (2001). Structure and autoregulation of the insulin-like growth factor 1 receptor kinase. *Nature Structural Biology* **8**(12): 1058-1063

Fortes M R, Li Y, Collis E, Zhang Y & Hawken R J (2013). The IGF1 pathway genes and their association with age of puberty in cattle. *Animal Genetics* **44**(1): 91-95

Humbel R E (1990). Insulin-like growth factors I and II. *European Journal of Biochemistry* **190**: 445-462

Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S & LeRoith D (2007). A familial IGF-1 receptor mutant leads to short stature: Clinical and biochemical characterization. *Journal of Clinical Endocrinology & Metabolism* **92**: 1542-1548

Keyhanfar M, Booker G W, Whittaker J, Wallace J C & Forbes B E (2007). Precise mapping of an IGF-I-binding site on the IGF-1R. *Biochemical Journal* **401**: 269-277

Kim J J, Farnir F, Savell J & Taylor J F (2003). Detection of quantitative trait loci for growth and beef carcass fatness traits in a cross between *Bos taurus* (Angus) and *Bos indicus* (Brahman) cattle. *Journal of Animal Science* **81**: 1933-1942

Le Roith D, Kavsan V M, Koval A P & Roberts C T Jr (1993). Phylogeny of the insulin-like growth factors (IGFs) and receptors: A molecular approach. *Molecular Reproduction and Development* **35**: 332-336

Liu J P, Baker J, Perkins A S, Robertson E J & Efstratiadis A (1993). Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75**: 59-72

Micke G C, Sullivan T M, McMillen I C, Gentili S & Perry V E A (2011). Protein intake during gestation affects postnatal bovine skeletal muscle growth and relative expression of IGF1, IGF1R, IGF2 and IGF2R. *Molecular and Cellular Endocrinology* **332**: 234-241

Moody D E, Pomp D & Barendse W (1996). Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. *Mammalian Genome* **7**: 168-169

Morris C A, Cullen N G, Pitchford W S, Hickey S M, Hyndman D L, Crawford A M & Bottema C D K (2003). QTL for birth weight in Bos taurus cattle. *Association for the Advancement of Animal Breeding and Genetics Proceedings* **15**: 400-403

NCBI (2015). Database of single nucleotide polymorphisms (SNPs) and multiple small-scale variations that include insertions/deletions, microsatellites, and non-polymorphic variants (dbSNP). Retrieved in Juni, 29, 2015 from http://www.ncbi.nlm.nih.gov/snp/

Plath-Gabler A, Gabler C, Sinowatz F, Beriska B & Schams D (2001). The expression of the IGF family and GH receptor in the bovine mammary gland. *Journal of Endocrinology* **168**: 39-48

Sell C, Dumenil G, Deveaud C, Miura M, Coppola D, DeAngelis T, Rubin R, Efstratiadis A & Baserga R (1994). Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts. *Molecular and Cellular Biology* **14**: 3604-3612

Shi J, Luo L, Eash J, Ibebunjo C & Glass D J (2011). The SCF-Fbxo40 complex induces IRS1 ubiquitination in skeletal muscle, limiting IGF1 signaling. *Developmental Cell* **21**: 835-847

Szewczuk M, Zych S, Wójcik J & Czerniawska-Piątkowska E (2013). Association of two SNPs in the coding region of the insulin-like growth factor 1 receptor (IGF1R) gene with growth-related traits in Angus cattle. *Journal of Applied Genetics* **54**: 305-308

Teglund S, McKay C, Schuetz E, Van Deursen J, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G & Ihle J N (1998). Stat5a and Stat5b proteins have essential and non-essential, or redundant, roles in cytokine responses. *Cell* **93**: 841-850

Udy G B, Towers R P, Snell R G, Wilkins R J, Park S H, Ram P A, Waxman D J & Davey H W (1997). Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 7239-7244

Ullrich A, Gray A, Tam A W, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J & Fujita-Yamaguchi Y (1986). Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO Journal* **5**: 2503-2512

Woelfle J, Chia D J & Rotwein P (2003). Mechanisms of growth hormone (GH) action. Identification of conserved Stat5 binding sites that mediate GH-induced insulin-like growth factor-I gene activation. *Journal of Biological Chemistry* **278**: 51261-51266

Zhang R & Li X (2010). Association between IGF-IR, m-calpain and UCP-3 gene polymorphisms and growth traits in Nanyang cattle. *Molecular Biology Reports* **38**(3): 2179-2184

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Assessment of the Effects of Some Bacterial Isolates and Hormones on Corm Formation and Some Plant Properties in Saffron (*Crocus sativus* L.)

Fazilet PARLAKOVA KARAGÖZ[a], Atilla DURSUN[a], Recep KOTAN[b], Melek EKİNCİ[a], Ertan YILDIRIM[a], Parisa MOHAMMADI[b]

[a]*Atatürk University, Faculty of Agriculture, Department of Horticulture, 25240, Erzurum, TURKEY*

[b]*Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240, Erzurum, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Fazilet PARLAKOVA KARAGÖZ, E-mail: f.parlakova@atauni.edu.tr, Tel: +90 (507) 640 25 07
Received: 20 April 2015, Received in Revised Form: 10 July 2015, Accepted: 10 July 2015

ABSTRACT

The saffron, from the Iridaceae family and an autumn-flowering geophytes, is one of cormous plants. The biggest obstacle in the development of this plant, production having the most economic value as one of the medicinal and aromatic plants, is the insufficient bulbous used for propagation. Bacterial isolates showing capacity to grow in nitrogen-free conditions, for hormones production (IAA, GA_3) and to solubilise phosphate as microbial fertilizer were used to reproduce the corms of saffron plants. Thus, the disappearance of saffron from the species that are under threat of extinction can be prevented and the continuation of the species can be provided by its widespread propagation as an ornamental plant. In this study, a total of ten treatments; (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C and (7) *Bacillus* GC group B strain TV119E, (8) Control (untreated bacteria or hormones) (9) Control 2 [100 mg L^{-1} *IBA* (indole-3 butyric acid)] and (10) Control 3 [100 mg L^{-1} GA_3 (gibberellic acid)] were tested to see their effects on the plant growth and development parameters of saffron. The number of cormlet, average cormlet diameter (mm), cormlet length (mm), cormlet weight (g), macro and micro plant nutrients (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B and Cd) contents of corms were determined in greenhouse assays. Some of the bacterial applications gave growth and yields of saffron equal to or higher than the hormones applied. Bio-fertilizers used in organic farming, increase in plant growth and development of saffron were concluded to have positive effect.

Keywords: PGPR; GA_3; IBA; Saffron; Cormlet

Bazı Bakteri İzolatları ve Hormon Uygulamalarının Safran (*Crocus sativus* L.) Bitkisinde Korm Oluşumu ve Kimi Bitki Özelliklerine Etkisi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Fazilet PARLAKOVA KARAGÖZ, E-posta: f.parlakova@atauni.edu.tr, Tel: +90 (507) 640 25 07
Geliş Tarihi: 20 Nisan 2015, Düzeltmelerin Gelişi: 10 Temmuz 2015, Kabul: 10 Temmuz 2015

ÖZET

Safran, süsengiller (*Iridaceae*) familyasından ve sonbaharda çiçek açan, soğanlı bitkilerden biridir. Ekonomik değeri yüksek, tıbbı ve aromatik bitkilerden biri olan bu bitkinin üretiminin geliştirilmesinde en büyük engel tohumluk olarak kullanılan soğanların yetersizliğidir. Safran bitkisinin kormlarının çoğalmasında mikrobiyal gübre olarak azot fiksasyonu yapabilme, fosfatı çözebilme ve hormon (IAA, GA_3) üretebilme özelliğine sahip bakteri izolatlarının kullanımı hedeflenmiştir. Böylece, nesli tehlike altında olan türlerden olan safranın yok olması önlenebilir ve bir süs bitkisi olarak üretiminin yaygınlaştırılmasıyla türlerin devamı sağlanabilir. Bu çalışmada, toplam on uygulama (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C and (7) *Bacillus* GC group B strain TV119E, (8) Kontrol (bakteri ve hormon uygulamasız) (9) Kontrol 2 [100 mg L^{-1} *IBA* (indole-3 butyric acid)] ve (10) Kontrol 3 [100 mg L^{-1} GA_3 (gibberellic acid)] safranın bitki büyüme ve gelişim parametreleri üzerindeki etkilerini belirlemek için test edilmiştir. Sera koşullarında, yavru korm sayısı, yavru korm çapı (mm), yavru korm uzunluğu (mm), yavru korm ağırlığı (g) ve kormların makro ve mikro (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B ve Cd) besin içerikleri belirlenmiştir. Safranın büyüme ve verim değerleri, bakteri uygulamalarının bazılarında hormon uygulamalarından daha yüksek veya eşit şekilde elde edilmiştir. Organik tarımda kullanılan biyo-gübrelerin, safranın bitki büyüme ve gelişimini artırması üzerine olumlu bir etkiye sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: PGPR; GA_3; IBA; Safran; Yavru korm

1. Introduction

Crocus sativus L. (saffron) belonging to Iridaceae family show spread in tropical and subtropical regions of the northern hemisphere of the world. This species has been cultivated for 4300 years (Escribano et al 2000). It is a perennial herbaceous plant cultured corm in many countries bordering the Mediterranean Sea especially in Italy, Spain, Greece and Turkey as well as in Japan, China, Iran and Azerbaijan (Vurdu et al 1997).

Saffron name is generally used both for the plant and spice. Saffron is known to be economically very important. The plant has been used by humans for centuries due to its smell, colour and curative effects (Tarantilis & Polissiou 1997; Lozano et al 1999; Carmona et al 2006). In the year of 1000 BC, saffron was rumoured to be used to paint the mummies that were stored in coffin-shaped crates or mummification in Egypt. Firstly, saffron was used as a hair dye in Romans and as a perfume later on (Basker & Negbi 1983).

C. sativus is to be grown in semi-arid climate having hot and dry winds, which are similar to habitat of the Mediterranean maquis and North America chaparral vegetation (similar to the Mediterranean maquis vegetation). Plants can survive cold winters and they may remain under snow until -10 °C in a short period of time (Chichiricco 1984; Amirghasemi 2001).

The *Crocus* genus includes approximately 80 species worldwide. There are about 32 species of *Crocus* genus in Turkey (Vurdu & Güney 2004). Some species also include subspecies between 2 and 10. A part of *Crocus* species bloom in autumn and some of them bloom in spring period. About 30 of these species are grown as ornamental plants (İpek et al 2009). Saffron cultivation area is limited in Turkey. Saffron has been cultured in an area of 1.5 ha in Karabük in Turkey (Kara 2010). The product obtained cannot even meet the domestic consumption. Therefore, Turkey needs the saffron corm. Some *Crocus* species are produced from both corm and seeds. But, *Crocus sativus* L. can only be produced from corm (Chichiricco 1984). Saffron flowers are sterile; i.e., the plant is not able to set viable seed. The pollen of Saffron sterility is auto triploid (2n= 24) (Chichiricco 1984). The propagation of corms is necessary because of the triploid nature of *Crocus sativus* L. (Warburg 1957).

Corms with 2-3 cm diameter have the best production of flowers and corms. Corm measurements

are important in terms of the relationship between flower number and weight of stigmas.

Modern agriculture is faced with increasing difficulty of growing worldwide, the decline in soil productivity and product quality, and due to rising consumer demand. Therefore, there is need for new and highly effective fertilizer to protect the ecological balance of nature. In this regard, plant growth-promoting bacteria (PGPR) could have a very important role.

Benefits of PGPRs on plants are the inclusion biocontrol, biological N_2 fixation, phosphorus solubilisation, production of siderophore and/or production of phytohormone encouraged directly to improve plant growth by means of bacteria (Mia et al 2012; Turan et al 2014). Bacteria are also encouraging to improve plant growth such as to suppress plant pathogens by indirect means (Kotan & Şahin 2002; Dobbelaere et al 2003; Şahin et al 2004; Çakmakçı et al 2006). Therefore, PGPR application is stated to increase the plant growth and yield as well as on improving the soil quality.

PGPRs produce phytohormones plant growth-promoting compounds- as auxins, cytokinins and gibberellins (Saikia et al 2006). Auxin causes cell expansion and growth, encourages cell elongation, tissue growth and root formation (Grunewald et al 2009). Auxin is an effective substance in cell volume and fraction (meristem formation). Therefore, it is effective in growth and development. In addition, it is used to end the dormancy in the some plants (Budak et al 1994; Kaynak & Ersoy 1997; Kaynak & Memiş 1997). Various development processes, such as stem elongation, control various aspects of seed germination, including dormancy break and mobilization of endosperm reserves, are influenced by endogenous gibberellins. Moreover, gibberellins influence the transition from juvenile stage to mature stage, induction of flowering, sex determination and fruit set establishment in the reproductive development (Taiz & Zeiger 2004).

Saffron cultivation area is limited in Turkey. Turkey has lost importance in saffron trade. The product obtained cannot even meet the domestic consumption. Therefore, Turkey needs the saffron corm. Saffron has several beautiful flowers and carries the perennial property due to corm. Because of this feature, the use of saffron as an ornamental plant in flower beds, among the grass, balconies and terraces, in the regulation of the roof garden will be of great importance in terms of providing the continuation of the species. There are few studies using PGPR as plant growth promoting agent in the cultivation of saffron (Sharaf-Eldin et al 2008; Parray et al 2013) around world and there is no study in Turkey. The aim of this study was to evaluate the plant growth parameters and corm formation on saffron by using PGPRs (*Achromobacter xylosoxidans* strain TV-42A, *Brevibacillus choshinensis* strain TV-53D, *Myroides odoratus* strain TV-85C*, Bacillus megaterium* strain TV-87A*, Colwellia psycrerytreae* strain TV-108G, *Kluyvera cryocrescens* strain TV-113C and *Bacillus* GC group B strain TV119E) and hormones (indole-3 butyric acid and gibberellic acid) treatments in saffron in greenhouse assays.

2. Material and Methods

2.1. Materials

2.1.1. Plant material

A total of 150 corms of saffron used in the experiments were obtained from the Safranbolu Directorate of District Food, Agriculture and Livestock, Karabük, Turkey. The corms were selected free of wounds and rots, and as homogeneous as possible in size (2.0 to 2.5 cm). They were stored at 1-4 ^{o}C until using. The study was conducted under the natural light in greenhouse condition at the Faculty of Agriculture at Atatürk University. Temperatures inside the greenhouse were determined as 15±2 ^{o}C at night and daytime temperatures were determined as 27±2 ^{o}C. Treated corms were planted on July 28, 2012 then the harvest was made on 08 May 2013.

2.1.2. PGPR bacteria and hormones

All of the bacterial strains (*Achromobacter xylosoxidans* strain TV-42A, *Brevibacillus choshinensis* strain TV-53D, *Myroides odoratus*

strain TV-85C, *Bacillus megaterium* strain TV-87A, *Colwellia psycrerytreae* strain TV-108G, *Kluyvera cryocrescens* strain TV-113C and *Bacillus* GC group B strain TV119E) were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University (Table 1). These non-pathogenic bacterial strains had been isolated from the rhizosphere and phyllosphere of wild and traditionally cultivated plants growing in Erzurum and Van Cities located in the Eastern Anatolia Region of Turkey (Kotan et al 2005; Erman et al 2010). The identity of all bacterial strains used in this study was confirmed according to fatty acid methyl esters (FAME) analysis by using Sherlock Microbial Identification System (Microbial ID, Newark, DE, USA) (Miller 1982). Bacterial cultures were grown on nutrient agar (NA; Difco™) for routine use, and maintained in Luria Broth (LB; Difco™) with 15% glycerol at -80 ºC for long-term storage. In the previous studies, all strains used in this study were determined that they showed capacity to grow in N-free conditions, for hormones production (IAA, GA_3) and to solubilise phosphate (Table 1) (Ekinci et al 2014; Kotan et al 2014; Turan et al 2014). In addition, the effectiveness of the commercially available indole-3 butyric acid (IBA; Indole-3 butyric acid/SIGMA/anhydrous, molecular weight 203.2 g) and gibberellic acids (GA_3 molecular weight 346.38 g) of hormones with the effectiveness of selected bacteria was used to compare and was aimed to determine the degree of activity.

2.2. Method

2.2.1. Corms surface disinfection with sodium hypochlorite

Corms were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the corm surface. Corms disinfection was performed by dipping the corm for 3 min. in 3% sodium hypochlorite and washing four times in sterilized and distilled water (sdH_2O). The corms were left to dry on sterile filter paper sheets overnight in the laminar flow hood to be used in further studies.

2.2.2. Media and growth condition

Tryptic Soy Agar (TSA, Oxoid) and Tryptic Soy Broth (TSB, Oxoid) medium were used in the experiments. All bacterial isolates were incubated in TSA at 27 °C for 24 h. After the incubation period, a single colony was transferred to 500 mL flasks containing TSB, and grown aerobically in the flasks on a rotating shaker (150 rpm) for at 27 °C for 48 h (Merck KGaA, Germany). Bacterial suspension was then diluted in sdH_2O to a final concentration of $1x10^8$ cfu mL^{-1} with a turbidimeter.

Table 1- Bacterial strains, their host, nitrogen fixation (N) and phosphate-solubilising activity (P) properties and hormones (IAA, GA_3) production (µg mL^{-1}) (Ekinci et al 2014; Turan et al 2014)

Çizelge 1- Kullanılan bakteri izolatlarının izole edildikleri bitki, azot fiksasyonu ve fosfat çözebilme özellikleri ve hormon (IAA, GA_3) üretimi (µg mL^{-1})

Bacterial strains	*Isolated from*	*N*	*P*	*GA_3*	*IAA*
Achromobacter xylosoxidans TV-42A	*Poaceae* sp.	S+	W+	299.532	1.402
Brevibacillus choshinensis TV-53D	*Taraxacum* sp.	S+	S+	362.206	1.140
Myroides odoratus TV-85C	Sugar beet	-	-	243.893	0.324
Bacillus megaterium TV-87A	Sugar beet	+	-	262.163	0.608
Colwellia psychrerytreae TV-108G	*Poaceae* sp.	+	-	166.856	0.000
Kluyvera cryocrescens TV-113C	*Allium* sp.	+	+	171.620	10.325
Bacillus GC group A TV-119E	*Poaceae* sp.	W+	+	290.349	0.509

+, positive; S+, strong positive; W+, weak positive; -, negative

2.2.3. Coating procedure of the bacteria on the corms

The bacteria were grown in TSB as described above. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and properly diluted to $1x10^8$ CFU mL^{-1} in sdH_2O. Approximately, 0.2 g of sucrose (10 mg mL^{-1}) was added to each Erlenmeyer flasks, and the surface-sterilized corms were soaked separately in this suspension. The corms were incubated in the flasks by shaking at 80 rpm for two hours at 28 ºC to coat the corms with the bacteria. As controls applications, untreated bacteria and hormones (100 mg L^{-1} IBA and 100 mg L^{-1} GA_3) were used in this study. Concentration of the hormone was prepared at the rate of 100 mg L^{-1} with drinking water. After then, the corms were soaked in these solutions for two hours to coat the corms with the hormones and untreated bacteria. After shaking, the corms were taken out and air-dried on sterile Whatman filter paper sheets overnight in the laminar flow hood.

2.2.4. Greenhouse studies

In the study, there were 10 treatments: (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C, (7) *Bacillus* GC group B strain TV119E, (8) Control 1 (untreated bacteria or hormones), (9) Control 2 (only IBA treatment) and (10) Control 3 (only GA_3 treatment). Research was established in a complete randomized design with 3 replications and each replication have 5 plants. For each application, 5 saffron corms were planted. A total of 150 corms were used, and the study was conducted under greenhouse conditions. Experimental soil composed of 1:1:1 ratio of soil, sand and farmyard manure. Treated corms were planted in black pouch having 3 litres volume. During the study period, considering the humidity and temperature values of greenhouse, irrigation was performed according to the irrigation requirement of the saffron plant.

2.2.5. Evaluation of the results

Vegetative growth of saffron plant such as the number of days between planting and emergence, emergence ratio (%), number of stems (number $plant^{-1}$), number of leaf (number $plant^{-1}$), the thickness of root collar (mm), number of cormlet, average cormlet diameter (mm), cormlet length (mm) and cormlet weight (g) of saffron plant were determined. Macro and micro nutrient (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B and Cd) contents of corms were also determined. Plant samples were oven-dried at 68 °C for 48 h and were then ground. Potassium (K), Ca and Mg were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-Salicylic acid mixture. Phosphorus (P) was determined spectrophotometrically by the vanadomolybdophosphoric-yellow method (Lott et al 1956). Potassium (K) and Ca were determined by flame photometry, and Mg, Cu, Fe, Mn, Na, Zn, Pb, B and Cd were determined by atomic absorption spectrometry using the methods of AOAC (1990). Boron was determined, after dry-ashing of plant samples, spectrophotometrically at 550 nm by the curcumin method (Odom 1992).

2.3. Statistical analysis

Data treated by the analysis of variance by using the SPSS version 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). For the significance level, 5% has been set to be the maximum acceptable limit to be considered as a significant result.

3. Results

The emergence dates of saffron subjected to different treatments were given in Table 2. The treatments affected the emergence dates of saffron. The earliest emergence date was obtained from *M. odoratus* TV-85C and *C. psycrerytrea* TV-108G bacterial strains as 48-53 days (Table 2). Emergence of plant continued from the 1st week of September to the 2nd week of October.

The resulting effects of the treatments on emergence ratio and some morphological values of saffron plant were given in Table 3. According

Table 2- The effect of different treatments on saffron-emergence dates

Çizelge 2- Farklı uygulamaların safran çıkış sürelerine etkisi

Treatments	*Number of days between planting and emergence*
A. xylosoxidans TV-42A	50-54
B. choshinensis TV-53D	48-60
M. odoratus TV-85C	48-53
B. megaterium TV-87A	49-60
C. psychrerytreae TV-108G	48-53
K. cryocrescens TV-113C	50-54
Bacillus GC group A TV-119E	51-65
Control 1 (untreated)	50-58
Control 2 (100 mg L^{-1} IBA)	51-58
Control 3 (100 mg L^{-1} GA_3)	52-65

to these results, there were no significant (P>0.05) differences in terms of IBA, GA_3 and all bacterial treatments when compared to the control treatment on emergence ratio (%). However, emergence ratio in GA_3 treatment (86.67%) was found low according to the other treatments. It was found that emergence ratio was approximately the same to the bacteria treatments and IBA treatment.

The effects of the all bacterial treatments on the number of saffron stems was significant (at P<0.001) according to the control 3 (100 mg L^{-1} GA_3). The average number of stem of all applications was 2.65 number $plant^{-1}$. Minimum number of stem (1.70 number $plant^{-1}$) was obtained from the GA_3 treatment. The maximum number of stems wre obtained from *A. xylosoxidans* TV-42A. The increase in *A. xylosoxidans* TV-42A application as the control 1, 2 and 3 were 73.9%, 60.0% and 135.3%, respectively. *B. choshinensis* TV-53D, *M. odoratus* TV-85C, *B. megaterium* TV-87A, *Bacillus* GC group A TV-119E, control 1 (untreated) and control 2 (100 mg L^{-1} IBA) were located in the same group. There were no significant (P>0.001) differences in terms of IBA, GA_3 and bacterial treatments when compared to the untreated control treatment on the number of saffron leaf. The highest number of leaf were obtained from *A. xylosoxidans* TV-42A (17.20 number $plant^{-1}$), *C. psychrerytreae* TV-108G (16.63 number $plant^{-1}$) and *K. cryocrescens* TV-113C (15.60 number $plant^{-1}$). Average number of leaf was 14.39 number $plant^{-1}$. There were no significant (at P>0.05) effects of treatments on the thickness of root collar. According to the control treatment, the maximum thickness of root collar (2.75 mm) was obtained from *C. psycrerytreae* TV-108G bacteria treatment.

Table 3- The effects of the bacteria and hormones treatments on the emergence ratio and some morphological values of saffron plant

Çizelge 3- Bakteri ve hormon uygulamalarının safran bitkisinin çıkış oranı ve kimi morfolojik özelliklerine etkisi

Treatments	*Emergence ratio (%)*	*Number of stems (number $plant^{-1}$)*	*Number of leaf (number $plant^{-1}$)*	*The thickness of root collar (mm)*
A. xylosoxidans TV-42A	100.00±0.00	4.00±0.20 a	17.20±0.72 a	2.32±0.36
B. choshinensis TV-53D	93.33±11.54	2.47±0.12 c	14.80±0.20 cd	2.57±0.19
M. odoratus TV-85C	93.33±11.55	2.40±0.00 c	12.40±0.60 f	2.42±0.10
B. megaterium TV-87A	93.33±11.55	2.33±0.08 c	13.80±0.00 def	2.61±0.13
C. psychrerytreae TV-108G	100.00±0.00	3.00±0.00 b	16.63±1.38 ab	2.75±0.22
K. cryocrescens TV-113C	93.33±11.55	3.20±0.00 b	15.60±0.80 bc	2.44±0.01
Bacillus GC group A TV-119E	100.00±0.00	2.60±0.20 c	13.00±0.60 ef	2.49±0.18
Control 1 (untreated)	100.00±0.00	2.30±0.30 c	12.40±1.00 f	2.62±0.07
Control 2 (100 mg L^{-1} IBA)	100.00±0.00	2.50±0.30 c	14.00±1.20 def	2.59±0.30
Control 3 (100 mg L^{-1} GA_3)	86.67±23.09	1.70±0.30 d	14.10±1.10 cde	2.52±0.33
Average	96.00±9.68	2.65±0.62	14.39±1.76	2.53±0.22
F-values	0.61ns	31.60***	11.16***	0.95ns

ns, non-significant; ***, significant at P<0.001; difference between the means with same letter in a column is not significant

The effects of the treatments on the number of cormlet, cormlet diameter, cormlet length and cormlet weight of saffron plant were given in Table 4. As shown, the bacteria except *B. megaterium* TV-87A and hormones treatments significantly ($P<0.001$) increased the number of saffron cormlets according to control 1 (untreated). When the values obtained in the yield are compared with the value before planting, a reduction is observed. The maximum number of cormlet was obtained from *A. xylosoxidans* TV-42A (2.8 number plant^{-1}) and *K. cryocrescens* TV-113C (2.4 number plant^{-1}) application. Cormlet diameter (mm) and cormlet length (mm) were found significant ($P<0.001$) in bacteria and hormones treatments when compared to the control 1. Also, effect of bacteria and hormones treatments on cormlet weight (g plant^{-1}) was found significant ($P<0.05$). The maximum cormlet diameter, cormlet length and cormlet weight were obtained from *B. megaterium* TV-87A. However, this application was in the same group with control 1 (untreated) treatment.

The treatments had significant effects on P, Ca, S, K, Mg (at $P<0.001$) and N (at $P<0.01$) (Table 5). The maximum P (1.98%) and K (1.85%) were found in *B. megaterium* TV-87A treatment while the maximum (1.80%) total N was found in *K. cryocrescens* TV-113C bacteria treatment. The maximum Mg (0.21%) was determined in *Bacillus* GC group TV-119E treatment. *K. cryocrescens* TV-113C, *Bacillus GC group* TV-119E bacteria treatments and GA_3 treatments were in the same group and it was concluded that there were no significant differences among these treatments (Table 5).

The treatments had significant effects on Fe, Na, Mn, Zn, Cu, Pb, Cd and B nutrient elements at $P<0.001$. The maximum total Na (225.25 mg kg^{-1}) was found in *B. choshinensis* TV-53D bacteria treatment while the maximum Fe (157.08 mg kg^{-1}) and Zn (54.60 mg kg^{-1}) were found in *B. megaterium* TV-87A bacteria treatment. The maximum (35.20 mg kg^{-1}) total Mn was obtained from *B. megaterium* TV-87A bacteria treatment, but there was no significant difference between it and the control. According to control 1 treatment, the maximum (52.80 mg kg^{-1}) total Cu and the maximum (8.80 mg kg^{-1}) total Pb were obtained from control 2 (100 mg L^{-1} IBA) treatment. According to the control treatment, the maximum (21.32 mg kg^{-1}) total B was determined in

Table 4- The effects of the bacteria and hormones treatments on some morphological values of saffron corms

Çizelge 4- Bakteri ve hormon uygulamalarının safran kormlarının kimi morfolojik özelliklerine etkisi

Treatments	*Number of cormlet (number plant^{-1})*	*Cormlet diameter (mm)*	*Cormlet length (mm)*	*Cormlet weight (g plant^{-1})*
A. xylosoxidans TV-42A	2.80±0.40 a	10.05±0.61 de	7.51±1.01 d	0.77±0.10 b
B. choshinensis TV-53D	2.10±0.10 bc	11.03±0.79 cd	8.42±0.85 cd	1.37±0.20 a
M. odoratus TV-85C	2.30±0.30 bc	11.23±0.28 bcd	8.28±0.45 cd	1.28±0.13 a
B. megaterium TV-87A	1.40±0.00 e	12.80±0.99 a	10.00±0.03 a	1.50±0.20 a
C. psychrerytreae TV-108G	2.20±0.20 bc	12.44±1.53 ab	9.69±0.33 ab	1.38±0.34 a
K. cryocrescens TV-113C	2.40±0.35 ab	11.21±0.65 bcd	8.38±0.61 cd	1.18±0.02 a
Bacillus GC group A TV-119E	1.60±0.00 de	8.87±0.13 e	8.69±0.48 bc	1.33±0.30 a
Control 1 (untreated)	2.30±0.30 bc	12.42±0.47 ab	9.98±0.81 a	1.28±0.14 a
Control 2 (100 mg L^{-1} IBA)	1.90±0.10 cd	11.67±0.49 abc	9.89±0.08 a	1.21±0.14 a
Control 3 (100 mg L^{-1} GA_3)	1.60±0.20 de	12.32±0.08 abc	9.98±0.71 a	1.44±0.28 a
Average	2.05±0.46	11.40±1.32	9.08±1.03	1.27±0.26
F-values	10.02***	8.44***	6.74***	2.97*

*, significant at $P<0.05$; ***, significant at $P<0.001$; difference between the means with same letter in a column is not significant

Table 5- Macronutrient concentrations of saffron corm (%)

Çizelge 5- Safran kormlarına makro besin elementi konsantrasyonları (%)

Treatments	*N*	*P*	*K*	*Ca*	*S*	*Mg*
A. xylosoxidans TV-42A	1.60±0.10 ab	0.24±0.02 e	1.56±0.04 c	0.62±0.03 f	0.51±0.02 d	0.33±0.02 b
B. choshinensis TV-53D	1.70±0.10 ab	0.24±0.01 e	1.44±0.08 d	0.62±0.02 f	0.42±0.05 e	0.37±0.02 a
M. odoratus TV-85C	1.60±0.20 ab	0.25±0.02 e	1.66±0.03 b	0.68±0.03 e	0.43±0.02 e	0.36±0.02 ab
B. megaterium TV-87A	1.60±0.10 ab	0.28±0.01 e	1.85±0.02 a	0.70±0.01 e	0.49±0.01 d	0.38±0.01 a
C. psychrerytreae TV-108G	1.30±0.10 c	0.17±0.01 f	1.12±0.02 e	0.37±0.02 g	0.35±0.01 f	0.25±0.02 cd
K. cryocrescens TV-113C	1.80±0.10 a	1.98±0.02 a	0.29±0.03 f	1.50±0.03 ab	0.67±0.02 a	0.20±0.01 d
Bacillus GC group A TV-119E	1.70±0.10 ab	1.66±0.08 d	0.24±0.02 f	1.47±0.03 b	0.63±0.02 b	0.21±0.01 d
Control 1 (untreated)	1.60±0.10 ab	1.85±0.04 b	0.24±0.01 f	1.42±0.03 c	0.62±0.01 bc	0.20±0.02 d
Control 2 (100 mg L^{-1} IBA)	1.50±0.10 b	1.76±0.03 c	0.26±0.02 f	1.52±0.02 a	0.69±0.01 a	0.21±0.02 d
Control 3 (100 mg L^{-1} GA_3)	1.60±0.10 ab	1.77±0.04 c	0.26±0.01 f	1.20±0.02 d	0.59±0.01 c	0.19±0.03 d
Average	1.60±0.16	1.02±0.80	0.89±0.67	1.01±0.44	0.54±0.11	0.27±0.08
F-values	4.10**	1725.00***	1240.68***	986.45***	90.96***	53.33***

, significant at P<0.01; *, significant at P<0.001; difference between the means with same letter in a column is not significant

A. xylosoxidans TV-42A bacteria treatment. However, *M. odoratus* TV-85C and *B. megaterium* TV-87A treatments and *A. xylosoxidans* TV-42A treatment were in the same group. The maximum (1.91 mg kg^{-1}) total Cd was determined in the control 3 (100 mg L^{-1} GA_3) treatment (Table 6).

Table 6- Micronutrient and heavy metal concentrations of saffron corm (mg kg^{-1})

Çizelge 6- Safran kormlarına mikro besin elementi ve ağır metal konsantrasyonları (mg kg^{-1})

Treatments	*Na*	*Fe*	*Mn*	*Zn*	*Cu*	*Pb*	*B*	*Cd*
A. xylosoxidans TV-42A	187.31±0.69 d	151.70±1.67 c	28.88±0.65 c	48.38±0.94 b	10.95±0.08 g	0.15±0.01 f	21.32±0.80 a	0.17±0.02 g
B. choshinensis TV-53D	225.25±3.28 a	128.70±0.33 g	30.81±0.17 bc	47.45±1.56 b	11.06±0.83 g	0.47±0.02 c	17,.26±0.28 b	0.35±0.01 e
M. odoratus TV-85C	182.78±1.20 e	139.04±0.97 d	31.08±1.01 b	48.98±0.42 b	12.64±0.43 f	0.37±0.01 e	20.54±0.55 a	0.22±0.01 fg
B. megaterium TV-87A	206.40±2.92 b	157.08±0.19 b	35.20±1.58 a	54.60±1.03 a	11.20±0.89 g	0.40±0.01 d	21.00±0.78 a	0.16±0.01 g
C. psychrerytreae TV-108G	191.73±3.59 c	185.64±1.34 a	14.40±0.96 d	27.38±0.99 e	7.20±0.39 h	0.07±0.01 g	13.34±0.81 c	0.29±0.01 ef
K. cryocrescens TV-113C	145.01±1.08 g	139.54±0.49 d	13.53±1.00 d	22.96±0.16 f	34.31±0.53 e	0.68±0.01 b	10.25±0.53 d	1.72±0.12 cd
Bacillus GC group A TV-119E	150.15±2.85 f	132.36±1.53 f	14.38±1.60 d	27.32±0.43 e	43.45±0.56 c	0.87±0.02 a	10.36±0.71 d	1.67±0.10 d
Control 1 (untreated)	138.48±1.57 h	135.00±3.01 e	12.88±0.68 d	33.18±0.60 c	39.50±0.53 d	0.67±0.02 b	10.16±0.58 d	1.85±0.04 ab
Control 2 (100 mg L^{-1} IBA)	144.00±1.64 g	139.00±2.03 d	14.08±1.5 d	31.08±1.01 d	52.80±0.24 a	0.88±0.02 a	10.34±0.25 d	1.80±0.05 bc
Control 3 (100 mg L^{-1} GA_3)	132.34±0.74 ı	121.00±0.91 h	14.28±1.30 d	26.85±1.16 e	45.20±1.15 b	0.69±0.01 b	10.34±0.50 d	1.91±0.01 a
Average	170.35±31.27	142.91±17.66	20.95±8.94	36.82±11.30	26.83±17.14	0.53±0.27	14.49±4.82	1.01±0.79
F-values	639.62***	449.43***	194.82***	483.11***	2317.43***	1072.2***	199.73***	689.959***

***, significant at P<0.001; difference between the means with same letter in a column is not significant

4. Discussion

There are several PGPR inoculants presently commercialized that seem to promote growth through at least one mechanism; improved nutrient acquisition (Biofertilizers) or phytohormone production (Biostimulants). In this study, a total of seven bacterial strains and hormone applications were tested to see their effects on plant growth promoters of saffron.

There has no information on the number of stems per plant so far. The number of stem is an important character of which there was a very close relationship between the number of stem and the number of corm per plant. As a matter of fact, İpek et al (2009) reported the same finding. Deo (2003) indicated that the large bulb had between 5-11 leaves. The average number of leaves was the same with the findings and the treatments had enhancing effect on the parameter. It is considered that there may be closely related to the number of leaves and the number of stems.

Frank (1986) stated that bulbous plants blooming in autumn are usually blooming in the second year. Soheilivand et al (2007), reported that Iranian origin saffron bulbous bloomed in the second year. Saffron bulbous of our research were not bloomed in the first year. Findings of our research were in accordance with these findings. As a matter of fact, the aim of our study was to get a large number of quality corms and to investigate the availability of the resulting quality corms as well as an ornamental plant. Based on the obtained results, we can interpret that the use of these bacterial isolates in the cultivation of ornamental plants may be one of the important tools for decreasing the maintenance cost and achieving sustainability in the landscapes. Benschop (1993) determined that *Crocus sativus* is the most important specie bloomed in autumn and Crocus species is used as a garden plant and pot. After all other flowering plants seeding in October, they bloom bright-coloured flowers with a darker purple colour from light pastel mauve (Willard 2002). Flowers are similar to lilies and they are at size of tulips (Safranbolu 2015).

Bacterial inoculations increased the plant nutrient element content. The plant nutrient elements Pb, Cd, S and Ca that obtained from GA_3 application were excluded. The highest content of K, Mg, Mn, Zn and B were obtained from *B. megaterium* TV-87A bacterial application. Furthermore, maximum cormlet diameter, cormlet length and cormlet weight values were obtained from this application. Corm diameter is the most important factor affecting the yield (Çavuşoğlu & Erkel 2005). Also, the highest content of N, P and S were obtained from *K. cryocrescens* strain TV-113C bacterial application. Increasing the mineral content may be explained by organic acids plant growth hormones and amino acids production by plant and bacterial inoculations (Table 1). In the study of Turan et al (2014), *B. megaterium* TV-87A inoculation increased the plant growth parameters such as fresh shoot weight, dry shoot weight, root diameter, root length, fresh root weight, dry root weight, plant height, stem diameter, leaf area and chlorophyll contents of cauliflower transplant. Except for indole acetic acid (IAA), the values of abscisic acid (ABA), gibberellic acid (GA) and salicylic acid (SA) increased in the applications by some ratio compared to the control.

Many publications have been previously documented on plant growth promoting bacteria (Erman et al 2010; Ekinci et al 2014; Turan et al 2014). However, the effects of the PGPR and hormones treatments on plant growth and formation of corm in saffron cultivation has not been enough studied. Sharaf-Eldin et al (2008) studied the effect of *B. subtilis* FZB24 on saffron (*Crocus sativus* L.) corms under *ex-vitro* conditions in Egypt and reported that inoculation of *B. subtilis* FZB24 significantly increased the leaf length, flowers per corm, weight of the first flower stigma, total stigma biomass and significantly decreased the time required for corms to sprout and the number of shoots. In addition, Parray et al (2013) stated that some important plant growth promoting bacteria as *B. subtilis* and *Pseudomonas* ssp., showed IAA production, phosphate solubilisation production and siderophore production can be used as biofertilizers and application of these rhizobacterial strains

may provide some benefit to saffron growers by speeding corm growth (earlier shoot emergence) and increasing stigma biomass. The present study showed that some of the bacterial application increased the plant growth parameters of saffron. These results are in agreement with the previous literature reports on bacteria. To our best knowledge, there is no investigation on the effects of PGPR on plant growth and quality of saffron in Turkey. For this reason, our study is one of the first of its kind in Turkey.

However, P, Ca, S, Mg, Mn, Cu, Pb and Cd content of the plants applied bacteria decreased whereas the uptake of K, Na, Fe, Zn and B slightly increased (Table 5 and 6). In recent years, the heavy metals pollution resulting from industrial and agricultural activities has emerged as a major problem. It was reported to be significant in the level of the metal products as Mn, Cu, Cd, Pb and Hg (Saharan & Nehra 2011). Contamination of soil with Cd can negatively affect biodiversity and the activity of soil microbial communities (Chen et al 2003). In this study, bacteria applied plants reduced Cu, Cd, P, Ca, S and Pb concentration while the Na, Fe, Zn, Mn, K, Mg and B were abundantly present as compared to control. The presence of these bacteria in the soil as biofertilizers can protect the plant from metal toxicity and stimulate plant growth. In addition, Mayak et al (2004) reported that *A. piechaudii* having ACC deaminase activity significantly increased the fresh and dry weights of tomato seedlings grown in the presence of NaCl. The bacterium reduced the production of ethylene in tomato seedlings, which was otherwise stimulated when seedlings were challenged with an increase in salt concentrations.

In conclusion, the main objective was to achieve the most and best quality corm by using some hormones and PGPR improving the quality, growth and the yield of saffron product. The results were statistically evaluated. From these results, it could be concluded that the tested bacterial strains have some important plant growth promoting traits that can be used as biofertilizers and application of these bacterial strains may provide some benefit to saffron growers by speeding corm growth (earlier shoot emergence), and increasing the stem and leaf biomass and number of cormlet and cormlet weight. In conclusion, the bacteria treatments used in research were determined as significant in terms of plant development and corm of saffron. The bacterial strain tested in this study may be a potential to be used as a biofertilizer in sustainable and organic common vetch production. The bacterial bio-formulations used in organic farming increase plant growth, and the development of saffron complete that positive affect. Saffron blooms several beautiful flowers and carries perennial feature due to the corm. Because of these properties, saffron can be used as ornamental plant in rock gardens, in flower beds, in grass, in balconies and terraces and in the regulation of the roof garden. Use of these bacterial isolates in the cultivation of ornamental plants may be one of the important tools for decreasing the maintenance cost and achieving sustainability in the landscapes. The effects of the PGPR and hormones treatments on plant growth and formation of corm in saffron cultivation has not been studied enough. Our study is one of the first of its kind in this sense. The bacteria treatments used in this study had significant effects on plant development and corm of saffron. Further, it is thought that further investigation should be made by using different bacterial breeds. Further similar study will be useful with more material. Results should be transferred into practice and converted into economic benefits by sharing with employees in the crop production sector**.**

References

Amirghasemi T (2001). Saffron, the red gold of Iran. Ayandegan publishing cultural Institution. Tehran, Iran

AOAC (1990). Official methods of Analysis of the AOAC. Volume 2 (No. Ed. 15). Association of Official Analytical Chemists Inc

Basker D & Negbi M (1983). Uses of Saffron. *Economic Botany* **37**(2): 228-236

Benschop M (1993). Crocus. The physiology of flower bulbs. pp. 257-272

Budak N, Çalışkan C F & Çaylak Ö (1994). Plant growth regulators and use of agricultural production. *Ege University Journal of Faculty of Agriculture* **31**: 289-296

Çakmakçı R, Dönmez F, Aydın A & Şahın F (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology & Biochemistry* **38**(6): 1482-1487

Carmona M, Zalacain A, Sanchez A M, Novella J L & Alonso G L (2006). Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS. *Journal of Agriculture and Food Chemistry* **54**: 973-979

Çavuşoğlu A & Erkel E İ (2005). The effect of different planting areas and corm size on yield and harvest period of saffron (*Crocus sativus* L.) in Kocaeli province. *Akdeniz University Journal of the Faculty of Agriculture* **18**(2): 179-184

Chen Y, Yang Y, Zheng S & Wong M (2003). Effect of cadmium on nodulation and N2 fixation of soyabean in contaminated soils. *Chemosphere* **50**: 781-787

Chichiricco G (1984). Karyotype and meiotic behaviour of the triploit *Crocus sativus* L. *Caryologia* **37**: 233-239

Deo B (2003). Growing saffron-the world's most expensive spice. *Crop and Food Research* **20**(1): 1-4

Dobbelaere S, Vanderleyden J & Okon Y (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences* **22**(2): 107-149

Ekinci M, Turan M, Yildirim E, Güneş A, Kotan R & Dursun A (2014). Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* l. var. *botrytis*) transplants. *Acta Scientiarum Polonorum Hortorum Cultus* **13**(6): 71-85

Erman M, Kotan R, Çakmakçı R, Çığ F, Karagöz K & Sezen M (2010). Effect of nitrogen fixing and phosphate-solubilizing rhizobacteria isolated from Van Lake Basin on the growth and quality properties in wheat and sugar beet. *Turkey IV. Organic Farming Symposium*, 28 June-1 July, Erzurum, Turkey, pp. 325-329

Escribano J, Diaz-Guerra M J M, Riese H H, Alvarez A, Proenza R & Fernandez J A (2000). The cytolytic effect of a glycoconjugate extracted from corms of saffron plant (*Crocus sativus* L.) on human cell lines in culture. *Planta Medica* **66**: 157-162

Frank R (1986). 'Zwiebel und Knollengewächse' Eugen Ulmer Verlag, Stuttgart, DE, ISBN 3-8001-6159-1

Grunewald W, Noorden G V, Isterdael G V, Beeckman T, Gheysen G & Mathesius U (2009). Manipulation of auxin transport in plant roots during rhizobium symbiosis and nematode parasitism. *The Plant Cell* **21**: 2553-2562

İpek A, Arslan N & Sarıhan E (2009). Effects of different planting depth and bulb sizes on yield and yield components of saffron (*Crocus sativus* L.). *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **15**(1): 38-46

Kara I (2010). Saffron Cultivation in Eskisehir. *Eskişehir Trade Exchange Journal* **1**(1): 29-31

Kaynak L & Ersoy N (1997). General properties of plant growth regulator and uses. *Akdeniz University Faculty Agricultural Journal* **10**: 223-236

Kaynak L & Memiş K (1997). Effect of plant growth blocker and delay mechanisms. *Akdeniz University Faculty Agricultural Journal* **10**: 237-248

Kotan R & Şahin F (2002). Use of bacterial organisms in biological control of plant disease. *Atatürk University Faculty Agriculture Journal* **33**: 111-119

Kotan R, Sahin F & Ala A (2005). Identification and pathogenicity of bacteria isolated from pome fruits trees in Eastern Anatolia Region of Turkey. *Journal of Plant Diseases and Protection* **113**: 8-13

Kotan R, Çakir A, Ozer H, Kordali Ş, Çakmakci R, Dadasoglu F, Dikbaş N, Aydin T & Kazaz C (2014). Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. *Scientia Horticulturae* **172**: 210-220

Lott W L, Nery J P, Gallo J R & Medcalf J C (1956). Leaf analysis technique in coffee research. *IBEC Research Institute* **9**: 21-24

Lozano P, Castellar M R, Simancas M J & Iborra J L (1999). Quantitative highperformance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L) products. *Journal of Chromatography A* **830**: 477-483

Mayak S, Tirosh T & Glick B R (2004). Plant growth-promoting bacteria confer resistance in tomato plants

to salt stress. *Plant Physiology and Biochemistry* **42**: 565-572

Mia M A B, Hossain M M, Shamsuddin Z H & Islam M T (2012). Plant-associated bacteria in nitrogen nutrition in crops with special reference to rice and banana. In: D. K. Maheshwari (ed.), *Bacteria in Agrobiology: Plant Nutrition.* pp. 97-126

Miller L T (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *Journal Clinical Microbiology* **16**: 584-586

Odom J W (1992). Determination of Total Boron in Plants by the Curcumin Method. Plant Analysis Reference Procedures for the Southern Region of the United States. Southern Cooperative Series Bulletin No. 368, The University of Georgia Crops and Soil Science Dept. Athens. GA

Parray J A, Kamili A N, Reshi Z A, Hamid R & Qadri R A (2013). Screening of beneficial properties of rhizobacteria isolated from Saffron (*Crocus sativus* L.) rhizosphere. *African Journal of Microbiology Research* **7**(23): 2905-2910

Safranbolu (2015). Safranbolu Kaymakamlığı. http://www.safranbolu.gov.tr/default_B0.aspx?content=86. (Erişim tarihi: 26.01.2015)

Saharan B S & Nehra V (2011). Plant growth promoting rhizobacteria: A critical review. *Life Sciences and Medicine Research* **2011**: LSMR-21

Saikia R, Kumar R, Arora D K, Gogoi D K & Azad P (2006). *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: Production of salicylic acid and peroxidases. *Folia Microbiologica* **51**: 375-380

Sharaf-Eldin M, Shereen E, Jose-Antonio F, Helmut J, Ronald C, Jose G & Pamela W (2008). *Bacillus subtilis* FZB24 affects flower quantity and quality of Saffron (*Crocus sativus*). *Planta Medica* **74**: 732-734

Soheilivand S, Agayev Y M, Shakib A M, Fathi M & Rezvani E (2007). Comparsion of diversity in flowering rate of two saffron (*Crocus sativus L.*) populations of Iran. *Acta Horticulturae* (ISHS) **739**: 303-306

Şahin F, Çakmakçı R & Kantar F (2004). Sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria. *Plant and Soil* **265**: 123-129

Taiz L & Zeiger E (2004). Fisiologia Vegetal. 3.ed. *Porto Alegre: Artmed*, 719 pp

Tarantilis P A & Polissiou M G (1997). Isolation and identification of the aroma components from saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry* **45**: 459-462

Turan M, Ekinci M, Yildirim E, Güneş A, Karagöz K, Kotan R & Dursun A (2014). Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. *Turkish Journal of Agriculture and Forestry* **38**: 327-333

Vurdu H & Güney K (2004). Kırmızı Altın. ISBN:975-92006-0-0, 108 s, Bizim Büro Basımevi, Ankara

Vurdu N, Allahverdiev S & Vurdu H (1997). The effects of hormones on the growth of the saffron crocus (*Crocus sativus* L.). *Kastamonu Education Journal* **4**: 85-89

Warburg E F (1957). Crocuses. *Endeavour* **16**: 209-216

Willard P (2002). Secrets of Saffron: The Vagabond Life of the World's Most Seductive Spice. Beacon Press

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Effect of *Lactobacillus plantarum* AK4-11 and Different Grape Varieties on the Properties of Hardaliye

Gülden BAŞYİĞİT KILIÇ[a], Kadir AĞDAŞ[b], Aynur Gül KARAHAN[c], Mehmet Lütfü ÇAKMAKÇI[d]

[a]*Mehmet Akif Ersoy University, Faculty of Engineering and Architecture, Department of Food Engineering, Burdur, TURKEY*

[b]*Agricultural Bank of the Republic of Turkey, Kelkit Branch, Kelkit, Gümüşhane, TURKEY*

[c]*Süleyman Demirel University, Faculty of Engineering, Department of Food Engineering, Isparta, TURKEY*

[d]*Ankara University, Faculty of Engineering, Department of Food Engineering, Ankara, TURKEY*

ARTICLE INFO

Research Article
Corresponding Author: Gülden BAŞYİĞİT KILIÇ, E-mail: gkilic@mehmetakif.edu.tr, Tel: +90 (248) 213 27 21
Received: 02 March 2015, Received in Revised Form: 10 July 2015, Accepted: 10 July 2015

ABSTRACT

This article reports the effects of using *Lactobacillus plantarum* AK4-11 and different grape varieties on some properties of hardaliye. The results showed that grape variety did not have any effect on pH during fermentation period, but using red grapes resulted in higher pH 4.10 in hardaliye after 90 day storage. On the other hand using white grape resulted in higher brix values ranged from 12.90 to 14.00 at the end of the 14th day of fermentation. The colour results indicated that CI and redness values were higher (2.01-2.90 and 41.84-44.50, respectively) and yellowness values were lower (41.71-43.15) in hardaliye samples produced with red grapes. Using red grapes also increased the amount of phenolic compounds in hardaliye samples. Results of this study indicated that using *L. plantarum* AK4-11 and different grape varieties in hardaliye manufacture affected some quality parameters of hardaliye.

Keywords*:* Hardaliye; Probiotic; Grape; Phenolic compounds

Lactobacillus plantarum AK4-11 ve Farklı Üzüm Çeşitlerinin Hardaliye Üzerine Etkisi

ESER BİLGİSİ

Araştırma Makalesi
Sorumlu Yazar: Gülden BAŞYİĞİT KILIÇ, E-posta: gkilic@mehmetakif.edu.tr, Tel: +90 (248) 213 27 21
Geliş Tarihi: 02 Mart 2015, Düzeltmelerin Gelişi: 10 Temmuz 2015, Kabul: 10 Temmuz 2015

ÖZET

Bu makale *Lactobacillus plantarum* AK4-11 ve farklı üzüm çeşitlerinin hardaliyenin bazı özellikleri üzerindeki etkisini açıklamaktadır. Elde edilen sonuçlar üzüm çeşitlerinin fermantasyon süresince pH'yı etkilemediğini ancak kırmızı üzüm kullanımının 90 gün depolama sonrasında pH'yı yükselttiğini (pH 4.10) göstermiştir. Diğer taraftan beyaz üzüm kullanımı ile 14 günlük fermantasyon sonunda 12.90 ile 14.00 arasında daha yüksek briks değeri ölçülmüştür. Kırmızı üzümle

üretilen hardaliye örneklerinin renk ölçüm sonuçlarına göre renk yoğunluğu ve kırmızılık değerleri yüksek (sırasıyla 2.01-2.90 ve 41.84-44.50) ve sarılık değeri ise düşük (41.71-43.15) bulunmuştur. Hardaliye örneklerinin kırmızı üzüm ile üretilmesi fenolik bileşenlerde artışa sebep olmuştur. Bu çalışmanın sonuçları, *L. plantarum* AK4-11 ve farklı üzüm çeşitleri kullanılarak yapılan üretimin hardaliyenin bazı kalite parametrelerini etkilediğini ortaya koymuştur.

Anahtar Kelimeler: Hardaliye; Probiyotik; Üzüm; Fenolik bileşikler

1. Introduction

Functional foods are thought to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of some diseases and other health conditions (IFICF 2011). Probiotics can be considered functional foods because they provide health benefits beyond the traditional nutrition function (Lin 2003). A probiotic is a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract (Salminen et al 1998). There is evidence that the oral consumption of probiotics might have beneficial effects on several microbial disorders of the gut and produces a protective effect on the gut flora (Dembele et al 1998). The most commonly used strains belong to the genera *Lactobacillus* and *Bifidobacterium* (Quwehand et al 2002). Lactic acid bacteria (LAB) are generally regarded as safe and widely used in fermentation of a variety of food for the flavor, texture and preservation purposes. Certain strains can be used as probiotic organisms possess some important properties to improve human health (Fuller 1989). It is well documented that probiotic bacteria inhibit the growth of various pathogenic bacteria by producing different organic acids such as lactic and acetic acid, hydrogen peroxide, bacteriocins, bacteriocin like substances and possibility biosurfactants (Gilliand & Speck 1977; Chang et al 2001). In addition, probiotic bacteria could prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract (Lee et al 2000). The mechanisms of these beneficial effects are related to exclusion of pathogenic bacteria by direct antagonism, competition for nutrients, adhesion receptors and stimulation of host immunity (Elmer et al 1996).

In the last decades there is a growing interest in traditional foods all over the world. Traditional fermented foods are essential for the well-being of many people of the world (Hesseltine & Wang 1980). Therefore many studies on traditional foods have been focused on improving health benefits, quality, safety and processing methods of these products. "Hardaliye" is also a traditional fermented beverage that has been produced and consumed since ancient times in Thrace region of Turkey. It is manufactured by lactic acid fermentation of red grape or grape juice (Arici & Coskun 2001). Due to the LAB flora of hardaliye; it has been classified as non-dairy probiotic beverage (Prado et al 2008). In hardaliye production, the grapes are washed and crushed in a jar or barrels and 0.3-0.4% of crushed mustard seeds and/or 0.1% of benzoic acid is added, the solution is left to fermentation at room temperature for 10 days. After fermentation, hardaliye is removed from mustard seeds, vine leaves and grape residues by filtration (Arici & Coskun 2001; Prado et al 2008; Gucer et al 2009). The color of hardaliye reflects the original color of the grapes and has a characteristic aroma (Arici & Coskun 2001; Coskun & Arici 2006; Prado et al 2008). Mustard seeds, K-benzoate or Na-benzoate are used as preservative agents. Mustard seeds and K-benzoate mixture inhibits the yeast growth and prevents the alcohol fermentation (Coşkun 2012).

It has already been reported that a moderate intake of grape products like wines or grape juices have health protection effects (Dani et al 2009). Because of the production technique and potentially high grape polyphenol content, hardaliye is hypothesized to provide antioxidative effects (Amoutzopoulos et al 2013). Grapes and grape juice contain many of the same biologically active phenolic compounds such

as catechins, epicatechins, epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins, all of which are antimutagenic and antiviral agents (Saito et al 1998). The health benefits of catechins and procyanidins have led to the use of grape seed extract as a dietary supplement (Soleas et al 1997).

The objective of this study was to investigate differences between traditional hardaliye production with natural fermentation, and controlled fermentation with probiotic *L. plantarum* AK4-11 in two different grape varieties, red (R) and white (W). Moreover, we determined the chemical, microbiological, sensory, and phenolic characteristics of hardaliye samples.

2. Material and Methods

2.1. Probiotic culture

A probiotic strain, *L. plantarum* AK4-11, was used as a starter culture in the production of hardaliye. The strain was isolated from feces samples and some probiotic properties of the strain were determined (Başyiğit 2004). This strain was also identified by 16S rRNA analysis (Başyiğit Kılıç & Karahan 2010). The strain was inoculated in de Man, Rogosa and Sharpe (MRS) broth and incubated at 37 °C for 24 h until the cell number reached 10^9 CFU mL^{-1}. The cells were pelleted by centrifugation at 5000 x *g* for 10 minutes at 20 °C, and the pellets were washed in phosphate-buffered saline solution (PBS, pH 7.4) twice. Finally, the probiotic bacterium was added in grape juices at the level of 10^6 CFU mL^{-1}.

2.2. Hardaliye production

In this study, red (Demre) (R) and white (Gimrik) (W) grape varieties were used. Hardaliye production was carried out with three different groups for each grape variety. The fresh grapes were collected from local markets in Isparta, Turkey during autumn season. The control group was produced using grape juice, crushed mustard seeds and cherry leaves, the first group was produced using grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves and the second group was produced using grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves. Groups were titled as CW: Control group produced with white grape; 1W: 1st group produced with white grape; 2W: 2nd group produced with white grape; CR: Control group produced with red grape; 1R: 1st group produced with red grape; 2R: 2nd group produced with red grape. Hardaliye production method is presented in Figure 1. Hardaliye was obtained 25 days after.

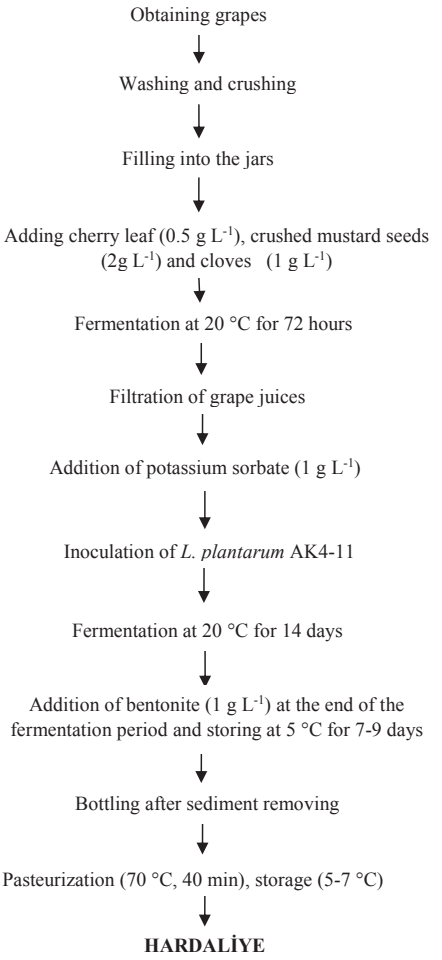


Figure 1- Hardaliye production process with grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves

Şekil 1- Üzüm suyu, L. plantarum AK4-11, ezilmiş hardal tohumu, kiraz yaprağı ve karanfilden hardaliye üretim işlemi

2.3. Chemical and microbiological analyses

The chemical analyses were performed after the addition of *L. plantarum* AK4-11, 7th and 14th days of the fermentation and 3 months of the storage. The pH of the hardaliye samples was measured with a pH-meter (InoLab WTW-537, Germany) and soluble solids (°Brix) was measured by using a hand refractometer (Atago, Japan) at 20 °C. The average values of two measurements for pH and soluble solids were recorded.

Microbiological analyses were carried out at the 1st, 7th and 14th days of fermentation. The preparation of the samples and dilutions for microbiological examinations was performed according to IDF standard 122C (Anonymous 1996; Karahan et al 2002). Ten (10) mL of hardaliye samples suspended in 90 mL of sterile 1/4 ringer solution. Decimal dilutions in ringer solution were made and plated on MRS agar (Merck, Germany) for lactobacilli counts. MRS plates were incubated for 48 h at 37 °C. Potato Dextrose agar pH 3.5 (Merck, Germany) was used to determine the yeast and molds. PCA agar was used for total mesophilic aerobic bacteria (Anonymous 1998a; APHA 2002). All analyses were performed in duplicate.

2.4. Color analyses

Spectrophotometric measurements of color were carried out by measuring the absorbance with a quartz cell of 1 mm path length at 420, 520 and 620 nm (SHIMADZU, UV-1601, Japan) at the end of the 90-day storage period. The color intensity (CI), proportions of red (R%), yellow (Y%) and blue (B%) were determined according to the Glories procedure (Glories 1984) by using Equations 1-4, respectively. All samples were analyzed in duplicate.

$$CI = A_{420} + A_{520} + A_{620} \quad (1)$$

$$R\% = A_{520} * 100/CI \quad (2)$$

$$Y\% = A_{420} * 100/CI \quad (3)$$

$$B\% = A_{620} * 100/CI \quad (4)$$

2.5. Determination of phenolic compounds

At the end of the 90-day storage period, phenolic compounds were evaluated by high performance liquid chromatography (RP-HPLC) (Shimadzu, Japan) with direct injection. Detection and quantification was carried out with a SCL-10Avp System controller, a SIL-10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector with wavelengths set at 278 nm. The 250 x 4.6 mm i.d., 5 µm column was used (Agilent Eclipse XDB-C18). The flow rate was 0.8 mL min^{-1}, injection volume was 20 µL and the column temperature was 30 °C. Gradient elution of two solvents was used. Solvent A consisted of acetic acid-water (3:100 v v^{-1}) and solvent B consisted of methanol. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The hardaliye samples, standard solutions and mobile phases were filtered by a 0.45-µm pour size membrane filter. The amount of phenolic compounds in the extracts was calculated as µg L^{-1} wine using external calibration curves, which were obtained for each phenolic standard. Standards were purchased from Sigma-Aldrich (Steinheim, Germany). Phenolic compositions of wines were determined by the modified method of Caponio et al (1999).

2.6. Sensory analyses

Sensory analyses was performed at the end of the storage period at the Department of Food Engineering at the Suleyman Demirel University by a group of eighteen non-smoker panellists experienced in the sensory evaluation of fruit juice. Hardaliye samples from each treatment was randomly chosen and served to the panelists. The taste (the taste of grape, clove taste, bitterness), smell, appearance (clarity), acidity and the overall acceptability of hardaliye samples were evaluated. Hardaliye attribute intensities were rated on 5 point scale.

2.7. Statistical analysis

The entire experiment was replicated two times on separate production days. Data collected for microbiological level, physicochemical properties and sensory attributes were analyzed by the statistical analysis system (Anonymous 1998b). The

generated data was analyzed by analysis of variance (ANOVA). Differences among mean values were established using the Duncan's multiple range test and were considered significant at $P<0.05$.

3. Results and Discussion

In this study, two varieties of grapes were used and three different combinations of hardaliye were produced for each grape variety. The results for the chemical and microbiological properties of hardaliye samples are shown in Table 1 and 2. The pH and brix values of the hardaliye samples produced by two different grapes decreased until the 14th day of the fermentation. However, the values increased at the 90th days of the storage ($P<0.05$). There were not any significant differences at pH values between the groups during the 1st and 14th days of fermentation. At the end of the 90 days of storage, the pH of hardaliye samples produced with red grapes was higher than that of hardaliye samples produced with white grapes ($P>0.05$). Similar to our results, Güven & Aksoy (2009) reported that the pH values of the hardaliye produced with only mustard seeds, and mustard seed and clove were 4.02-3.94 and 4.06-3.91, respectively, during period between 3rd and 21st day of fermentation. In another study conducted by Aydoğdu et al (2014), the pH value of hardaliye produced from the Alphonse Lavallée grapes was 4.27 on 1st day of fermentation and 3.96 on 10th day.

The initial brix values in hardaliye samples produced with red grapes were higher than those produced with white grapes. However, the brix values in hardaliye samples produced with white grapes were found to be higher than those produced with red grapes at the 14th day of fermentation ($P<0.05$). In this study, even though there was no significant difference in LAB counts among the groups of hardaliye samples produced with white grapes ($P>0.05$), LAB counts were approximately 1 log CFU mL^{-1} higher in 1R and 2R groups compared to CR group ($P<0.05$). The activity of the LAB makes this beverage safe in terms of pathogenic microorganisms (Aydoğdu et al 2014). In this study the number of yeast in all groups was found to be around 6 log CFU mL^{-1} and mold growth were not observed in the hardaliye samples. No significant changes were determined in all R and W hardaliye groups for the number of total

Table 1- Changes in pH and solid content of hardaliye samples during storage days (5 ºC)

Çizelge 1- Depolama süresince (5 ºC) hardaliye örneklerinde meydana gelen pH ve kurumadde değişimi

Groups	*Day*	*pH*	*Brix*
CW	1	3.7±0.15^{a*}	16.3±2.12^{ab}
CW	7	3.6±0.32^{ab}	15.7±2.51^{ab}
CW	14	3.3±0.19^{b}	14.0±0.84^{b}
CW	90	3.8±0.10^{a}	15.2±3.64^{ab}
1W	1	3.7±0.14^{a}	16.2±4.06^{ab}
1W	7	3.5±0.14^{ab}	15.6±2.27^{ab}
1W	14	3.3±0.30^{b}	13.1±0.98^{b}
1W	90	3.8±0.19^{a}	15.4±4.87^{abc}
2W	1	3.7±0.08^{a}	16.9±2.89^{ab}
2W	7	3.6±0.20^{a}	16.7±3.71^{ab}
2W	14	3.3±0.30^{b}	12.9±0.42^{b}
2W	90	3.8±0.14^{a}	16.2±3.71^{ab}
CR	1	3.8±0.03^{a}	18.6±1.62^{a}
CR	7	3.7±0.03^{a}	17.5±0.70^{a}
CR	14	3.5±0.42^{ab}	9.7±0.63^{c}
CR	90	4.1±0.36^{c}	11.9±6.36^{abc}
1R	1	3.8±0.06^{a}	18.7±1.94^{a}
1R	7	3.7±0.05^{a}	17.4±0.98^{a}
1R	14	3.5±0.45^{ab}	9.7±0.84^{c}
1R	90	4.1±0.39^{c}	11.5±7.03^{abc}
2R	1	3.7±0.04^{a}	19.8±1.37^{a}
2R	7	3.7±0.02^{a}	19.2±1.14^{a}
2R	14	3.5±0.35^{ab}	9.1±0.91^{c}
2R	90	4.1±0.30^{c}	13.2±9.82^{abc}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; *, values within columns with different superscript letter are significantly different ($P<0.05$) (n= 4)

mesophilic aerobic bacteria throughout the 14th days of storage (P>0.05). Contrary to the expectations of this study, the addition of mustard seeds, cloves and potassium sorbate did not show a significant inhibitory effect on the microbial count. Arıcı & Coşkun (2001) investigated physicochemical and microbiological properties of 26 days aged hardaliye samples collected from Kırklareli region in Turkey and researchers reported that the LAB count ranged $1.0x10^2$ and $4.0x10^4$ CFU mL^{-1}. The same researchers reported that pH of hardaliye samples manufactured in laboratory conditions dropped from 3.86 to 3.39 during the 7 day fermentation period. Coşkun & Arıcı (2011) reported that there were no significant difference in total mesophilic aerobic bacteria and LAB counts between hardaliye samples containing white (*Brassica alba* (L.) Boiss) or black (*Brassica nigra* (L.) Koch) mustard seeds. On the other hand the researchers observed lower yeast and mold counts in hardaliye samples containing black mustard seeds. As a result of different varieties, the year, region, and juice content in relevant studies it is natural to see different microbiological/chemical results (Aydoğdu et al 2014). Aydoğdu et al (2014) observed a progressive reduction/increase/reduction pattern for the aerobic mesophilic bacteria and lactic acid bacteria colony counts.

Table 2- Microbiological changes in hardaliye samples (log CFU g^{-1}) during storage days (5 ºC)

Çizelge 2- Depolama süresince hardaliye örneklerinde meydana gelen mikrobiyolojik değişimler (log KOB g^{-1}) (5 ºC)

Groups	*Day*	*TMAB*	*LAB*	*Y*
CW	1	6.6±0.53^{b*}	6.5±0.41^{a}	6.4±0.37^{a}
CW	7	7.3±0.56^{a}	6.9±0.19^{a}	6.0±0.03^{a}
CW	14	7.2±0.44^{ab}	6.6±0.08^{a}	5.9±0.74^{a}
1W	1	6.7±0.002^{ab}	6.8±0.49^{ab}	6.4±0.03^{a}
1W	7	6.9±0.27^{ab}	6.4±0.94^{ab}	6.5±0.55^{a}
1W	14	7.1±0.30^{ab}	6.1±0.79^{ab}	6.9±0.62^{a}
2W	1	6.8±0.22^{ab}	6.8±0.53^{ab}	6.3±0.12^{a}
2W	7	7.2±0.46^{ab}	7.3±0.42^{a}	6.0±0.56^{a}
2W	14	7.3±0.24^{ab}	6.7±0.47^{a}	5.3±0.93^{a}
CR	1	6.6±0.11^{ab}	5.8±0.79^{ab}	6.3±0.22^{a}
CR	7	6.5±0.40^{ab}	5.5±0.92^{b}	6.4±0.35^{a}
CR	14	6.5±0.12^{ab}	5.8±0.18^{ab}	6.6±0.03^{a}
1R	1	6.8±0.24^{ab}	6.7±0.23^{ab}	6.4±0.24^{a}
1R	7	6.8±0.01^{ab}	6.8±0.04^{ab}	6.7±0.12^{a}
1R	14	6.4±0.12^{b}	6.5±0.25^{ab}	6.2±0.07^{a}
2R	1	6.7±0.399^{ab}	6.7±0.37^{ab}	6.4±0.28^{a}
2R	7	6.8±0.38^{ab}	6.6±0.61^{ab}	6.3±0.56^{a}
2R	14	6.8±0.15^{ab}	6.6±0.11^{ab}	6.3±1.82^{a}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves, 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; TMAB, total mesophilic aerobic bacteria; LAB, lactic acid bacteria; Y, yeast; *, values within columns with different superscript letter are significantly different (P<0.05) (n= 4)

The color measurement results of hardaliye samples are presented in Table 3. Results indicated that different grape varieties and treatments affected the CI values of hardaliye samples (P<0.05). The CI of R groups was higher than that of W groups (P<0.05). However, these values did not show any

Table 3- Colour changes in hardaliye samples

Çizelge 3- Hardaliye örneklerinde meydana gelen renk değişimi

Groups	*CI*	*R%*	*Y%*	*B%*
CW	1.1±0.08^{c*}	29.1±0.38^{b}	51.6±1.06^{a}	19.2±0.68^{a}
1W	0.7±0.02^{c}	29.2±0.58^{b}	51.3±1.51^{a}	19.4±0.93^{a}
2W	0.8±0.09^{c}	27.8±0.56^{b}	54.4±2.50^{a}	17.7±1.93^{ab}
CR	2.0±0.16^{b}	44.5±3.39^{a}	42.5±2.81^{b}	12.9±0.57^{b}
1R	2.9±0.36^{a}	41.8±1.89^{a}	41.7±0.53^{b}	16.4±1.36^{ab}
2R	2.4±0.22^{a}	43.1±1.24^{a}	43.1±0.07^{b}	13.6±1.31^{b}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4–11, crushed mustard seeds, cherry leaves and cloves; CI, colour intensity; R%, proportions of red values; Y%, proportions of yellow values; B%, proportions of blue values; *, values within columns with different superscript letter are significantly different (P<0.05) (n= 4)

significant difference among treatment groups for each grape variety. As expected yellowness value was clearly higher in W groups and red and yellow components were also higher in R groups.

In this study, separation was achieved for 19 components including phenolic acids and flavonoids. A previous study revealed that dark fruit products such as juice or red wine have, on the average, several-fold greater concentration of polyphenols than light-coloured juices or white wines (Makris et al 2003). Phenolic contents of six different hardaliye (mg L^{-1}) were shown in Table 4. In our study, as expected, phenolic compounds in the sample groups from red grape juice (R) were higher than in the sample groups from white grape juice (W) ($P<0.05$). The bioavailability of phenolic compounds can also be affected by differences in cell wall structures, location of glycosides in cells and binding of phenolic compounds within the food matrix (Hollman et al 1997). While the major compounds in R groups were gallic, cafeic, syringic, and coumaric acid. Gallic acid and syringic acid were observed in all R groups and coumaric acid was also observed in all W groups. On the other hand, catechin, chlorogenic acid, epicatechin, rutin, resveratrol, hesperidin, apigenin-7-glucoside, rosmarinic acid, eriodictyol, quercetin, naringenin, luteolin, apigenin, ferulic acid and acacetin were not detected in any of the hardaliye samples.

Since there has not been any information in the literature for the phenolic compounds of hardaliye, it is difficult to compare the results for the phenolic content determined in our study. Therefore, results of phenolic content are compared with other beverages. The amount of phenolic compounds of hardaliye produced with red grapes in our study was lower than the phenolic compounds of red wine samples reported by Del Alamo et al (2004). Balasundram et al (2006) mentioned in his review article that red wines contain more than 1000 mg gallic acid equivalents (GAE) L^{-1} of total phenolics, compared to less than 500 mg GAE L^{-1} for most white wines. Anthocyanins from grape skins are the major component responsible for the color and the higher phenolic content of red wines compared to white wines (Mazza et al 1999). Özkan & Baydar (2006) mentioned that the most abundant phenolic were catechin (17.82-33.59 mg L^{-1}) as flavonoid and gallic acid (13.25-16.39 mg L^{-1}) as phenolic acid in red wines from four different Turkish grape cultivars. There are wide variations between the total phenolic contents of the different fruits or vegetables, or even for the same fruits or vegetables reported by different authors (Balasundram et al 2006). These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis (Kalt 2001).

Table 4- Phenolic compounds of hardaliye groups (mg L^{-1})

Çizelge 4- Hardaliye gruplarının fenolik bileşenleri (mg L^{-1})

Groups	*Gallic acid*	*Cafeic acid*	*Syringic acid*	*Coumaric acid*
CW	nd[¥]	nd	nd	0.02±0.002[a]
1W	nd	0.1±0.04[a]	0.1±0.07[a]	0.02±0.003[a]
2W	nd	nd	nd	0.03±0.003[a]
CR	0.5±0.25[a*]	nd	1.9±0.69[b]	0.07±0.006[a]
1R	0.3±0.12[a]	0.7±0.35[b]	1.7±0.54[b]	0.02±0.005[a]
2R	0.3±0.17[a]	0.5±0.33[b]	1.2±0.74[b]	nd

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; [¥]nd, not detected; *, values within columns with different superscript letter are significantly different ($P<0.05$) (n= 4)

Data of sensory evaluation is presented in Table 5. The results showed that the addition of clove affected some of the sensory attributes compared with the C group. The odour acceptance of the groups was not different among C and 1st groups, but a 2nd group has higher odor intensity compared with the other groups. This may be explained by the addition of cloves into these groups. The CW and 2R groups were received the higher taste scores and the acidity was also higher in these groups. The overall

Table 5- Sensory quality of hardaliye groups

Çizelge 5- Hardaliye örneklerinin duyusal özellikleri

Sensory quality	*CW*	*1W*	*2W*	*CR*	*1R*	*2R*
Clarity	3.3±1.2^{ab*}	3.6±0.9^{b}	3.6±1.4^{b}	2.6±0.8^{a}	2.5±1.2^{a}	3.0±0.7^{ab}
Odor	3.3±0.9^{b}	3.1±1.1^{ab}	3.6±0.9^{c}	2.8±0.7^{a}	3.1±0.8^{ab}	3.8± 1.2^{c}
Taste	4.3±1.2^{a}	3.8±1.4^{b}	3.8±1.1^{b}	2.6±1.1^{c}	3.0±1.3^{c}	4.0±1.2^{a}
Bitterness	1.8±0.5^{ab}	2.1±0.9^{b}	2.0±0.7^{b}	1.1±0.8^{a}	2.0±0.9^{b}	1.1±0.9^{a}
Acidity	3.3±1.1^{ab}	3.1±1.3^{ab}	2.8±1.0^{a}	3.0±1.3^{ab}	3.3±1.2^{b}	3.6±1.4^{b}
Clove taste	2.5±1.1^{a}	2.3±0.9^{a}	3.5±1.4^{b}	2.5±0.9^{a}	2.3±1.1^{a}	3.3±1.3^{b}
Grape taste	3.8±1.3^{a}	3.0±1.2^{b}	3.5±1.3^{a}	3.6±1.5^{a}	3.0±1.1^{b}	4.0±0.9^{a}
Appearance	3.6±1.3^{a}	3.6±1.4^{a}	4.0±1.3^{a}	3.8±1.2^{a}	3.8±0.9^{a}	4.6±0.8^{b}
The overall acceptability	3.1±1.1^{ab}	2.8±1.1^{a}	3.8±0.9^{b}	3.8±1.3^{b}	2.8±0.8^{a}	4.6±0.9^{c}

CW, control group produced with white grape juice, crushed mustard seeds and cherry leaves; 1W, 1st group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2W, 2nd group produced with white grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; CR, control group produced with red grape juice, crushed mustard seeds and cherry leaves; 1R, 1st group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds and cherry leaves; 2R, 2nd group produced with red grape juice, *L. plantarum* AK4-11, crushed mustard seeds, cherry leaves and cloves; *, values within rows with different superscript letter are significantly different (P<0.05)

acceptability scores of 1st groups were lower than that of groups C and 2. Panelists indicated that in both W and R groups, the clove added (2nd) groups received the highest scores for overall acceptability. The results of sensory evaluation revealed that 2R group had the best acceptability.

4. Conclusions

The effects of *L. plantarum* AK4-11 and grape varieties on some properties of hardaliye were investigated. The results of this study showed that using red grape in hardaliye production resulted in higher phenolic compounds, CI and redness values and lower brix and yellowness values. The addition of *L. plantarum* AK4-11 and clove caused one log unit increase on LAB counts in hardaliye produced with red grapes. No significant differences were determined among groups for total mesophilic bacteria and yeast during the fermentation and storage period.

References

Amoutzopoulos B, Löker G B, Samur G, Çevikalp S A, Yaman A, Köse T & Pelvana E (2013). Effects of a traditional fermented grape-based drink 'hardaliye' on antioxidant status of healthy adults: A randomized controlled clinical trial. *Journal of the Science of Food and Agriculture* **93**: 3604-3610

Anonymous (1996). Preparation of samples and dilutions for microbiological examination. International IDF Standard 122C:1996, FIL-IDF. Brussels, Belgium

Anonymous (1998a). SAS Institute Inc. SAS user's guide, version 7 ed. SAS Institute Inc. Cary, N. C

Anonymous (1998b). Merck Gıda Mikrobiyolojisi 98. pp 68. Ankara, Turkey. Orkim Limited Şirketi

APHA (American Public Health Association) (2002). In F. P. Downes & K. Ito (Eds.), Compendium of methods for the microbiological examination of foods (4th ed.). Washington: APHA

Arici M & Coskun F (2001). Hardaliye: Fermented grape juice as a traditional Turkish beverage. *Food Microbiology* **18**(4): 417-421

Aydoğdu H, Yıldırım Ş, Halkman A K & Durgun T (2014). A study on production and quality criteria of hardaliye; a traditional drink from Thrace region of Turkey. *Gıda* **39**(3): 193-145

Balasundram N, Sundram K & Samman S (2006). Phenolic compounds in plants and agro-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry* **99**: 191-203

Başyiğit G (2004). The probiotic properties of some lactic acid bacteria. MSc Thesis, Suleyman Demirel Univesity, Research Project Grants SDU-BAP-517

Başyiğit Kılıç G & Karahan A G (2010). Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia and determination of their pH, bile and antibiotic tolerance properties. *Journal of Molecular Microbiology and Biotechnology* **18**: 220-229

Caponio F, Alloggio V & Gomes T (1999). Phenolic compounds of virgin olive oil: Influence of paste preparation techniques. *Food Chemistry* **64**: 203-209

Chang H, Kim J, Kim W, Kim K & Park W (2001). Selection of potential probiotic lactobacillus strain and subsequent in vivo studies. *Antonie van Leeuwenhoek* **80**: 193-199

Coşkun F (2012). Farklı yöntemler kullanılarak üretilen hardaliyelerin bazı özelliklerinde depolama sonunda meydana gelen değişmeler. *Tekirdağ Ziraat Fakültesi Dergisi-Journal of Tekirdag Agricultural Faculty* **9**(3): 62-67

Coskun F & Arici M (2006). The effects of using different mustard seeds and starter cultures on some properties of hardaliye. *Annals of Microbiology* **56**: 335-338

Coşkun F & Arıcı M (2011). Hardaliyenin bazı özellikleri üzerine farklı hardal tohumları ve üzüm çeşitleri kullanımının etkisi. *Academic Food Journal-Akademik GIDA* **9**(1): 6-11

Dani C, Oliboni L S, Vanderlinde R, Pra D, Dias J F, Yoneama M L, Bonatto D, Salvador M & Henriques J A P (2009). Antioxidant activity and phenolic and mineral content of rose grape juice. *Journal of Medicinal Food* **12**(1): 188-192

Del Alamo S, Fernández M, Escudero J A & De Castro R (2004). Changes in phenolic compounds and colour parameters of red wine aged with oak chips and in oak barrels. *Food Science and Technology International* **10(**4): 233-241

Dembele T, Obdrzalek V & Votava M (1998). Inhibition of bacterial pathogens by lactobacilli. *Zentralbl Bakteriology* **288**: 395-401

Elmer G, Surawicz C & McFarland L (1996). Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. *Journal of the American Medical Association* **275**(11): 870-876

Fuller R (1989). A Review. Probiotics in man and animals. *Journal of Applied Microbiology* **66**: 365-378

Gilliand S E & Speck M L (1977). Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. *Journal of Food Protection* **40**: 820-823

Glories Y (1984). La colour des vins rouges. 20 Partie. *Conn Vigne Vin* **18**: 253-271

Gucer Y, Aydogdu H & Durgun T A (2009). Traditional Thracian beverage; "Hardaliye". *Thrace Journal of Sciences* **7**(2): 208-210

Güven S & Aksoy M (2009). Hardaliye üretiminde bazı modifikasyonlar. *II. Geleneksel Gıdalar Sempozyumu*, s. 675-678

Hesseltine C W & Wang H L (1980). The importance of traditional fermented foods. *Food from Microbes* **30**(6): 402-404

Hollman P C H, van Trijp J M P, Buysman M N C P, Gaag M S, Mengelers M J B & de Vries J H M (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters* **418**: 152-156

IFICF (2011). Functional foods. Retrieved in January, 03, 2015 from http://www.foodinsight.org/Content/3842/Final%20Functional%20Foods%20Backgrounder.pdf

Kalt W (2001). Interspecific variation in anthocyanins, phenolics and antioxidant capacity among genotypes of high bush and low bush blueberries (*Vaccinium* section *cyanococcus* spp.). *Journal of Agricultural and Food Chemistry* **49**: 4761-4767

Karahan A G, Arıdoğan-Cicioğlu B & Çakmakçı M L (2002). Genel Mikrobiyoloji Uygulama Kılavuzu. Süleyman Demirel Üniversitesi, Isparta, Türkiye

Lee Y K, Lim C Y, Teng W L, Ouwehand A C, Tuomola E M & Salminen S (2000). Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. *Applied and Environmental Microbiology* **66**: 3692-3697

Lin C D (2003). Probiotics as functional foods. *Nutrition in Clinical Practice* **18**: 497-506

Makris D P, Psarra E, Kallithraka S & Kefalas P (2003). The effects of polyphenolic composition as related to antioxidant capacity in white wines. *Food Research International* **36**: 805-814

Mazza G, Fukumoto L, Delaquis P, Girard B & Ewert B (1999). Anthocyanins, phenolics and colour of Cabernet franc, Merlot and Pinot Noir wines from

British Columbia. *Journal of Agricultural and Food Chemistry* **47**(10): 4009-4017

Özkan G & Baydar N G (2006). A direct RP-HPLC determination of phenolic compounds in Turkish red wines. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi* **19**(2): 229-234

Prado F C, Parada J L, Pandey A & Soccol C R (2008). Trends in non-dairy probiotic beverages. *Food Research International* **41**: 111-123

Quwehand A C, Salminen S & Isolauri E (2002). Probiotics: An overview of beneficial effects. *Antonie van Leeuwenhoek* **82**: 279-289

Saito M, Hosoyama H, Ariga T, Kataoka S & Yamaji N (1998). Antiulcer activity of grape seed extract and proanthocyanidins. *Journal of Agricultural and Food Chemistry* **46**: 1460-1464

Salminen S, Bouley C, Boutron-Ruault M C, Cummings J H, Franck A, Gibson G R, Isolauri E, Moreau M C, Roberfroid M & Rowland I (1998). Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition* **80**(1): 147-171

Soleas G J, Diamandis E P & Goldberg D M (1997). Wine as a biological fluid: History, production and role in diseae prevention. *Journal of Clinical Laboratory Analysis* **11**: 287-313

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

A Comparison of Natural *Eimeria spp.* and Gastrointestinal Nematode Infections of Goat Breeds

Cemil TÖLÜ[a], Türker SAVAŞ[a]

[a]*Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Animal Science, 17020, Çanakkale, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Cemil TÖLÜ, E-mail: cemiltolu@comu.edu.tr, Tel: +90 (286) 218 00 18
Received: 03 December 2014, Received in Revised Form: 06 July 2015, Accepted: 19 July 2015

ABSTRACT

The number of *Eimeria* oocysts per gram faeces (OPG) and number of gastrointestinal nematod (GIN) eggs per gram faeces (EPG) depend on some factors such as gender, season and production systems. In order to determine the *Eimeria* infection and some gastrointestinal nematode burdens in Maltese, Gökçeada and Turkish Saanen goats, OPG, EPG and packed cell volume (PCV) were investigated. Maltese breed tended to have lower parasite burden than other goat breeds ($P \leq 0.05$). In the first observation, the prevalence of *Eimeria* and nematodes was 100% in Gökçeada breed, while the prevalence of *Eimeria* and the prevalence of GIN infection were 98% and 78%, respectively, in Maltese. It was determined that OPG and EPG burdens were significantly affected by the age of goats and the sampling date ($P \leq 0.0193$). It was seen that OPG burden decreased as the goats get older ($P = 0.0157$), while EPG value varied by the age of a goat in an unsteady manner ($P < 0.0001$). The PCV values determined in the breeds ranged from 0.23 to 0.31. Statistically significant and positive correlation coefficients were determined between OPG and EPG $r = 0.20$ ($P = 0.0036$), and between the PCV value and OPG $r = 0.41$ and PCV and EPG $r = 0.37$ ($P < 0.0001$).

Keywords: Gökçeada; Maltese; Turkish Saanen; Packed cell volume; Age

Keçi Genotiplerinin *Eimeria* Türleri ve Mide-Bağırsak Nematodları ile Doğal Enfestasyonlarının Karşılaştırılması

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Cemal TÖLÜ, E-posta: cemiltolu@comu.edu.tr, Tel: +90 (286) 218 00 18
Geliş Tarihi: 03 Aralık 2014, Düzeltmelerin Gelişi: 06 Temmuz 2015, Kabul: 19 Temmuz 2015

ÖZET

Keçilerde koksidiyal ookist (OPG) ve parazit yumurtası (EPG) yükü ırk, cinsiyet, mevsim, yetiştirme sistemi gibi etmenlere göre değişebilmektedir. Bu çalışmada Gökçeada, Malta ve Türk Saanen keçi genotiplerinde üç yıl süreyle OPG, EPG ve kan hematokrit değeri (PCV) takip edilmiştir. Malta genotipi iç parazit yumurtası bakımından diğer keçi genotiplerinden önemli derecede düşük düzeyde yüke sahip olmuştur ($P \leq 0.05$). Gökçeada keçilerinin ilk gözlemde *Eimeria* ve mide bağırsak kıl kurtları bakımından prevalansı % 100 olurken, Malta genotipinde *Eimeria*'da % 98, mide

bağırsak kıl kurtları içinse % 78'lik bir prevalans tespit edilmiştir. OPG ve EPG yükü keçilerin yaşı ve örnekleme tarihine göre istatistiksel olarak önemli düzeyde farklılık göstermiştir (P≤0.0193). Keçi yaşı ilerledikçe OPG yükü azalırken (P= 0.0157) EPG değerinin keçi yaşlarında sistemli olmasa da farklılaştığı görülmüştür (P<0.0001). Keçilerde, PCV değeri 0.25-0.31 arasında değişmiştir. Çalışmada, OPG ile EPG arasındaki korelasyon katsayısı r= 0.20 (P= 0.0036) olarak gerçekleşmiş, PCV ile OPG (r= 0.41) ve PCV ile EPG arasında da (r= 0.37) önemli derecede pozitif ilişki tespit edilmiştir (P<0.0001).

Anahtar Kelimeler: Gökçeada; Maltız; Türk Saanen; Hematokrit; Yaş

1. Introduction

Parasite diseases are the leading cause of the losses in sheep husbandry. It was reported that 60% of the losses in sheep husbandry in USA are due to parasite diseases (Charon 2004). Thus, research on the parasites has been steadily increasing lately. The most common method of controlling parasites is the use of drugs. However, factors such as parasite resistance and risk of residues make these control methods questionable. Therefore, in the recent years, different approaches have been adopted to determine resistance or tolerance to parasites among breeds or individuals. There are reports of genetic variation of resistance on gastrointestinal parasites between and within goat breeds (Chhabra & Pandey 1991; Pralomkarn et al 1997; Baker et al 1998; Baker et al 2001). The studies in the last twenty years have shown that, by selection, it may be possible to get resistant or tolerant animals to parasite diseases (Bishop & Stear 1997; Gauly & Erhardt 2002; Cardellino et al 2002).

Coccidiosis, caused by the protozoa of *Eimeridae* family, is a contagious disease that may lead to mortalities particularly among young animals (Gül & Değer 2002). The disease occurs more and progresses more gravely in young animals. This disease, encountered frequently in the kid growing period, hinders the progress of performance in kids and, in some cases, it may result in death (Daş et al 2012). It is guessed that, apart from the known clinical effects of the disease, its subclinical effects are also high (Dinçer 2001). The number of coccidial oocysts per gram faeces (OPG) is a good parameter to observe its subclinical stage. The OPG burden is higher in young animals (Balicka-Ramisz 1999). It has been determined that high values might also be reached in old animals in some regions (Harper & Penzhorn 1999). There are also reports of breed differences of *Eimeria* resistance. Chhabra & Pandey (1991) express that Zimbabwean native goats are more resistant to coccidiosis than Boer goats. EPG (the number of parasite eggs per gram of faeces) is a good parameter when determining the presence and burden of gastrointestinal nematodes (GIN) infection in a host. Besides, the packed cell volume value, an indication of anemia, is regarded as an essential means particularly when revealing the effects of some endoparasites which feed by sucking blood on the animal (Gauly & Erhardt 2002). Pralomkarn et al (1997) reported that goats were more sensitive to gastrointestinal nematodes than sheep.

In order to determine the *Eimeria* infection and some gastrointestinal nematode burdens in three goat breeds, OPG and EPG were examined in this study. We also investigated the relationship between OPG and EPG, and packed cell volume (PCV).

2. Material and Methods

The study was conducted on each thirty Gökçeada, thirty Maltese and thirty Turkish Saanen goats in the small ruminant husbandry unit at the Center of Technological and Agricultural Research at Çanakkale Onsekiz Mart University for 30 months. The center had a fenced area of about 0.30 ha. Gökçeada goat is an autochthonous breed from the Island of Gökçeada, located in the North Aegean Sea. The breed is maintained under near feral conditions. Maltese goats were imported into Anatolia during

the Ottoman era. It is grown under extensive husbandry conditions and is now widespread in the Coastal Region of Aegean. The Turkish Saanen goat breed is a backcross between Saanen bucks and local does. The breed has been developed in the last 30 years and is a relatively high yielding genotype under intensive husbandry conditions. All goats used in the study were adult animals with ages between 1 and 6 years. The average live weights (± standard deviation) were 35.9±3.1 kg for Gökçeada goats, 41.7±7.3 kg for Maltese goats and 47.8±6.4 kg for Turkish Saanen goats. The average daily milk yield values of the breeds were found 1.25±0.27 kg in 240 days, 1.52±0.31 kg in 250 days and 2.18 kg±0.42 in 274 days for the Gökçeada, Maltese and Turkish Saanen, respectively. The goats were milked twice a day, at 7 am and 7 pm. They were fed with a concentrate in pellet form (890 g DM kg^{-1}, 210 g CP kg^{-1} DM, 2.8 Mcal ME kg^{-1} DM; 1.0 kg $goat^{-1}$ per day) and oat hay (890 g DM kg^{-1}, 82 g CP kg^{-1} DM, 2.1 Mcal ME kg^{-1} DM; 0.3 kg $goat^{-1}$ per day) throughout the study. Concentrate feed was given in two equal portions during each milking, while the oat hay was given only before daily grazing period in group conditions. The does stayed in the pasture between 9 am and 5 pm hours and were housed in the barn for the rest of the day. The goats treated for endoparasites in October 2006, June 2007, January 2008 and May 2008 (Tölü 2009). In the current study, the number of coccidial oocysts (OPG) and the number of gastrointestinal nematode eggs (EPG) per gram of faeces and the packed cell volume (PCV) have been determined immediately before treatment for endoparasites in all examined goats. For this purpose, the related parameters were observed after the first introduction of Gökçeada and Maltese breeds to the establishment (September 2006) and all of the three breeds in May 2007 and May 2008. Since endoparasite treatment was performed in Turkish Saanen, no faecal samples were taken from these animals during the measurements in 2006. The faecal sample was taken from the rectum, brought to the laboratory and subjected to analysis. The samples were preserved at +4 ºC and, OPG and EPG were determined with the modified McMaster method without any distinction between species (MAFF 1986; Cork & Halliwell 2002). A saturated salt solution was used and the flotation technique was utilized in faecal analyses.

The faecal consistency was also checked when taking faeces for parasite observations. In addition, the PCV was detected with the blood samples taken simultaneously from *vena jugularis*. The PCV was determined by means of capillary tubes and hematocrit centrifuge (5 min. at 5,000 rpm turns) (Cork & Halliwell 2002).

In order to fulfill the preconditions for the analysis of variance, the OPG and EPG values were subjected to logarithmic (Log (OPG+100); Log (EPG+100)) transformation. A linear model including breed (Gökçeada, Maltese, Turkish Saanen), age (1,...,6) and sampling day (1, 2, 3) was utilized in the repeated measurement variance analyses for all traits. TUKEY test was utilized in the *post-hoc* analyses. The Pearson correlation coefficient (r) was used to determine the relationship among the parameters concerned. SAS (1999) package program was used for the statistical analyses.

3. Results and Discussion

It was determined that the number of OPG tended to decrease in goats over the experiment years, while fluctuations were seen in the gastrointestinal parasite EPG (Table 1). The highest OPG value in the study was in the Maltese in 2006, whereas the highest EPG value was in the Turkish Saanen in 2007. In the first observation, the prevalence of *Eimeria* and gastrointestinal nematodes (GIN) was 100% in the Gökçeada breed, while the prevalence of *Eimeria* was detected as 98% and the prevalence of GIN was 78% in the Maltese breed. The prevalence of GIN for the years 2007 and 2008, was 45-36%, 47-70% and 70-62% for Gökçeada, Maltese and Turkish Saanen breeds, respectively. In the other studies conducted in Turkey, 9 to 10 *Eimeria* species were determined in farm animals, while the prevalence was around 80% (Arslan & Tüzer 1998; Değer et al 2003; Gül 2007). It might be stated that subclinical coccidiosis is quite common in goats. Değer et al

Table 1- The least square mean (LSM) and standard error (SE) values for the OPG, EPG and PCV values determined in different periods according to goat breeds

Çizelge 1- Irklara göre farklı dönemlerde belirlenen OPG, EPG ve PCV değerlerine ait en küçük kareler ortalama (LSM) ve standart hata (SE) değerleri

Traits	*Breed*	*Gökçeada*		*Maltese*		*Turkish Saanen*	
	Year	*LSM*	*SE*	*LSM*	*SE*	*LSM*	*SE*
OPG	2006	3.46^{a}	0.08	3.11^{b}	0.08	-	-
	2007	3.22^{a}	0.09	3.06^{a}	0.09	2.79^{b}	0.09
	2008	3.11^{a}	0.08	2.68^{b}	0.10	2.71^{b}	0.09
EPG	2006	2.89^{a}	0.06	2.47^{b}	0.06	-	-
	2007	2.18^{a}	0.06	2.22^{ab}	0.07	2.36^{b}	0.07
	2008	2.57	0.12	2.52	0.08	2.59	0.08
PCV	2006	0.31^{a}	0.006	0.25^{b}	0.007	0.25^{b}	0.007
	2007	0.25^{a}	0.001	0.23^{b}	0.006	0.24^{ab}	0.006
	2008	0.24	0.007	0.25	0.007	0.24	0.006

OPG, number of oocysts per gram of faces; EPG, number of parasite eggs per gram of faces; PCV, packed cell volume; $^{a-b}$, values in the same row without a common superscript letter are significantly different (P≤0.05)

(2003) reported that the prevalence of *Eimeria* oocysts ranged from 53.3% to 94.8% for goat herds in Turkey. Arslan & Tüzer (1998) determined the rate of cattle's free from *Eimeria* species as 32% and reported that the correlation coefficient between bloody diarrhea and soft faeces and the OPG value was 0.96.

It was determined that the OPG and EPG burdens were significantly affected by the age of goats and the sampling date (P≤0.0193). It was seen that the OPG burden decreased as the goat got older (P= 0.0157), while the EPG value varied by the age of a goat, although not in a systematic way (P<0.0001). Harper & Penzhorn (1999) determined the prevalence of *Eimeria* species as 88-100% in their study performed in three different regions in Africa. The authors reported that the highest OPG value was detected in the goats older than 1 year old in a region, and the highest value was in the goats younger than 1 year old in another region. Balicka-Ramisz (1999) determined that the goats had a lower OPG value than kids and the values ranged from 50 to 2500. Sharkhuu (2001) reported that the highest average number of parasites per gram of faeces as 2634 for the Mongolian goat.

The Maltese breed, which had a lower average than the Gökçeada breed in terms of EPG and OPG in the first observation, had also low values in the other observations (Table 1). In the first observation, the median values were determined as 2784 and 923 for OPG and as 70 and 300 for EPG in Gökçeada and Maltese breeds, respectively (P≤0.05). The Gökçeada breed had a significantly lower mean EPG value than the other breeds in the measurements performed in 2007 after the parasite treatment in 2006 (P≤0.05). The OPG values of the Turkish Saanen were slightly lower than those of the other breeds, whereas the EPG value was at slightly higher levels (P≤0.05). In a study on goats aged more than one year old, conducted in three different regions of South Africa, the researcher found similar OPG values in native goats and Saanen breeds, while they found significantly higher values in the crossbreeds (Harper & Penzhorn 1999). The authors could not see any significant difference among the breeds, aged less than one year. It is seen that there is a similar case in our study. Usually intensive husbandry conditions are more contaminated with *Eimeria spp.* than extensive husbandry conditions. Therefore, Turkish Saanen goats are more confronted with *Eimeria spp.* oocysts than the other genotypes and

for this reason Turkish Saanen goats could develop a higher resistance to *Eimeria spp*. On the contrary, the GIN eggs are more common in pastures than in barns. This resulted with a higher infection pressure in pasture based livestock systems. Moreover, the Gökçeada goats are kept outdoors year around. The lower value of the EPG's of the Gökçeada breed maybe an adaptation to this condition.

The PCV values determined by the breeds ranged from 0.23 to 0.31 (Table 1). A significant and positive relationship was determined between the PCV and OPG (r= 0.41), and PCV and EPG (r= 0.37) (P<0.0001). The mean PCV value in goats ranged from 0.24 to 0.28. It has been shown that the PCV value decreases in the groups infected with various endoparasite species (Baker 1998; Mandonn et al 2005; Eguale et al 2009). Goossens et al (1998) stated that PCV value ranged from 0.22 to 0.24 in the goats infected with *Trypanosoma congolense,* and that it was 0.30 in the control group. Baker et al (1998) reported significant differences among breeds for endoparasite burden and PCV value. It seems that an endoparasite infection to an organism causes a decline in the PCV value. However, the positive relationship of PCV with OPG and EPG in this study contradicts with this argument. That might be because the infection was not at clinical level, and therefore, anemia had not been formed yet. In addition, the humoral immunity mechanism was probably activated, so that the number of eosinophils in infected goats increased (Mandonnet et al 2005). Furthermore, the anemia was probably formed in the presence of bloodsucking parasites and as no distinction between species was made in this study, it is not known by which gastrointestinal nematodes the goats were infected. On the other hand, especially the Gökçeada goats came in a far better environment than on the island, where the animals may face insufficient nutrition. This could mislead the expected relationship between OPG and PCV, or EPG and PCV. In this regard, another problem may be the relatively small variation of the measured variables in the study. Therefore, the relationships of EPG with OPG and PCV should be repeated with larger data records.

4. Conclusions

In this study it was seen that the OPG and EPG values which were used to observe the *Eimerian* and nematode burden varied significantly by year, age and breed. It can be speculated that animals from Maltese breed would have lower OPG and EPG values than the other breeds. The low values in Maltese suggest that this breed, reared in Western Anatolia for long years, is well adapted to the environment in terms of endoparasites.

Acknowledgements

The authors thank TÜBİTAK, which provided support for the supply of animals within the scope of Project No. 106O411, and Gökçe GAMLI, Halil TUNCA, Tamer GARAGON, Elif DALAR (AKYÜZ), Ercan DIBEK and Raziye IŞIK for their assistance in the collection and analysis of the samples. The last but not the least, we thank Dr. Cem Ömer EGESEL for his valuable contribution.

References

Arslan M Ö & Tüzer E (1998). Prevalence of bovine eimeridosis in thracia, Turkey. *Turkish Journal of Veterinary and Animal Science* **22**: 161-164

Baker R L, Mwamachi D M J, Audho J O, Aduda E O & Thorpe W (1998). Resistance of galla and Small East African goats in the sub-humid tropics to gastrointestinal nematode infections and the peri-parturient rise in faecal egg counts. *Veterinary Parasitology* **79**: 53-64

Baker R L, Audho J O, Aduda E O & Thorpe W (2001). Genetic resistance to gastro-intestinal nematode parasites in Galla and Small East African goats in the sub-humid tropics. *Animal Science* **73**: 61-70

Balicka-Ramisz A (1999). Studies on coccidiosis in goats in Poland. *Veterinary Parasitology* **81**: 347-349

Bishop S C & Stear M J (1997). Modelling responses to selection for resistance to gasto-intestinal parasites in sheep. *Animal Science* **64**: 469-478

Cardellino R A, Nari A & Castells D (2002). Animal health links to recording systems. Resistance to Internal Parasites in Sheep. ICAR technical series No:8 129-141 URL: http://www.icar.org/docs/technical_series/tec_series_8_interlaken.pdf

Charon K M (2004). Genes controlling resistance to gastrointestinal nematodes in ruminants. *Animal Science Paper and Reports* **22**(1): 135-139

Chhabra R C & Pandey V S (1991). Coccidia of goats in Zimbabwe. *Veterinary Parasitology* **39**: 199-205

Cork S C & Halliwell R W (2002). The veterinary laboratory and field manual. Nottingham University Press. ISBN: 1-897676-49-2 UK

Daş G, Ataşoğlu C, Akbağ H I, Tölü C, Yurtman İ Y & Savaş T (2012). Effects of kefir on coccidial oocysts excretion and performance of dairy goat kids following weaning. *Tropical Animal Health and Production* **44**: 1049-1055

Değer S, Gül A, Ayaz E & Biçek K (2003). The prevalence of eimeria species in goats in Van. *Turkish Journal of Veterinary Animal Science* **27**: 439-442

Dinçer Ş (2001). Coccidiosis. *12. Ulusal Parazitoloji Kongresi Kongre Kitabı*. Türkiye Parazitoloji Derneği Yayın No: 17, İzmir

Eguale T, Mekonnen G A & Chaka H (2009). Evaluation of Variation in susceptibility of three Ethiopian faecal sheep breeds to experimental infection with *Fasciola hepatica*. *Small Ruminant Research* **82**: 7-12

Gauly M & Erhardt G (2002). Changes in faecal trichostrongyle egg count and haematocrit in naturally infected Rhon sheep over two grazing periods and associations with biochemical polymorphisms. *Small Ruminanat Research* **44**: 103-108

Goossens B, Osaer S, Kora S & Ndao M (1998). Haematological changes and antibody response in trypano tolerant sheep and goats following experimental *Trypanosoma congolense* infection. *Veterinary Parasitology* **79**: 283-297

Gül A (2007). The prevalence of *Eimeria* species in goats in Iğdır. *Turkish Journal of Veterinary and Animal Science* **31**(6): 411-414

Gül A & Değer S (2002). Van yöresinde koyunlarda bulunan *Eimeria* türleri ve bunların prevalansı. *Turkish Journal of Veterinary and Animal Sci*ence **26**: 859-864

Harper C K & Penzhorn B L (1999). Occurrence and diversity of coccidia in indigenous, Saanen and crossbred goats in South Africa. *Veterinary Parasitology* **82**(1-2): 1-9

MAFF (1986). Manual veterinary parasitological laboratory techniques. 3rd edition. Ministry of Agriculture, Fisheries and Food. London

Mandonnet N, Bachand M, Mahieu M, Arquet R, Baudron F, Abinne-Molza L,Varo H & Aumont G (2005). Impact on productivity of peri-parturient rise in faecal egg counts in Creole goats in the humid tropics. *Veterinary Parasitology* **134**: 249-259

Pralomkarn W, Pandey V S, Ngampongsai W, Choldumrongkul S, Saithanoo S, Rattaanachon L & Verhulst A (1997). Genetic resistance of three genotypes of goats to experimental infection with *Haemonchus contortus*. *Veterinary Parasitology* **68**: 79-90

SAS (1999). Institute Inc., *SAS OnlineDoc®,* Version 8, Cary, NC

Sharkhuu T (2001). Helminths of goats in Mongolia. *Veterinary Parasitology* **101**: 161-169

Tölü C (2009). Farklı keçi genotiplerinde davranış, sağlık ve performans özellikleri üzerine araştırmalar. Doktora tezi, Çanakkale Onsekiz Mart Üniversitesi, Fen Bilimleri Enstitüsü (Basılmamış), Çanakkale

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

The Effect of Saline and Non-Saline Soil Conditions on Yield and Nutritional Characteristics of Some Perennial Legumes Forages

Süleyman TEMEL[a], Bilal KESKİN[a], Uğur ŞİMŞEK[b], İbrahim Hakkı YILMAZ[a]

[a]*Iğdır University, Faculty of Agriculture, Department of Field Crops, Iğdır, TURKEY*

[b]*Iğdır University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Iğdır, TURKEY*

ARTICLE INFO

Research Article
Corresponding Author: Süleyman TEMEL, E-mail: stemel33@hotmail.com, Tel: +90 (533) 437 87 03
Received: 05 April 2015, Received in Revised Form: 04 August 2015, Accepted: 05 August 2015

ABSTRACT

Salinity is one of the important environmental stress factors restricting agricultural productivity and sustainability, particularly in arid and semi-arid regions. In the evaluation of saline soils, growing of salt tolerant or resistant plants is recently a widespread implementation. The aim of this study was to compare some yield and nutritional properties of alfalfa (*Medicago sativa* L.), sainfoin (*Onobrychis sativa* Lam.) and bird's foot trefoil (*Lotus corniculatus* L.) species cultivated in extreme saline-soil (9.80 EC dS m^{-1}) and non-saline soil (0.43 EC dS m^{-1}) conditions. For this purpose, this research was conducted in randomized blocks design with three replications on the Iğdır Plain, located in eastern Turkey, between the years of 2011-2013. Plants were sown under irrigable conditions in 2011, and data were obtained from the examined plants during three years including the year of sowing. In the study, leaf area index (LAI), crude protein (CP), fresh hay and hay yields differed significantly ($P<0.01$) in terms of species x soil type x year interaction. In respect to plant height, all the paired interactions, but only soil type x year interaction in terms of neutral detergent fiber (NDF) were found statistically significant. According to these results, maximum fresh hay yields and LAI were obtained from alfalfa grown on non-saline soils in the maintenance years (2012-2013), and maximum hay yields were determined again in alfalfa grown on non-saline soils for each of the three years and also on saline soil in 2012. However, minimum fresh hay and hay yields were measured under saline soil conditions in the establishing year for each of the three species. Maximum and minimum CP contents were found in alfalfa and sainfoin grown on saline soil conditions in the establishing year (2011), respectively. Along with changing as per species, plant heights increased in the years following the establishing year, but decreased on saline soil compared to non-saline soil. In respect of NDF content, the highest values were determined under non saline-soils in 2012, and the lowest ones were obtained from saline soil conditions in 2011 and 2013. As conclusion, it was determined that all species can easily grow without too much yield and quality loss in salt-affected areas and can provide enough forage production for livestock feding.

Keywords: Feed value; Forage yield; Halomorphic soils; Salinity; Leaf area index

Bazı Baklagil Yem Bitkisi Türlerinin Verim ve Besin Özellikleri Üzerine Tuzlu ve Tuzsuz Toprak Koşullarının Etkisi

ESER BİLGİSİ

Araştırma Makalesi
Sorumlu Yazar: Süleyman TEMEL, E-posta: stemel33@hotmail.com, Tel: +90 (533) 437 87 03
Geliş Tarihi: 05 Nisan 2015, Düzeltmelerin Gelişi: 04 Ağustos 2015, Kabul: 05 Ağustos 2015

ÖZET

Tuzluluk özellikle kurak ve yarı kurak bölgelerde tarımsal verimlilik ve sürdürülebilirliği kısıtlayan önemli çevresel stres faktörlerinden birisidir. Tuzlu toprakların değerlendirilmesinde tuza toleranslı ya da tuza dayanıklı bitkilerin yetiştirilmesi son zamanlarda oldukça yaygın uygulamalardandır. Bu çalışmada amaç, çok (aşırı) tuzlu (9.80 EC dS m^{-1}) ve tuzsuz (0.43 EC dS m^{-1}) toprak koşullarında yetiştirilen yonca (*Medicago sativa* L.), korunga (*Onobrychis sativa* Lam.) ve sarıçiçekli gazalboynuzu (*Lotus corniculatus* L.) türlerinin bazı verim ve besin özelliklerini karşılaştırmaktır. Bu amaçla, 2011-2013 yılları arasında Türkiye'nin Doğusunda yer alan Iğdır Ovasında tesadüf blokları deneme desenine göre üç tekrarlamalı olarak bu araştırma yürütülmüştür. Bitkiler 2011 yılında sulu şartlarda ekilmiş ve ekim yılı da dahil 3 yıl boyunca veri alınmıştır. Araştırmada yaprak alan indeksi (YAI), ham protein (HP), yaş ot ve kuru ot verimleri, tür x toprak tipi x yıl interaksiyonu açısından önemli bir şekilde farklılık göstermiştir ($P<0.01$). Bitki boyu bakımından tüm ikilli interaksiyonlar, nötr çözücülerde çözünemeyen lif (NDF) açısından ise sadece toprak tipi x yıl interaksiyonu istatisitiksel olarak önemli bulunmuştur. Bu sonuçlara göre maksimum yaş ot verimleri ve YAI, bakım yıllarında (2012-2013) tuzsuz toprak koşullarında yetişen yonca bitkisinde, maksimum kuru ot verimleri ise her üç yılda da yine tuzsuz toprak koşullarında yetişen yonca bitkisinden elde edilmiştir. Oysa minimum yaş ot ve kuru ot verimleri her üç türde de tesis yılında tuzlu topraklarda ölçülmüştür. Maksimum ve minimum HP içerikleri ise sırasıyla 2011 yılında tuzlu toprak koşullarında yetişen yonca ve korunga bitkisinde bulunmuştur. Türlere göre değişmekle birlikte bitki boyları tesis yılını müteakiben artmış, oysa tuzsuz toprağa göre tuzlu toprakta azalmıştır. NDF içeriği bakımından en yüksek değerler 2012 yılında tuzsuz toprak koşullarında, en düşük değerler 2011 ve 2013 yılında tuzlu topraklarda elde edilmiştir. Sonuç olarak çalışmada, tüm türlerin tuzdan etkilenmiş alanlarda çok fazla verim ve kalite kaybı olmadan kolay bir şekilde yetiştirilip, hayvan beslenmesi için yeterli yem üretimi sağlayabildiği tespit edilmiştir.

Anahtar Kelimeler: Besin değeri; Yem verimi; Halomorfik topraklar; Tuzluluk; Yaprak alan indeksi

1. Introduction

Soil salinity is one of the most serious abiotic factors restricting productivity of field, plant diversity and plant growth in arid and semi-arid regions, where soil salt content is high and precipitation is insufficient (Kazemi & Eskandari 2011). Salt affected area throughout the world is estimated at over 12.78 million hectares (FAO 2011). In Turkey, there is salinity problem in 1.5 million hectares of soils due to improper management of irrigation and inadequate drainage (Kendirli et al 2005). In Iğdır Plain (92,200 ha), which has a microclimate feature, more than 1/3 of the total cultivable lands have been affected by salinity. Consequently, soil salinity is particularly a great socioeconomic problem in Iğdır, resulting in the dislocation of populations and forcing farmers to subsistence level of living in the salt-affected areas (Temel & Şimşek 2011). In these areas, desired yields can not be obtained and also can not meet roughage needs. Therefore, the quantities of marginal areas within the suitable farmlands are gradually increasing. Bringing these areas back into production and ameliorating salt-affected soils by leaching and drainage is difficult, very expensive and has a high cost of maintenance (Hanay et al 2004). Thus, the uses of salt tolerant plants present a useful approach in bringing these areas back into production (Qureshi & Barrett-Lennard 1998; Temel et al 2013). Salt tolerant plants also have the great potential to ameliorate the saline soils. Of course, yield and quality losses range depending on the type of crop and the severity of the salinity problem (Karakullukçu & Adak 2008; Özaslan Parlak & Parlak 2008). However, there are many forage species that grow under saline conditions, and lot of these have been opportunistically used as fodder for grazing livestock and sustaining livestock production (Grattan et al 2004).

Fodder crops not only bring the salt-affected areas into production, but also provide a good source of roughage for livestock feeding. For this reason, identification and selection of salt-tolerant forage crop varieties offers a useful approach for increasing yield and improving the salt-affected

areas (Qureshi & Barrett-Lennard 1998; Hameed & Ashraf 2008). Therefore, studies for the identification of salt tolerant plants must be carried out in different ecolgies. For this purpose, a study was conducted to performance and to compare some yield and nutritional properties of some perennial legumes forage species grown at extreme saline soil and non-saline soil in Iğdır conditions.

2. Material and Methods

2.1. Experimental site

The research was conducted on irrigable lands of the Iğdır Plain, located in eastern Turkey where more than 1/3 of the total cultivable lands have been affected by salinity. Long term annual precipitation, relative humidity and temperature are 264.0 mm, 51.2% and 12.5 °C, respectively (MGM 2014). Throughout the research years of 2011, 2012 and 2013, total annual precipitation amounts were 340.0 mm, 237.2 mm and 226.9 mm, respectively. Lowest temperatures during winters of 2011, 2012 and 2013 were recorded as -9.0 °C, -2.9 °C and -4.0 °C, respectively. In establishment year, average annual total precipitation amount and relative humidity were found relatively higher than those in 2012 and 2013 while annual average temperature was lower in 2011 when compared to 2012 and 2013. Moreover, long-term average annual precipitation, average temperature and relative humidity are higher when compared to three-year average climate data of trial years (MGM 2014).

Initial chemical and physical characteristics of the soil (0-30 cm) belonging to the site were determined according to the fallowing methods, and soil characteristics of trial areas were presented in Table 1. Soils were air dried and ground to pass a 2 mm sieve opening. Particle size distribution was determined by the Bouyoucos hydrometer method (Gee & Baunder 1986). The pH was measured in 1:2.5 (soil:water) extracts according to Rhoades (1996). The organic matter (OM) of the soil was performed by using the Smith-Weldon method (Kacar 1994). Available phosphorus (P) content was determined by sodium bicarbonate ($NaHCO_3$) extraction and subsequent spectrophotometry (Olsen & Summers 1982). Exchangeable potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were determined using an ammonium acetate extraction followed by the atomic absorption method (Rhoades 1982a). Boron (B) was determined as described by John et al (1975). Electrical conductivity (EC) was determined by a conductivity meter in saturation extract (Rhoades 1982b).

2.2. Experimental design

A randomized block experiment with three replicates was conducted between 2011-2013 years. Research was established at two locations with different soil properties, extreme saline soil (coordinates 39°55'31.47'' N, 44°27'05.54'' E, EC 9.80 dS m^{-1}, ESP 11.9%) and non-saline soil (coordinates 39°54'57.36'' N, 44°28'25.26'' E, EC 0.43 dS m^{-1}, ESP 8.9%), but the similar climate properties. As the test plants, three perennial legumes forage species with different degrees of salt tolerance were used in this study. Alfalfa (*Medicago sativa* L.) and bird's foot trefoil (*Lotus corniculatus* L.) have moderate salinity tolerance. Sainfoin (*Onobrychis sativa* Lam.) has low salinity tolerance. In the study,

Table 1- Some physical and chemical soil characteristics of the experimental locations

Çizelge 1- Deneme alanı toprakların bazı fiziksel ve kimyasal özellikleri

Soil type	*Texture*	*EC*	*pH*	*OM*	*P*	*Ca*	*Mg*	*Na*	*K*	*B*	*ESP*
		dS m^{-1}		*%*	*mg kg^{-1}*						*%*
Non saline	Clay loam	0.43	8.2	4.4	27.9	3640	537	552	1251	4.3	8.9
Saline	Loamy sand	9.80	8.5	2.1	33.8	3680	549	759	1329	12.4	11.9

ESP, exchangeable sodium percentage; OM, organic matter

30, 20 and 120 kg of seeds per hectare were used for alfalfa, bird's foot trefoil and sainfoin species, respectively. The fodder crops were manually sown in 30 cm row spacing with a seeding depth of 2 cm. Sowings were done on trial plots prepared in 3 m x 4 m dimensions on 20th of April 2011. In each year, weeds were controlled with hand hoeing as needed.

For the fertilization, the amount of nutrient elements in soil were neglected. For the fertilization, 40 kg ha^{-1} N (ammonium sulphate) and 80 kg ha^{-1} P_2O_5 (triple superphosphate) were given to legume forages at sowing in the first year of the study. For the subsequent years, 150 kg ha^{-1} P_2O_5 (triple superphosphate) for alfalfa and bird's foot trefoil and 50 kg ha^{-1} P_2O_5 (triple superphosphate) for sainfoin were applied between row after the last harvest in season of autumn for maintenance years, but N fertilizer was not applied. Irrigation periods of the plants were determined with "Soil Water Potential Measurement Device" (WaterScout SM 100 Sensor), taking soil texture classes into account. Irrigation was started when the available moisture level in soil dropped to 50%. Plants were irrigated five times within one year and in every irrigation period, about 75 mm of water was given by means of surface irrigation method which is widely and practically used in the region.

2.3. Data collection

Plants were harvested at the convenient growth stages. Alfalfa, bird's foot trefoil and sainfoin were cut at early flowering, full flowering stages and between early flowering and 50% flowering stages, respectively according to the procedure described by Tan & Serin (2012). In both locations, alfalfa and bird's foot trefoil were harvested three times in 2011, but four times in the maintenance years. On the other hand, sainfoin was cut three times in 2012, but twice in 2011 and 2013 years. All the characteristics investigated in the study were given as average of the cuttings. Before each harvest, plant height was determined by taking measurements on 10 stems selected randomly in each plot. For leaf area index (LAI), above-ground biomass of plants grown on a 30 cm x 30 cm area was cut out from ground level at the convenient growth stages of every plant and immediately delivered to the laboratory. Leaf blades were separated from the point where the leaf sheaths combined and leaf areas were measured with a portable leaf area measurement device (CI-202 Portable Area Meter Model). Then, measured leaf areas were transformed to unit areas (Yunusa & Sedgley 1992). At each harvest, a square meter of area was cut from the middle of each trial plot when plants reached the planned harvest stage, and the fresh hay weight was immediately determined. Then, a representative sub-sample (0.5 kg) of the cut material at each cutting period was dried at 70 ^{o}C in an oven for 48 h and the hay yields were calculated in metric tons ha^{-1} by comparing dried hay with fresh hay yield. Later, dried samples ground in a Wiley mill to pass through a 1 mm screen prior to analyses (CP and NDF). All analyses were carried out on duplicate samples. The nitrogen content of the forage was measured by the Kjeldahl method and crude protein (CP) of plant samples were calculated by multiplying N with 6.25. Neutral detergent fibre (NDF) was measured using the procedure described by (Van Soest et al 1991).

2.4. Statistical analysis

The results were statistically evaluated by using JMP 5.1 software (JMP, A Business Unit of SAS, Cary, NC, 2003) and mean separations were made on the basis of Duncan's Multiple Range tests.

3. Results and Discussion

The fresh hay and hay yields differed significantly ($P<0.01$) in terms of plant x soil type x year interaction (Table 2). According to these results, maximum fresh hay yields were measured in the maintenace years (2012 and 2013) from alfalfa grown under the non-saline soil conditions, and this followed the establishing year (2011) of alfalfa plant (55.97 t ha^{-1}) grown on non saline-soils. Maximum hay yields were determined in alfalfa plant grown on the non-saline soil conditions for each of the three years. This can be explained with its genetic characteristics depending on the properties for each species which are able to get and store the nutritional

elements from the soil. In addition, the differences between species have been attributed to their cutting numbers within the year of the plants and the hay amount attained per cutting. Regarding this issue, Avcıoğlu et al (2009) stated that the yields in alfalfa changed depending on the varieties, climate, cultural procedures and the number of cuttings made during a season. In the studies conducted previously related to the salinity, it was also reported that the yield parameters varied among the forage species and its cultivars (Mlay et al 2006; Tavirimirwa et al 2012; Mohajer et al 2013).

As shown in Table 2, minimum fresh hay yields were determined in sainfoin (9.26 t ha^{-1}) followed by bird's foot trefoil (15.25 t ha^{-1}) and alfalfa (21.25 t ha^{-1}) grown under saline soils in the year of 2011, and the yield values of both species (bird's foot trefoil and sainfoin) were under the same statistical group (Table 2). This can be attributed to the difference of the plants in their tolerance to salinity depending on the genetic characteristics of the species and the increase in the amount of the salt ions in the soil. In addition, this may be caused by the fact that the examined forage species have a weak seedling development in the first year and their tolerances to salinity are low in the seedling stage (Torabi et al 2011; Yarnia et al 2011). Because, while the plants with less resistance against salinity reflects significant yield decreases even under low salinity, the plants with high resistance against salinity may not reflect significant yield decreases even under high salinity (Yurtseven et al 1996). Hence, in many researches performed, it has been reported that low yield in the plants is more along with increasing salinity (Li et al 2010; Kandil et al 2012).

The results showed that minimum fresh hay and hay yields were obtained from saline soil conditions in the establishing year (2011) for each of the three species. The reason of low yields in the establishing year compared to the following years may arise from the condition that the chemical and physical properties of soils is worse in the first years compared to the following years. Because, at the saline soil conditions chosen as trial area in our present study, no crop production had been conducted for a long time. Therefore, this may be caused by the fact that the soluble salts are excessively available especially at saline soils in the establishing year. As it is known, depending on the soil salinity, increases in the amount of cations such as magnesium, potassium, sodium, and in the amount of anions such as chlorine, sulphate, carbonate and bicarbonate are being observed. Soluble salts which are excessively available in saline soils can be taken easily by the plants. The salt compounds being taken by the plant might cause toxic effect

Table 2- The fresh hay and hay yields (t ha^{-1}) of forage legume species grown on saline and non-saline soil conditions during the three years

Çizelge 2- Üç yıl boyunca tuzlu ve tuzsuz toprak koşulunda yetiştirilen baklagil yem bitkisi türlerinin yaş ot ve kuru ot verimleri (t ha^{-1})

Soil types		*Fresh hay yield (t ha^{-1})*			*Hay yield (t ha^{-1})*		
	Years	*M.s*	*L.c*	*O.s*	*M.s*	*L.c*	*O.s*
NSS	2011	55.97 b	34.54 fg	26.16 h	14.34 a	8.76 de	8.18 ef**
	2012	61.26 a	51.42 cd	49.74 d	15.03 a	10.60 c	9.55 cde
	2013	63.16 a	45.08 e	33.55 g	14.89 a	10.09 cd	8.64 ef
SS	2011	21.25 ı	15.25 j	9.26 k	6.11 g	3.53 h	3.10 h
	2012	54.44 bc	37.15 fg	24.94 hı	14.44 a	9.39 cde	7.37 fg
	2013	53.31 bcd	38.12 f	37.75 fg	12.75 b	8.83 de	9.06 de
Interaction LSD		P x S: 2.62*, Y x S: 2.62** P x Y: ns, P x S x Y: 4.54**			P x S: 0.80*, Y x S: 0.80** P x Y: ns, P x S x Y: 1.39**		

**, difference between same letters is not significant; NSS, non-saline soil; SS, saline soil; *M.s, Medicago sativa; L.c, Lotus corniculatus* and *O.s, Onobrychis sativa;* P, plant; S, soil type; Y, year

in plant through nutrition and deterioration of metabolism depending on the type and amount of salt. Moreover, excessive salt at saline soils hampers the plant to take water from the soil, and slows down the growth of plant by deteriorating the structure of soil and stops it in the following stages (Güngör & Erözel 1994). Despite having sufficient water in soil under saline conditions, the plants are unable to take the water due to high osmotic pressure. This condition is being called physiological drought, and it negatively affects the growth of plant (Ayyıldız 1990). Hence, in many researches performed, it has been reported that serious decreases are experiences in the fresh hay and hay yields of the forage species being cultivated on the saline soils (Hussain et al 2009; Kandil et al 2012).

In the study, plant x soil type interaction was found significant (P<0.01) in terms of plant height (Figure 1). In this context, which *M. sativa* grown under the non-saline soil had the highest plant height (78.6 cm), the lowest plant height (33.0 cm) was measured in *L. corniculatus* cultivated in the extreme saline soil conditions. This difference arises from the condition that plant species react different against soil salinity (Pessarakli et al 1991). Generally, the saline soils have a higher concentration of salt when compared to the normal agriculture soils. Consequently, along with increasing salinity, the physical and chemical structure of soil is negatively affected, and the growth and elongation of plant slow down or even remains (Kanber et al 1992; Güngör & Erözel 1994; Sima et al 2013).

The plant heights were found significant (P<0.01) in terms of plant x year interaction and the lowest plant height was determined in *L. corniculatus* (33.66 cm) in 2011 while the highest plant height was obtained from *M. sativa* (77.64 cm) in 2013 (Figure 2). This may have resulted from the difference of species-variety depending on the genetic characteristics of the plants. In the studies performed regarding the subject, it was revealed that the plant heights were different among forage species and even among varieties of the same species (Mlay et al 2006; Tavirimirwa et al 2012).

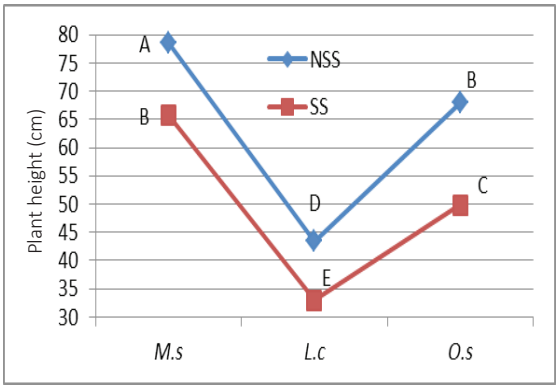


Figure 1- The effects of species x soil type interaction on the plant height (cm). Plots followed by the same letter are not significantly different. NSS, non-saline soil; SS, saline soil; *M.s, Medicago sativa*; *L.c, Lotus corniculatus* and *O.s, Onobrychis sativa*

Şekil 1- Bitki boyu üzerine tür x toprak tipi interaksiyonunun etkileri. Aynı harfleri takip eden çizimler arasındaki fark önemli değildir. NSS, tuzsuz toprak; SS, tuzlu toprak; M.s, Medicago sativa; L.c, Lotus corniculatus and O.s, Onobrychis sativa

Figure 2- The effects of species x year interaction on the plant height (cm). Plots followed by the same letter are not significantly different. *M.s, Medicago sativa*; *L.c, Lotus corniculatus* and *O.s, Onobrychis sativa*

Şekil 2- Bitki boyu üzerine tür x yıl interaksiyonunun etkileri. Aynı harfleri takip eden çizimler arasındaki fark önemli değildir. M.s, Medicago sativa; L.c, Lotus corniculatus and O.s, Onobrychis sativa

In addition, this may be caused by the fact that the examined forage species have a weak seedling development in the first year. Generally, because seedlings of the forage crops develop weakly in the first year, their gradations are low (Tan & Serin 2012). However, thanks to the strong root systems that the forage crops develop in the years following the establishing year, they can establish a better height increase (Torabi et al 2011; Yarnia et al 2011).

In addition, the plant height differed significantly ($P<0.01$) with respect to year x soil type interaction (Figure 3). According to these results, while the highest plant height was measured in the non-saline soils in 2013, the lowest plant height was determined in the saline soil conditions in 2011. And these results obtained are in agreement with reports of Özaslan Parlak & Parlak (2008). After all, as non-saline soils are more suitable for growth of plants, the plants are able to be a stronger growth and are able to show a better elongation (Li et al 2010). In addition, it has been reported by different researchers that many plant species are more sensitive to salinity in the establishing year according to the next years and the negative impact of the salinity is seen especially

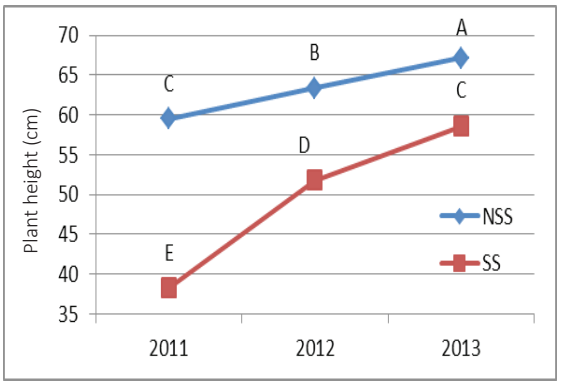


Figure 3- The effects of soil type x year interaction on the plant height (cm). Plots followed by the same letter are not significantly different. NSS, non-saline soil; SS, saline soil

Şekil 3- Bitki boyu üzerine toprak tipi x yıl interaksiyonunun etkileri. Aynı harfleri takip eden çizimler arasındaki fark önemli değildir. NSS, tuzsuz toprak; SS, tuzlu toprak

in the germination and seedling period (Torabi et al 2011; Yarnia 2011; Temel et al 2015). Due to these reasons, it may be differed the plant heights of soil types according to years.

In the study, the leaf are index (LAI) was found significiant ($P<0.01$) in terms of species x soil type x year interaction (Table 3). According to these results, the highest LAI was obtained from *M. sativa* plant grown under non saline-soil conditions in the years of 2013 and 2012 that followed by *O. sativa* growing on non saline-soil conditions in 2011. This may have resulted from the difference of species-variety depending on the genetic characteristics of the plants, or the difference of tolerance degrees of plants against saline stress. When Table 3 is examined, it has also been seen that LAI values of alfalfa grown on non saline soils were at the same statistical group in the years of 2013 and 2012. On the other hand, the lowest LAI values were determined in *L. corniculatus* (1.37 cm^2) cultivated under saline soil conditions in 2011. It can be said that this differences stem from the increase in the amount of the salt ions causing to the salinity of the soils (Table 1). In addition, this can be due to the weak growth of forage species in the first year and their strong growth depending on in the following years. Generally, the saline soils have higher ion concentration compared to normal soil. Therefore, along with the increase of salt concentrations in the soil, it becomes hard for the plants to get water from the soil and the enlargement of cell and development of shoots slow down. As a consequence, the plants produce both less amounts of organic matter and decrease their leaf sizes so as to decrease the loss of water with transpiration (Tuteja 2007).

It has also been reported in the previous studies that LAI differed among forage species and varieties according to locations and years (Açıkgöz 1991). For example, it has been stated that LAI is required to be 3-5 cm^2 in flat leaf plants and 5-6 cm^2 in alfalfa (Nelson 1995). In fact, high LAI of sainfoin plant grown on non saline-soils in establishing year could be due to its large seeds compared to other species and thus due to a better seedling growth in the first year. As it is known, the forage crops show a weak development in the first year, but a much better development in the

Table 3- Leaf area index (cm^2) and the crude protein contents (%) of forage legume species grown on saline and non-saline soil conditions during the three years

Çizelge 3- Üç yıl boyunca tuzlu ve tuzsuz toprak koşulunda yetiştirilen baklagil yem bitkisi türlerinin yaprak alan indeksi (cm^2) ve ham protein içerikleri (%)

Soil types		*Leaf area index (cm^2)*			*Crude protein (%)*		
	Years	*M.s*	*L.c*	*O.s*	*M.s*	*L.c*	*O.s*
NSS	2011	5.02 c	2.19 h	5.72 ab	16.62 ab	15.62 bcdef	14.34 gh**
	2012	6.14 a	3.85 de	5.13 bc	15.63 bcdef	14.78 efgh	14.94 defgh
	2013	6.28 a	2.95 fg	3.29 ef	16.28 bc	15.05 defg	14.63 fgh
SS	2011	4.47 cd	1.37 ı	4.49 cd	17.55 a	15.93 bcd	13.33 ı
	2012	4.90 c	2.49 gh	3.06 fg	15.65 bcde	14.91 efgh	14.45 gh
	2013	4.56 c	2.90 fg	2.88 fg	15.78 bcde	13.97 hı	15.51 cdef
Interaction LSD		P x S: ns, Y x S: 0.39**			P x S: ns, Y x S: ns		
		P x Y: 0.49**, P x S x Y: 0.69**			P x Y: 0.71**, P x S x Y: 1.01**		

**, difference between same letters is not significant; NSS, non-saline soil; SS, saline soil; *M.s, Medicago sativa; L.c, Lotus corniculatus* and *O.s, Onobrychis sativa;* P, plant; S, soil type; Y, year

years following the establishing year (Tan & Serin 2012). This arises from the condition of small seed of forage species and of their low degree of competition against weeds in the first year. On the other hand, it has been seen that LAI in sainfoin decreased in years following the establishing year in both soil types (Table 3). This may have resulted from its lowering of plant frequency due to the root rots and decrease of area covered on soil surface at unit area.

As shown in Table 3, the crude protein (CP) contents of the fodders obtained significantly ($P<0.01$) changed in terms of species x soil type x year interaction. While maximum CP content (17.55%) was obtained from *M. sativa* grown under saline soil conditions in 2011, this followed again *M. sativa* (16.62%) grown on non saline-soils in the year of 2011 (Table 3). This may cause that forage species examined have higher leaf-to-stem ratio and weaker seedling growth in the establishing year when compared with the maintenance years. Moreover, this may be caused by the fact that the forage species are in tendency to show a worse growth and a lower height increase in the soils in which the stress factors take place. Thus, these results obtained are in agreement with reports of Zandi Esfahan et al (2010), Panahi et al (2012) and Sayar et al (2014), who stated that the forage quality of species changed depending on the characteristics of the soil and years. For instance, Canpolat & Karaman (2009) in their study intended to determine the feed value of some legume fodders determined that alfalfa had the highest CP rate (17.84%). Minimum CP values were determined in *O. sativa* (13.33%) grown on saline soils in 2011 (Table 3). As it is known, sainfoin is a perennial species that has an economic life of 3-4 years and show a better seedling development and height increase in the first year when compared to alfalfa (Tan & Serin 2012). Thus, the sainfoin plant may have had lower CP content due to having higher stem-to-leaf ratio in the year of establishment. In addition, this can be explained by the genetic properties depending on the ability of each species to take the nutritional element from soil and their ability to store. Because in coarse fodder there are many factors affecting the feeding value. These are plant characteristics, environmental factors and cultural practices. And among plant factors, species and variety have a significant position (Schut et al 2010; Panahi et al 2012). In the studies performed regarding the subject, it has been revealed that CP contents were different among forage species and even among varieties of the same species (Mlay et al 2006; Tavirimirwa et al 2012).

NDF content of the fodders has been found to be significant ($P<0.01$) in respect of year x soil

type interaction. According to this, while maximum NDF percentage was found under non-saline soil conditions in 2012 followed by 2011, minimum values were determined under saline soil conditions in 2011 followed by 2013 (Figure 4). Similar results have been revealed by different researchers, and they stated that the NDF ratio in fodders decreased along with the increase of salinity in soil (Valipoor Dastenai et al 2012) and also changed depending on the years (Zandi Esfahan et al 2010; Panahi et al 2012). Generally, the plants are in tendency to show a much better growth and height increase in the year following the establishing year and in the soils in which the stress factors do not take place. However, decreases occur in the leaf/stem ratio with height increase. Therefore, the decrease of NDF content can be attributed to the decrease in the quality of the stem elements and the decrease in the leaf/stem ratio. Because physiologically, the leaves of the plants are the organs of the plants that produce the most organic matter and have the least structural composites due to the density of the ratio of the chlorophyll. However, the ratio of fibers is more in the stems of the plants (Nelson & Moser 1994).

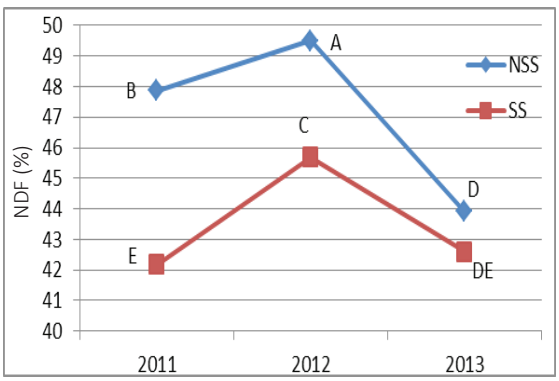


Figure 4- The effects of soil type x year interaction on the NDF content (%) of the fodders. Plots followed by the same letter are not significantly different. NSS, non-saline soil; SS, saline soil

Şekil 4- Yemlerin NDF içeriği (%) üzerine toprak tipi x yıl interaksiyonunun etkileri. Aynı harfleri takip eden çizimler arasındaki fark önemli değildir. NSS, tuzsuz toprak; SS, tuzlu toprak

4. Conclusions

As the conclusion of this 3 year study, the salinity of soil-along with changing as per species-caused decreases in the yield and yield parameters of plants and a partial increase in the quality components. Especially when alfalfa is compared with other species, it was the least affected species under saline soil conditions in respect of all the examined characteristics for each of the three years. Moreover, in years following the establishing year, significant increases were recorded in yield and yield components while decreases were found in the quality components of the fodders obtained along with changing as per species. Consequently, it can be said that the examined species at saline soils of Iğdır Plain-which has an arid climate and where many crop plants cannot be cultivated economically-can be easily cultivated without high yield and quality loss, and that it can be contributed to coarse fodder required by the animals.

Acknowledgements

This research was supported by the Scientific and Technological Research Council of Turkey with Project No. 110O259. The authors thank TUBITAK for its funding.

References

Açıkgöz E (1991). Yem Bitkileri. Uludağ Üniversitesi Yayınları: 633: 2, Bursa, pp. 456

Avcıoğlu R, Geren H, Tamkoç A & Karadağ Y (2009). Yonca (*Medicago sativa* L.). (Ed: Avcıoğlu R, Hatipoğlu R, Karadağ Y), *Yem Bitkileri*, Vol II. TUGEM, Emre Basımevi, İzmir. pp. 290-316

Ayyıldız M (1990). Sulama Suyu Kalitesi ve Tuzluluk Problemleri. Ankara Üniversitesi Ziraat Fakültesi Kültürteknik Bölümü, Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1196, Ders Kitabı: 344, Ankara, pp. 282

Canpolat Ö & Karaman Ş (2009). Bazı baklagil kaba yemlerinin *in vitro* gaz üretimi, organik madde sindirimi, nispi yem değeri ve metabolik enerji içeriklerinin karşılaştırılması. *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **15**: 188-195

FAO (2011). Food and Agricultural Organization. *Land and plant nutrition management*, Rome

Gee G W & Baunder J W (1986). Particle- Size Analysis. In: Methods of Soil Analysis. Part 1. *Physical and Minerological Methods*, 2nd ed. (Ed. A Klute). American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin pp. 383-441

Grattan S R, Grieve J A, Poss P H, Robinson D L & Benes S E (2004). Evaluation of salt-tolerant forages for sequential water reuse systems. I. Biomass production. *Agricultural Water Management* **70**: 109-120

Güngör Y & Erözel Z (1994). Drenaj ve Arazi Islahı. Ankara Üniversitesi Ziraat Fakültesi Yayınları No: 1341, Ders Kitabı: 389, Ankara, pp. 232

Hameed M & Ashraf M (2008). Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the salt range (Pakistan) to salinity stress. *Flora* **203**: 683-694

Hanay A, Büyüksönmez F, Kızıloğlu F M & Canpolat M Y (2004). Reclamation of saline-sodic soils with Gypsum and MSW Compost. *Compost Science and Utilization* **12**: 175-179

Hussain K, Majeed A, Nawaz K, Bhatti K H & Nisar F K (2009). Effect of different levels of salinity on growth and ion contents of black seeds (*Nigella sativa* L.). *Current Research Journal of Biological Science* **1**: 135-138

John M K, Chuah H H & Neufeld J H (1975). Application of improved azomethine-H method to the determination of boron in soils and plants. *Analytical Letters* **8**: 559-568

Kacar B (1994). Bitki ve Toprağın Kimyasal Analizleri III. Ankara Üniversitesi Ziraat Fakültesi Eğitim Araştırmaları Vakfı Yayınları No: 3, Ankara

Kanber R, Kırda C & Tekinel O (1992). Sulama Suyu Niteliği ve Sulamada Tuzluluk Sorunları. Çukurova Üniversitesi Ziraat Fakültesi Genel Yayın No: 21, Ders Kitapları Yayın No: 6, Adana

Kandil A A, Sharief A E, Abido W A E & Ibrahim M M (2012). Effect of salinity on seed germination and seedling characters of some forage sorghum cultivars. *International Journal of Agricultural Sciences* **4**: 306-311

Karakullukçu E & Adak M S (2008). Determination of salinity tolerance of some chickpea (*Cicer arietinum* L.) varieties. *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **14**: 313-319

Kazemi K & Eskandari H (2011). Effects of salt stress on germination and early seedling growth of rice (*Oryza sativa*) cultivars in Iran. *African Journal of Biotechnology* **10**: 17789-17792

Kendirli B, Çakmak B & Ucar Y (2005). Salinity in the Southeastern Anatolia Project (GAP), Turkey: Issues and Options. *Irrigation and Drainage* **54**: 115-122

Li R, Shi F, Fukuda K & Yang Y (2010). Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). *Soil Science and Plant Nutrition* **56**: 725-733

MGM (2014). Başbakanlık DMİ Genel Müdürlüğü Meteoroloji Bültenleri, Ankara

Mlay P S, Pereka A, Phiri E C, Balthazary S, Igusti J & Hvelplund T (2006). Feed value of selected tropical grasses, legumes and concentrates. *Veterinarski Arhiv* **76**: 53-63

Mohajer S, Taha R M, Khorasani A & Mubarak E E (2013). Comparative studies of forage yield and quality traits among proso millet, foxtail millet and sainfoin varieties. *International Journal of Environment Science and Development* **4**: 465-469

Nelson C J (1995). Photosynthesis and Carbon Metabolism In Foreges. (Ed: Barnes R F, Miller D A, Nelson C J), *An Introduction to Grassland Agriculture*, Vol I. lowa State University Press, Ames, Iowa, USA, pp. 31-44

Nelson C J & Moser L E (1994). Plant Factors Affecting Forage Quality. (Ed: Fahey G C, Collins M, Mertens D R, Moser L E), *Forage Quality, Evaluation and Utilization*, ASA, CSSA, SSSA, Madison, Wisconsin, USA, pp. 998

Olsen S R & Summers L E (1982). Phosphorus. In: Methods of Soil Analysis. Part 2. *Chemical and Microbiological Properties*. 2nd ed. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin pp. 403-427

Özaslan Parlak A & Parlak M (2008). Effect of salinity in irrigation water on some plant development parameters of sainfoin (*Onobrychis viciifolia* Scop.) and soil salinity. *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **14**: 320-325

Panahi F, Assareh M H, Jafari M, Jafari A, Arzani H, Tavili A & Zandi Esfahan E (2012). Phenological effects on forage quality of *Salsola arbuscula*, *Salsola orientalis* and *Salsola tomentosa* in three habitats in the central part of Iran. *Middle East Journal of Scientific Research* **11**: 800-807

Pessarakli M, Tucker T C & Nakabayashi K (1991). Growth response of barley and wheat to salt stress. *Journal of Plant Nutrition* **14**: 331-340

Qureshi R H & Barrett-Lennard E G (1998). Saline Agriculture for Irrigated Land in Pakistan. *A handbook*, ACIAR Monograph No. 50 Canberra, Australian

Rhoades J D (1982a). Exchangeable cations. In: Methods of Soil Analysis. Part 2. *Chemical and Microbiological Properties.* 2nd ed. American Society of Agronomy, Soil Science Society of America*,* Madison, Wisconsin pp. 159-164

Rhoades J D (1982b). Soluble salts. In: Methods of Soil Analysis. Part 2. *Chemical and Microbiological Properties*. 2nd ed. American Society of Agronomy, Soil Science Society of America, Madison pp. 167-179.

Rhoades J D (1996). Salinity: Electrical conductivity and total dissolved solids. In: Methods of Soil Analysis. Part 3. *Chemical Methods*. Sparks D.L. (Ed.), American Society of Agronomy, Soil Science Society of America, Madison pp. 417-436

Sayar M H, Han Y, Yolcu H & Yücel H (2014). Yield and quality traits of some perennial forages as both sole crops and intercropping mixtures under irrigated conditions. *Turkish Journal of Field Crops* **19**: 59-65

Schut A, Gherardi S & Wood D (2010). Empirical models to quantify the nutritive characteristics of annual pastures in south-west Western Australia. *Crop and Pasture Science* **61**: 32-43

Sima N A K K, Ahmad S T & Pessarakli M (2013). Comparative study of different salts (sodium chloride, sodium sulfate, potassium chloride and potassium sulfate) on growth of forage species. *Journal of Plant Nutrition* **36**: 214-230

Tan M & Serin Y (2012). Baklagil Yem Bitkileri. Atatürk Üniversitesi Ziraat Fakültesi Ders Yayınları No: 190, Eruzurm.

Tavirimirwa B, Manzungu E & Ncube S (2012). The evaluation of dry season nutritive value of dominant and improved grasses in fallows in Chivi District, Zimbabwe. *Online Journal of Animal and Feed Research* **2**: 470-474

Temel S & Şimşek U (2011). Desertification process of Iğdır Plain soils and solution suggestions. *Alınteri Journal of Agricultural Science* **21**: 53-59

Temel S, Şimşek U, Keskin B & Yılmaz İ H (2013). Effectiveness of forage grasses which have different degrees of salt tolerance as phytoremediation for reclamation of saline soils. *10th Field Crops Congress*, 10-13 September 2013 Konya, Vol 3, pp. 242-250

Temel S, Keskin B, Şimşek U & Yılmaz İ H (2015). Bazı çok yıllık yem bitkisi türlerinin m^2'deki bitki çıkışına halomorfik toprak koşullarının etkisi. *Tekirdağ Ziraat Fakültesi Dergisi* **12**: 46-54

Torabi M, Halim R A, Sinniah U R & Choukan R (2011). Influence of salinity on the germination of Iranian alfalfa ecotypes. *African Journal of Agricultural Research* **6**: 4624-4630

Tuteja N (2007). Mechanisms of high salinity tolerance in plants. *Methods in Enzymology* **428**: 419-438

Valipoor Dastenai M, Mirhadi M J & Mehrani A (2012). The study and comparison of 3 foxtail millet (*Setaria italica* L.) cultivars in different phenological stages in Karaj Region. *Technical Journal of Engineering and Applied Science* **2**: 62-68

Van Soest P J, Robertson J D & Lewis B A (1991). Methods for diatery fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal's nutrition. *Journal of Dairy Science* **74**: 3583-3597

Yarnia M, Heidari Sharif Abad H, Hashemi Dezfuli S A, Rahimzade Khoei F & Ghalavand A (2011). Evaluation of alfalfa (*Medicago sativa* L.) lines to salinity tolerance. *Iranian Journal of Crop Science* **3**: 12-26.

Yunusa I A M & Sedgley R H (1992). Reduced tillering spring wheats for heavy textured soils in a semiarid Mediterranean environment. *Journal of Agronomy and Crop Science* **168**: 159-168

Yurtseven E, Öztürk A, Kadayıfçı A & Ayan B (1996). Sulama suyu tuzluluğunun biberde (*Capsium annuum*) farklı gelişme dönemlerinde bazı verim parametrelerine etkisi. *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **2**: 5-9

Zandi Esfahan E, Assareh M H, Jafari M, Jafari A A, Javadi S A & Karimi G (2010). Phenological effects on forage quality of two halophytes species *Atriplex leucoclada* and *Suaeda vermiculata* in four saline rangelands of Iran. *Journal of Food Agriculture and Environment* **8**: 999-1003

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Effects of Lead (Pb) and Cadmium (Cd) Elements on Lipid Peroxidation, Catalase Enzyme Activity and Catalase Gene Expression Profile in Tomato Plants

Semra SOYDAM AYDIN[a], İlker BÜYÜK[b], Esra GÖKÇE GÜNDÜZER[b], Burcu Pelin BÜYÜK[b], İrfan KANDEMİR[b], Demet CANSARAN-DUMAN[c], Sümer ARAS[b]

[a]*Ministry of Health of Turkey, Turkish Medicines and Medical Device Agency, Ankara, TURKEY*

[b]*Ankara University, Faculty of Science, Department of Biology, Ankara, TURKEY*

[c]*Ankara University, Biotechnology Institute, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Semra SOYDAM AYDIN, E-mail: semrasoydam@gmail.com, Tel: +90 (312) 565 53 60
Received: 19 November 2014, Received in Revised Form: 09 August 2015, Accepted: 09 August 2015

ABSTRACT

Heavy metals are significant abiotic stress factor, affecting various response mechanisms in plants. These responses include: changes in membrane composition, production of small molecules and free radicals, and alterations in the activities of antioxidant enzymes and their gene expressions. For this reason, lipid peroxidation levels (MDA), catalase enzyme activity, and gene expression profiles, quantified by real-time PCR, were analyzed in tomato plants exposed to various concentrations (0, 80, 160, 320, 640 and 1280 µM) of Cd^{2+} and Pb^{2+}. All concentration of Cd^{+2} or Pb^{+2} contamination led to increased lipid peroxidation and catalase enzyme activity, except for 320 and 640 µM Cd^{+2} contamination levels. As a result, gene expression patterns at the mRNA level and changes in MDA content under different concentrations of Pb^{+2} and Cd^{+2} contamination revealed a positive correlation, although no correlation was found between gene expression patterns at the mRNA level and catalase enzyme activity. These results might be explained by the regulation of genes at the transcriptional, posttranscriptional, and also translational or posttranslational levels.

Keywords: Tomato (*Solanum lycopersicum* L.); Lipid peroxidation; CAT enzyme activity; Gene expression; qRT-PCR

Domates Bitkisinde Kurşun (Pb) ve Kadminyumun (Cd) Lipid Peroksidasyonu, Katalaz (CAT) Enzim Aktivitesi ve Gen Ekpresyon Profiline Etkisi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Semra SOYDAM AYDIN, E-posta: semrasoydam@gmail.com, Tel: +90 (312) 565 53 60
Geliş Tarihi: 19 Kasım 2014, Düzeltmelerin Gelişi: 09 Ağustos 2015, Kabul: 09 Ağustos 2015

ÖZET

Önemli bir abiyotik stres faktörü olan ağır metaller bitkilerde çok çeşitli yanıt mekanizmalarını uyarabilirler. Bu yanıt mekanizmaları; membran kompozisyonunda değişiklik, küçük molekül ve serbest radikallerin üretimi, antioksidant enzimlerin aktivitelerinin ya da gen ekspresyonlarının değişimini içerir. Bu sebeple, bu araştırmada çeşitli konsantrasyonlardaki (0, 80, 160, 320, 640 ve 1280 µM) Pb^{2+}ve Cd^{2+} kontaminasyonuna maruz kalan domates bitkilerinde lipid peroksidasyon seviyesi (MDA), katalaz enzim aktivitesi ve real-time PCR aracılığı ile katalaz gen ekpresyon seviyesi belirlenmiştir. 320 ve 640 µM Cd^{+2} kontaminasyonu hariç tüm Cd^{+2} ve Pb^{+2} kontaminasyonları lipid peroksidasyonuna ve katalaz enzim aktivitesinde artışa neden olmuştur. Sonuç olarak; çeşitli konsantrasyonlarda Pb^{2+} ve Cd^{2+} kontaminasyonuna maruz kalan domates bitkisinde, CAT gen ekpresyonu ve lipid peroksidayonu arasında pozitif korelasyon bulunurken, CAT gen ekpresyonu ve enzim aktivitesi arasında korelasyon tespit edilememiştir. Bu durum genlerin transkripsiyonel, posttranskripsiyonel ve aynı zamanda translasyonel veya posttranslasyonel seviyelerdeki regülasyonu ile açıklanabilir.

Anahtar Kelimeler: Domates (*Solanum lycopersicum* L.); Lipid peroksidasyonu; CAT enzim aktivitesi; Gen ekpresyonu; Real-time PCR

1. Introduction

Pollution of the environment by heavy metals is a serious problem throughout the world. The most common heavy metals in the environment are cadmium, chromium, copper, mercury, lead and zinc and some of their main sources are emissions from burning fossil fuels, industrial activities, automotive emissions, pesticides usage and domestic wastes (Kabata-Pendias & Pendias 1989).

Heavy metal contamination of economically important plants, such as fruits and vegetables, poses a threat to their quality and leads to alterations in the health statuses of humans and animals. Several researches have indicated that consumption of vegetables loaded with heavy metals such as Cd^{+2} and Pb^{+2} can generate carcinogenic effects (Trichopoulos 1997; Türkdoğan et al 2002). Climatic changes, nature of the soil, time of harvest, and concentrations of heavy metals in the soil are significant impacts on assumed threats of heavy metals in vegetables (Lake et al 1984; Scott et al 1996; Voutsa et al 1996). In addition, post-harvest vegetables could be influenced by air pollution during transportation and marketing, which can lead to elevated levels of heavy metals (Sinha et al 2005; Sharma et al 2006; 2007).

Abiotic stress factors, such as soil salinity, drought, high temperatures, and heavy metals are known to cause oxidative stress in plants by the production of reactive oxygen species (ROS), such as O_2^-, H_2O_2, $^1O_2$, HO_2^-, OH, ROOH, ROO, and RO (Smirnoff 1993). The most important intracellular generators of ROS are chloroplasts, mitochondria, and peroxisomes. As a result of stomatal closure and limited CO_2 availability, chloroplasts generate O_2^- and H_2O_2 by the electron acceptor of photosystem I, while singlet oxygen is produced by the transfer of an electron from an activated chlorophyll molecule to molecular oxygen (Asada & Takahashi 1987; Hernandez et al 1995). It has been estimated that, under stress conditions, 1-2% of O_2 consumption leads to the formation of ROS, which causes lipid peroxidation, membrane defects, and instability of enzymes in higher plants (Mittler 2002; El-Beltagi et al 2010). On the other hand, it is important to emphasize that ROS can act not only as damaging factors, but also as protective or signaling factors, which depend on the equilibrium between ROS production and scavenging mechanisms (Gratao et al 2005).

Plants are able to develop antioxidant defense systems to protect themselves against ROS and cope with different stress factors (Rao et al 2006). The antioxidant system restricts and removes ROS damage and maintains ROS homeostasis in plant cells. The components of this system are enzymatic, such as monodehydro ascorbate reductase

(MDHAR), dehydro ascorbate reductase (DHAR), glutathione reductase (GR), ascorbate peroxidase (APX), superoxide reductase (SOD), catalase (CAT) and non-enzymatic antioxidants, such as glutathione (GSH), proline, carotenoids, and tocopherol (Mittler et al 2004).

The various antioxidants have partially overlapping functions and can functionally compensate for each other. In this regard, SOD dismutases O_2^- into H_2O_2, which, in turn, is detoxified by CAT, APX, or PRX. CAT is one of the enzymatic antioxidants with the highest turnover rates among all enzymes, and it is located mainly in the peroxisomes. One molecule of CAT can convert approximately 6 million molecules of H_2O_2 to O_2 and H_2O per minute (Lee & An 2005). In this manner, it prevents longer H_2O_2 action, which could lead to cell disturbances and DNA damage (Shim et al 2003). There have been many reports regarding catalase enzyme activity, lipid peroxidation (MDA), and gene expression levels in several different plant species under stress conditions, such as salt stress (Mittova et al 2003), boron (B) toxicity (Cervilla et al 2007), Cu toxicity (Cui et al 2010), and Pb toxicity in tomatoes (Wang et al 2008), low temperature stress in wheat (*Triticum aestivum* L.) (Matsumura et al 2002), Mn toxicity in spruce trees (*Picea abies* L.) (Polle et al 1992), pecan trees [*Caryaillinoinensis* (Wangenh.) C. Koch, cv. Kiowa] (Henriques 2003), and Cd and Cu toxicity in mouse-ear cress (*Arabidopsis thaliana*) (Skorzynska-Polit et al 2010).

The control of gene expression in all eukaryotic cells is a complex process that involves molecules such as RNA polymerases, numerous transcription factors, the DNA template, RNA produced by transcription, and protein produced by translation, with its attendant processing. The examination of gene expression often involves quantifying the abundance of a particular transcript. Contemporary methods, such as real-time PCR, that examine gene expression reveal the dynamic nature of this biochemical process. Real-time PCR allows precise measurements of mRNA steady-state levels and provides advantages, such as very high sensitivity and precise quantification of expression levels under different conditions or treatments. This method only measures immediate levels or final accumulation of RNA in the cell. Also other modern methods, such as microarray hybridization, or more conventional methods, like Northern hybridization, do not provide information about the transcriptional activity of genes. These methods fail to quantify the stability of the RNA or the ratio of transcription at the specific loci under investigation. In order to detail understanding the nature of gene expression modulation, it is essential to evaluate alteration of transcript and corresponding protein levels which both are associated with a phenotypic change (Farrel 2007). As such, the experiments in the current study were conducted in three phases. In the first phase, after Cd^{+2} and Pb^{+2} stress treatments, MDA levels were determined in order to obtain evidence that the plants were in stress as a result of the treatments. In the second phase of the study, the steady-state level of CAT mRNA was determined by quantitative real-time PCR. Finally, in the third phase, CAT enzyme activity was determined in order to obtain an idea about the final stage of CAT gene expression in tomato samples exposed to different concentrations of Cd^{+2} and Pb^{+2} stress.

2. Material and Methods

2.1. Plant material, growth conditions, and stress treatment

Plant growth conditions and stress treatments were performed as previosly reported by Soydam Aydın et al (2013). Tomato (*Solanum lycopersicum* L. 'Falcon') seeds were germinated and grown hydroponically in pots containing 0.2 L of modified 1/10 Hoagland's solution. After germination, seedlings were transferred to pearlite and watered with 1/10 Hoagland's media in regular time interval (daily). Twenty-five-day-old six tomato plants grown in 1/10 Hoagland's media were used for the stress treatments. For the heavy metal application, Pb^{+2} and Cd^{+2} were added to the hydroponic solution for 24 h at concentrations of 0 (control), 80, 160, 320, 640 and 1280 µM. Harvested tomato leaves were ground in liquid nitrogen and used for estimation

of lipid peroxidation, CAT enzyme activity assay, RNA extraction, and gene expression analysis.

2.2. Estimation of lipid peroxidation

Malondialdehyde (MDA) content is a marker of oxidative lipid injury was performed as described and previously reported by Hodges et al (1999) and Soydam Aydın et al (2013). ELISA microtiter reader (SpectraMax M2) was used to read absorbance at 440 nm, 532 nm, and 600 nm. MDA equivalents were calculated as described by Hodges et al (1999) using Equation 1, 2 and 3.

$$[(Abs532_{+TBA}) - (Abs600_{+TBA}) - (Abs532_{-TBA}\text{-}Abs600_{-TBA})] = A \quad (1)$$

$$[(Abs440_{+TBA}\text{-}Abs600_{+TBA}) \times 0.0571] = B \quad (2)$$

$$\text{MDA equivalents (nmol mL}^{-1}) = (A\text{-}B/157000) \times 10^6 \quad (3)$$

2.3. RNA extraction and cDNA preparation

RNA extraction was performed using a TRIzol protocol followed by RNeasy mini cleanup kit (Qiagen, Cat no: 74104). RNA quantity and quality were measured with a NanoDropND-1000 spectrophotometer. Quality of RNA was also confirmed by gel electrophoresis, containing 1.5% agarose and formaldehyde. The cDNA synthesis based on reverse transcription reactions were performed with 2 µg of RNA and a high fidelity cDNA synthesis kit (Roche) containing 2.5 µM anchored oligo(dT)18, 1X transcriptor high-fidelity reverse transcriptase reaction buffer, 20 U protector Rnase inhibitor, 1 mM deoxynucleotide mix, 5 mM DTT, and 10 U transcriptor high-fidelity reverse transcriptase at final concentration. And, the program applied was 65 °C, 10 min; 55 °C, 30 min; 85 °C, 5 min.

2.4. Real-time RT-PCR and quantification of mRNA levels

Quantitative real-time PCR was performed with a LightCycler® 480 System (Roche) thermal cycler. The sequences of primers and probes (Table 1) of the target gene catalase (CAT) and actin (ACT), which is used for normalization, were designed based on the sequences of tomato genes available in the databank (NCBI 2013a; NCBI 2013b). Amplifications of PCR product were monitored via intercalation of hybridization probes (HyProbe, FRET probes) that allow exact and specific identification of the target gene. After pre-denaturation at 95 °C for 10 min, 45 cycles of 95 °C 10 s, 60 °C 30 s, and 72 °C 15 s were applied. Data collection for quantification was accomplished during the annealing period. Copy numbers of the genes (CAT, ACT) under stress treatment were determined by using standard curves. PCR efficiency for CAT standard curve was 1.937, while ACT was found to be 1.954.

Table 1- Primer and probe sequences of CAT and Actin

Çizelge 1- CAT ve Actin için primer ve prob sekansları

Primer and probe name	*Sequences*
CAT2 F	*CTTTCCTCTTCGACGATATTGGTA*
CAT2 S	*TATTCCCCAAGATTACAGGCAT*
CAT2 A	*CCGACTCGGATTGCCTT*
CAT2 R	*GTGATTTGCTCCTCCGACTC*
CAT2 FL	*CAACAGGGCTGGAAAATCAACTTATGT-FL*
CAT2 LC 640	*AAGTTCCACTGGAAGCCCACATGT p*
Actin S Fw	*TCTGTTTCCCGGTTTTGCTATTAT*
Actin R Rev	*TGCATCAGGCACCTCTCAAG*
Actin FL	*ATTCATAGCCCCCACCACCAAAC-FL*
Actin LC 640	*TCTCCATCCCATCAAAAAAACAAATTGACTp*

2.5. Catalase enzyme activity assay

Catalase enzyme activities were performed as previosly reported by Aebi (1984) and Soydam Aydın et al (2013). Powdered tomato tissues were suspended in extraction buffer and homogenates were centrifuged at 15000 *g* for 20 min, and the supernatant fraction was used for the enzyme activity assays (Jovanovic 2006). All steps were carried out at 4 °C. The catalase activity assay was performed according to the method reported by Aebi (1984), based on 240 nm absorbance.

2.6. Statistical methods

The abundance of target gene transcripts was normalized to ACT and set relative to the control plants (no stress exposure), according to the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001). Changes in

relative expression levels (REL) of the CAT gene were checked for statistical significance according to one-way ANOVA. The results were considered statistically significant if the P value was <0.05 in Dunnett's test.

3. Results and Discussion

In the current study, the effects of heavy metal stress (Pb^{+2} and Cd^{+2}) on cell membrane, gene expression and CAT enzyme activity profiles in tomato plants (*Solanum lycopersicum* L.) were analyzed. It is known that heavy metal contamination can lead to an increase in different forms of ROS.

Malondialdehyde (MDA) analysis was used as a marker of oxidative lipid injury, which might have changed in response to Cd^{+2} and Pb^{+2} heavy metals that led to stress in tomato seedlings. Changes in MDA content were observed in the tomato seedlings treated with different concentrations of Pb^{+2}, indicating that the samples were under stress. It has been demonstrated that increased lipid peroxidation is a characteristic feature of oxidative stress caused by unbalanced equilibrium between ROS production and scavenging or defense mechanisms under heavy metal stress (Smirnoff 1993; Mittler 2002; Lima et al 2006).

Small hydrocarbon fragments, such as ketones and MDAs, which are considered the first evidence of stress in plants, have been used as indicators of lipid peroxidation or membrane damage, (Lyons 1973). In recent studies, increased lipid peroxidation has been observed in plants under heavy metal stress, such as tomato (Krupa & Baszynski 1985; 1989; Quariti et al 1997; Ben Ammar et al 2005), wheat (Malik et al 1992), barley (Vassilev 2004), and mustard (Gaur & Grupa 1994; Nouairi et al 2006). Results of the current study indicate that MDA content substantially increased with all concentrations of Pb^{+2} contamination. The maximum level of MDA content was determined in the samples exposed to 320 µM concentrations of Pb^{+2} contamination while a significant decrease was measured at 1280 µM among treated samples (Figure 1). The data also show that ROS-induced lipid peroxidation began in a short period of time and that the level of injury was dependent on the exposure concentrations of Pb^{+2} contamination. However, MDA contents, which were obtained in the samples exposed to Cd^{+2} contamination, showed an increase only with the 80 µM and 1280 µM Cd^{+2} concentrations. The highest level of inhibition in MDA content was observed in the treatment with 1280 µM Cd^{+2}. In addition, the maximum decrease in MDA content was observed in the tomato seedlings exposed to the 320 µM concentration of Cd^{+2}. Surprisingly, lower MDA levels were observed at 160, 320, and 640 µM of Cd^{+2} treatment compared to the control samples (Figure1).

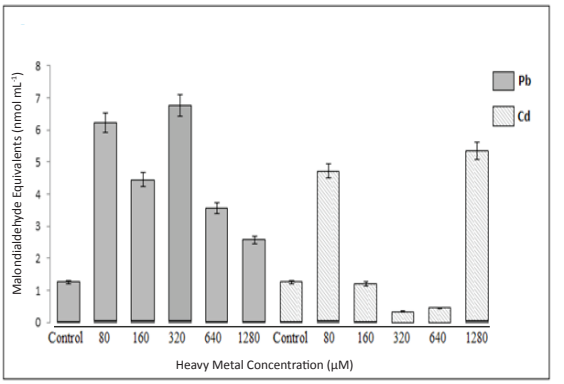


Figure 1- Lipid peroxidation (malondialdehyde, MDA content) in the total tissues of tomato plants exposed to different concentrations of Pb^{2+} and Cd^{2+}

Şekil 1- Farklı konsantrasyonlarda Pb^{2+} ve Cd^{2+}'ye maruz kalan domates bitkisinin tüm dokularındaki lipid peroksidasyonu (malondialdehit, MDA içeriği)

When evaluated SOD enzyme activity and gene expression results of tomato samples exposed to same concentration of Cd^{+2} contamination in another study conducted in our laboratory (unpublished data), it was difficult to suggest that prevention of lipid peroxidation in the cells of the tomato samples exposed to 160 µM, 320 µM, and 640 µM Cd^{+2} concentrations in the current study might result from increased activation of antioxidant mechanisms. Further analysis is necessary to explain this complex connection between lipid peroxidation and antioxidant mechanisms. Results also revealed that lipid peroxidation induced by ROS was observed after contamination of low (80 µM) and high

(1280 µM) Cd^{+2} concentrations, which generated an imbalance between production of ROS and antioxidant enzyme activity or gene expression as a component of scavenging and defense mechanisms of ROS.

ROS have a dual action, not only acting as protective or signaling factors, but also as oxidative damaging factors at the cellular level due to an imbalance between the production and removal of ROS under stress conditions (Mittler 2002). To combat the negative effects of this imbalance, an enzymatic antioxidant defense system is activated to protect plants from cellular injury with removing excessively produced H_2O_2 with the enzymes, such as peroxidase (POD), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) (Qilin et al 2009). In recent years, genes involved in response to abiotic stress have become the main field for biotechnological studies, as it constitutes the basis for improving the stress tolerance of plants. Many genes play a crucial role in responding to environmental stresses at the post-transcriptional level, and CAT is one of these genes that encode the catalase enzyme. Studies about the protective activity of CAT in response to different abiotic stresses have been demonstrated by previous research studies conducted with *Coffea* sp., oilseed rape (*Brassica napus* L.), pea (*Pisum sativum* L.), and tomato (*Lycopersicum esculentum* L.) plants (Goupil et al 2009; Qilin et al 2009; Fortunato et al 2010).

In the current study, exposure of tomato seedlings to Pb^{+2} and Cd^{+2} led to significant changes in the abundance of CAT gene transcripts, suggesting the involvement of the gene in heavy metal tolerance (Figure 2). To evaluate the stability of our results, CAT transcript levels of all samples were measured three times for each stress condition, which indicated steady-state mRNA levels in the cells for both CAT and the reference gene ACT. With regard to the control and to each other, different expression levels were recorded in all stress conditions applied. The highest level of transcript was recorded at 320 µM of Pb^{+2} and 160 µM of Cd^{+2} concentrations, and these results were found to be statistically significant. The lowest levels of mRNA were recorded at the 640 µM of Pb^{+2} and 1280 µM of Cd^{+2} concentrations.

Contamination with different concentrations of Pb^{2+}, 80 and 320 µM Pb^{+2} led to a statistically significant increase in mRNA level in the tomato seedlings compared to the control, while the 640 µM concentration led to a reduction in the expression of the gene; these results were statistically significant (P_1= 0.001; P_2= 0.001; P_3= 0.000). Changes in the rate of expression level in the catalase gene compared to the control were statistically insignificant upon exposure to 160 and 1280 µM of Pb^{+2} concentrations. CAT expression levels decreased significantly in response to 640 µM of Pb^{+2}, which indicates that CAT was strongly downregulated by this concentration (Figure 2). CAT mRNA levels in the tomato seedlings increased in response to 80 µM and 160 µM of Cd^{+2} treatments; these results were statistically significant (P_1= 0.000; P_2= 0.000) when checked for significance according to one-way ANOVA. The 320, 640, and 1280 µM of Cd^{+2} treatments led to a decrease in catalase gene expression level compared to the control, but only the 640 µM and 1280 µM of Cd^{+2} treatment results were statistically significant (P_1= 0.005; P_2= 0.000) (Figure 2). When we analyzed this complex profile of gene expression patterns at the mRNA level and changes in MDA content under different concentrations of Pb^{+2} and Cd^{+2} contamination, the results revealed positive correlations between each other (Figure 1 and 2).

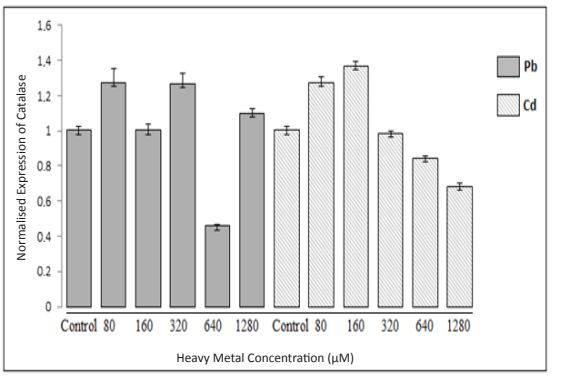


Figure 2- Catalase mRNA levels in the total tissues of tomato plants exposed to different concentrations of Pb^{2+} and Cd^{2+}

Şekil 2- Farklı konsantrasyonlarda Pb^{2+} ve Cd^{2+}'ye maruz kalan domates bitkisinin tüm dokularındaki katalaz mRNA seviyesi

Also, catalase enzyme activity assay revealed different data from gene expression patterns at the mRNA level in tomato seedlings treated with different concentrations of Pb^{+2} and Cd^{+2}. In particular, 640 µM Pb^{+2} and 80 µM Cd^{+2} contamination levels led to a significant increase in catalase enzyme activity, while gene expression levels under these contamination levels were under the control level or slightly increased (Figure 2 and 3). Increased activity of CAT enzyme indicates the ability of the cell to scavenge increased concentrations of H_2O_2; this ability was not weakened by Pb^{+2} or Cd^{+2} treatment according to the results of the current study, in accordance with previous reports (Mead 1976). The differences recorded in the enzyme activities of the plants under Pb^{+2} and Cd^{+2} stress may be due to the balance of ROS generation and antioxidant activities that must be achieved to enhance the protection of the cell.

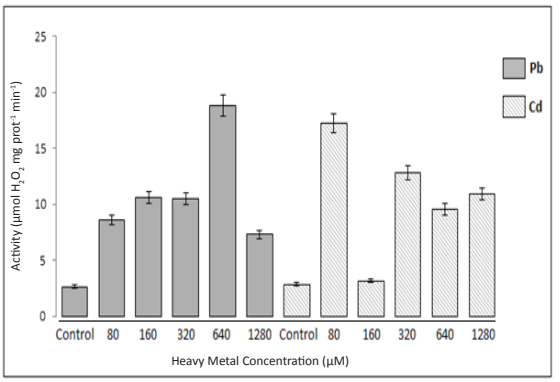


Figure 3- Catalase enzyme activity in the total tissues of tomato plants exposed to different concentrations of Pb^{2+} and Cd^{2+}

Şekil 3- Farklı konsantrasyonlarda Pb^{2+} ve Cd^{2+}'ye maruz kalan domates bitkisinin tüm dokularındaki katalaz enzim aktivitesi

The differential recovery in CAT enzyme activity at various concentrations of Pb^{+2} treatments, 640 Pb^{+2} and 1280 µM Pb^{+2}, might imply that the enzyme contributes to cellular defense with differing performances, depending on the concentrations of the heavy metals. In the current study, CAT gene expression patterns at the mRNA level and changes in CAT enzyme activities under different concentrations of heavy metal treatment revealed no positive correlation or inverse proportion. For example, although the steady-state level of mRNA did not change after the 160 µM Pb^{+2} treatment (compared to control), a significant increase was observed in enzyme activity levels at the same concentration. In addition, while the mRNA level of CAT decreased in tomato plants exposed to the 640 µM Pb^{+2} treatment, enzyme activity significantly increased, and while the mRNA level of CAT increased in tomato plants exposed to the 1280 µM Pb^{+2} treatment, enzyme activity slightly increased compared to the control. The results suggest that a translationalpost-translational level of control for CAT gene expression or enzyme activity might be regulated by the reversible modulation of the functionality of its mRNA, depending on the supply of the methyl group donors from glycine and serine and that production of these amino acids is greatly enhanced by photo respiratory carbon flow. Therefore, catalase protein synthesis is associated with the photo respiratory and photosynthetic pathways (Schmidt et al 2002).

4. Conclusions

Results of the current research verified that oxidative stress alerts plant antioxidant defense systems in unsupportable concentrations of heavy metals, and that in most cases, the CAT gene is induced to encode the CAT enzyme to scavenge ROS generated under stress conditions (Lee & An 2005). The data on gene expression and enzyme activity of CAT obtained in the current study could provide a better understanding of the antioxidant mechanisms in tomato plants.

References

Aebi H (1984). Catalase in vitro. *Methods in Enzymology* **105**: 121-126

Asada K & Takahashi M (1987). Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CJ, Artzen CJ, editors. Photoinhibition: Topics in Photosynthesis. Amsterdam, Elsevier, pp. 227-287

Ben Ammar W, Nouairi I, Tray B, Zarrouk M, Jemal F & Ghorbel M H (2005). Cadmium effects on mineral nutrition and lipid contents in tomato leaves (in French). *Journal of Social Biology* **199**: 157-163

Cervilla L M, Blasco B, Rios J J, Romero L & Ruiz J M (2007). Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to boron toxicity. *Annals of Botany* **100**: 747-756

Cui L, Lee H S, Ndinteh D T, Mbafor J T, Kim Y H, Tv L E, Nguyen P H & Wk O H (2010). New prenylated flavanones from *Erythrina abyssinica* with protein tyrosine phosphatase 1B (PTP1B) inhibitory activity. *Planta Medica* **76**: 713-718

El-Beltagi H S, Mohamed A A & Rashed M M (2010). Response of antioxidative enzymes to cadmium stress in leaves and roots of radish (*Raphanus sativus* L.). *Notulae Scientia Biologicae* **2**: 76-82

Farrel R E (2007). The regulation of gene expression in plants and animals. In: Bassett CL, editor. Regulation of Gene Expression in Plants: The Role of Transcript Structure and Processing. New York, NY: Springer, pp. 1-34

Fortunato A, Lidon F C, Batista-Santos P, Leitão A E, Pais I P, Ribeiro A I & Ramalho J C (2010). Biochemical and molecular characterization of the antioxidative system of *Coffea sp.* under cold conditions in genotypes with contrasting tolerance. *Journal of Plant Physiology* **167**: 333-342

Gaur A & Grupa S K (1994). Lipid components of mustard seeds (*Brassica juncea* L.) as influenced by cadmium levels. *Plant Food for Human Nutrition* **46**: 93-102

Goupil P, Souguir D, Ferjani E, Faure O, Hitmi A & Ledoigt G (2009). Expression of stress-related genes in tomato plants exposed to arsenic and chromium in nutrient solution. *Journal of Plant Physiology* **166**: 1446-1452

Gratao P L, Polle A, Lea P J & Azevedo R A (2005). Making the life of heavy metal-stressed plants a little easier. *Functional Plant Biology* **32**: 481-494

Henriques F (2003). Gas exchange, chlorophyll a fluorescence kinetics and lipid peroxidation of pecan leaves with varying manganese concentrations. *Plant Science* **165**: 239-244

Hernandez J A, Olmos E, Corpas F J, Sevilla F & De1 Rio L A (1995). Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Science* **105**: 151-167

Hodges D M, Delong J M, Forney C F & Prange R K (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **207**: 604-611

Jovanovic Z, Milosevic J & Radovic S (2006). Antioxidative enzymes in the response of buckwheat (*Fagopyrum esculentum* Moench) to ultraviolet B radiation. *Journal of Agriculture and Food Chemistry* **54**: 9472-9478

Kabata-Pendias A & Pendias H (1989). Trace Elements in the Soil and Plants. Boca Raton, CRC Pres

Krupa Z & Baszynski T (1985). Effects of cadmium on the acyl lipid content and fatty acid composition in thylakoid membranes isolated from tomato leaves. *Acta Physiology Plantarum* **7**: 55-64

Krupa Z & Baszynski T (1989). Acyl lipid composition of thylakoid membranes of cadmium-treated tomato plants. *Acta Physiology Plantarum* **11**: 111-116

Lake D L, Kirk P W W & Lester J N (1984). Fractionation, characterization, and speciation of Heavy metals in sewage and sludge amended soil: A review. *Jornal of Environmental Quality* **13**: 175-183

Lee S H & An C S (2005). Differential expression of three catalase genes in hot pepper (*Capsicum annuum* L.*). Molecules and Cells* **20**: 247-255

Lima L, Seabra A, Melo P, Cullimore J & Carvalho H (2006). Phosphorylation and subsequent interaction with 14-3-3 protein regulates plastid glutamine synthetase in *Medicago truncatula*. *Planta* **223**: 558-567

Livak K J & Schmittgen T D (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* **25**: 402-408

Lyons J M (1973). Chilling injury in plants. *Annual Review of Plant Physiology* **24**: 445-466

Malik D, Sheoran I S & Singh R (1992). Lipid composition of thylakoid membranes of cadmium treated wheat seedlings. *Indian Journal of Biochemistry & Biophysics* **29**: 350-354

Matsumura H, Xie Y, Shirakata S, Inoue T, Yoshinaga T, Ueno Y, Izui K & Kai Y (2002). Crystal structures of C4 form maize and quaternary complex of E. coli phosphoenolpyruvate carboxylases. *Structure* **10**: 1721-1730

Mead J F (1976). Free radical mechanisms of lipid damage and consequences for cellular membranes. In: WA Pryor, editor. Free Radicals in Biology, Vol 1. New York, Academic Press, pp. 51-68

Mittler R (2002). Oxidative stress, antioxidants and stres tolerance. *Trends in Plant Science* **7**: 405-410

Mittler R, Vanderauwera S, Gollery M & Van Breusegem F (2004). The reactive oxygen gene network in plants. *Trends in Plant Science* **9**: 490-498

Mittova V, Theodoulou F L, Kiddle G, Gomez L, Volokita M, Tal M, Foyer C H & Guy M (2003). Coordinate induction of glutathione biosynthesis and glutathione-metabolizing enzymes is correlated with salt tolerance in tomato. *FEBS Letter* **554**: 417-421

NCBI (2013a). Nucleotide. http://www.ncbi.nlm.nih.gov/nuccore/M93719.1 (Erişim tarihi: 10.01.2014)

NCBI (2013b). Nucleotide http://www.ncbi.nlm.nih.gov/nuccore/NM_001308447.1 (Erişim tarihi: 17.01.2014)

Nouairi I, Ben Ammar W, Ben Youssef N, Ben Mileddaoud D, Habibghorbal M & Zarrouk M (2006). Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. *Plant Science* **170**: 511-519

Polle A, Chakrabarti K, Chakrabarti S, Seifert F, Schramel P & Rennenberg H (1992). Antioxidants and manganese deficiency in needles of Norway spruce *(Picea abies* L.) trees. *Plant Physiology* **99**: 1084-1089

Qilin D, Jin W, Bin F, Tingting L, Chen C, Honghui L & Shizhang D (2009). Molecular cloning and characterization of a new peroxidase gene (OvRCI) from *Orychophragmus violaceus*. *African Journal of Biotechnology* **8**(23): 6511-6517

Quariti O, Boussama N, Zarrouk M, Cherif A & Ghorbalm H (1997). Cadmium and copper-induced changes in tomato membrane lipids. *Phytochemistry* **45**: 1343-1350

Rao A Q, Hussain S S, Shahzad M S, Bokhari Sy A, Raza M H, Rakha A, Majeed A, Shahid A A, Saleem Z, Husnain T & Riazuddin S (2006). Somatic embryogenesis in wild relatives of cotton (*Gossypium* spp.). *Journal of Zhejiang University Science B* **7**(3): 1673-1581

Schmidt M, Dehne S & Feierabend J (2002). Post-transcriptional mechanisms control catalase synthesis during its light-induced turnover in rye leaves through the availability of the hemin cofactor and reversible changes of the translation efficiency of mRNA. *Plant Journal* **31**: 601-613

Scott D, Keoghan J M & Allen B E (1996). Native and low input grasses-a New Zealand high country perspective. *New Zealand Journal of Agricultural Research* **39**: 499-512

Sharma R K, Agrawal M & Marshall F (2006). Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. *Bulletin of Environmental Contamination and Toxicology* **77**: 312-318

Sharma R K, Agrawal M & Marshall F M (2007). Heavy metals contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicology and Environmental Safety* **66**: 258-266

Shim I S, Momose Y, Yamamoto A, Kim D W & Usui K (2003). Inhibition of catalase activity by oxidative stress and its relationship to salicylic accumulation in plants. *Plant Growth Regulation* **39**: 285-292

Sinha S, Saxena R & Singh S (2005). Chromium induced lipid peroxidation in the plants of *Pistias tratiotes* L., role of antioxidants and antioxidant enzymes. *Chemosphere* **58**: 595-604

Skorzynska-Polit E, Drazkiewicz M & Krupa Z (2010). Lipid peroxidation and antioxidative response in *Arabidopsis thaliana* exposed to cadmium and copper. *Acta Physiology Plantarum* **32**: 169-175

Smirnoff N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytology* **125**: 27-58

Soydam Aydın S, Büyük İ, Çelikkol B P & Aras S (2013). A role of catalase (CAT) in detoxification of reactive oxygen species (ROS) in tomato (*Lycopersicum esculentum*) contaminated with manganese (Mn^{2+}). *Biological Diversity and Conservation* **6**: 140-145

Trichopoulos D (1997). Epidemiology of Cancer. In: DeVita VT editor. Cancer: Principles and Practice of Oncology, Philadelphia, Lippincott Company, pp. 231-258

Türkdoğan M K, Kilicel F, Kara K, Tuncer I & Uygan I (2002). Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environmental Toxicology and Pharmacology* **13**: 175-179

Vassilev A (2004). Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. *Biologia Plantarum* **48**: 153-156

Voutsa D A, Grimanis A & Samara C (1996). Trace elements in vegetables grown in industrial areas in relation to soil and air particulate matter environ. *Environmental Pollution* **94**: 325-335

Wang D, Heckathorn S A, Barua D, Joshi P, Hamilton E W & Croix J L A (2008). Effects of elevated CO_2 on the tolerance of photosynthesis to acute heat stress in C_3, C_4 and CAM species. *American Journal of Botany* **95**: 165-176

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

TARIM BİLİMLERİ DERGİSİ — JOURNAL OF AGRICULTURAL SCIENCES 22 (2016) 548-554

Effect of Heat Shock Treatment on Microspore Embryogenesis in *Brassica oleracea* Species

Burcu TUNCER[a], Arzu ÇIĞ[b], Ruhsar YANMAZ[c], Fikret YAŞAR[a]

[a]*Yuzuncu Yil University, Faculty of Agriculture, Department of Horticulture, 65080, Van, TURKEY*

[b]*Siirt University, Faculty of Agriculture, Department of Horticulture, 56100, Siirt, TURKEY*

[c]*Ankara University, Faculty of Agriculture, Department of Horticulture, 06110, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Burcu TUNCER, E-mail: brctuncer@gmail.com, Tel: +90 (432) 225 17 01
Received: 18 February 2015, Received in Revised Form: 06 August 2015, Accepted: 09 August 2015

ABSTRACT

Heat shock treatments are widely used to induce microspore embryogenesis in *Brassica* species. In this study, the effect of high temperature treatment (32 °C and 35 °C for 2 days) on microspore embryogenesis was investigated in six genotypes of Turkish white head cabbage (Yalova-1, Ercis, 177 C, 177 T, 531 C, 538 C), three genotypes of Turkish kale (Balkaya, Yanmaz, Karadere 077) and five commercial F_1 ornamental kale hybrids (Red Piegon, Victoria Piegon, Red Chidori, white Kamome, and Pink Kamome). Microspore-derived embryos formation differed depending on genotype and high temperature. The highest embryo yield was obtained as 9.92 embryo per petri dish in cv. Yalova-1, 11.13 embryo per petri dish in Pink Kamome F_1 at 32 °C, and 5.63 embryo per petri dish in cv. Karadere 077 at 35 °C.

Keywords: Embryo; Genotype; High temperature; Microspore culture; White cabbage; Kale

Brassica oleracea Türlerinde Sıcaklık Şoku Uygulamalarının Mikrospor Embriyogenesis Üzerine Etkisi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Burcu TUNCER, E-posta: brctuncer@gmail.com, Tel: +90 (432) 225 17 01
Geliş Tarihi: 18 Şubat 2015, Düzeltmelerin Gelişi: 06 Ağustos 2015, Kabul: 09 Ağustos 2015

ÖZET

Sıcaklık şoku uygulamaları *Brassica* türlerinde mikrospor embriyogenesisi uyarmak amacıyla yaygın olarak kullanılmaktadır. Bu çalışmada, 6 Türk beyaz baş lahana (Yalova-1, Ercis, 177 C, 177 T, 531 C, 538 C), 3 Türk yaprak lahana (Karadere 077, Balkaya, Yanmaz) çeşit ve genotipi ile 5 ticari F_1 hibrid süs lahanası çeşidinde (Red Piegon, Victoria Piegon, Red Chidori, White Kamome, and Pink Kamome) yüksek sıcaklık uygulamalarının (32 °C ve 35 °C, 2 gün) mikrospor embriyogenesisi üzerine etkisi araştırılmıştır. Mikrospor kökenli embriyo oluşumu genotip ve yüksek sıcaklığa göre farklılık göstermiştir. En yüksek embriyo oluşumu 32 °C'de 9.92 embriyo petri^{-1} değeri ile Yalova-1 çeşidinden, 11.13 embriyo petri^{-1} değeri ile Pink Kamome F_1 çeşidinden ve 35 °C'de ise 5.63 embriyo petri^{-1} Karadere 077 çeşidinden elde edilmiştir.

Anahtar Kelimeler: Embriyo; Genotip; Yüksek sıcaklık; Mikrospor kültürü; Beyaz baş lahana; Yaprak lahana

1. Introduction

Microspore culture can be utilized to shorten the duration of plant breeding programs and to obtain homozygous pure lines in *Brassica* species. Different stress treatments can be applied to flower buds, anthers or isolated microspores to stimulate microspore derived embryo formation (Shariatpanahi et al 2006; Tuncer & Yanmaz 2007; Yuan et al 2012; Cristea 2013). In all these cases, conversion to the sporophytic pathway can be induced by subjecting microspores to various stresses. The most effective and common stress treatment in *Brassica* species is the short-term heat shock. Heat shock is more effective when applied after microspore isolation. Gametophytic developmental pathway can be diverted from sporophytic pathway when optimal heat shock is applied to the isolated microspores, and microspore based embryo development increases (Ferrie & Caswel 2011).

Optimal heat shock regimes vary from species to species. For example, more successful results are noted to be attained at 32.5 °C for 1 days in broccoli (Carlos & Dias 2001; Yuan et al 2011) 32.5 °C for 1 days in *B. oleracea* var. *costata* (Dias & Correia 2002), 30 °C for 2 days (Wan et al 2011), 32.5 °C for 2 days (Kim et al 2012), 30 °C for 6 days (Ahmadi et al 2012), and 32 °C for 2 days in *B. napus* (Prem et al 2012; Wen et al 2012); 30.5 °C for 2 days in *B. oleracea* (Winarto & Teixeira da Silva 2011), 35 °C for 1 days in *B. rapa* (Zhang et al 2011), 32.5 °C for 10-15 days in *B. juncea* (Prem et al 2005) and 32 ºC for 3 days in *B. carinata* (Abraha et al 2008). *Brassica* crops such as cabbage and kales widely grown in Turkey. But breeding studies on these species have progressed slightly due to cross pollination and take many years of breeding studies. Microspore culture technique is effective to accelerate the *Brassica* breeding process abroad countries. However, this technology can not to be used effectively in Turkey. In this study, the applicability of this technology has been researched in Turkish cabbage and kale genotypes/breeding lines which still continue breeding studies in Turkey.

The present study aimed to investigate effective heat shock treatment in order to promote microspore derived embryo formation by microspore culture in different cultivars of Turkish white head cabbage and kale and ornamental kale.

2. Material and Methods

2.1. Plant material and growing donor plants

Four breeding lines (177 C, 177 T, 531 C, 538 C), one variety (cv. Yalova-1) and one genotype (Ercis genotype) of Turkish white head cabbage, three varieties of Turkish kale (Balkaya cv., Yanmaz cv., and Karadere cv.) and five ornamental kale hybrids (Red Piegon F_1, Victoria Piegon F_1, Red Chidori F_1, white Kamome F_1, and Pink Kamome F_1) were used as plant material. The plants were grown in open field conditions. The seeds of white head cabbage and kale were sown in May 2011 and the seeds of ornamental kale were sown in July 2012 in peat filled multipots. The seedlings of cabbage and kale were planted in the field, while the ornamental kale seedlings were planted in pots filled with peat. The donor plants in field were watered with drip irrigation system, the plants in 15-cm plastic pots irrigated with tap water as required and fertilized as necessary with N:P:K (6:4:6) fertilizer. Cabbage heads and kale plants were harvested in October 2011 and were stored in an unheated plastic greenhouse during the winter months. Ornamental kales in the pots were kept in the unheated greenhouse during winter. Buds were harvested at the beginning of flowering. The flower buds were collected from 80 healthy plants for each genotype.

2.2. Isolation of microspores

Flower buds including at the late uninucleate stage were harvested from white head cabbage (2.5-3.5 mm length), kale and ornamental kale (4.0-4.5 mm length). A modified method of Tuncer (2010) was used for microspore isolation. The harvested buds were surface sterilized in 10% (v v^{-1}) bleach (sodium hypochlorite) with a few drops of Tween-20 for 10 minutes and then rinsed three times in distilled water (6 minutes each time). The buds (35-40 buds per

isolation) were crushed in 3.5 mL cold NLN medium (Lichter 1982) with 13% (w v^{-1}) sucrose (hormone-free, pH 6.1). They were then filtered through a 40 mm nylon mesh and collected in a glass beaker. The meshes and beakers were rinsed with 6.5 mL of the cold NLN-13 medium, and the final volume was made up to 10 mL. The resultant suspension was centrifuged at 4 °C, 900 rpm speed three times for three minutes in order to increase the microspore purity. The last pellet was re-suspended at a density of about 4×10^4 microspores mL^{-1} in cold NLN-13 liquid medium (1 mL cold NLN-13 bud^{-1}). Five mL of the suspension were cultured in a sterilized glass petri dish (6 cm diameter, 1.5 cm height).

2.3. Heat shock treatment and culture of microspores

For each treatment and replication, 5 mL aliquots of microspore suspension were dispensed into 60 mm × 15 mm sterile petri dishes (200,000 microspore $petri^{-1}$). In the heat shock experiment, isolated microspores were incubated under dark conditions at 32 °C and 35 °C for 2 days, and then maintained at 25±1 °C under dark conditions. Development stage of the embryos was observed with a binocular microscope (Leica mark, ICC50 HD model) at the end of culture period (three weeks after the isolation). petri dishes were taken on to the 60 rpm orbital shaker when globular and heart shaped embryos were visible with naked eyes and were kept shaken for 3 weeks.

2.4. Embryogenic capacity

Three weeks after the isolation, embryos were counted per petri and embryo development stages were determined as percentages.

2.5. Experimental design and statistical analysis

This experiment was designed as a factorial experiment based on a completely randomized design with 3 replications (8 petri dish was a replication, total 24 petri per treatment). The data was subjected to analysis of variance using SPSS software (ver. 13) and means were separated by Duncan's multiple range test ($P<0.05$). The results of microspore embryogenesis were quantified in terms of number of embryos produced per petri dish.

3. Results and Discussion

Heat shock treatment induced the embryo number in white head cabbage (*B. oleracea* var. *capitata* subs. *alba*) variety and breeding lines (Table 1). The difference between the temperature degrees were found to be statistically significant in all varieties and breeding lines except Ercis population ($P<0.05$) (Table 1).

The highest embryo yield at 32 °C for 2 days was obtained from Yalova-1 cultivars (9.92 embryo per petri) and 177 C breeding line (6.13 embryo per petri), while 531 C (8.29 embryo per petri), 538 C (7.13 embryo per petri) and 177 T (6.00 embryo per petri) breeding lines were more successful at 35 °C for 2 days. It could be seen that all the varieties and breeding lines except for Ercis population were temperature selective (Table 1).

Table 1- Effect of heat shock treatment on microspore embryogenesis in white head cabbage (*B. oleracea* var. *capitata* subs. *alba*)

Çizelge 1- Beyaz baş lahanada (B. oleracea var. capitata subs. alba) sıcaklık şoku uygulamalarının mikrospor embriyogenesisi üzerine etkisi

Accessions	*32* °C	*35* °C
	Embryos petri^{-1}	*Embryos petri^{-1}*
Yalova-1	9.92±0.71 aA*	3.96±0.55 bcdB
Ercis	3.88±0.56 cA	2.08±0.21 dA
177 C	6.13±1.13 bA	2.79±0.33 cdB
177 T	2.54±0.29 cB	6.00±1.79 abcA
531 C	0.00±0.00 dB	8.29±0.29 aA
538 C	3.21±0.61 cB	7.13±1.52 abA

*, different small letters in the same column show significant differences among the species ($P<0.05$) and different capital letters in the same line show significant differences among the temperatures ($P<0.05$)

The effect of heat shocks on microspore embryogenesis in kale (*B. oleraceae* var. *acephala*) was given in Table 2. The temperature differences were statistically significant except for 'Balkaya' variety, and 35 °C treatment was more promising in terms of microspore embryogenesis. The highest embryo yield obtained from Karadere 077 (5.63

embryo per petri) and Yanmaz (5.33 embryo per petri) varieties at 35 °C (Table 2).

Table 2- Effect of heat shock treatment on microspore embryogenesis in kale (*B. oleraceae* var. *acephala*)

Çizelge 2- Yaprak lahanada (B. oleraceae var. acephala) sıcaklık şoku uygulamalarının mikrospor embriyogenesisi üzerine etkisi

Accessions	*32* °C	*35* °C
	Embryos petri^{-1}	*Embryos petri*$^{-1}$
Balkaya	1.91±0.29 bA*	1.88±0.43 bA
Yanmaz	2.38±0.44 abB	5.33±0.72 aA
Karadere 077	3.59±0.49 aB	5.63±0.81 aA

*, different small letters in the same column show significant differences among the species (P<0.05) and different capital letters in the same line show significant differences among the temperatures (P<0.05)

In all the varieties of the ornamental kale, heat shock was found to be significant (P<0.05), except Red Chidori F_1 and Red Piegon F_1. The highest embryo number was attained from Pink Kamome F_1 variety (11.13 embryo per petri), followed by Victoria Piegon F_1 variety (8.37 embryo per petri) at 32 °C. The temperature shock at 32 °C was found to be more effective in the ornamental kale except for white Kamome F_1 variety (Table 3).

Table 3- Effect of heat shock treatment on microspore embryogenesis in ornamental kale *(B. oleraceae var. acephala)*

Çizelge 3- Süs lahanasında (B. oleraceae var. acephala) sıcaklık şoku uygulamalarının mikrospor embriyogenesisi üzerine etkisi

Accessions	*32* °C	*35* °C
	Embryos petri^{-1}	*Embryos petri*$^{-1}$
Pink Kamome F_1	11.13±2.46 aA*	4.00±0.52 abB
White Kamome F_1	1.92±0.62 dB	4.00±1.37 abA
Red Chidori F_1	4.13±1.85 cdA	5.13±2.38 aA
Victoria Piegon F_1	8.37±2.01 abA	3.16±0.11 bB
Red Piegon F_1	2.08±0.33 dA	0.79±0.40 cA

*, different small letters in the same column show significant differences among the species (P<0.05) and different capital letters in the same line show significant differences among the temperatures (P<0.05)

Globular (Figure 1a), heart-shaped (Figure 1b, 1c) and torpedo-shaped (Figure 1d, 1e) embryos were observed in the microscopic examination under microscope three weeks after the isolation. Variation of embryo development stages in varieties and genotypes with respect to heat shocks were given in Table 4. It could be seen that most of the microspores derived embryos were globular and hearth shaped, while the formation rates of torpedo embryos, which was the previous stage of cotyledon embryo, were found to be low. Torpedo shaped embryos were only observed in 538 C (12.2%) white head cabbage variety at 35 °C, in Pink Kamome F_1 (8.8%) and in Yalova-1 variety (3.3%) at 32 °C, while cotyledon shaped embryos were not observed at all the species (Table 4).

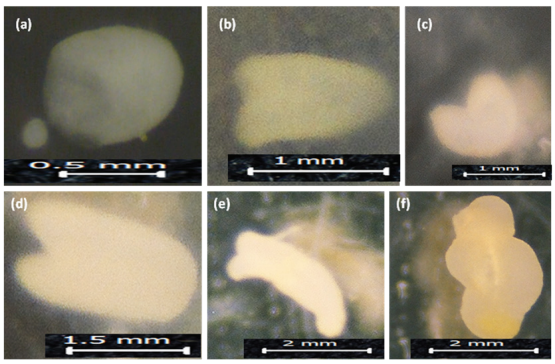


Figure 1- Embryo stages observed at 3 weeks after the isolation; a, Globular embryo (35 °C, Karadere 077); b, heart-shaped embryo (Yanmaz, 35 °C); c, heart-shaped embryo (32 °C, 177 C); d, torpedo-shaped embryo (32 °C, Yalova-1); e, torpedo-shaped embryo (35 °C, 538 C); f, embryoid (32 °C, Pink Kamome F_1)

Şekil 1- İzolasyondan 3 hafta sonraki embriyo gelişim aşamaları; a, Globular embriyo (35 °C, Karadere 077); b, Yürek şekilli embriyo (Yanmaz, 35 °C); c, Yürek şekilli embriyo (32 °C, 177 C); d, torpido şekilli embriyo (32 °C, Yalova-1); e, torpido şekilli embriyo (35 °C, 538 C); f, embriyoid (32 °C, Pink Kamome F_1)

Heat shock has been used as a trigger to induce embryogenesis in *Brassica* microspores. In *Brassica*

Table 4- The effect of heat shock on development stages of the embryos in *Brassica* species

Çizelge 4- Brassica türlerinde sıcaklık şoku uygulamalarının embriyo gelişim dönemi üzerine etkisi

Accessions	*32 °C*			*35 °C*		
	Percent of embryos			*Percent of embryos*		
	G	*H*	*T*	*G*	*H*	*T*
			White head cabbage			
Yalova-1	45.8	50.8	3.3	58.3	41.6	0.0
Ercis	100.0	0.0	0.0	100.0	0.0	0.0
177 C	53.3	46.6	0.0	100.0	0.0	0.0
177 T	38.8	61.1	0.0	44.0	55.9	0.0
531 C	0.0	0.0	0.0	25.0	75.0	0.0
538 C	36.1	63.9	0.0	27.7	60.0	12.2
			Kale			
Balkaya	100.0	0.0	0.0	100.0	0.0	0.0
Yanmaz	100.0	0.0	0.0	47.2	52.7	0.0
Karadere 077	100.0	0.0	0.0	49.9	50.0	0.0
			Ornamental kale			
Pink Kamome F_1	30.6	60.4	8.8	53.3	46.6	0.0
White Kamome F_1	100.0	0.0	0.0	100.0	0.0	0.0
Red Chidori F_1	100.0	0.0	0.0	100.0	0.0	0.0
Victoria Piegon F_1	52.0	47.9	0.0	100.0	0.0	0.0
Red Piegon F_1	100.0	0.0	0.0	100.0	0.0	0.0

G, globular-shaped embryos; H, heart-shaped embryos; T, torpedo-shaped embryos

microspores, heat shock treatments led to influence on the cell-cycle events and synthesis of heat-shock proteins (Hsp). These proteins interfere with the synthesis of gametophytic proteins while those induce synthesis of sporophytic proteins (Segui-Simarro et al 2003; Shariatpanahi et al 2006). In previous research, mostly the 30-35 °C heat shocks were suggested for different time periods (18-72 h) to stimulate microspore embryogenesis (Ferrie & Caswell 2011; Wan et al 2011; Zhang et al 2011; Kim et al 2012; Prem et al 2012; Cristea 2013). Optimal heat shock regimes differed according to the species and genotypes. In this study, these source statements were take into consideration and 32 °C and 35 °C temperature treatments were applied to microspores. Tuncer (2010) reported that treatment at 35 °C treatment is more effective in kale, and 32 °C treatment is more effective in white head cabbage (Ercis genotype), while ornamental kale cv. Red Chidori F_1 are not temperature selective for inducing microspore embryogenesis. The researcher also emphasized the requirement of repetitive studies on higher number of genotypes in order to determine the effect of 32 °C and 35 °C of temperature heat shocks clearly (Tuncer 2010). The present study aimed to examine the effects of heat shock on microspore embryogenesis more clearly by increasing the number of varieties and genotypes as suggested by Tuncer (2010). It was observed that 32 °C of temperature was more effective in cv. Yalova-1 and breeding line 177 C, while 177 T, 531 C and 538 C breeding line of white head cabbage produced more embryo at 35 °C. Although 35 °C temperature treatment was more effective in kale varieties, 32 °C temperature shock was more effective in terms of embryo stimulation for the ornamental kale.

Ferrie & Caswell (2011) reported that plant regeneration can be provided only with healthy embryos in cotyledon stage. Moreover, in some studies, some exogenous factors affecting plant

regeneration such as gibberellins, abscisic acid, antiauxin p-chlorophenoxyisobutyric acid (PCIB), 2,3,5-triiodobenzoic acid (TIBA), osmotic pressure, quality and age of embryos, embryo desiccation, and cotyledon excision, were studied to identify their influence in improving the rate of plant regeneration (Zhang et al 2006; Haddadi et al 2008; Feng et al 2009; Zhang et al 2011). In *Brassica juncea*, adding PCIB to the embryo-inducing medium not only increased the embryo yield but also played a key role in plant regeneration (Agarwal et al 2006; Zhang et al 2011). Zhang et al (2011) found that a 9.6-fold increase in plant regeneration was observed after treatment with 40 µM of PCIB. In *B. juncea*, the addition of 20 µM of PCIB led to a 5-fold increase in the frequency of microspore embryogenesis (Agarwal et al 2006).

In the present study, different rates of microspore embryo formation based on the species and varieties were obtained however plant transformation was not ensured since the embryos were not healthy cotyledonary embryos. In some cultures, embryo germination experiments could not be established due to infections, while in other cultures the infection was not occur, embryos in which liquid NLN-13 media were too small to be transferred to the solid germination media. In order to overcome sterilization problems, making different dose and duration treatments on bud sterilization in future studies can be recommended. Although studies conducted on different *Brassica* species (Agarwal et al 2006; Zhang et al 2011), it is thought to be useful addition certain antiauxin to induction medium (NLN-13) in terms of embryo maturation and plant regeneration. Genotype is the most important factor affecting the success in tissue culture techniques. Further steps might focus on foreign origin genotypes determined to be successful in microspore embryogenesis for achieving success in embryo yield and plant regeneration in vitro.

4. Conclusions

In conclusion, it was determined that effective temperature regime to stimulate microspore embryogenesis in Turkish white head cabbage, Turkish kale and ornamental kale varied depending on the species and breeding lines. These results indicate that although microspore embryogenesis was induced from microspores, it is still difficult to apply the microspore culture technique to practical breeding of *Brassica oleracea* L. genotypes with Turkish origin. Plant regeneration could not be achieved and therefore we are planning to do studies towards solving this problem in the future.

Acknowledgements

We would like to express our gratitude to Prof. Dr. Ahmet BALKAYA for providing seeds of white head cabbage and kale varieties. This study was supported by Yüzüncü Yıl University Scientific Research Project Council (YYU BAP, Project No: 2011-ZF-B005).

References

Abraha E, Bechyne M, Klima M & Vyvadilova M (2008). Analysis of factors affecting embryogenesis in microspore cultures of *Brassica carinata*. *Agricultura Tropica et Subtropica* **41**: 53-60

Agarwal P K, Agarwal P, Custers J B M, Liu C M & Bhojwani S S (2006). PCIB an antiauxin enhances microspore embryogenesis in microspore culture of *Brassica juncea*. *Plant Cell, Tissue and Organ Culture* **86**: 201-210

Ahmadi B, Ghadimzadeh M, Moghaddam A F, Alizadeh K & Teixeira da Silva J A (2012). Bud length, plating density and incubation time on microspore embryogenesis in *Brassica napus*. *International Journal of Vegetable Science* **18**: 346-357

Carlos J & Dias S (2001). Effect of incubation temperature regimes and culture medium on broccoli microspore culture embryogenesis. *Euphytica* **119**: 389-394

Cristea T O (2013). The influence of pH on microspore embryogenesis of white cabbage (*Brassica oleracea* L.). *Notulae Scientia Biologicae* **5**(4): 485-489

Dias J S & Correia M C (2002). Effect of medium renovation and incubation temperature regimes on tronchuda cabbage microspore culture embryogenesis. *Scientia Horticulturae* **93**: 205-214

Feng H, Guo S, Feng J Y & Wang Y S (2009). Microspore-derived embryos and plant regeneration in edible

kale (*Brassica oleracea* L. var. *acephala* DC). *Acta Horticulturae* **4**: 587-592

Ferrie A M R & Caswell K L (2011). Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. *Plant Cell, Tissue and Organ Culture* **104**: 301-309

Haddadi P, Moieni A, Karimzadeh G & Abdollahi M R (2008). Effects of gibberellin, abscisic acid and embryo desiccation on normal plantlet regeneration, secondary embryogenesis and callogenesis in microspore culture of *Brassica napus* L. cv PF704. *International Journal of Plant Production* **2**: 153-162

Kim K S, Lee Y H, Cho H J, Jang Y S & Park K G (2012). Effects of culture condition on microspore culture of *Brassica napus* L. domestic cultivar 'Tammiyuchae'. *Korean Journal Crop Science* **57**: 317-323

Lichter B (1982). Induction of haploid plants from isolated pollen of *B. napus*. *Zeitschrift für Pflanzenphysiologie* **105**: 427-434

Prem D, Gupta K & Agnihotri A (2005). Effect of various exogenous and endogenous factors on microspore embryogenesis in Indian mustard (*Brassica juncea* L. Czern and Coss). *In Vitro Cellular & Developmental Biology* **41**: 266-273

Prem D, Solís M T, Bárány I, Rodríguez-Sanz H, Risueño M C & Testillano P S (2012). A new microspore embryogenesis system under low temperature which mimics zygotic embryogenesis initials, expresses auxin and efficiently regenerates doubled-haploid plants in *Brassica napus*. *BMC Plant Biology* **12**: 127

Segui-Simarro J M, Testillano P S & Risueno M C (2003). Hsp70 and Hsp90 change their expression and subcellular localization after microspore embryogenesis induction in *Brassica napus* L. *Journal of Structural Biology* **142**: 379-381

Shariatpanahi M E, Bal U, Heberle-Boors E & Touraev A (2006). Stresses applied for the re-programming of plant microspores towards in vitro embryogenesis. *Physiologia Plantarum* **127**: 519-534

Tuncer B (2010). The effect of different treatments on embryo induction of cabbages via microspore culture. PhD thesis, Ankara University (Unpublished), Ankara, Turkey

Tuncer B & Yanmaz R (2007). Haploid bitki elde etme yollarından biri: Mikrospor kültürü. *Alatarım* **6**: 1-8

Wan G L, Naeem M S, Geng X X, Xu L, Li B, Jilani G & Zhou W J (2011). Optimization of microspore embryogenesis and plant regeneration protocols for *Brassica napus*. *International Journal of Agriculture and Biology* **13**: 83-88

Wen Y, Fui T, Wen J, Tu J, Ma C, Shen J, Zhang S & Bin Y **(**2012). Profound improvements of isolated microspores culture techniques in winter *Brassica napus* L. *African Journal of Biotechnology* **11**: 1617-1623

Winarto B & Teixeira da Silva J A (2011). Microspore culture protocol for Indonesian *Brassica oleracea*. *Plant Cell, Tissue and Organ Culture* **107**: 305-315

Yuan S X, Liu Y M, Fang Z Y, Yang L M, Zhuang M, Zhang Y Y & Sun P T (2011). Effect of combined cold pretreatment and heat shock on microspore cultures in broccoli. *Plant Breeding* **130**: 80-85

Yuan S X, Su Y B, Liu Y M, Fang Z Y, Yang L M, Zhuang M, Zhang Y Y & Sun P T (2012). Effects of pH, MES, arabinogalactan-proteins on microspore cultures in white cabbage. *Plant Cell, Tissue and Organ Culture* **110**: 69-76

Zhang G Q, Zhang D Q, Tang G X, He Y & Zhou W J (2006). Plant development from microspore-derived embryos in oilseed rape as affected by chilling, desiccation and cotyledon excision. *Biologia Plantarum* **50**(2): 180-186

Zhang Y, Wang A, Liu Y, Wang Y & Feng H (2011). Effects of the antiauxin PCIB on microspore embryogenesis and plant regeneration in *Brassica rapa*. *Scientia Horticulturae* **130**: 32-37

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Primary Production Estimation of Çankırı Province's Rangelands Using Light Use Efficiency (LUE) Model with Satellite Data and AgrometShell Module

Ediz ÜNAL[a], İlhami BAYRAMİN[b]

[a]*Field Crops Central Research Institute, Şehit Cem Ersever Street, No: 9-11, Ankara, TURKEY*

[b]*Ankara University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 06110, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Ediz ÜNAL, E-mail: eunal@tagem.gov.tr, Tel: +90 (312) 343 10 50
Received: 25 February 2014, Received in Revised Form: 14 August 2015, Accepted: 14 August 2015

ABSTRACT

In this study, monthly and annual gross primary production (GPP) of rangelands in Çankırı province for the period of 2000-2009 was calculated using light use efficiency (LUE) model with the inputs of satellite data and AgrometShell module. The average production of rangelands varied seasonally and annually (from 12630 to 37701 tons) and was approximately 17800 tons for the last ten years. The amount of rainfall and changing number of animal grazing in the region probably led to the variation. Model performance was tested with integrated normalized difference vegetation index (INDVI) approach which produced a moderate significant correlation (R^2= 0.69, P<0.05) between LUE model gross primary productivity (GPP) output and INDVI values. On the other hand, comparison of modelled results of annual gross primary production (GPP) with above ground measurements, indicated that correlation between the variables were insignificant (r = 0.60, P>0.05 for 2008, r= 0.41, P>0.05 for 2009) due to some factors such as sampled plant type, scale differences between satellite data and ground sample size, and subjective sampling errors. This study indicates that LUE Model together with the inputs of AgrometShell module is suitable tool for estimation of rangeland primary production.

Keywords: Biomass; Çankırı; Range; Remote sensing; Vegetation

Uydu Verisi ve AgrometShell Modülü ile Işık Kullanım Etkinliği (LUE) Modeli Kullanarak Çankırı İli Meralarının Birincil Üretim Tahmini

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Ediz ÜNAL, E-posta: eunal@tagem.gov.tr, Tel: +90 (312) 343 10 50
Geliş Tarihi: 25 Şubat 2014, Düzeltmelerin Gelişi: 14 Ağustos 2015, Kabul: 14 Ağustos 2015

ÖZET

Bu çalışmada, Çankırı meralarının 2000-2009 arasındaki aylık ve yıllık toplam birincil üretimleri ışık kullanım etkinliği modeli ile hesaplanmıştır. Elde edilen bulgulara göre il sınırları içinde kalan meraların son on yıllık ortalama birincil üretimi yaklaşık 17877 tondur ve bu üretim hem mevsimsel hem de yıllık olarak (12630-37701 ton arası) değişkenlik

göstermektedir. Bu değişkenliğin ana sebepleri içinde bölgeye düşen yağış miktarı ve otlayan hayvan sayısındaki değişimler gösterilebilir. Model performansı, toplanmış normalize edilmiş farklılık indeksi (INDVI) ile test edilmiştir. Test sonucuna göre, INDVI ve toplam birincil üretim arasında orta seviyede bir ilişki (R^2= 0.69, P<0.05) bulunmuştur. Uygulanan hassaslık analizi sonuçları, orantılı fotosentetik aktif radyasyonun (FPAR) en hassas değişken olduğunu göstermiştir. Diğer taraftan, modelden hesaplanan yıllık birincil üretim (GPP) değerleri ve arazi çalışmaları ile hesaplanan biyokütle arasında önemsiz ilişki bulunmuştur (r = 0.60, P>0.05, 2008; r= 0.41, P>0.05, 2009). Örneklenen bitki türleri, kişisel örnekleme hataları ve uydu verileri ile örnekleme alanı arasındaki ölçek farklılığı ilişki çıkmamasının ana sebepleri olarak gösterilebilir. Bu çalışma, AgrometShell girdilerini kullanan LUE modelinin meralarda birincil üretim miktarının tahmin edilmesinde iyi bir araç olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Biyokütle; Çankırı; Mera; Uzaktan algılama; Vejetasyon

1. Introduction

Rangelands are important natural resources for livestock feeding and providing habitats for biological diversity. Being a challenging issue, assessing productivity and gross primary production (GPP) of rangelands is important for their efficient management. The employment of remote sensing which has been the most frequently used method utilizes two approaches; a) establishing relationships between spectral reflectance and biomass (Tucker et al 1983) and b) modelling GPP from remotely sensed spectral reflectance to estimate the amount of absorbed photosynthetically active radiation (APAR) (Brogaard et al 2004). A light use efficiency (LUE) approach is widely applied concept for modelling the GPP (Goetz et al 1999; Hilker et al 2008), and expresses the GPP as a product of the APAR. This approach is the main component of the current study based on the idea that biological production is directly proportional to the photosynthetically active radiation (PAR) absorbed by the green vegetation (Monteith 1972).

The revised model of Seaquist et al (2003) presented in Equation 1 was used in this study, because it includes environmental effects (drought, temperature, pollution, nutrient deficiency, illness etc.) as stress factors which play an important role in biological activities of the plant and hence in the GPP.

$$GPP = \sum_{i=1}^{n} \varepsilon p \times \varepsilon \times FPAR \times PAR \qquad (1)$$

Where; GPP, gross primary production (g m^{-2}) converted to dry plant matter (DM) through photosynthesis; Ɛp, LUE factor (g DM MJ^{-2}) expressing conversion of light energy into dry mass; Ɛ, unitless environmental stress factor; PAR, photosynthetic active radiation (MJ m^{-2}) of sun light in the spectral range of 400-700 nm and FPAR, fraction of absorbed light by vegetation.

The main objective of this study was to estimate and map annual and monthly GPPs of rangelands in Çankırı province using a light use efficiency model. The following steps were also achieved by reaching the main objective; 1) a validation of calculated GPP by ground data and 2) performing a sensitivity analysis of the model variables.

2. Material and Methods

2.1. Study area

The study area is Çankırı province located in the Central Anatolia, Turkey (Figure 1). The landscape of Çankırı is mostly mountainous with hilly topography covering approximately 60% of the province. The average elevation is 723 m with hills and plateaus interrupted by Ilgaz Mountain ranges. Continental climate dominates the region with long term average rainfall of 500 mm. The land is mostly bare on the hills and plateaus, while the mountains are covered with coniferous trees. Foot lands are generally used for grain cultivation. Soil erosion is prevalent across the province, which explains why the non-cropped lands are used mostly as rangelands.

These rangelands have some characteristics of desert plant species, resulting from low rainfall of 300-500 mm and over grazing (Ketenoğlu et al 1983). The botanical composition of rangelands generally consists of short grass (*Festuca sp, Poa sp*.), broad leaves (*Medicago sp*.) and various thorny species (*Astragalus sp.*) (Kurt et al 2006).

In this study, the GPP was calculated both monthly and annually for 10 years (2000-2009) for the active vegetation period of a growing season that starts with first leaf appearance and ends with senescence. The growing season was divided into 10-day periods (dekad) totaling to 14 dekads for each year. The first dekad starts on March 20th and ends on March 31st. The last dekad spans from August 1st to August 10th.

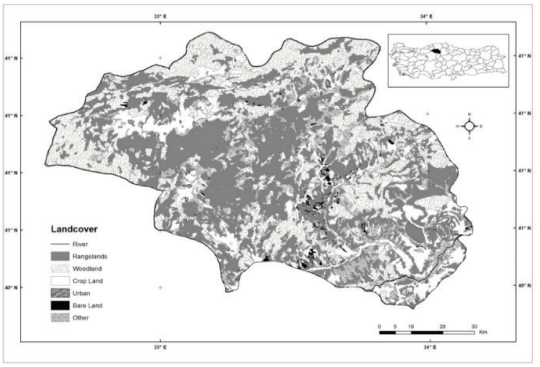


Figure 1- Location of study area and land cover classes

Şekil 1- Çalışma alanı ve arazi örtüsü sınıfları

2.2. Used data

The meteorological data stored in AgrometShell module retrieved from 36 automatic weather stations (AWS) distributed over the province of Çankırı and neighboring provinces (Figure 2). The AWSs measured many weather parameters some of which were the inputs in database of AgrometShell module developed by FAO Environment and Natural Resources Service (SDRN). The module provides a toolbox for agro-meteorological crop monitoring and forecasting (Mukhala & Hoefsloot 2004) and includes a database of weather data as 10-day average of temperature (°C), solar radiation (Cal m^{-2}), wind speed (m s^{-1}) and 10-day sum of rainfall (mm) as well as crop specific information such as crop type, crop cycle length, irrigation, etc. AgrometShell module runs a water balance model to produce actual evapotranspiration (AET) and potential evapotranspiration (PET) which were used later for water stress calculation in LUE model. Calculated PET and AET values were then converted into grid format by the inverse distance weighting (IDW) kriging interpolation method (Ha et al 2011) to generate surfaces with same cell size of 1 km of NDVI data.

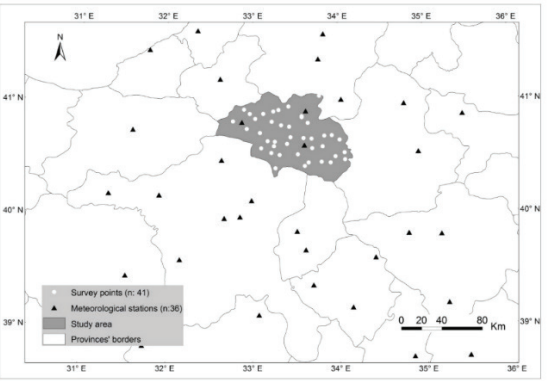


Figure 2- Meteorological stations and survey points

Şekil 2- Meteoroloji istasyonları ve sörvey noktaları

NDVI images were used as satellite data, which is widely regarded as measurements of surface vegetation condition and dynamics and indicates the greenness of live vegetation (Huete et al 2002). NDVI is numerically calculated from (NIR-RED/NIR+RED). RED and NIR stand for the spectral reflectance measurements acquired in red and near-infrared regions of the spectrum, respectively. SPOT-Vegetation (SPOT-Veg) 10-days maximum value composite (MVC) NDVI images (S-10 product) were used in the model. S10 data were derived from physical products, which were surface reflectance's corrected for molecular and aerosol scattering, water vapor, ozone and other gas absorption (Holben 1986). A total of 140 composited

SPOT 10-day MVC NDVI images, hereafter called NDVI, covering a 10 year (2000-2009) time period was obtained from ARTEMIS Project of Food and Agricultural Organization (FAO) of United Nations. The NDVI images coincided with growing season dekads.

Vector dataset (shapefile polygon) of rangeland borders produced earlier throughout the National Rangeland Project (Mermer et al 2012) was used as a mask file to exclude the areas other than rangelands for which the GPP was calculated.

Reference data were obtained from the field surveys in both July of 2008 and 2009. Stratified random sampling method was applied to determine field visit locations. 1/25000 scaled topographic map grids were used as sampling frame laid over the rangeland polygons as upper layer in a geographic information software (GIS). Automatically generated random points intersecting both rangeland polygons and map grids were selected as sampling points. The number of sampling points was determined by the approach that each map grid had at least two survey locations representing rangelands in the grid (Anonymous 2012). A total of 41 points were identified (Figure 2) for field measurements which included registering botanical composition of 1 m^2 quadrats and cutting the live vegetation in each quadrat. Clipped vegetation was dried at sun for 7-10 days and then weighed as a dry mass (g m^{-2}).

2.3. Model application

Primary production considers biomass accumulation in vegetation as the results of succession stages during which sun light energy is intercepted (Monteith 1972). Primary production is deduced as the product of radiation energy (PAR), fraction of PAR by the plant (FPAR), conversion efficiency of absorbed radiation into biomass (LUE factor) and environmental factor (stress factor). Therefore, the primary production is assumed to be proportional to these variables (Equation 1).

Methodology used similar to the one that Seaquist et al (2003) and Brogaard et al (2004) employed was revised for potential evapotranspiration (PET) and actual evapotranspiration (AET) calculations. For the PET calculations, the FAO Penman-Monteith (Allen et al 1998) method was used, while AET was calculated by FAO AgrometShell water balance model. 10-day average PAR, FPAR and stress factors rasterized as grid format with the same spatial size of NDVI data (1 km^2) were used in the model, while LUE factor was used as constant. Graphical representation of the LUE model is shown in Figure 3.

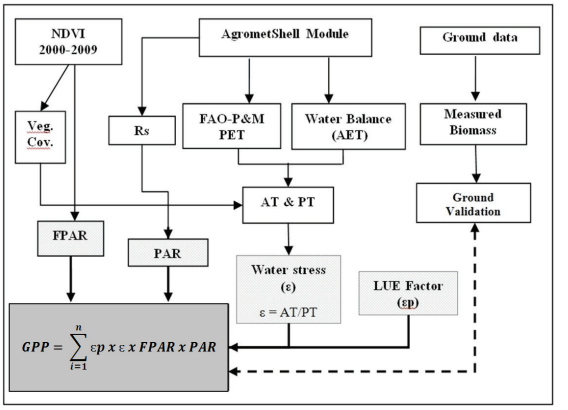


Figure 3- Graphical representation of LUE model

Şekil 3- LUE Modelinin grafiksel gösterimi

2.3.1. Photosynthetic active radiation (PAR)

PAR is a part of total incoming solar radiation in the visible spectrum (400-700 nm), which shows distinct temporal and spatial patterns due to varying atmospheric conditions (Uzun & Demir 2012). Only the PAR of total solar radiation can be used by green vegetation to produce organic matter through photosynthesis. The common and simple method to calculate the PAR is to proportionate solar radiation by total radiation received at the surface. The PAR was calculated from the solar radiation (MJ m^{-2}) which was measured at meteorological station. The solar radiation incident on canopies tends to contain a relatively constant fraction of PAR varying from 45% to 50% depending on location and sky conditions (Le Roux et al 1997). On average, 48% of PAR ratio was used in calculations.

2.3.2. Fractioned photosynthetic active radiation (FPAR)

FPAR is the fraction of the absorbed PAR by the plant canopy and can generally be calculated by either physical models (Los et al 2005) or empirical methods (Huete et al 2002) taking into account of spectral vegetation indexes. The most known index is NDVI which relates to the FPAR given in Equation 2 (Goetz et al 1999).

$$FPAR = a \pm b \times NDVI \quad (2)$$

Where; *a* and *b*, correlation coefficients. For the FPAR calculation, empirical method was used in this study as used by Seaquist et al (2003) and Brogaard et al (2004). The only difference was the use of additional coefficients. Background and dead materials play a substantial role on the FPAR, especially during senescence period of the plant. The FPAR absorbed by dead material reaches by 20% (Le Roux et al 1997) and thus, the FPAR value decreases gradually after development stage. We therefore added the coefficient of 0.80 to the FPAR equation to compensate dead material effects for the period of senescence which corresponds to between July 1st and August 10th (Equation 3). During the initial and development stage (March 20th-July 31st), no additional coefficient was added to FPAR equation (Equation 4).

$$FPAR = (1.67x(NDVI) - 0.07) \times 0.80 \quad (3)$$

$$FPAR = 1.67x(NDVI) - 0.07 \quad (4)$$

2.3.3. LUE factor (εp)

LUE factor, regarded as empirical constant, represents the actual efficiency of a absorbed radiation energy used by plants to produce biomass. Seasonal changes in LUE factor are closely related to soil water content and phenological stage of the plant (Prince 1991). In the case that available water in the soil is adequate, the value of LUE factor in early stage is higher than the one in development stage of the plant (Le Roux et al 1997). The available water is assumed to be adequate in the early stages of rangeland plants in the study area. According to Sims & Singh (1978), mean LUE values measured for short grass species ranged between 0.42 and 0.62. Therefore a higher LUE value of 0.62 for early stage corresponding the period of March 20-May 10 was used, while the lower LUE value of 0.42 was used for the remaining stages of May 10-August 10. These values (Table 1) were used in the model, because there were not any measured LUE values for our study area where the botanical compositions consisted of mostly short grass steppe.

Table 1- Measured mean LUE values (Sims & Singh 1978)

Çizelge 1- Ölçülmüş ortalama LUE değerleri

Species	*Location*	*Mean LUE*
Desert	New Mexico	0.10
Desert	New Mexico	0.07
Short	Texas	0.51
Short	Texas	0.62
Short	Colorado	0.51
Short	Colorado	0.42
Herbaceous	Washington	0.06
Herbaceous	Washington	0.04
Mixed	South Dakota	0.84
Mixed	South Dakota	0.79
Mixed	North Dakota	1.74
Mixed	North Dakota	2.00
Mixed	Kansas	1.02
Mixed	Kansas	0.93
Tall grass	Oklahoma	0.88
Tall grass	Oklahoma	1.15

2.3.4. Stress factor (ε)

The stress component of the model is presented by lack of adequate soil moisture which plays an important role in biological activities of the plant. A stress factor was used in the LUE models as a part of a scalar environment factor (ε) in which drought, temperature, pollution, nutrient deficiency, illness and other elements were covered (Prince 1991). The stress factor was calculated by the ratio of actual transpiration (AT) to potential transpiration (PT) (Equation 5).

$$\varepsilon = \frac{AT}{PT} \quad (5)$$

Where; epsilon, ε, stress factor in the model; AT (mm), term emphasizes evaporated water during respiration process of plant, and PT, potential transpiration (mm) when there is no soil water deficit. The AT was calculated by multiplying actual evapotranspiration (AET) with fraction of vegetation cover (FVC) (Equation 6).

$$AT = AET \times FVC \quad (6)$$

Where; AET (actual evapotranspiration, mm), total water loss from both soil and plant during respiration. It was calculated based on the water balance model that FAO AgrometShell module performs (Mukhala & Hoefsloot 2004). The FVC was calculated from NDVI data (Equation 7).

$$FVC = \left(\frac{NDVI - NDVI_s}{NDVI_v - NDVI_s} \right)^2 \quad (7)$$

Where; FVC, values range between 0 and 1 corresponding non vegetation and full vegetation cover, respectively; $NDVI_s$, pixel value of NDVI corresponding non vegetation; $NDVI_v$, full vegetation cover of the NDVI and NDVI, cell-specific NDVI value in the vegetation map.

The PT is the rate of transpiration that occurs in a large area completely covered with vegetation with access to unlimited water supply and calculated by multiplying the FVC with the potential evapotranspiration (PET) and crop coefficient (Kc) (Equation 8).

$$PT = Kc \times PET \times FVC \quad (8)$$

Kc incorporates crop characteristics and effects of evaporation from the soil. A value of 0.85 was used for the development stage of vegetation, during which the Kc corresponds to amount of ground cover and plant development (Wight & Hanks 1981). For the calculation of PET, FAO's Penman-Monteith method was used, which considers many parameters related to the evapotranspiration process such as solar radiation, air temperature, vapor pressure deficit and wind speed (Allen et al 1998). All these parameters were composited as 10-day average to coincidence with NDVI data.

3. Results and Discussion

3.1. Monthly and annual production

Monthly and annual primary production calculations with LUE model were presented in Table 2. The rangelands were estimated to produce 17877 tons of 10 year mean annual production in approximately 301939 hectares of range area.

The 10 year figures illustrated the least production was observed in both central and southern region of the province (Figure 4a). Field surveys showed that the rangelands were mostly covered by stones and the plants were of a steppe character resulting in very low canopy cover. On the other hand, in the northern part and northwest regions of Çerkeş and Ilgaz counties, the rangelands produced greater biomasses than the rest of the region (Figure 4a). Ranges in these regions were calculated to have higher canopy covers and

Table 2- Modelled monthly and annual summed GPP

Çizelge 2- Aylık ve yıllık toplam GPP

	GPP (ton)									
Months	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*	*2009*
March	156	223	96	228	261	300	303	54	113	142
April	2484	2347	2044	2090	2038	1840	3342	2992	1839	1525
May	15439	4204	7765	7478	9873	7528	7038	5720	5782	6681
June	17688	4076	6492	6987	7322	8027	5871	3912	6227	3659
July	1665	249	572	502	655	789	92	146	375	529
August	269	62	121	95	151	137	3	22	52	100
TOTAL	37701	111618	170906	173801	203004	186214	166490	128463	14388	12636

dry yields which was confirmed with the field survey results. The differences in 10 year average production may be resulted from the changes in the amount of rainfall and the frequency of animal grazing. The average rainfall during growing season (throughout 2000-2009) was 156 mm, which explains the low yield in the southern and eastern regions. However in the northern parts of the province, where the yield was relatively higher, average rainfall was 200 mm. This situation supported the general conclusion that the higher rainfall is, the more biomass the plant produce (Figure 4a and 4b).

The number of grazing animals can be another factor effecting variation in biomass production. There was a gradual decrease in the animal number for 9 year-period of 2000-2008 (Figure 5). Unfortunately, there weren't any statistical records of number of grazing animals for the 2009. In the 9 years, the total number of ruminants has been decreased by 21.347% totaling to 90539 in 2008 (Table 3). Figure 4c shows percentage change of the animal number with respect to the province's counties. Positive variations in the period explained the increases in animal number, while negatives indicated the decreases. The animal numbers decreased in 9 out of 12 counties, but increased in 3 counties (Figure 4c).

It was seen that average GPP was higher in the counties which had negative variations in the number of animal than those which had positive variations. Besides, the northern counties (Çerkes, Bayramören, Ilgaz, Kurşunlu and Atkaracalar) had higher GPP values as a result of higher amount of precipitation (Figure 4b) compared to the southern counties and lower number of grazing animals resulting in lower grazing pressures on the live vegetation. The results showed both rainfall and animal number exhibited an interactive role in rangelands' production.

3.2. Evaluation of model performance

Model performance was tested to determine how well the LUE model estimated the GPP of rangelands. Two approaches were used for performance evaluation; a) comparison of field biomass measurements with the LUE model's GPPs and b) regression analysis between integrated

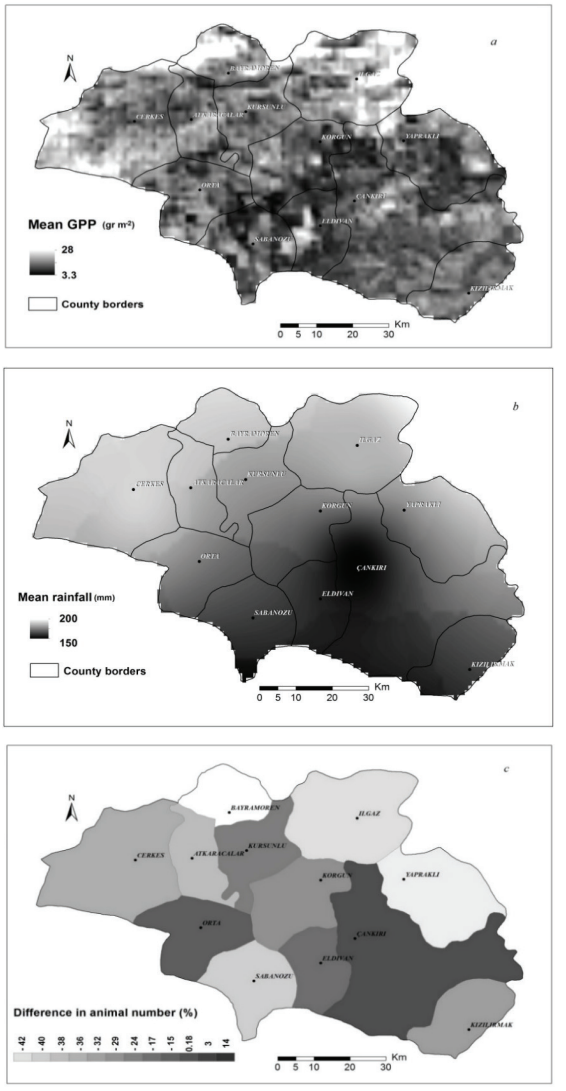


Figure 4- a, average GPP; b, average rainfall; c, animal number variation for 10 years (2000-2009) by counties

Şekil 4- a, 10 yıllık ortalama GPP üretimi; b, ortalama yağış; c, ilçelere göre hayvan sayısındaki 10 yıllık varyasyon

normalized difference vegetation index (INDVI) and LUE model's GPPs.

Relationships between field measurements of biomass and LUE model GPPs were tested by

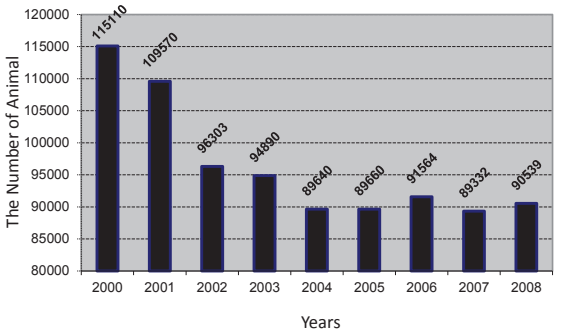


Figure 5- The number of animal between 2000-2008 in Çankırı province (TUIK 2009)

Şekil 5- 2000-2008 yılları arası Çankırı ilindeki hayvan sayısı (TUIK 2009)

Table 3- The number of animals by counties in 2000 and 2008 (TUIK 2009)

Çizelge 3- 2000 ve 2008 yıllarında ilçelere göre hayvan sayısı (TUIK 2009)

Counties	*The number of animal*		*Difference (%)**
	2000	*2008*	
Atkaracalar	4438	3011	-32.15
Bayramören	3609	2092	-42.03
Çerkeş	24808	17463	-29.60
Eldivan	3218	3224	0.18
Ilgaz	11572	7095	-38.68
Kızılırmak	9759	7350	-24.68
Korgun	5265	4335	-17.66
Kurşunlu	9425	7986	-15.26
Merkez	14337	16360	14.11
Orta	9439	9766	3.46
Şabanözü	9900	6324	-36.12
Yapraklı	9340	5533	-40.76
Total	115110	90539	-21.34

*, positive variations in the period explained the increases in animal number, while negatives indicated the decreases.

correlation analysis for both years. There were moderate correlations between variables (r= 0.60 and r= 0.41 for 2008 and 2009, respectively). The relationships were insignificant for both years (P>0.05). Unfortunately the correlations were not very high because of the various factors such as sampled plant types, scale differences between sampling area and pixel size of satellite image, and measurement errors. The sampled plant type was the most important factor making the biomass measurements inconsistent. For instance, herbaceous plants (*Festuca sp., Poa sp., Bromus sp.*) had high ground coverage due to their extensive spread habit causing high NDVI values which drove the model to calculate higher GPPs than the that of woody plants such as thyme (*Thymus sp.*), and astragalus (*Astragalus sp.*). Scale differences caused location errors between ground observations and satellite data (NDVI) and thus low correlations, because biomass measurements were taken from very small site (1 m^2) which was approximately 1/1.000.000 of one pixel size of NDVI data.

The second method for model evaluation was the use of INDVI accounting for the summed NDVI of during growing stage. The INDVI was regressed with the GPPs calculated from the LUE model (LUE-GPPs). The correlation was significantly high (r= 0.83) by giving the regression coefficient of 0.69 which means that approximately 69% of total variation in the LUE-GPPs can be explained by the linear relationship between these variables (Figure 6). Significance test of regression explained that there was a relationship between the variables in the linear regression model (t= 7.4, P<0.05). According to ANOVA test, the INDVI and the LUE-GPP were statistically significant (Table 4).

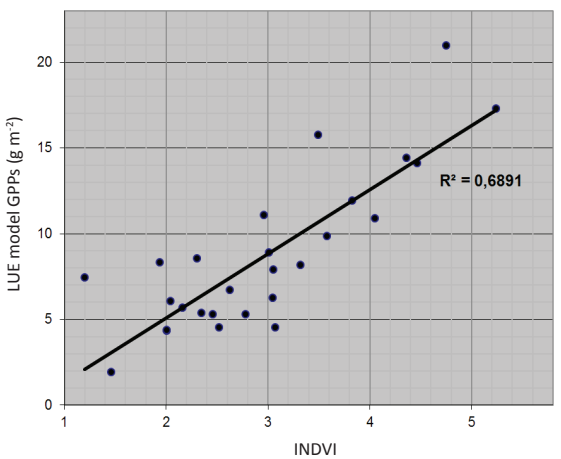


Figure 6- Correlation between INDVI and LUE model GPPs

Şekil 6- INDVI ve LUE modeli GPP arasındaki korelasyon

Table 4- Significance test statistics for linear regression of INDVI and model GPP

Çizelge 4- INDVI ve modelin ürettiği GPP arasındaki doğrusal regresyonun anlamlılık testi

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	371.75	371.75	55.40	0.00
Residual	25	167.73	6.70		
Total	26	539.49			
	Coefficients	*Standard error*	*t Stat*	*P-value*	
Intercept	-2.39	1.57	-1.52	0.14	
INDVI	3.74	0.50	7.44	0.00	

3.3. Sensitivity analysis of the model

Sensitivity analysis was performed to determine to what extent the choice of variables affected the model output. There is a large body of scientific literature on various methods of sensitivity analysis (Morgan & Henrion 1990; Shevenell & Hoffman 1993). Of the approaches for sensitivity analysis, no single method serves as the best analysis for all modelling efforts. The sensitivity ratio, also known as elasticity equation, is used for the analysis in this study (Anonymous 2001). The sensitivity ratio (SR) shown in Equation 9 is the percentage change in output divided by the percentage change in input for a specific input variable. When the sensitivity ratio is higher, the more sensitive the model output is.

$$SR = \left(\frac{y2-y1}{y1}\right) \Big/ \left(\frac{x2-x1}{x1}\right) \quad (9)$$

Where; SR, sensitivity ratio; *x1*, average value calculated from the 10 year data of model variables (*FPAR*, *PAR* and ε); *x1* value for the *LUE* variable was taken as 0.500 approximating the average of highest and lowest values for the short grass steppe (Table 1); *x2*, can take values of *x1* variable's minimum and maximum values for the "worst" and "best" case scenarios, respectively; *y1*, represents the average GPP calculated from variables' means; *y2*, represents the GPP calculated from the variables one of whose value is changed in accordance with scenarios. The minimum and maximum values of the stated variables were used to build "Best Case" and "Worst Case" scenarios for calculation of sensitivity ratios (Table 5). Best and worst case scenarios respectively denote minimum and maximum GPP to be produced, which is directly proportional to degree of variables in GPP equation. The minimum and maximum values of *FPAR*, *PAR* and ε variables were derived from 10 year grid data used, while the minimum and maximum LUE values were directly taken from empirical measurements given in the Table 1 (Sims & Singh 1978).

For the worst case scenario, all variables have the SR value of around 1.00, which means that biomass produced is dominated by all variables almost equally (Table 5). As for the best case scenario, similar results were obtained that SR values are smaller than 1.00 but around it. The most sensitive variable is *FPAR* (SR= 0.99). It is because the *FPAR*

Table 5- Sensitivity ratios (SR) for LUE model variables for the best case and the worst case scenarios

Çizelge 5- En iyi ve en kötü senaryolara göre LUE modeli değişkenlerinin hassasiyet oranı (SR)

	Worst case scenario					*Best case scenario*				
Variables	*X1*	*X2*	*Y1*	*Y2*	*SR*	*X1*	*X2*	*Y1*	*Y2*	*SR*
FPAR	0.261	0.003	0.592	0.0043	1.00	0.261	0.588	0.592	1.330	0.99
PAR	8.660	6.050	0.592	0.4101	1.02	8.660	10.915	0.592	0.741	0.96
ε	0.529	0.061	0.592	0.0666	1.00	0.529	0.979	0.592	1.082	0.97
LUE	0.500	0.420	0.592	0.4883	1.01	0.505	0.620	0.592	0.720	0.94

is positively related to the vegetation index which is an estimate of how much photosynthetically active vegetation is present. As a general conclusion for sensitivity analysis, the variables are each important factors affecting biomass production and this conclusion is supported by high SR values.

4. Conclusions

This study combines the outputs of AgrometShell module and satellite NDVI data using the LUE model approach to estimate annual GPP of Çankırı rangelands. The 10-year average modelled GPPs point out that annual production was approximately 17880 tons which corresponds to 11.4 g m^{-2} of dry yield. The GPP production of the region varied seasonally and annually. The main reasons of this variability were rainfall and the number of animals grazed.

Comparison of model results of annual primary production (GPP) with ground truthing showed that the model unfortunately did not produce significant correlations ($P>0.05$) due to several sources of uncertainty and inconsistencies such as sampled plant types, erratic LUE values, residues' background effect, estimation of incident PAR and scale differences between satellite data and ground sample size. On the other hand, integrated NDVI approach produced higher correlation ($r= 0.83$, $P<0.05$) between the LUE model GPP output and the INDV values. According to sensitivity analysis, the model variables almost equally affect the GPP production in the worst case scenario. On the other hand the FPAR variable was the most significant factor affecting the biomass production in the best case.

The main advantage of the model used in this study was that it simulates primary production by considering the water used by plants (actual transpiration), which was difficult parameter to measure needing extensive ground based measurement equipment. However, AgrometShell module used here easily calculated this parameter through the weather data.

The results are encouraging that the LUE model could be applied in any rangeland region where conventional weather and satellite NDVI data exist. On the other hand, accurate estimation of GPP over large areas as rangelands, pastures, and forest does still have some drawbacks such as the effects of varying environmental conditions on vegetation canopy reflectance issues and the estimation of empirical LUE factor and other stress factors. It is apparent that GPP modelling studies will be active research area in future to overcome those handicaps.

Acknowledgements

This research study was supported by the project (No: 106G017) of "National Rangeland Management" funds of the Scientific and Technological Research Council of Turkey (TUBITAK). We express our gratitude to all staff for helping during the field visit and office work.

References

Allen R G, Pereira L S, Raes D & Smith M (1998). Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. United Nations Food and Agriculture Organization (FAO), Irrigation and Drainage Paper 56, Rome, Italy

Anonymous (2001). Risk assessment guidance for superfund (RAGS). In: Volume III-Part A. *Process for Conducting Probabilistic Risk Assessment*. U.S. Environmental Protection Agency Washington, DC, pp. 385-387

Anonymous (2012). Ulusal Mera Kullanım ve Yönetim Projesi sonuç raporu. Proje No: 106G017. TÜBİTAK Kamu Kurumları Araştırma ve Geliştirme Projelerini Destekleme Programı (1007 Programı)

Brogaard S, Runnström M & Seaquist J A (2004). Primary production of inner Mongolia, China between 1982 and 1999 estimated by a satellite-driven light use efficiency model. *Global and Planetary Change* **45**: 313-332

Goetz S J, Prince S D, Goward S N, Thawley M M & Small J (1999). Satellite remote sensing of primary production: an improved production efficiency modelling approach. *Ecological Modelling* **122**: 239-255

Ha W, Gowda P, Oommen T, Marek T, Porter D & Howell T (2011). Spatial interpolation of daily reference evapotranspiration in the Texas High Plains. In: *World Environmental and Water Resources Congress*, May 22-26, Palm Springs, CA, pp. 2796-2804

Hilker T, Nicholas C C, Wulder M A, Black T A & Guy R D (2008). The use of remote sensing in light use efficiency based models of gross primary production: A review of current status and future requirements. *Science of the Total Environment* **404**: 411-423

Holben B N (1986). Characteristics of maximum-value composite images from temporal AVHRR data. *International Journal of Remote Sensing* **7**: 1417-1434

Huete A, Didan K, Miura T & Rodriguez E (2002). Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment* **83**: 195-213

Ketenoğlu O, Quezel P, Akman Y & Aydoğdu M (1983). New syntaxa on the gypsaceous formation in the Central Anatolia. *Ecologia Mediterranea* **9**(3-4): 211-221

Kurt L, Tuğ G N & Ketenoğlu O (2006). Synoptic view of the steppe vegetation of Central Anatolia (Turkey). *Asian Journal of Plant Sciences* **5**(4): 733-739

Le Roux H X, Gauthier A, Begue H & Sinoquet H (1997). Radiation absorption and use by humid savannah grassland: assessment using remote sensing and modelling. *Agricultural and Forest Meteorology* **85**: 117-132

Los S O, North P R J, Grey W M F & Barnsley M J (2005). A method to convert AVHRR normalized difference vegetation index time series to a standard viewing and illumination geometry. *Remote Sensing of Environment* **99**(4): 400-411

Mermer A, Ünal E, Aydoğdu M, Urla Ö, Yıldız H & Torunlar H (2012). Determining rangeland areas by satellite images. *TABAD-Research Journal of Agricultural Sciences* **5**: 107-110

Monteith J L (1972). Solar radiation and productivity in tropical ecosystems. *Journal of Applied Ecology* **9**: 747-766

Morgan G M & Henrion M (1990). Uncertainty: A Guide to Dealing with Uncertainty in Quantitative Risk and Policy Analysis. Cambridge University Press, NY

Mukhala E & Hoefsloot P (2004). AgrometShell Manual. Agrometeorology Group, Environment and Natural Resources Service, Food and Agricultural Organization Rome, Italy

Prince S D (1991). Satellite remote sensing of primary production: comparison of results for Sahelian grasslands 1981-1988. *International Journal of Remote Sensing* **12**: 1301-1311

Seaquist J W, Olsson L & Ardö J (2003). A remote sensing based primary production model for grassland biomes. *Ecological Modelling* **169**: 131-155

Shevenell L & Hoffman F O (1993). Necessity of uncertainty analyses in risk assessment. *Journal of Hazardous Materials* **35**: 369-385

Sims P L & Singh J S (1978). The structure and function of ten western North America grasslands. II. Intra-seasonal dynamics in primary producer compartments. *Journal of Ecology* **66**: 251-285

Tucker C J, Vanpraet C, Boerwinkel E & Gaston A (1983). Satellite remote sensing of total dry matter production in the Senegalese Sahel. *Remote Sensing of Environment* **13**: 461-474

TUIK (2009). Livestock statistics. Retrieved in July, 18 2009 from http://www.tuik.gov.tr

Uzun B & Demir V (2012). Fotosentetik aktif radyasyon (FAR) ölçümlerinde LED ve foto diyotların hassas tarım açısından kullanılabilirliği üzerine bir araştırma. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **18** (3): 214-225

Wight J R & Hanks R J (1981). A water-balance, climate model for range herbage production. *Journal of Range Management* **4**: 307-311

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

The Effect of Harvesting Time on Seed Oil Content and Fatty Acid Composition of Some Lemon and Mandarin Cultivars Grown in Turkey

Muharrem GÖLÜKCÜ[a], Ramazan TOKER[a], Haluk TOKGÖZ[a], Orçun ÇINAR[a]

[a]*Batı Akdeniz Agricultural Research Institute, Antalya, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Muharrem GÖLÜKCÜ, E-mail: muharrem98@yahoo.com, Tel: +90 (242) 321 67 97
Received: 04 December 2014, Received in Revised Form: 04 March 2015, Accepted: 14 April 2015

ABSTRACT

Citrus fruits usually processed into juice. The main residues are peel and seeds for citrus fruits after juice production. In order to evaluate the seeds for alternative usages; the oil content and fatty acid compositions of four mandarin (*Citrus reticulata*) and three lemon cultivars (*Citrus limon*) were determined with respect to their harvesting times. Oil content and fatty acid compositions of the samples were significantly ($P<0.05$) varied depends on cultivars and their harvesting times for each citrus species. Oil content ranged from 21.66 to 37.75% for these seeds. These results revealed that citrus seeds contain much more oil than many oil seeds. The citrus seeds oil combined from eight different fatty acids. The highest fatty acid was determined as linoleic acid (35.64-37.39%) for mandarin and oleic acid (32.99-36.39%) for lemon seed oil. These results revealed that citrus seeds could be valued as an edible oil source and other industrial area with respect to fatty acid composition.

Keywords: Citrus seed; Fatty acid; Cultivar; Harvesting time

Türkiye'de Yetiştirilen Bazı Limon ve Mandalina Çeşitlerinin Çekirdek Yağları ve Yağ Asidi Bileşimleri Üzerine Hasat Zamanının Etkisi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Muharrem GÖLÜKCÜ, E-posta: muharrem98@yahoo.com, Tel: +90 (242) 321 67 97
Geliş Tarihi: 04 Aralık 2014, Düzeltmelerin Gelişi: 04 Mart 2015, Kabul: 14 Nisan 2015

ÖZET

Turunçgiller endüstriyel olarak genellikle meyve suyuna işlenmektedir. Turunçgillerin meyve suyuna işlenmesi sonucunda ortaya atık olarak önemli miktarda kabuk ve çekirdek çıkmaktadır. Bu çalışmanın amacı bazı turunçgil çekirdeklerinin alternatif değerlendirme yöntemlerine yol gösterecek veriler ortaya koymaktır. Bu amaçla dört mandalina (*Citrus reticulata*) ve üç limon (*Citrus limon*) çeşidine ait çekirdeklerde hasat zamanına bağlı yağ miktarı ve yağ asitleri bileşenlerinin değişimi incelenmiştir. Örneklerin yağ miktarları ve yağ asitleri bileşimleri her bir tür için çeşit ve hasat

zamanına göre önemli oranda ($P<0.05$) farklılık göstermiştir. Analiz edilen örneklerin yağ içerikleri % 21.66-37.75 arasında dağılım göstermektedir. Bu veriler turunçgil çekirdeklerinin birçok yağlı tohuma göre daha zengin yağ içeriğine sahip olduğunu göstermektedir. Örneklere ait yağlarda sekiz farklı yağ asidinin varlığı tespit edilmiştir. Mandalina çekirdek yağları için oransal en yüksek yağ asidi linoleik asit (% 35.64-37.39) iken limonlar için oleik asit (% 32.99-36.39) olmuştur. Araştırma sonuçları turunçgil çekirdek yağlarının yağ asitleri bileşimi bakımından başta alternatif yemeklik yağ kaynağı olmak üzere farklı endüstriyel alanlarda değerlendirilebilecek önemli bir kaynak olduğunu göstermiştir.

Anahtar Kelimeler: Turunçgil çekirdeği; Yağ asidi; Çeşit; Hasat zamanı

1. Introduction

Citrus species are recognized as one of the major cultivated fruit crops. They are cultivated in tropical or subtropical climate regions of the world (Liu et al 2012). The main producers for citrus are Brazil, China, Japan, Mexico, Pakistan, USA and the Mediterranean countries. The citrus fruit production of the world was 131,283,333 tons in 2012. Turkey has an important place in the world citrus production by 3,556,407 tons in 2012. Lemon and mandarin production amounts of Turkey were 759,711 tons and 889,293 tons in 2012, respectively (FAO 2013).

Citrus fruits have great economic value because of their varied uses. Although many citrus fruits, such as orange, mandarin, and grapefruit could be eaten fresh, about one third of citrus fruit is utilized in food industry in the worldwide (Liu et al 2012). The residues of the juice industry are peels and seeds. These residues, represent about 50% of the raw processed fruit, are potential sources of valuable by-products (Matthaus & Özcan 2012). The peels could be processed into jam, marmalade, essential oil, pectin, food additive (Baker 1994, Kim et al 2004; Saidani et al 2004; Shahidi & Zhong 2005; Yoshikawa et al 2006; Avula et al 2007; Yalçin et al 2011). On the other hand, citrus seeds generally are not valued in food industry.

There are a lot of cultivars for each citrus species in the world. The composition of each citrus seeds could be significantly varied depends on their species, cultivar, environmental conditions and other cultural practices. The main chemical component is oil for citrus seeds (Gorinstein et al 2001; Moufida & Marzouk 2003; Mathhaus & Özcan 2012). Citrus fruits are not grown for their seed oils. Conventional vegetable oil sources are sunflower, soybean, peanut, palm, rapeseed, corn and olive oils (Flickinger & Matsuo 2003). These sources are unable to meet demands of domestic and industrial sectors. Therefore, the need exists to investigate new alternative oil sources to supplement the supplies from natural sources. Citrus fruit seeds could be a good alternative to conventional edible oil sources (Kulkarni et al 2012).

Citrus seeds contain 20.0-78.9% oil according to the species, cultivar, and growing area (Habib et al 1986; Ajewole & Adeyeye 1993; Saidani et al 2004; Anwar et al 2008; Waheed et al 2009; Habila et al 2012). Fatty acid composition is the main quality parameter for edible oil. Mathhaus & Özcan (2012) were stated that oleic, linoleic and palmitic acids in 17 different citrus seeds oils ranged between 12.8-70.1%, 19.5-58.8%, 5.1-28.3%, respectively. Saidani et al (2004) were also found differences in fatty acid composition in seed oils of five varieties of Tunisian citrus fruits. Palmitic (21.40-39.40%), oleic (14.90-36.60%) and linoleic acids (23.80-40.30%) were detected as major fatty acids in all samples. To the best of our knowledge, there are no reports on the oil content and fatty acid composition of lemon and mandarin cultivars studied in this research. Additionally, there are comparative studies on the effects of harvesting time on oil content and composition of citrus seeds oil. Generally, harvesting time of these citrus fruits could be changed according to the marked demand. And, harvesting time could lead to significant changes in chemical composition of these fruits and their seeds.

The aim of this study was to compare oil content and fatty acid composition of seeds obtained from four mandarins and three lemons cultivars during their harvesting time.

2. Material and Methods

2.1. Material

Four mandarin (Batem Göral, Yerli Apireno, Nova and Klemantin Fina) and three lemon cultivars (Batem Pınarı, Interdonato, Meyer) were selected for this experiment. For each replication, 20 fruits, from all mandarin and lemon cultivars, were collected by hand from citrus trees growing in Bati Akdeniz Agricultural Research Institute citrus orchard in Antalya. The same amount of samples were collected for three harvesting times. Samples harvesting time were given in Table 1.

2.2. Methods

In order to determine some physical properties of the samples; fruit weight, seed content, seed weight were measured. The fruits were firstly peeled by hand. After that their seeds were removed from the fruit pulps. The seeds were washed and removed excess water from the seeds. After that the moisture content of the seeds were determined by drying them up to reach constant weight in an oven at 72 °C. And, the dried seeds were milled with a laboratory type miller (Retch GM200) than oil of seeds was extracted successively with petroleum ether using a soxhlet extractor (Gerhardt, Soxtherm 2000) for 3 h. Oil content was calculated as % on dry matter bases.

The fatty acid composition of the samples was analyzed by gas chromatography (Agilent 5975C) coupled to flame ionization detector and mass spectrometry (Agilent 5975C) (GC-MS-FID). Firstly, fatty acid methy esters (FAMEs) were prepared (Garces & Mancha 1993) and then injected in to GC-MS/FID. Separations were performed using an HP innowax capillary column (60 m, 0.25-mm i.d., 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 0.8 mL min^{-1}. Injector and detector temperatures were 250 and 260 °C, respectively. The temperature programming for the column was applied as follow; started from 150 °C and raised to 200 °C with an increment of 10 °C/minute, hold at 200 °C for 5 minute, then increased to 250 °C with 5 °C/minute increments and hold 250 °C at 10 minute (totally 30 minutes). Sample of 1 µL was injected by auto sampler with a split mode (1:50). The content (percentage by weight) of fatty acids was calculated from their corresponding integration data. MS spectra were monitored between 35-450 amu and the ionization mode used was electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas. FAMEs were identified by comparison of their retention times with those of the reference standards. FAMEs were further identified by using WILEY and NIST libraries of the GC-MS system.

2.3. Statistical analysis

Statistical analysis were performed using SAS program to evaluate the significance of differences in the analyzed quality parameters between cultivars and their harvesting times at the level of $P<0.05$.

Table 1- Citrus cultivars and their harvesting times

Çizelge 1- Turunçgil çeşitleri ve hasat zamanları

	Cultivars	*1st harvesting*	*2nd harvesting*	*3rd harvesting*
Mandarin	Batem Göral	25 October 2013	10 November 2013	25 November 2013
	Klemantin Fina	25 October 2013	10 November 2013	25 November 2013
	Nova	5 November 2013	20 November 2013	5 December 2013
	Yerli Apireno	5 January 2014	20 January 2014	4 February 2014
Lemon	Batem Pınarı	15 September 2013	30 September 2013	15 October 2013
	Interdonato	15 September 2013	30 September 2013	15 October 2013
	Meyer	10 October 2013	25 October 2013	10 November 2013

The experiment was conducted in randomized design with three replications (the fruit was picked from five different trees for each replication). Data were expressed as means ± standard error (SE).

3. Results and Discussion

In order to evaluate samples, some basic parameters of the samples were analyzed. These physical and chemical properties of each lemon and mandarin fruits, depends on cultivars and harvesting time, are presented in Table 2 and Table 3.

Seed content was significantly affected by cultivars and their harvesting time for mandarin. Not only amount of seeds but also total seeds' weight per fruit were found as the highest level for Yerli Apireno, which is well known characteristic for this cultivar, followed by Klemantin Fina, Nova and Batem Göral in descending order. The dry matter content of these seeds ranged between 38.57% (Nova) and 49.06%

Table 2- Some physical and chemical properties of mandarin depending on cultivars and harvesting time (mean±SE)

Çizelge 2- Mandalina çeşitlerinin hasat zamanlarına göre bazı fiziksel ve kimyasal özellikleri (ortalama±SH)

*Cultivar**	*Harvesting time*	*Fruit weight (g)*	*Seed weight (g fruit^{-1})*	*Seed number fruit^{-1}*	*Seed dry matter (%)*	*Seed oil content (%, DM)*
Batem Göral	1st	85.99^{e}±1.46	2.84^{a}±0.10	11.30^{d}±0.90	46.88^{b}±0.08	35.79b^{c}±1.29
	2nd	87.21^{e}±1.47	2.07^{c}±0.12	12.00^{cd}±0.80	49.50^{ab}±0.11	37.79^{ab}±0.27
	3rd	88.43^{e}±0.58	1.29^{d}±0.01	11.80^{cd}±1.00	50.81^{a}±0.96	39.67^{a}±2.03
Klemantin Fina	1st	94.81^{cd}±1.12	2.41^{b}±0.07	15.20^{cb}±0.40	47.15^{b}±0.22	31.22^{d}±1.20
	2nd	95.96^{c}±2.69	2.43^{b}±0.11	15.20^{cb}±0.80	47.26^{b}±0.99	35.92^{bc}±0.54
	3rd	104.21^{b}±1.70	2.69^{a}±0.07	15.20^{cb}±1.00	49.40^{ab}±0.17	36.37^{abc}±1.22
Nova	1st	125.70^{a}±0.67	2.11^{c}±0.05	18.50^{ab}±1.30	37.45^{c}±0.23	32.17^{d}±0.74
	2nd	122.71^{a}±3.75	2.10^{c}±0.05	17.40^{ab}±0.60	39.51^{c}±0.64	30.67^{d}±0.66
	3rd	126.93^{a}±2.57	2.11^{c}±0.03	18.30^{ab}±1.10	38.75^{c}±0.67	33.35^{cd}±0.04
Yerli Apireno	1st	90.97^{cde}±1.55	2.87^{a}±0.11	20.60^{a}±1.00	39.87^{c}±0.40	30.15^{d}±1.67
	2nd	95.10^{cd}±1.81	2.89^{a} ±0.02	20.60^{a}±0.80	40.19^{c}±1.81	30.26^{d}±0.13
	3rd	92.17^{cde}±3.02	2.75^{a}±0.10	20.10^{a}±2.10	39.69^{c}±1.25	29.89^{d}±1.17

*, mean in the same column with different letters are significantly different (P<0.05); DM, dry matter

Table 3- Some physical and chemical properties of lemon depending on cultivars harvesting time (mean±SE)

Çizelge 3- Limon çeşitlerinin hasat zamanlarına göre bazı fiziksel ve kimyasal özellikleri (ortalama±SH)

*Cultivar**	*Harvesting time*	*Fruit weight (g)*	*Seed weight (g fruit^{-1})*	*Seed number fruit^{-1}*	*Seed dry matter (%)*	*Seed oil content (%, DM)*
Batem Pınarı	1st	139.61^{a}±4.88	1.77^{c}±0.00	10.00^{b}±1.00	44.91^{ef}±0.51	22.00^{b}±1.95
	2nd	142.70^{a} ±2.22	1.70^{c}±0.06	10.20^{b}±0.40	46.34^{e}±0.81	21.73^{b}±1.18
	3rd	134.24^{ab}±3.28	1.71^{c}±0.03	8.90^{b}±0.30	48.47^{d}±0.59	21.26^{b}±0.78
Interdonato	1st	96.57^{d}±1.78	1.28^{d}±0.08	9.20^{b}±0.20	43.16^{f}±0.15	22.76^{b}±0.70
	2nd	100.53^{d}±3.26	1.39^{d}±0.10	9.15^{b} ±0.05	45.57^{e}±1.13	21.38^{b}±1.10
	3rd	99.95^{d}±0.68	1.29^{d}±0.05	9.40^{b}±0.40	49.05^{cd}±0.18	23.65^{b}±1.11
Meyer	1st	117.25^{c}±5.65	2.95^{a}±0.03	20.70^{a}±1.10	50.69^{bc}±0.30	36.26^{a}±0.85
	2nd	122.40^{bc}±4.04	2.72^{b}±0.11	19.00^{a}±1.20	51.69^{b}±0.46	37.62^{a}±0.88
	3rd	123.45^{bc}±5.99	2.62^{b}±0.06	20.40^{a}±1.20	53.66^{a}±0.14	37.17^{a}±0.46

*, mean in the same column with different letters are significantly different (P<0.05); DM, dry matter

(Batem Göral). Average total dry matter content of Batem Göral and Klemantin Fina were higher than the other mandarin cultivars. Additionally, small differences in dry matter content were observed between each harvesting time for the same cultivars. Dry matter content of the seeds slightly increased from first harvesting to third harvesting time in Batem Göral and Klemantin Fina. On the other hand, differences in dry matter content of Yerli Apireno and Nova were not statistically important between their harvesting times. Total oil contents were varied with respect to cultivars and their harvesting time on dry matter base. The highest oil content was determined in Batem Göral (37.75%) and followed by Klemantin Fina (34.50%), Nova (32.06%) and Yerli Apireno (30.10%) in descending order. Total oil content of the seeds increased steadily from first harvesting to third harvesting time for Batem Göral and Klemantin Fina. Total oil contents of the other two cultivars were similar between their harvesting times. As a consequence, the oil content of mandarin seeds (30.10-37.75%) was higher than cotton (15-24%) and soybean (17-21%) (Pritchard 1991). Anwar et al (2008) were analyzed oil content and fatty acid composition of four different citrus species. One of them was mandarin (Kinnow cultivar) and it's oil content was determined as 31.15%. Oil contents of Nova and Yerli Apireno seeds were similar with this literature finding.

Both amount of seeds and total seeds weight per fruit of Meyer cultivar were extremely higher than other two lemon cultivars. The dry matter content of the lemon seeds ranged from 43.16% (Interdonato) to 53.66% (Meyer). The total oil content of the seeds changed significantly with respect to cultivars and their harvesting times. The average highest oil content was determined in Meyer (37.02%) and followed by Interdonato (22.59%), Batem Pınarı (21.66%) in descending order. Total dry matter content of the lemon seeds steadily increased from first harvesting to third harvesting time in all lemon cultivars. Oil content of the seeds was not affected by harvesting time for each cultivar. Reda et al (2005) determined the oil content of lemon as 38.30%. This value showed similarity with Meyer seed oil content in this study. On the other hand, this value was higher than Interdonato and Batem Pınarı samples. This could be sourced by mainly cultivar differences. Growing condition could also affect their composition.

The fatty acid composition of the samples was depicted in Table 4, 5 for mandarin and Table 6,

Table 4- Unsaturated fatty acid composition of mandarin seed oils depending on cultivars and harvesting time (%, mean±SE)

Çizelge 4- Mandalina çekirdek yağlarının hasat zamanlarına göre doymamış yağ asitleri bileşimi (%, ortalama±SH)

*Cultivar**	*Harvesting time*	*Palmitoleic*	*Oleic*	*Linoleic*	*Linolenic*	*Gadoleic*
Batem Göral	1st	0.55^{b}±0.000	25.56^{h}±0.040	35.65^{d}±0.010	3.87^{f}±0.005	0.17^{b}±0.025
	2nd	0.49^{f}±0.000	25.58^{h}±0.010	35.99^{d}±0.035	4.35^{b}±0.005	0.24^{ab}±0.050
	3rd	0.45^{h}±0.000	27.73^{e}±0.080	35.28^{e}±0.165	3.88^{f}±0.005	0.28^{a}±0.050
Klemantin Fina	1st	0.43^{i}±0.000	27.76^{d}±0.045	35.10^{e}±0.095	3.90^{e}±0.010	0.22^{ab}±0.015
	2nd	0.48^{g}±0.000	26.39^{f}±0.050	37.12^{c}±0.270	4.22^{d}±0.005	0.17^{b}±0.030
	3rd	0.54^{c}±0.000	25.93^{g}±0.025	36.87^{c}±0.030	4.23^{d}±0.010	0.22^{ab}±0.015
Nova	1st	0.58^{a}±0.010	23.18^{j}±0.045	37.06^{c}±0.160	4.29^{c}±0.010	0.22^{ab}±0.050
	2nd	0.52^{d}±0.050	24.27^{i}±0.005	37.12^{c}±0.025	4.36^{b}±0.005	0.17^{b}±0.015
	3rd	0.50^{e}±0.000	24.17^{i}±0.020	37.98^{a}±0.000	4.55^{a}±0.010	0.23^{ab} ±0.015
Yerli Apireno	1st	0.31^{j}±0.000	28.99^{b}±0.010	34.95^{e}±0.050	3.06^{h}±0.000	0.30^{a}±0.015
	2nd	0.30^{k}±0.000	29.18^{a}±0.010	35.64^{d}±0.020	2.97^{i}±0.005	0.23^{ab}±0.025
	3rd	0.29^{l}±0.000	27.97^{c}±0.015	37.61^{b}±0.020	3.26^{g}±0.015	0.24^{ab} ±0.020

*, mean in the same column with different letters are significantly different (P<0.05)

Table 5- Saturated fatty acid composition of mandarin seed oils depending on cultivars and harvesting time (%, mean±SE)

Çizelge 5- Mandalina çekirdek yağlarının hasat zamanlarına göre doymuş yağ asitleri bileşimi (%, ortalama±SH)

*Cultivar**	*Harvesting time*	*Palmitic*	*Margaric*	*Stearic*	*Arachidic*	*Behenic*
Batem Göral	1st	26.51^{a}±0.005	0.40^{c}±0.005	6.52^{c}±0.020	0.61^{e}±0.015	0.19^{fg}±0.005
	2nd	25.81^{b}±0.065	0.38^{cd}±0.010	6.18^{f}±0.000	0.64^{d}±0.000	0.36^{b}±0.005
	3rd	24.61^{d}±0.020	0.36^{e}±0.000	6.76^{c}±0.005	0.72^{c}±0.005	0.45^{a}±0.000
Klemantin Fina	1st	24.94^{c}d±0.030	0.35^{e}±0.000	6.58^{d}±0.010	0.56^{fg}±0.000	0.18^{g}±0.015
	2nd	24.00^{e}±0.370	0.37^{de}±0.005	6.51^{e}±0.010	0.55^{gh}±0.005	0.21^{ef}±0.005
	3rd	25.28^{c}±0.000	0.35^{e}±0.000	5.88^{g}±0.005	0.53^{h}±0.005	0.19^{fg}±0.010
Nova	1st	26.82^{a}±0.270	0.47^{a}±0.000	6.58^{d}±0.005	0.62^{de}±0.010	0.19^{fg}±0.000
	2nd	26.54^{a}±0.030	0.45^{b}±0.005	5.78^{h}±0.000	0.58^{f}±0.005	0.25^{d}±0.005
	3rd	25.78^{b}±0.005	0.44^{b}±0.000	5.59^{i}±0.005	0.56^{fg}±0.005	0.22^{de}±0.010
Yerli Apireno	1st	23.35^{f}±0.015	0.48^{a}±0.005	7.59^{a}±0.015	0.70^{c}±0.010	0.29^{c}±0.010
	2nd	22.96^{f}±0.030	0.46^{ab}±0.005	7.21^{b}±0.005	0.77^{b}±0.015	0.31^{c}±0.015
	3rd	21.73^{g}±0.050	0.46^{ab}±0.015	7.23^{b}±0.000	0.86^{a}±0.005	0.37^{b}±0.000

*, mean in the same column with different letters are significantly different (P<0.05)

7 for lemon seeds oils. Ten different fatty acids were examined in all samples. Statistical analysis of the data showed fatty acid composition varied significantly (P<0.05) with in the cultivars and their harvesting time of the samples.

The main fatty acids determined as linoleic and oleic acids as unsaturated and palmitic acid as saturated fatty acid in the mandarin seed oils. Linoleic acid, is an essential fatty acid, was the most abundant fatty acids in all mandarin cultivars and ranged between 34.95 (Yerli Apireno)-37.98% (Nova). Significant differences determined between cultivars and their harvesting time. Small increasing was observed in linoleic acid content of the samples from first harvesting to third harvesting time in each mandarin cultivar. But these differences were small according to differences between mandarin and lemon species. The relatively few researches were reported for fatty acids composition of citrus seeds oil to date. El-Adawy et al (1999) analyzed the fatty acid composition of the mandarin seed oil. They detected eight different fatty acids, palmitic

Table 6- Unsaturated fatty acid composition of lemon seed oils depending on cultivars and harvesting time (%, mean±SE)

Çizelge 6- Limon çekirdek yağlarının hasat zamanlarına göre doymamış yağ asitleri bileşimi (%, ortalama±SH)

*Cultivar**	*Harvesting time*	*Palmitoleic*	*Oleic*	*Linoleic*	*Linolenic*	*Gadoleic*
Batem Pınarı	1st	0.33^{d}±0.000	36.93^{a}±0.000	28.67^{g}±0.000	4.64^{d}±0.000	0.25^{cd}±0.000
	2nd	0.52^{a}±0.000	36.16^{b}±0.005	28.86^{f}±0.030	3.99^{g}±0.005	0.23^{cd}±0.050
	3rd	0.52^{a}±0.025	36.10^{b}±0.055	28.88^{f}±0.045	4.04^{g}±0.085	0.19^{d}±0.020
Interdonato	1st	0.33^{d}±0.000	36.82^{a}±0.095	28.54^{h}±0.005	4.63^{d}±0.020	0.25^{cd}±0.000
	2nd	0.33^{d}±0.005	35.09^{c}±0.025	29.19^{e}±0.000	4.96^{b}±0.005	0.72^{a}±0.005
	3rd	0.33^{d}±0.005	34.12^{d}±0.125	31.26^{a}±0.085	4.79^{c}±0.010	0.38^{b}±0.000
Meyer	1st	0.44^{b}±0.000	33.92^{e}±0.020	29.34^{d}±0.015	4.40^{e}±0.010	0.23^{cd}±0.045
	2nd	0.41^{c}±0.005	32.18^{g}±0.015	30.97^{b}±0.020	5.13^{a}±0.015	0.20^{cd}±0.020
	3rd	0.43^{bc}±0.000	32.86^{f}±0.050	29.89^{c}±0.010	4.24^{f}±0.005	0.29^{c}±0.015

*, mean in the same column with different letters are significantly different (P<0.05)

(28.12%), oleic (24.89%) and linoleic (38.26%) acids were major fatty acids in the seed oils. Anwar et al (2008) studied on the fatty acid composition of mandarin seed oil. Linoleic acid (39.55%) was determined as the highest fatty acid. Our results were in agreement with the results of these studies. There were some researches on harvesting time effects on fatty acid composition of some plants. Ozdemir & Topuz (2004) stated that oleic acid contents of two avocado cultivars' oil were increased from 47.2-59.3% to 59.5-73.0% at three different harvesting times. Fatty acid composition of almond cultivars was affected significantly from harvesting time (Piscopo et al 2010). Sakouhi et al (2011) and Dag et al (2011) found that fatty acid composition was affected significantly from harvesting time. Our results also showed similarities with these reports.

Another higher unsaturated fatty acid was oleic in mandarin seed oil. There were significant differences in oleic acid content of the samples depend on cultivars and their harvesting time. The average highest oleic acid content was determined in Yerli Apireno (28.71%), and followed by Klemantin Fina (26.69%), Batem Göral (26.12%) and Nova (23.87%) cultivars in descending order. Some changes were observed according to harvesting time of the samples. While oleic acid content of the Batem Göral sample increased from first harvesting to third harvesting time, this fatty acid ratio decreased from first harvesting to last harvesting time in Klemantin Fina seed oil. Another quantitatively high unsaturated fatty acid was linolenic acid, and it ranged from 2.97% (Yerli Apireno) to 4.55% (Nova). Palmitoleic and gadoleic acids also were detected in the seeds oils, but totally lower than 1% in these samples.

The main saturated fatty acid was palmitic acid in mandarin seed oils and ranged between 21.73% and 26.82%. This fatty acid ratio was significantly different between cultivars and their harvesting time. The highest palmitic acid ratio was determined in Nova and followed by Batem Göral, Klemantin Fina, Yerli Apireno cultivars, in descending order. And, palmitic acid ratios of these cultivars were decreased from first harvesting to third harvesting time except for Klemantin Fina. The other quantitatively higher saturated fatty acid was stearic acid, ranged from 5.59 (Nova) and 7.59% (Yerli Apireno). There were small differences in stearic acid content between harvesting time of each cultivar. This fatty acid ratio decreased from first harvesting to third harvesting time except for Batem Göral cultivar. Margaric, arachidic and behenic acids contents were lower than 1% in these samples. Margaric and behenic acids were not detected in citrus seeds oil in previous studies (El-Adawy et al 1999; Saidani et al 2004; Anwar et al 2008;

Table 7- Saturated fatty acid composition of lemon seed oils depending on cultivars and harvesting time (%, mean±SE)

Çizelge 7- Limon çekirdek yağlarının hasat zamanlarına göre doymuş yağ asitleri bileşimi (%, ortalama±SH)

*Cultivar**	*Harvesting time*	*Palmitic*	*Margaric*	*Stearic*	*Arachidic*	*Behenic*
	1st	23.01^{e}±0.000	0.53^{b}±0.000	5.18^{e}±0.000	0.46^{e}±0.000	trace
Batem Pınarı	2nd	24.36^{c}±0.010	0.52^{b}±0.005	4.94^{g}±0.010	0.44^{e}±0.005	trace
	3rd	24.26^{c}±0.075	0.54^{b}±0.015	5.05^{f}±0.070	0.45^{e}±0.020	trace
	1st	22.95^{e}±0.060	0.54^{b}±0.005	5.50^{d}±0.000	0.46^{e}±0.005	trace
Interdonato	2nd	22.90^{e}±0.050	0.49^{c}±0.005	5.72^{c}±0.010	0.63^{b}±0.005	trace
	3rd	22.91^{e}±0.020	0.56^{a}±0.000	5.18^{e}±0.005	0.49^{d}±0.000	trace
	1st	24.65^{b}±0.035	0.32^{d}±0.005	5.99^{a}±0.010	0.58^{c}±0.005	0.16^{b}±0.025
Meyer	2nd	24.06^{d}±0.020	0.32^{d}±0.000	6.00^{a}±0.000	0.59^{c}±0.005	0.16^{b}±0.000
	3rd	25.08^{a}±0.035	0.33^{d}±0.000	5.85^{b}±0.000	0.66^{a}±0.000	0.39^{a}±0.005

*, mean in the same column with different letters are significantly different (P<0.05)

Matthaus & Özcan 2012). These results could be sourced from cultivar differences.

The main fatty acids for lemon cultivar seed oils in the present study were determined oleic and linoleic acids as unsaturated and palmitic acid as saturated fatty acid. While linoleic acid was the highest fatty acid in mandarin cultivars, oleic acid was the main fatty acid in lemon seed oils and ranged from 32.18% to 36.93%. This fatty acid ratio was affected significantly from cultivars and their harvesting time. The average highest oleic acid ratio was determined as 36.39% in Batem Pınarı cultivars, followed by Interdonato (35.34%) and Meyer (32.99%), in descending order. Small decreasing was observed in oleic acid content of the samples from first harvesting to third harvesting time in lemon cultivars except for Meyer. Matthaus & Özcan (2012) reported oleic acid content of lemon seed oils to be 38.5-63.6%. The oleic acid ratio in the present study was lower than these results. On the other hand, the present results for lemon seed oils were quite comparable with those of reported (36.60%) by Saidani et al (2004).

Linoleic acid content was also high in the lemon seed oils. The average highest linoleic acid (30.07%) was determined in Meyer cultivar seed oil and followed by Interdonato and Batem Pınarı cultivars in descending order. Linoleic acid content of the samples showed significant differences between harvesting time of each cultivar. This fatty acid ratio increased from first harvesting to third harvesting time in Batem Pınarı and Interdonato cultivars. On the other hand, the highest linoleic acid ratio was determined in second harvest sample for Meyer. The linoleic acid content of the lemon cultivars analyzed in the present study agreed well with those reported (31.40%) by Saidani et al (2004). However, our results differed from the findings (26.8-44.5%) of Matthaus & Özcan (2012). Besides the cultivars, differences in linoleic acid content could be resulted from ecology, cultural practices, harvesting time etc. Another determined fatty acid in the lemon seeds oils was linolenic acid. This fatty acid ratio was significantly different between samples and ranged from 3.99% to 5.13%. The average highest linoleic acid ratio was determined as 4.79% for Interdonato and this one was followed by Meyer (4.59%) and Batem Pınarı (4.22%). Some differences were observed according to harvesting time of each cultivar. While the highest linolenic acid ratios were determined in second harvest samples for Interdonato and Meyer cultivars, the highest value was determined in first harvest sample for Batem Pınarı. Palmitoleic and gadoleic acids were lower than 1% in lemon seeds oils.

The main saturated fatty acid was also palmitic acid in lemon cultivars seed oil and ranged between 22.90% (Interdonato) and 25.08% (Meyer). Palmitic acid ratios showed significant differences between samples. The highest palmitic acid for cultivars was determined in Meyer (24.59%), followed by Batem Pınarı (23.88%) and Interdonato (22.92%), in descending order. This saturated fatty acid ratio was significantly different between their harvesting times except for Interdonato. The lemon seeds oil had important level stearic acid and ranged from 4.94% to 6.00% in lemon cultivars. This fatty acid ratio was significantly different between cultivars and their harvesting times. The highest stearic acid ratio was determined in first and second harvest samples of Meyer cultivar. And the lowest value was observed in second harvest sample of Batem Pınarı. Margaric, arachidic and behenic acids contents were lower than 1% in these samples. Qualitative differences among the studied lemon cultivars were only found for behenic acid that was not detected in two of the cultivars. Behenic acid was only detected in Meyer cultivars. This fatty acid content was significantly increased from second harvesting to third harvesting time in this cultivar.

4. Conclusions

Citrus seeds have considerable amounts of oil. As a result of general evaluation, the highest oil content could be obtained from Klemantin Fina cultivars. Oil content of the Klemantin Fina was calculated as 4.15 g for 1 kg fresh fruit. This cultivar was followed by Batem Göral, Yerli Apireno and Nova cultivars in descending order. Total oil contents of these cultivars showed differences between each

harvesting time of these cultivars based on fresh fruit weight. And, the highest oil content was determined as 4.83 g for 1 kg fresh fruit on last harvesting time samples of Klemantin Fina. For lemon cultivars, the average highest oil content was calculated as 5.32 g for 1 kg fresh fruit. Fatty acid composition of the mandarin and lemon seeds showed significantly differences in terms of the cultivars and their harvesting time, and the main fatty acid were determined to be oleic, linoleic and palmitic acid for all samples. Fatty acids have importance in human nutrition and quality of edible oils. The seeds, remaining after processing the fruits into juice, could be subjected to oil extraction. This is important for making additional profit from these plants. Furthermore, the data could be useful for biochemical characterization of different mandarin and lemon cultivars.

References

Ajewole K & Adeyeye A (1993). Characterization of Nigerian citrus seed oils. *Food Chemistry* **47**: 77-78

Anwar F, Naseer R, Bhanger M I, Ashraf S, Talpur F N & Aladedunye F A (2008). Physicochemical characteristics of citrus seeds and seed oils from Pakistan. *Journal of American Oil Chemists' Society* **85**: 321-330

Avula B, Upparapalli S K, Navarrete A & Khan I A (2007). Simultaneous analysis of adrenergic amines and flavonoids in citrus peel jams and fruit juices by liquid chromatography: Part 2. *Journal of AOAC International* **90**: 633-640

Baker R A (1994). Potential dietary benefits of citrus pectin and fiber. *Food Technology* **48**: 133-137

Dag A, Kerem Z, Yogev N, Zipori I, Lavee S & Ben-David E (2011). Influence of time of harvest and maturity index on olive oil yield and quality. *Scientia Horticulturae* **127**: 358-366

El-Adawy T A, Rahman E H, El-Bedawy A A & Gafar A M (1999). Properties of some citrus seeds. Part 3. Evaluation as a new source of protein and oil. *Nahrung* **43**: 385-391

FAO (2013). Crops production database. Food and Agricultural Organisation of United Nations, Rome (http://faostat.fao.org/)

Flickinger B D & Matsuo N (2003). Nutritional characteristics of DAG oil. *Lipids* **38**: 129-132

Garces R & Mancha M (1993). One step lipid extraction and fatty acids methyl esters preparation from tree plant tissues. *Analytical Biochemistry* **211**: 139-143

Gorinstein S, Martin-Belloso O, Park Y S, Haruenkit R, Lojek L, Ciz M, Caspi A, Libman I & Trakhtenberg S (2001). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry* **74**: 309-315

Habib M A, Hammam M A, Sakr A A & Ashoush Y A (1986). Chemical evaluation of Egyptian citrus seeds as potential sources of vegetable oils. *Journal of American Oil Chemists' Society* **63**: 1192-1196

Habila N, Inuwa H M, Aimola I A, Agbaji A S, Ladan Z, Shangodare R, Williams I S, Odjobo O B & Ogabiela E (2012). Variation of fatty acids and vitamin E composition in seed oils of some plant species. *Journal of Plant Studies* **1**: 55-60

Kim W C, Lee D Y, Lee C H & Kim C W (2004). Optimization of narirutin extraction during washing step of the pectin production from citrus peels. *Journal of Food Engineering* **63**: 191-197

Kulkarni A S, More V I & Khotpal R R (2012). Composition and lipid classes of orange, tomato and pumpkin seed oils of Vidarbha region of Maharashtra. *Journal of Chemical and Pharmaceutical Research* **4**: 751-753

Liu Y, Heying E & TAnumihardjo S A (2012). History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety* **11**: 530-545

Matthaus B & Ozcan M M (2012). Chemical evaluation of citrus seeds, an agro-industrial waste, as a new potential source of vegetable oils. *Grasas Y Aceites* **63**: 313-320

Moufida S & Marzouk B (2003). Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. *Phytochemistry* **62**: 1283-1289

Ozdemir F & Topuz A (2004). Changes in dry matter, oil content and fatty acid composition of avocado during harvesting time and post-harvesting ripening period. *Food Chemistry* **86**: 79-83

Piscopo A, Romeo F V, Petrovicova B & Poiana M (2010). Effect of the harvest time on kernel quality of several almond varieties (*Prunus dulcis* (Mill.) D.A. Webb). *Scientia Horticulturae* **125**: 41-46

Pritchard J L R (1991). Analysis and properties of oilseeds. In: Analysis of Oilseeds, Fats and Fatty Foods (J.B. Rossell and J.L.R. Pritchard, eds), pp. 39-102. Elsevier Applied Sciences, New York

Reda S Y, Sauer E L, Batista A E C, Barana A C, Schnitzel E & Carneiro P I B (2005). Characterization of rangpur lime (*Citrus limonia* Osbeck) and "Sicilian" lemon (*Citrus limon*) seed oils, an agro-industrial waste. *Ciencia e Technologia de Alimentos* **25**: 672-676

Saidani M, Dhifi W & Marzouk B (2004). Lipid evaluation of some Tunisian citrus seeds. *Journal of Food Lipids* **11**: 242-250

Sakouhi F, Herchi W, Sebei K, Absalon C, Kallel H & Boukhchina S (2011). Accumulation of total lipids, fatty acids and triacylglycerols in developing fruits of *Olea europaea* L. *Scientia Horticulturae* **132**: 7-11

Shahidi F & Zhong Y (2005). Citrus oils and essences. In: Bailey's Industrial Oil and Fat Products, Sixth Edition (F. Shahidi, Ed), pp. 49-66. John Wiley & Sons, Inc

Waheed A, Mahmud S, Saleem M & Ahmad T (2009). Fatty acid composition of neutral lipid: Classes of citrus seed oil. *Journal of Saudi Chemical Society* **13**: 269-272

Yalçin H, Karaman S & Öztürk İ (2011). Evaluation of antioxidant efficiency of potato and orange peels and apple pomace extracts in sunflower oil. *Italian Journal of Food Science* **23**: 55-61

Yoshikawa H, Ogawa A, Fukuhara K & Kondo S (2006). Antioxidant activity of tropical fruit jam and marmalade processed with different combinations of peel and flesh in citrus fruit. *Journal of Food and Agriculture Environment* **4**: 78-84

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Cadmium Toxicity and its Effects on Growth and Metal Nutrient Ion Accumulation in *Solanaceae* Plants

Yakup ÇIKILI[a], Halil SAMET[b], Sevda DURSUN[a]

[a]*Düzce University, Çilimli Vocational School, Department of Crop and Animal Production, 81750, Düzce, TURKEY*

[b]*Kocaeli University, Vocational School of Food and Agriculture, Department of Crop and Animal Production, 41285, Kocaeli, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Yakup ÇIKILI, E-mail: yakupcikili@gmail.com, Tel: +90 (380) 681 73 12
Received: 14 July 2014, Received in Revised Form: 11 August 2015, Accepted: 20 August 2015

ABSTRACT

The effect of cadmium (Cd) toxicity was studied in four *Solanaceae* plants (tomato, *Solanum lycopersicum* L.; pepper, *Capsicum annuum* L.; eggplant, *Solanum melongena* L., and goldenberry, *Physalis peruviana* L.) grown in greenhouse under natural light conditions. The soil was treated with five levels of Cd (0, 2.5, 5, 10 and 20 mg kg^{-1}). Except for the tomato, the shoot and root dry biomass decreased with increasing Cd. Plant growth, bioaccumulation and translocation of Cd and accumulation of metal nutrient ions [potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn)] were investigated. On the basis of the percent reductions in the shoot dry biomass, the tomato was determined to be Cd-tolerant, and the other plants Cd-sensitive. The shoot and root Cd contents, uptakes, and total accumulation rate (TAR) were increased with increasing rate of Cd applied, except for the shoot Cd content and root uptake of the goldenberry. The bioconcentration factor (BCF) and the translocation factor (TF) of Cd diminished at all plants, with the exception of the TF for tomato. With respect to Cd translocation, plant species showed a ranking as follows: goldenberry<pepper<eggplant<tomato. The accumulation of all metal nutrient ions increased with Cd applications in the goldenberry shoots. While the accumulation of divalent metal nutrient ions, except for Zn and Cu, increased for the pepper and eggplant, the accumulation of K as monovalent metal nutrient ion decreased for only the pepper.

Keywords: Cadmium; *Solanaceae*; Accumulation; Bioconcentration; Translocation; Metal nutrient ions

Kadmiyum Toksisitesi ve Kadmiyumun *Solanaceae* Bitkilerinde Gelişim ve Metal Besin İyonu Akümülasyonuna Etkisi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Yakup ÇIKILI, E-posta: yakupcikili@gmail.com, Tel: +90 (380) 681 73 12
Geliş Tarihi: 14 Temmuz 2014, Düzeltmelerin Gelişi: 11 Ağustos 2015, Kabul: 20 Ağustos 2015

ÖZET

Serada ve doğal ışık koşulları altında yetiştirilen dört farklı *Solanaceae* familyası bitkisinde (domates, *Solanum lycopersicum* L.; biber, *Capsicum annuum* L.; patlıcan, *Solanum melongena* L. ve altınçilek, *Physalis peruviana* L.)

kadmiyum (Cd) toksisitesinin etkisi ve bitki gelişimi, Cd'un biyoakümülayonu, Cd'un translokasyonu ile metal besin iyonlarının [potasyum (K), kalsiyum (Ca), magnezyum (Mg), sodyum (Na), demir (Fe), mangan (Mn), bakır (Cu) ve çinko (Zn)] akümülasyonu araştırılmıştır. Bunun için, deneme toprağına beş farklı düzeyde Cd (0, 2.5, 5, 10 ve 20 mg kg^{-1}) uygulanmıştır. Domates hariç, diğer bitkilerin gövde ve kök kuru biyokütleleri artan Cd düzeylerine bağlı olarak azalmıştır. Gövde kuru biyokütlesindeki yüzde azalma temel alındığında; domatesin Cd'a toleranslı ve diğer bitkilerin ise Cd'a duyarlı olduğu tespit edilmiştir. Altınçilek bitkisinde gövde Cd içeriği ve kök Cd alımı hariç, bitkilerde gövde ve kökün Cd içerikleri, Cd alımları ve toplam akümülasyon oranları artan Cd düzeylerine bağlı olarak artmıştır. Domates için translokasyon faktörü hariç tüm bitkilerde, Cd'un biyokonsantrasyon faktörü ve translokasyon faktörü azalmıştır. Kadmiyumun translokasyonuna göre bitkiler; altınçilek<biber<patlıcan<domates olarak sıralanmıştır. Altınçileğin gövdesinde, tüm metal besin iyonlarının akümülasyonu Cd uygulamalarıyla artmıştır. Biber ve patlıcanda, Zn ve Cu hariç iki değerlikli metal besin iyonlarının akümülasyonu artarken, tek değerlikli metal besin iyonu olarak K akümülasyonu sadece biberde azalmıştır.

Anahtar Kelimeler: Kadmiyum; *Solanaceae*; Akümülasyon; Biyokonsantrasyon; Translokasyon; Metal besin iyonları

1. Introduction

Cadmium which is a non-essential element for plants, animals and humans has been a major pollutant in both terrestrial and aquatic environments for decades. Recent advances in industry and agriculture have led to an increased level of Cd in agricultural soils. Cadmium enters agricultural soil primarily through various anthropogenic sources, such as phosphate fertilizers, waste water, sewage sludge, and manure (Alloway & Steinnes 1999), and in emissions from metal-working industries, cement industries, power stations and urban traffic (Sanitá di Toppi & Gabbrielli 1999; Wu et al 2004). The accumulation of Cd in soil is dangerous for most living organisms. Despite being a non-essential element for plants, Cd is easily absorbed and accumulates in different plant parts. The accumulation/mobility of Cd seems to depend on the plant species and growth stage, the concentration of added nutrients, plant growth conditions, and/or metal combinations used (Murillo et al 2002). In plants, the accumulation of Cd can cause many morphological, physiological, biochemical and structural changes including plant growth retardation, chlorosis, necrosis (Benavides et al 2005), reduction of root growth, biomass production (Sanitá di Toppi & Gabbrielli 1999; Moussa 2004), chlorophyll content (Fatoba & Udoh 2008), inhibition of photosynthesis and transpiration (Mobin & Khan 2007; Shi et al 2010), imbalance of water and mineral nutrition (Gouia et al 2000; Sengar et al 2008), induction of oxidative stress (Shamsi et al 2007; Sandalio et al 2009), and affects on membrane structure and permeability (Sengar et al 2008).

Cadmium is easily taken up both active and passive pathways by plants and affects several metabolic activities in different cell compartments, especially in the chloroplasts. These deleterious effects include the inhibition of photosynthesis, such as biosynthesis of chlorophyll (Stobart et al 1985; Padmaja et al 1990; Ekmekçi et al 2008) and functioning of photochemical reactions (Krupa & Moniak 1998). Cadmium reduces plant growth by interrupting the plant photosynthetic activity and nutrient balance (Zhang et al 2002; Shamsi et al 2010) and also interferes with the uptake, translocation, and plant use of water and mineral nutrients (Shamsi et al 2007). Cadmium ions compete with most nutrients such as K, Ca, Mg, Fe, Mn, Cu, Zn, and nickel (Ni) across the same trans-membrane carriers (Clarkson & Luttge 1989; Rivetta et al 1997; Sanitá di Toppi & Gabbrielli 1999).

The accumulation of and tolerance to Cd differs considerably among plant species and their genotypes (Römer et al 2002; Metwally et al 2005). Variations in Cd accumulation have been observed among wheat cultivars (Stolt et al 2002), soybean (Shamsi et al 2008), pea (Belimov et al 2003), and maize cultivars (Ekmekçi et al 2008). According to the Cd accumulation level, Kuboi et al (1986) identified three general classes of plants: low accumulators

(*Leguminosae*), moderate accumulators (*Graminae*, *Liliaceae*, *Cucurbitaceae* and *Umbelliferae*) and high accumulators (*Solanaceae, Chenopodiaceae*, *Cruciferae* and *Compositae*). Many *Solanaceae* plants with a high accumulation of Cd are demanding plants grown in areas of sensitive agriculture with a high input of organic and mineral fertilizer.

In this study, the accumulation of Cd was examined in four plants belonging to different genera of the *Solanaceae* family. The plants include three widely-grown vegetables, tomato, pepper, and eggplant, and goldenberry, a lesser-known plant that is being introduced in several regions as an alternative crop. The main objective of the current study was to determine the effects of Cd toxicity on plant growth, the bioaccumulation and translocation of Cd and the accumulation of metal nutrient ions in these plants.

2. Material and Methods

Tomato (*Solanum lycopersicum* L., cv. H-2274), pepper (*Capsicum annuum* L., cv. Yalova Çorbacı-12), eggplant (*Solanum melongena* L., cv. Kemer), and goldenberry (*Physalis peruviana* L.) were used for the experiment, which was carried out in a greenhouse under natural light conditions. Seeds of each plant were germinated in seedling vials filled with peat. Three-week-old seedlings were transplanted at a rate of one plant per pot filled with 2 kg of air-dried soil. Some physical and chemical characteristics of the soil used in the experiment are presented in Table 1. The soil characteristics were determined according to methods detailed in Page et al (1982).

In a factorial (Cd levels and plant genera) pot experiment, five levels of Cd (0, 2.5, 5, 10, and 20 mg kg^{-1}) as cadmium chloride ($CdCl_2$) were added to the soil. The experiment was designed as complete randomized design with three replications. For basal fertilization; N, P and K, as ammonium nitrate (NH_4NO_3), potassium dihydrogen phosphate (KH_2PO_4), and potassium sulfate (K_2SO_4) was applied at 150, 75 and 150 mg kg^{-1}, respectively. All the supplementary ($CdCl_2$, NH_4NO_3, KH_2PO_4 and K_2SO_4) were incorporated into the soil by spraying the solutions before the planting and thoroughly mixed. During the experiment, pots were watered daily to 70% of water holding capacity by weighing the pots randomly.

Table 1- Some physical and chemical characteristics of the soil used in experiment

Çizelge 1- Denemede kullanılan toprağın bazı fiziksel ve kimyasal özellikleri

Soil properties	*Method/ fraction*	*Amount*
pH	1:2.5 soil/water extraction	7.34
EC (µS cm^{-1})	Saturation extraction	508
$CaCO_3$ (g kg^{-1})	Calcimeter	17.29
Sand (g 100 g^{-1})	Hydrometer	35.8
Clay (g 100 g^{-1})	Hydrometer	21.7
Silt (g 100 g^{-1})	Hydrometer	42.5
Soil texture	-	loam
Org C (g kg^{-1})	Walkley-Black	6.25
N (g kg^{-1})	Kjeldahl	0.86
P (mg kg^{-1})	$NaHCO_3$-available	12.43
K (mg kg^{-1})	NH_4OAc-extractable	100
Ca (mg kg^{-1})	NH_4OAc-extractable	2151
Mg (mg kg^{-1})	NH_4OAc-extractable	124
Na (mg kg^{-1})	NH_4OAc-extractable	64
Fe (mg kg^{-1})	DTPA-extractable	24.28
Mn (mg kg^{-1})	DTPA-extractable	65.27
Zn (mg kg^{-1})	DTPA-extractable	2.09
Cu (mg kg^{-1})	DTPA-extractable	1.17
Cd (mg kg^{-1})	DTPA-extractable	0.04
B (mg kg^{-1})	Hot water-extractable	1.64

A fresh leaf sample was taken from the youngest fully expanded leaf for photosynthetic pigment analysis before harvest. After six weeks of Cd treatment, the shoots were harvested, washed with running tap water and three-times rinsed with de-ionized water to remove any soil particles attached to the plant surfaces. The roots were carefully seperated from the soil and dipped into an aerated

0.5 mM $CaCl_2$ solution for 15 minutes in order to eliminate adsorbed nutrients from the root surface. The roots were quikly washed with running tap water and then rinsed with de-ionized water. All shoot and root samples were dried at 65 °C to their constant weight, and weighed for dry weight (DW) determination and ground to powder for Cd and metal ion nutrient analysis.

The dried tissues were digested by dry-ashing method in a muffle furnace at 500 °C for 6 hours (Miller 2004). The concentrations of Cd and metal nutrient ions were determined by ICP-OES (Perkin-Elmer Optima 2100 DV; Waltham, MA).

The Cd distribution, the Cd uptake, bioconcentration factor (BCF), translocation factor (TF) and total accumulation rate (TAR) were calculated by the Equation 1, 2, 3, 4 and 5, respectively (Ait Ali et al 2002; Shi et al 2010).

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

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

$$BCF = [Cd]_{\text{shoot or root}}/[\text{total Cd}]_{\text{soil}} \quad (3)$$

Where; $[\text{total Cd}]_{\text{soil}}$, present Cd concentration in experimental soil+added Cd concentration for each Cd level

$$TF\ (\%) = 100x[Cd]_{\text{shoot}}/[Cd]_{\text{root}} \quad (4)$$

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

Photosynthetic pigments were measured in fresh leaf samples before harvest. The fresh leaf samples (500 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).

Statistical analysis of the experimental data was performed by using ANOVA with the MINITAB package program (Minitab Corp., State College, PA). Multiple comparisons of means among different Cd treatments were performed using Duncan's Multiple Range Test at the significance level (α: 0.05).

3. Results and Discussion

3.1. Plant growth and biomass

Visual toxicity symptom of Cd occurred as reduction of shoots and root growth and discernible browning and decomposing in main roots in experimental plants, except for tomato. It was observed that these symptoms are intensified in pepper and goldenberry, especially at the 20 mg Cd kg^{-1} rate.

Both genera and Cd treatment significantly affected the shoot and root dry biomass (Figure 1). With the increasing applications of Cd, the changes in plant growth differed in the various *Solanaceae* plants. Except for tomato, the shoot and root dry biomass of the *Solanaceae* family plants significantly decreased with increasing of Cd in comparison with the control (Figures 1a and 1c). At 2.5 mg kg^{-1} Cd rate, shoot biomass decreased by 23.5% and 26.5% for pepper and goldenberry, respectively, but increased by 7.4% and 3.9% for tomato and eggplant, respectively. Moreover, shoot biomass decreased by 8.2%, 86.4%, 90.1%, and 65.5% for tomato, pepper, eggplant and goldenberry, respectively, at the highest amounts of added Cd. Similarly, with increasing rate of Cd, the root biomass diminished for pepper, eggplant, and goldenberry, but increased for tomato (Figure 1b and 1d). The reduction of shoot and root dry biomass as a result of the increasing Cd supply might be attributed to prominent decreases in shoot height and root length and changes in the rate of net photosynthesis that reduces the supply of carbohydrates or proteins.

Major differences among *Solanaceae* plants in biomass production under increasing supply of Cd to the soil might be associated with the possible presence of differing mechanisms among plants in the accumulation and translocation process, and might be strongly related to genetics. On the basis of the reduction rate in the shoot dry biomass of the *Solanaceae* plants, tomato was determined to be Cd-tolerant and the other plants Cd-sensitive

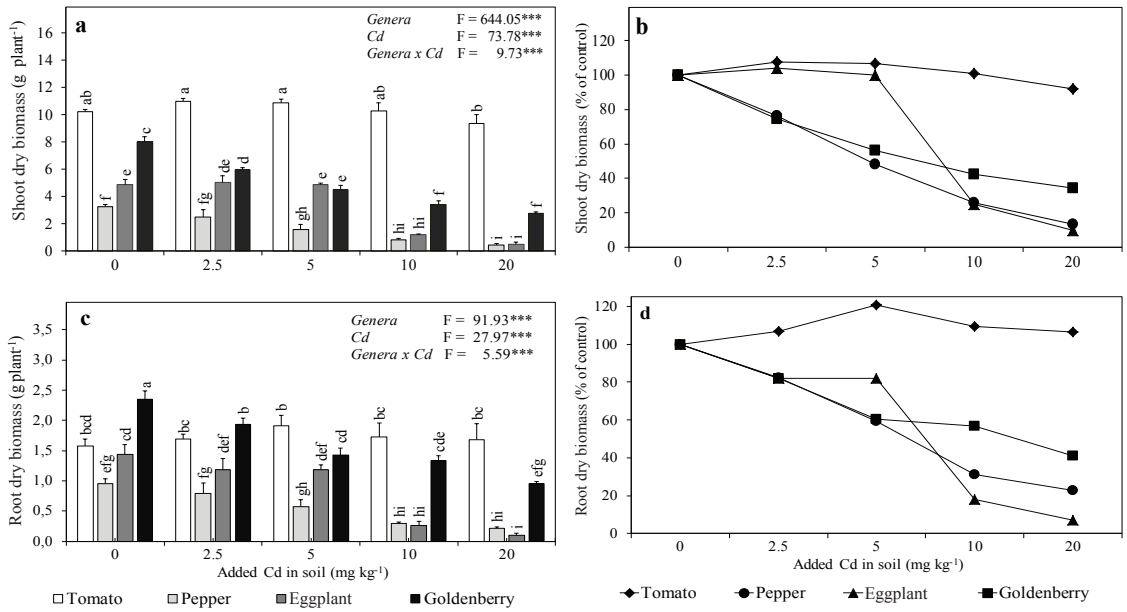


Figure 1- Effects of cadmium on shoot and root dry biomass of *Solanaceae* plants (mean±SE, *n*= 3). The bars followed by the same letter are not significantly different for genera x Cd interaction (Duncan's Multiple Range Test, α: 0.05); ANOVA shows significant difference at ***, P<0.001

*Şekil 1- Kadmiyumun Solanaceae bitkilerinin gövde ve kök kuru biyokütlelerine etkisi (ortalama±standart hata, n= 3). Çubukları izleyen aynı harfler, cins x Cd interaksiyonu için farkın önemli olmadığını gösterir (Duncan Çoklu Karşılaştırma Testi, α: 0.05). ANOVA'ya göre; ***, P<0.001*

according to the scale suggedted by Shahbaz et al (2011) as tolerant, moderately tolerant, and sensitive for the reduction rate of <30%, 30-60% and >60%, respectively. In support of these findings, the reduction of shoot and root dry biomass caused by Cd application has been demonstrated in many plants, including tomato (Haouari et al 2012), eggplant (Arao et al 2008), transgenic and wild type tobacco (Dağhan et al 2013), soybean (Shamsi et al 2010), safflower (Shi et al 2010), and maize (Ekmekçi et al 2008).

3.2. Cadmium accumulation

The shoot and root Cd contents of the four *Solanaceae* plants significantly (P<0.001) increased with elevated rates of Cd application as compared to the control, except for the shoot Cd content of goldenberry (Table 2). The shoot Cd contents of goldenberry, pepper, tomato and eggplant increased in response to the increased additions of Cd and reached to 5.0, 32.1, 69.4 and 109.3 mg kg^{-1}, respectively at the highest Cd application rate. Obviously, the root Cd contents were much higher than those of the shoots. Considering the Cd distribution in shoots and roots, the goldenberry accumulated much lower Cd in the shoots than the other experimental plants with the increasing Cd supply (Table 2). Differences in Cd distribution among different plants could be explained by the presence of different mechanisms of tolerance, physiology of transport, and accumulation. Many researchers have reported that Cd is accumulated more in the roots than in the shoots of plants such as sunflower (De Maria et al 2013), tomato (Haouari et al 2012), safflower (Shi et al 2010), soybean (Shamsi

et al 2010), eggplant (Arao et al 2008), and some *Solanaceae* plants (Thiebeauld et al 2005). Furthermore, the shoot Cd uptake of tomato and eggplant significantly increased with increments of Cd supply (Table 2). The maximum shoot Cd uptake was observed in tomato, followed by eggplant, pepper, and goldenberry, respectively. At all Cd levels, the root Cd uptake significantly increased in tomato and goldenberry.

The shoot and root BCF significantly diminished at all Cd levels as compared to the control (Figure 2a). The present results showed that the BCF was greater in the roots than in the shoots (Figure 2b). Furthermore, except for the shoot BCF of goldenberry, both the shoot and the root BCF exceeded the critical level for a Cd-hyperaccumulator, currently accepted as BCF> 1 (Baker 1981; Ma et al 2001).

Table 2- Effects of cadmium on Cd content and Cd uptake in shoots and roots of *Solanaceae* plants

Çizelge 2- Kadmiyumun Solanaceae bitkilerinde gövde ve kökün Cd içerikleri ve Cd alımlarına etkisi

Added Cd to soils ($mg\ kg^{-1}$)	*Cd content* ($mg\ kg^{-1}$ *DW*)		*Cd distribution (%)*		*Cd uptake* ($\mu g\ plant^{-1}$)	
	Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
			Tomato			
0	1.1 j*	7.3 j	13.1	86.9	11.5 i	11.7 j
2.5	15.3 i	166.5 hi	8.4	91.6	168.3 d	280.4 de
5	24.3 gh	319.2 ef	7.1	92.9	264.4 c	611.8 b
10	43.8 d	215.1 gh	16.9	83.1	449.9 b	372.8 cd
20	69.4 c	247.6 fg	21.9	78.1	647.3 a	394.1 c
			Pepper			
0	2.2 j	3.0 j	42.3	57.7	7.1 i	2.9 i
2.5	18.7 hi	86.2 ij	17.8	82.2	46.2 g	65.9 hij
5	28.7 fg	257.8 fg	10.0	90.0	43.4 gh	141.8 ghi
10	36.1 e	342.2 e	9.5	90.5	30.1 ghi	102.8 g-i
20	32.1 ef	873.0 a	3.5	96.5	14.3 hi	189.8 efg
			Eggplant			
0	1.3 j	2.3 j	36.1	63.9	6.1 i	3.4 j
2.5	26.8 fg	40.9 j	39.6	60.4	135.1e	48.4 ij
5	37.5 e	139.4 hi	21.2	78.8	182.6 d	163.6 fgh
10	78.4 b	596.5 c	11.6	88.4	96.0 f	157.3 fgh
20	109.3 a	753.1 b	12.7	87.3	52.7 g	73.4 hij
			Goldenberry			
0	0.5 j	3.4 j	12.8	87.2	4.1 i	7.9 j
2.5	2.1 j	132.7 hi	1.6	98.4	12.4 i	256.2 ef
5	3.1 j	274.5 efg	1.1	98.9	14.1 hi	388.4 c
10	4.3 j	483.2 d	0.9	99.1	14.3 hi	638.6 b
20	5.0 j	873.0 a	0.6	99.4	13.9 hi	843.6 a
ANOVA: F values						
Genera	493.82^{***}	34.37^{***}			1049.90^{***}	132.04^{***}
Cd	436.69^{***}	409.06^{***}			200.75^{***}	85.08^{***}
Genera x Cd	87.27^{***}	35.79^{***}			181.78^{***}	21.84^{***}

*, values are mean of three replicates and means followed by the same letter are not significantly different for genera x Cd interaction (Duncan's Multiple Range Test, α: 0.05); ANOVA shows significant difference at ***, P<0.001

The TF can be described as the translocation of heavy metals in plants. Significant variations were found in the TF of the *Solanaceae* plants. The TF decreased from 74.5% to 3.6% for pepper, from 14.8% to 0.6% for goldenberry, and from 65.5% to 14.6% for eggplant with increasing Cd levels, but the TF of tomato increased at the higher Cd levels (Figure 2c). The TF of all plants was much lower than the critical level (TF> 100%). Similar results were found for safflower (Shi et al 2010) and sunflower (De Maria et al 2013). When the *Solanaceae* plants classified with respect to Cd translocation at the highest Cd level, plant species showed a ranking as follows: goldenberry<pepper<eggplant<tomato.

The total accumulation rate (TAR) of Cd differed among all the plants. While the TAR value of tomato and goldenberry significantly increased with all Cd levels in comparison with the control, the TAR of pepper and eggplant increased up to the 5 mg kg^{-1} Cd treatment (Figure 2d). On the other hand, the TAR for tomato was higher than for the other plants. Similar results were obtained by, Sharma & Agrawal (2006), who stated that the TAR and Cd uptake are significantly increased in carrot at the excess Cd level.

3.3. Photosynthetic pigments

Between 2.5 and 10 mg kg^{-1} Cd treatments, non-significant changes in the photosynthetic pigment were observed for pepper and eggplant compared to the control (Figure 3). But the highest level of Cd significantly decreased the Chl *a*, Chl *a+b*

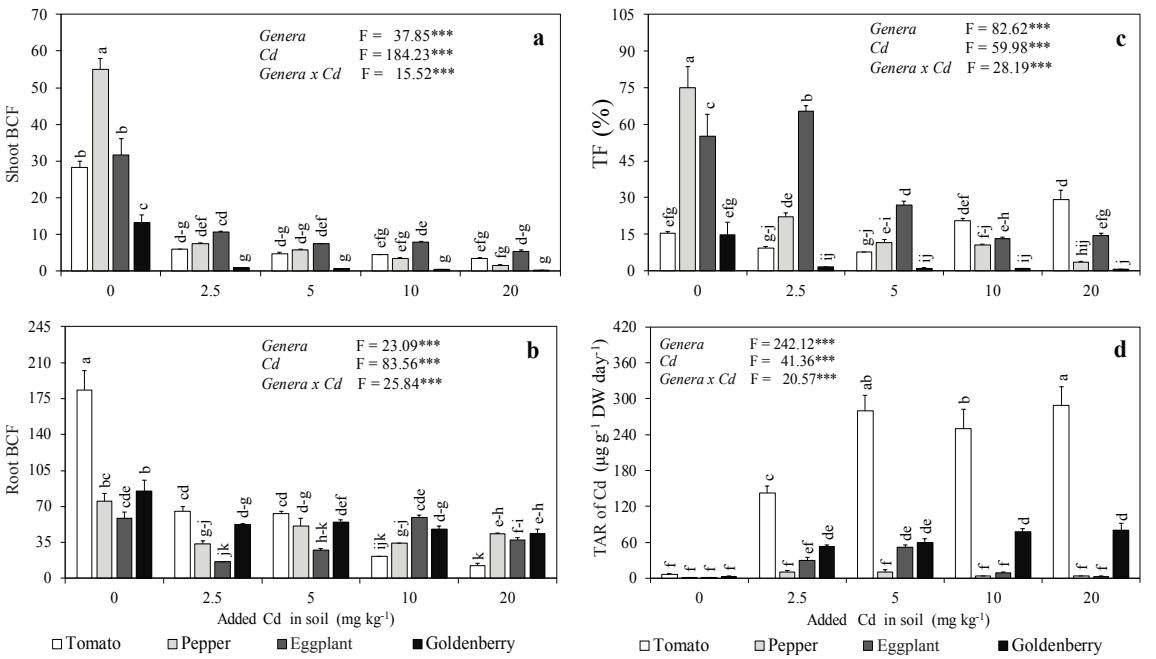


Figure 2- Effects of cadmium on a, shoot BCF; b, root BCF; c, TF and d, TAR of Cd in *Solanaceae* plants (mean±SE, *n*= 3); the bars followed by the same letter are not significantly different for genera x Cd interaction (Duncan's Multiple Range Test, *α*: 0.05); ANOVA shows significant difference at ***, P<0.001

*Şekil 2- Kadmiyumun Solanaceae bitkilerinde a, gövde biyokonsantrasyon faktörü; b, kök biyokonsantrasyon faktörü; c, translokasyon faktörü ve d, toplam akümülasyon oranına etkisi; (ortalama±standart hata, n= 3); çubukları izleyen aynı harfler, cins x Cd interaksiyonu için farkın önemli olmadığını gösterir (Duncan Çoklu Karşılaştırma Testi, α: 0.05); ANOVA'ya göre ***, P<0.001*

and Car content by 19.8%, 18.3%, 20.4% for tomato, and 21.2%, 21.4%, 22.8% for goldenberry, respectively. Additionally, any interaction of genera and Cd affecting the Chl *b* content was statistically non-significant in all plants. The reduction of chlorophyll content in Cd-treated plants is related to chlorophyll degradation of and/or disorders in its biosynthesis and with the reduction of thylakoid membrane integrity (Sandalio et al 2001). The present findings support other research which has revealed that reduction of photosynthetic pigments with an increasing Cd content occurs in some plants, including tomato (Haouari et al 2012), eggplant (Arao et al 2008) and some *Solanaceae* plants (Thiebeauld et al 2005).

3.4. Metal nutrient ion accumulation

Interaction of the genera and Cd resulted in significant variations in shoot K and Na contents for all plants (P<0.001) (Table 3). The shoot K content significantly increased for tomato, eggplant, and goldenberry, reaching to levels as high as 40.65, 59.24, and 66.92 g kg^{-1}, respectively. A reduction was observed in the shoot K content of pepper; however, this reduction was only significant for soil treated at the level of 10 mg Cd kg^{-1}. The effect of the interaction of the genera and Cd addition on the shoot Ca and Mg contents was not significant, whereas the effect of increasing Cd supply on shoot Ca content (P<0.05) and the effect of the genera on shoot Mg content (P<0.001) were found to be

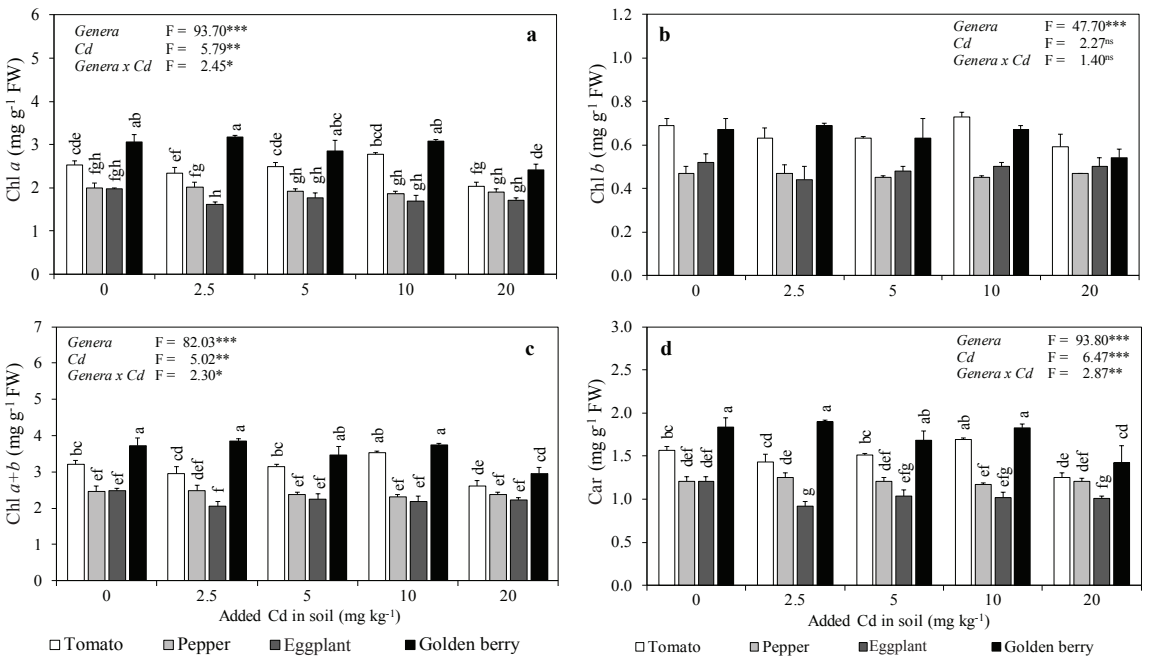


Figure 3- Effects of cadmium on the photosynthetic pigments in leaves of *Solanaceae* plants (mean±SE, *n*= 3). The bars followed by the same letter are not significantly different for genera x Cd interaction (Duncan's Multiple Range Test, α: 0.05); ANOVA shows significant difference at ***, P<0.001; **, P<0.01; *, P<0.05; ns, not significant

*Şekil 3- Kadmiyumun Solanaceae bitkilerinin yapraklarında fotosentetik pigmentlere etkisi; (ortalama±standart hata, n= 3). Çubukları izleyen aynı harfler, cins x Cd interaksiyonu için farkın önemli olmadığını gösterir (Duncan Çoklu Karşılaştırma Testi, α: 0.05); ANOVA'ya göre ***, P<0.001; **, P<0.01; *, P<0.05; ns, önemli değil*

significant (Table 3). Except for tomato, all plants exhibited significant changes in shoot Na content. For instance, compared to the control, while there was no significant increase in shoot Na content of tomato at the highest level of Cd, this parameter in pepper, eggplant, and goldenberry significantly increased, by 2.2-, 1.9-, and 2.4-fold, respectively. In contrast, a significant reduction was observed in the shoot Na content of pepper at 5 and 10 mg Cd kg^{-1} treatments as compared to control.

Of all plants in soil untreated with Cd, the shoot Fe content was the highest in goldenberry, followed by tomato, pepper, and eggplant, respectively (Table 3). The shoot Fe contents of tomato tended to decrease with increasing rate of Cd being significant only at 2.5 mg Cd kg^{-1} level. Significant increases in the shoot Fe content were found for goldenberry and pepper at 2.5 and 20 mg Cd kg^{-1}, respectively. The shoot Mn content of pepper showed significant linear increases at all Cd levels and rose up to 232.5 mg kg^{-1}, while those of tomato, eggplant, and goldenberry significantly increased at the highest Cd treatment, and reached to 159.0, 192.1, and 89.3 mg kg^{-1}, respectively (Table 3). On the other hand, the shoot Mn content exhibited a slight decline in tomato and eggplant at 2.5-5 mg Cd kg^{-1} range.

Table 3- Effects of cadmium on shoot metal nutrient ion contents of *Solanaceae* plants

Çizelge 3- Kadmiyumun Solanaceae bitkilerinin gövdesinde metal besin iyonu içeriklerine etkisi

Added Cd to soil (mg kg^{-1})	*K*	*Ca*	*Mg*	*Na*	*Fe*	*Mn*	*Zn*	*Cu*
	--------- g kg^{-1} DW ---------			*---------- mg kg^{-1} DW ----------*				
				Tomato				
0	33.15 j[x]	13.25	3.69	673 c-f	97.8 de	129.7 def	33.1 hij	17.1 a
2.5	32.85 j	11.81	3.28	680 c-f	82.1 efg	116.3 e-h	35.4 gh	15.8 abc
5	35.53 ij	11.81	3.54	653 def	86.3 ef	101.7 gh	40.9 ef	14.5 bcd
10	39.86 hi	13.33	3.06	780 cd	87.1 ef	117.1 e-h	41.6 ef	14.5 bcd
20	40.65 ghi	14.24	4.35	840 c	85.1 efg	159.0 c	41.6 ef	15.2 a-d
				Pepper				
0	50.68 def	10.74	3.53	573 fg	86.7 ef	119.9 efg	59.2 a	13.1 de
2.5	51.76 cde	11.96	3.53	473 ghi	75.1 fgh	143.0 cde	36.3 gh	7.5 g
5	44.64 fgh	12.48	3.66	400 hi	84.7 efg	151.6 cd	34.7 ghi	5.2 h
10	41.96 gh	14.00	3.86	367 hi	78.3 fgh	154.0 cd	27.9 k	3.9 h
20	44.65 fgh	14.15	4.02	1282 a	107.5 cd	232.5 a	16.8 l	4.6 h
				Eggplant				
0	49.60 def	12.85	2.20	573 fg	77.3 fgh	113.3 fgh	51.4 bc	14.7 bcd
2.5	57.92 bc	14.15	2.56	593 efg	81.3 efg	101.1 gh	52.5 b	14.9 a-d
5	52.76 cde	13.56	2.61	560 fg	67.4 gh	91.4 hi	47.0 cd	13.0 de
10	59.32 b	14.09	2.62	760 cde	81.7 efg	155.3 cd	47.6 cd	15.5 abc
20	59.24 b	12.31	2.01	1084 b	61.8 h	192.1 b	45.5 de	10.4 f
				Goldenberry				
0	46.60 efg	9.02	3.93	313 i	118.1 bc	45.5 k	30.2 ijk	11.3 ef
2.5	50.44 def	10.16	4.08	367 i	143.1 a	64.4 jk	38.9 fg	14.5 bcd
5	49.52 def	11.77	4.57	687 c-f	130.0 ab	53.6 k	35.6 gh	13.9 cd
10	53.80 bcd	13.77	4.49	527 fgh	126.9 ab	67.6 ijk	29.4 jk	16.4 ab
20	66.92 a	14.96	4.67	747 cde	122.9 bc	89.3 hij	29.1 jk	16.1 abc
ANOVA: F values								
Genera	88.75***	1.28ns	47.10***	16.16***	90.09***	108.11***	109.32***	153.76***
Cd	9.16***	3.30*	1.73ns	56.83***	0.22ns	43.47***	26.61***	8.81***
Genera x Cd	5.89***	1.18ns	1.66ns	10.80***	3.86***	4.72***	32.11***	13.01***

[x], values are mean of three replicates and means followed by the same letter are not significantly different for genera x Cd interaction (Duncan's Multiple Range Test, α: 0.05); ANOVA shows significant difference at ***, P<0.001; *, P<0.05; ns, not significant

While a significant reduction in shoot Zn and Cu content with increasing additions of Cd was observed in pepper, this reduction was significant only at 20 mg Cd kg^{-1} level for eggplant (Table 3). Compared to control, goldenberry shoot Cu content increased by all Cd treatments, but for Zn such increases were detected only at 2.5 and 5 mg Cd kg^{-1} levels. Although the shoot Cu content of tomato showed a significant reduction at 5 and 10 mg Cd kg^{-1} levels, the shoot Zn content of tomato significantly increased parallel with the Cd levels, except for 2.5 mg Cd kg^{-1} level.

Cadmium may interfere with nutrient uptake due to its effect on the permeability of plasma membranes. Toxic heavy metals, like Cd, are regarded as competitive ion with the transport systems operating for divalent cations such as Ca, Fe, Mg, Cu and Zn, as they use the same trans-membrane carriers (Llamas et al 2000; Roth et al 2006). Therefore, the sensitivity of some dicotyledonous plants to Cd toxicity might be associated with Cd effects on the influx and transport of Fe, Mn, Ca, and Mg (Yang et al 1996). Yang et al (1996) indicated that the influx and transport of Ca, Mg, Fe, Mn, Zn, and Cu in plants including ryegrass, maize, white clover, and cabbage were decreased by additional Cd. López-Millán et al (2009) stated that, with Cd treatment, Fe, Mn and Zn accumulation increased in tomato stems, while in the leaves only Mn accumulation increased. The same researchers determined a reduction of K, Fe and Cu accumulation in the leaves, and Cu accumulation in the stems of tomato. Moreover, Sandalio et al (2001) reported that K, Ca, Mg, Fe, Mn, Zn, and Cu contents of pea shoots decreased with increases of Cd in an aerated full-nutrient media. However, Zhang et al (2002) found that, while K, Fe, Mn, Zn, and Cu concentrations increased in wheat genotypes at the seedling stage, there was a reduction of Ca and Mg concentrations. As reported by Jiang et al (2004), the nutrients mainly affected by Cd in Indian mustard were K, Ca, Fe, and Zn in the roots, and K, Ca and Cu in the shoots. Obata & Umebayashi (1997) revealed that K concentrations decreased in Cd-sensitive kidney bean and pea with increasing of Cd, while Mn concentrations decreased in semi-resistant rice and maize.

4. Conclusions

The response of plants to excess Cd varied. The phenomena of reduction of root and shoot biomass, uptake of Cd, and bioaccumulation and translocation of Cd were all dependant on Cd concentration and species. On the basis of the percent reductions in the shoot dry biomass of the four *Solanaceae* plants, tomato was determined to be Cd-tolerant, and the other plants Cd-sensitive. Moreover, when classified by translocation of Cd at the highest level, the order was observed as goldenberry<pepper<eggplant<tomato. Goldenberry and pepper exhibited poor accumulation of Cd in their shoots. Thus, these plants might be appropriate for cultivation in Cd-contaminated soils. Moreover, the accumulation of all metal nutrient ions increased in the goldenberry shoots. The results showed that, except for Zn and Cu, there was an increment in accumulation of divalent metal nutrient ions for pepper and eggplant; however, the accumulation of K as monovalent metal nutrient ion decreased for only the pepper. Further investigation is needed to identify the mechanism depending on concentration and species which is responsible for the low Cd translocation.

References

Ait Ali N, Bernal M P & Ater M (2002). Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant and Soil* **239**: 103-111

Alloway B J & Steinnes E (1999). Anthropogenic additions of cadmium to soils. In: M J Laughlin & B R Singh (Eds), *Cadmium in Soil and Plants,* Kluwer Academic Publishers, Dordrecht (The Netherlands), pp. 97-123

Arao T, Takeda H & Nishihara E (2008). Reduction of cadmium translocation from roots to shoots in eggplant (*Solanum melongena*) by grafting onto *Solanum torvum* rootstock. *Soil Science and Plant Nutrition* **54**: 555-559

Baker A J M (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643-654

Belimov A A, Safronova V I, Tsyganov V E, Borisov A Y, Kozhemyakov A P, Stepanok V V, Martenson A M, Gianinazz-Pearson V & Tikhonovich I A (2003). Genetic variability in tolerance to cadmium and accumulation of heavy metals in pea (*Pisum sativum* L.). *Euphytica* **131**: 25-35

Benavides M P, Gallego M S & Tomaro M L (2005). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology* **17**: 21-34

Clarkson D T & Luttge U (1989). Mineral nutrition -Divalent cations, transport and compartmentalization. *Progress in Botany* **51**: 93-112

Dağhan H, Uygur V, Köleli N, Arslan M & Eren A (2013). Transgenik ve transgenik olmayan tütün bitkilerinde ağır metal uygulamalarının azot, fosfor ve potasyum alımına *etkisi. Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **19**: 129-139

De Maria S, Puschenreiter M & Rivelli A R (2013). Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. *Plant, Soil and Environment* **59**(6): 254-261

Ekmekçi Y, Tanyolaç D & Ayhan B (2008). Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. *Journal of Plant Physiology* **165**: 600-611

Fatoba P O & Udoh E G (2008). Effects of some heavy metals on chlorophyll accumulation. *Ethnobotanical Leaflets* **12**: 776-783

Gouia H, Ghorbal M H & Meyer C (2000). Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiology and Biochemistry* **38**: 629-638

Haouari C C, Nasraoui A H, Bouthour D, Houda M D, Daieb C B, Mnai J & Gouia H (2012). Response of tomato (*Solanum lycopersicon*) to cadmium toxicity: Growth, element uptake, chlorophyll content and photosynthesis rate. *African Journal of Plant Science* **6**(1): 1-7

Jiang X J, Lou Y M, Liu Q, Liu S L & Zhao Q G (2004). Effect of cadmium on nutrient uptake and translocation by Indian mustard. *Environmental Geochemistry and Health* **26**: 319-324

Krupa Z & Moniak M (1998). The stage of leaf maturity implicates the response of the photosynthetic apparatus to cadmium toxicity. *Plant Science* **138**: 149-156

Kuboi T, Noguchi A & Yazaki J (1986). Family-dependent cadmium accumulation characteristics in higher plants. *Plant and Soil* **92**: 405-415

Lichtenthaler H K (1987). Chlorophylls and carotenoids: pigments of photosynthetic biometers. *Methods in Enzymology* **148**: 350-382

Llamas A C, Ullrich I & Sanz A (2000). Cd^{2+} effects on transmembrane electrical potential difference, respiration and membrane permeability of rice (*Oryza sativa* L.) roots. *Plant and Soil* **219**(1-2): 21-28

López-Millán A, Sagardoy R, Solanas M, Abadía A & Abadía J (2009). Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environmental and Experimental Botany* **65**: 376-385

Ma L Q, Komar K M, Tu C, Zhang W, Cai Y & Kenelly E D (2001). A fern that hyper accumulates arsenic. *Nature* **409**: 579-582

Metwally A, Safronova V I, Belimov A A & Dietz K J (2005). Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *Journal of Experimental Botany* **56**: 167-178

Miller R O (2004). High-temperature oxidation: Dry ashing. In: Kalra Y P (Eds), *Handbook of Reference Methods for Plant Analysis.* CRC Press, Boca Raton, Florida, pp. 53-56

Mobin M & Khan N A (2007). Photosynthetic activity, pigment composition and anti oxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *Journal of Plant Physiology* **164**: 601-610

Moussa H R (2004). Effect of cadmium on growth and oxidative metabolism of faba bean plants. *Acta Agronomica Hungarica* **52**: 269-276

Murillo B, Troyo-Diéguez E, García-Hernández J L, Larrinaga-Mayoral J A, Nieto-Garibay A & López-Cortés A (2002). Efecto dela salinidad en genotipos de chicharo de vaca *Vigna unguiculata* (L.) Walp. durante la etapa de plántula. *Agrochimica* **46**(1-2): 73-87

Obata H & Umebayashi M (1997). Effects of cadmium on mineral nutrient concentrations in plants differing in tolerance for cadmium. *Journal of Plant Nutrition* **20**(1): 97-105

Padmaja K, Parsad D D K & Parsad A R K (1990). Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* L. seedling by cadmium acetate. *Photosynthetica* **24**: 399-404

Page A, Miller R & Keeney D (1982). Methods of Soil Analysis. American Society of Agronomy, Inc., Madison WI (USA)

Rivetta A, Negrini N & Cocucci M (1997). Involvement of Ca^{2+}-calmodulin in Cd^{2+} toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination. *Plant Cell and Environment* **20**: 600-608

Roth U, Roepenack-Lahaye E V & Clemens S (2006). Proteome changes in *Arabidopsis thaliana* roots upon exposure to Cd^{2+}. *Journal of Experimental Botany* **57**(15): 4003-4013

Römer W, Egle K, Kang D K, Keller H & Gerke J (2002). L'absorption de Cd par différentes variétés de lupins (*Lupinus albus* et *Lupinus angustifolius*) comparéé á celle du ray-grass (*Lolium multiflorum*) en function de la fertilisation phosphatéé. *Agronomie* **22**: 431-442

Sandalio L M, Dalurzo H C, Gomez M, Romero-Puertas M C & del Rio L A (2001). Cadmium induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany* **52**: 2115-2126

Sandalio L M, Rodríguez-Serrano M, Río L A & Romero-Puertas M C (2009). Reactive oxygen species and signaling in cadmium toxicity. In: L A del Rio & A Puppo (Eds), *Reactive Oxygen Species in Plant Signaling*, Springer-Verlag, Berlin, Heidelberg, pp. 175-189

Sanitá di-Toppi L & Gabrielli R (1999). Response to cadmium in higher plants. *Environmental and Experimental Botany* **41**: 105-130

Sengar R S, Gautam M, Sengar R S, Garg S K, Sengar K & Chaudhary R (2008). Lead stress effects on physiobiochemical activities of higher plants. *Reviews of Environmental Contamination and Toxicology* **196**: 73-93

Shahbaz M, Ashraf M, Akram N A, Hanif A, Hameed S, Joham S & Rehman R (2011). Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). *Acta Physiologiae Plantarum* **33**: 1113-1122

Shamsi I H, Wei K, Jilani G & Zhang G P (2007). Interactions of cadmium and aluminum toxicity in their effect on growth and physiological parameters in soybean. *Journal of Zhejiang University Science* B **8**: 181-188

Shamsi I H, Wei K, Zhang G P, Jilani G & Hassan M J (2008). Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. *Biologia Plantarum* **52**: 165-169

Shamsi I H, Jiang L, Wei K, Jilani G, Hua S & Zhang G P (2010). Alleviation of cadmium toxicity in soybean by potassium supplementation. *Journal of Plant Nutrition* **33**: 1926-1938

Sharma R K & Agrawal M (2006). Single and combined effects of cadmium and zinc on carrots: Uptake and bioaccumulation. *Journal of Plant Nutrition* **29**: 1791-1804

Shi G, Liu C, Cai Q, Liu Q & Hou C (2010). Cadmium accumulation and tolerance of two safflower cultivars in relation to photosynthesis and antioxidative enzymes. *Bulletin of Environmental Contamination and Toxicology* **85**: 256-263

Stobart A K, Griffiths W T, Ameen-Bukhari I & Sherwood R P (1985). The effect of Cd^{+2} on the biosynthesis of chlorophyll in leaves of barley. *Physiologia Plantarum* **63**: 293-298

Stolt P, Hultin S & Asp H (2002). Genetic variation in wheat regarding cadmium accumulation in relation to soil, soil solution cadmium concentration and growing season. In: P Stolt (Eds), Cadmium, a Challenge for Organisms-Agricultural Perspectives, PhD thesis, Dept. of Crop Science, Swedish Uni. of Agric. Sci., Alnarp, Sweden.

Thiebeauld O, Soler S, Raigón M D, Prohens J & Nuez F (2005). Variation among *Solanaceae* crops in cadmium tolerance and accumulation. *Agronomy for Sustainable Development* **25**: 237-241

Wu F B, Chen F K, Wei K & Zhang G P (2004). Effects of cadmium on free amino acids, glutathione, and ascorbic acid concentration in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. *Chemosphere* **57**: 447-454

Yang X, Baligar V C, Martens D C & Clark R B (1996). Cadmium effects on influx and transport of mineral nutrients in plant species. *Journal of Plant Nutrition* **19**(3-4): 643-656

Zhang G P, Fukami M & Sekimoto H (2002). Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Plants Research* **77**: 93-98

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Solid Matrix Priming of Cabbage Seed Lots: Repair of Ageing and Increasing Seed Quality

Sıtkı ERMİŞ[a], Fatih KARA[b], Eren ÖZDEN[b], İbrahim DEMİR[b]

[a]*Republic of Turkey, Ministry of Food, Agriculture and Livestock, Variety Registration and Seed Certification Centre, Ankara, TURKEY*

[b]*Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, TURKEY*

ARTICLE INFO

Research Article
Corresponding Author: Sıtkı ERMİŞ, E-mail: seedman37@gmail.com, Tel: +90 (312) 315 46 05
Received: 23 January 2015, Received in Revised Form: 03 September 2015, Accepted: 07 September 2015

ABSTRACT

This study was conducted to determine the effect of solid matrix priming treatment on 25 cabbage seed lots of various ages in terms of enhanced germination, emergence, mean germination and emergence time, and electrical conductivity. Solid matrix priming at a seed: vermiculite: water rate of 1:2:2.5 (w:w:w) was applied at 25 °C for 16 hours in the dark. Matrix priming was found to increase germination and emergence, reduced mean germination, emergence times and solute leakage. The advantages of solid matrix priming were observed more in aged than fresh seeds. The results indicated that SMP may enhance aged cabbage seed quality.

Keywords: Seed pretreatment; Electrical conductivity; Mean germination time; Germination; Emergence

Lahana Tohum Partilerinde Katı Madde ile Priming: Yaşlılığın Tamiri ve Tohum Kalitesine Olan Etkisi

ESER BİLGİSİ

Araştırma Makalesi
Sorumlu Yazar: Sıtkı ERMİŞ, E-posta: seedman37@gmail.com, Tel: +90 (312) 315 46 05
Geliş Tarihi: 23 Ocak 2015, Düzeltmelerin Gelişi: 03 Eylül 2015, Kabul: 07 Eylül 2015

ÖZET

Bu çalışmada, katı matriks priming uygulamalarının yaşlandırılmış 25 lahana tohum partisinde çimlenme, çıkış, ortalama çimlenme ve çıkış hızı ile birlikte elektiriksel iletkenlik testine olan etkisi incelenmiştir. Katı matriks priming koşullarında 1:2:2.5 tohum, vermikülit ve su karışımı ortamında olmak üzere tohumlar 16 saat karanlık ve 25 °C'de uygulanmıştır. Matriks priming uygulamasının çimlenme ve çıkışı artırdığı, ortalama çimlenme, çıkış hızı ile akıntıyı azalttığı tespit edilmiştir. Matriks priming'in etkisinin yaşlanmamış tohumlara oranla yaşlı tohumlarda daha avantajlı olduğu belirlenmiştir. SMP'nin yaşlı lahana tohumlarında tohum kalitesini artırdığı sonucuna varılmıştır.

Anahtar Kelimeler: Tohum ön uygulamaları; Elektriksel iletkenlik; Ortalama çimlenme süresi; Çimlenme; Çıkış

1. Introduction

Seed priming in solutions of low water potential e.g. polyethylene glycol salts, has been used extensively to increase germination and emergence percentages, and to reduce germination and emergence times (Heydecker & Coolbear 1977). Solid carriers, such as vermiculite, expanded calcined clay, agro-lig and synthetic calcium silicates have also been used for preplant seed conditioning, which is also referred to as solid matrix priming (Khan 1992; Parera & Cantliffe 1992; Hacısalihoglu 2007). Both the holding capacity and density of solid carriers and the amount of carrier relative to seed and water used for optimum conditioning have differed greatly (Khan et al 1992). Solid matrix priming has been proven to increase seed emergence percentages and seedling growth in various vegetables (Kubik et al 1988; Khan et al 1992; Carlos et al 1993; Jett et al 1996; Wang et al 2003; Pandita et al 2010), including, tomato, pepper, broccoli, okra and snap bean. Solid matrix priming increased germination at sub-optimal temperatures and increased anti-oxidative enzyme activity and levels of antioxidants in treated seeds (Wang et al 2003; Kepczynska et al 2007).

Cabbage seeds are intolerant to seed ageing in suboptimal storage conditions and the vigor of seed lots may be lost in a short time (Matthews et al 2009). Seed quality may be lost in a year or so in the event of seed storage conditions not being ideal, which is common in the less-developed regions of Turkey. Seed deterioration is major cause of poor emergence in modules and erratic emergence (Powell et al 2000), resulted in the low quality transplant production (Matthews et al 2012). Priming treatments are used to rejuvenate deteriorated seed after natural or artificial aging in different seeds (Taylor et al 1988; Powell et al 2000; Sung & Chiu 2001). However, solid matrix priming has been insufficiently tested for the reversal of seed deterioration in vegetable seeds. Solid matrix priming (SMP) can be a good means of repairing deterioration and enhancing seedling emergence potential, which is of great concern to transplant producers who often use left-over seed lots (one or two-year-old seeds). SMP offers some advantages, including the ease at which priming can be combined with such growth regulators as GA_3 (Pill & Kilian 2000), beneficial bacteria, fungicides (Parera & Cantliffe 1992) and better aeration (Khan et al 1992).

This study was conducted to test the effect of SMP on cabbage seeds of various ages in terms of enhanced germination, emergence percentages and the rate of germination and emergence, along with electrical conductivity, as an indicator of solute leakage amounts.

2. Material and Methods

Samples of 25 cabbage (*Brassica oleracea* var. capitata) seed lots belong to the Yalova-1 cultivar were obtained from different seed companies over the last three years. The production years of the cabbage lots as follows: 1 and 10 in 2014, 11 and 18 in 2013, 19 and 25 in 2012. The seeds were stored in laminated aluminum foil papers at 5 °C until use. The germination percentage of the seed lots ranged between 68 and 98%. Experiments were conducted on 50 seed samples on total, 25 of which were SMP treated and 25 were untreated.

The solid matrix priming (SMP) treatment carried out by mixing seed, vermiculite (No: 5) and water at the rate of 1:2:2.5 for 16 hours at 25 °C in 100 mL plastic cups (tightly closed), stored in the dark. After the treatment, the seeds were separated from the vermiculite and then dried at 25 °C down to initial seed moisture. Germination, emergence and electrical conductivity tests were conducted within three days of the treatment, during which the seeds were kept at 5 °C.

The treated and untreated seeds were germinated at 20 °C between wet paper towels (20 x 20 cm, Filtrak, Germany) for 14 days (ISTA 2008) in the dark. In each germination test, three replications of 50 seeds were used. Paper towels were rolled and placed into plastic bags in order to prevent water loss. The germinated seeds (2 mm radicle protrusion) were counted daily, and normal and abnormal seedlings were determined after 14 days.

The seedling emergence percentages of both the SMP treated and untreated samples were conducted in a peat moss:perlite mixture of 2:1. Then, three

replications of 50 seeds were sown in seedling trays (25 cm x 34 cm x 6 cm) at a 1 cm depth. The seedling trays were watered everyday and emerge tests were carried out at 20±1 °C for 20 days at controlled climatic room. Light intensity was 72 µmol m^2 s^{-1} in a cycle of 16 h of light and 8 h of dark. The emerged seedlings were controlled daily (cotyledons parallel to surface) for 20 days. The mean germination and mean emergence times were calculated on the basis of daily counts using the Equation 1 and 2 (Mavi et al 2010).

$$MGT/MET= \Sigma (nd)/\Sigma n \quad (1)$$

Where; n, number of seeds newly emerged at time d; d, days from sowing, beginning of tests.

$$\Sigma n= \text{final germination/emergence (\%)} \quad (2)$$

For the electrical conductivity test, two replicates of 50 seeds in SMP treated and untreated seed samples were weighed and soaked in 40 mL of distilled water for 8 hours at 20 °C in the dark. The electrical conductivity of the seed soak water was measured using a conductivity meter (Schott-Gerate, GmbH Hofheim) and expressed as per gram of seeds (µS cm^{-1} g^{-1}). There is no accredited procedure in ISTA rules for Brassica seeds. Therefore procedure was conducted according to Mathews et al (2009).

A statistical analysis was performed using SPSS to carry out (for Windows 15.0) to test to compare mean of treated and untreated cabbage lots. The regression values (R^2) between the various germination and emergence criteria were calculated and the percentages were angle transformed prior to analysis.

3. Results

SMP was found to increase the germination and emergence percentages except lot 1, 2, 8, 10 in germination test and lot 1 in emergence test (Figures 1 and 2). The difference between the treated and untreated seed lots was greater in those lots that had lower germination and were untreated. The difference between the SMP-treated seed germination and untreated lots was found to be significant in 13 lots ($P<0.05$) in the germination test, and in 22 lots in the emergence test (Figures 1 and 2). A greater effect of SMP was seen in the older seeds lots that recorded seed germination at lower percentages. SMP treatment increased germination rates, indicated by a reduced time to germination and emergence (Figures 3 and 4). MGT values ranged from 39 h to 138 h in 25 seed lots. In the treated seed lots, MGT values were reduced to 31 h and 69 h in the fastest and the lowest germinating lots. Then the advantage of the treatment on MGT was changed between 8 and 69 hours. Similar effects were seen also in the mean emergence time values, which were lower in the treated seed lots than in the untreated ones, in all lots except lot 22.

The electrical conductivity (EC) of solute leakage after treatment was reduced, and a higher germination was accompanied with lower EC values in not only the treated, but also the untreated lots (Figure 5). In all seed lots, SMP treatment reduced the amount of solute leakage from the seeds. EC affected the germination percentages in both the untreated and treated lots, and the relationship was highly significant ($P<0.001$, $R^2= 0.60$ in untreated lots, $R^2= 0.49$ in treated lots).

The MGT and MET values were highly related to the EC levels in the cabbage seeds (Figures 6 and 7). The fast germinating or emerging of seed lots showed less solute leakage, indicating that good-quality seeds have the potential to emerge faster, which is related to the EC level in cabbage lots.

4. Discussion

The results of present study showed that solid matrix priming increased germination and seedling emergence percentages (Figures 1 and 2), reduced mean germination and emergence times in a large number of cabbage seed lots (Figures 3 and 4). The seed lots used in this study were obtained from different companies and different production years. Solid matrix priming was found to enhance the germination and emergence percentages in various crop seeds, either in modules in greenhouse or under field conditions (Parera & Cantliffe 1991; Khan et al 1992; Jett et al 1996; Pill & Kilian 2000;

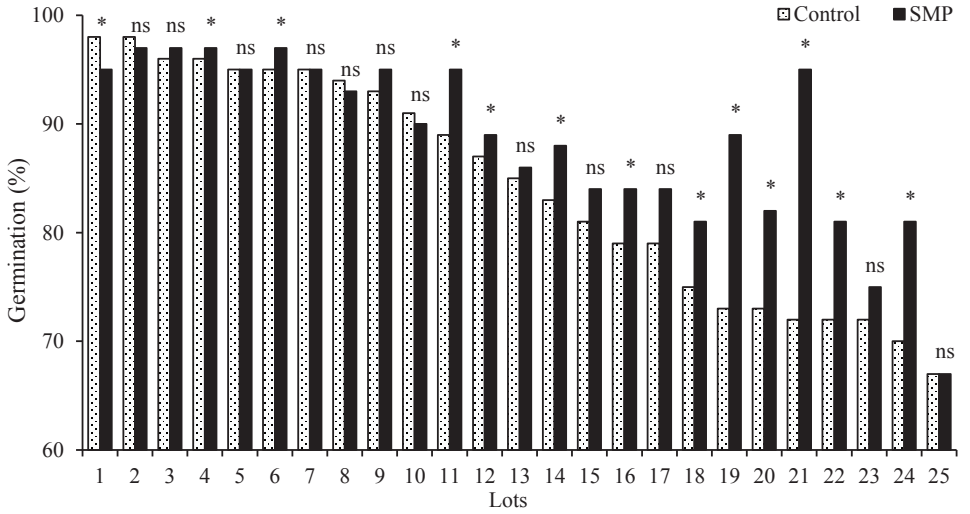


Figure 1- The effect of solid matrix primed (SMP) treatments on germination percentages of 25 different cabbage seed lots. An asterisks next to a lot indicates that the difference between the SMP and control samples was significant (P<0.05)

Şekil 1- Katı madde priming'in (KMP) 25 farklı lahana tohum partisinde çimlenme oranına etkisi. Her tohum partisinin üzerindeki yıldız işaretleri KMP ile kontrol örnekleri arasındaki farkın istatistiksel olarak (P<0.05) anlamlı olduğunu göstermektedir

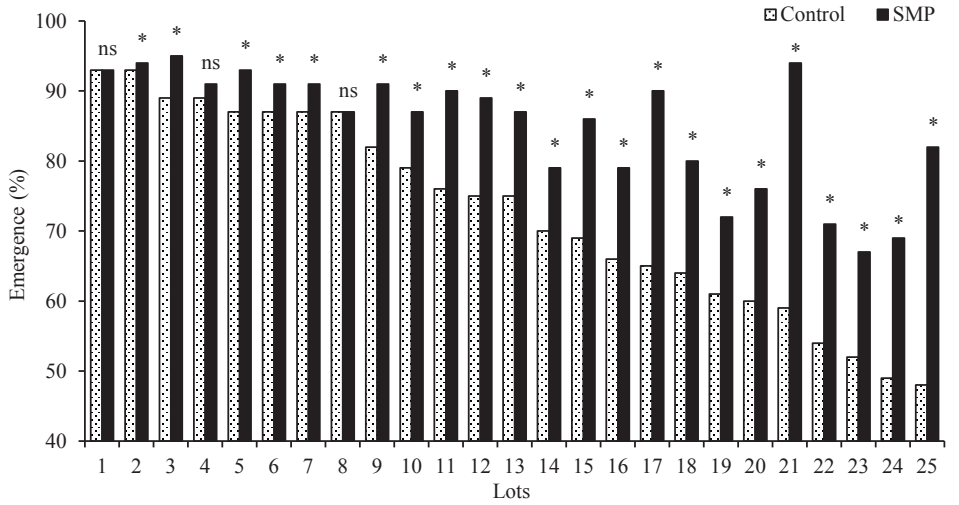


Figure 2- The effect of SMP on the emergence percentages of 25 cabbage seed lots. An asterisks next to a lot indicates that the difference between the SMP and control samples was significant (P<0.05)

Şekil 2- Katı madde priming'in (KMP) 25 farklı lahana tohum partisinde çıkış oranına etkisi. Her tohum partisinin üzerindeki yıldız işaretleri KMP ile kontrol örnekleri arasındaki farkın istatistiksel olarak (P<0.05) anlamlı olduğunu göstermektedir

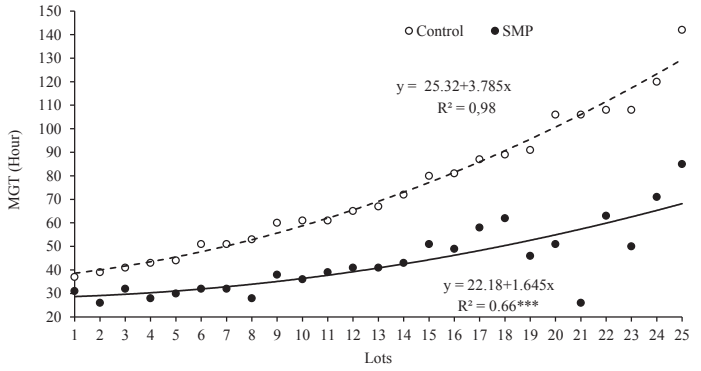


Figure 3- Changes in mean germination times (MGT) of SMP (●) and control (○) cabbage seed lots

Şekil 3- Lahana tohum partilerinde KMP (●) ile kontrol (○) arasında ortalama çimlenme hızı arasındaki değişim

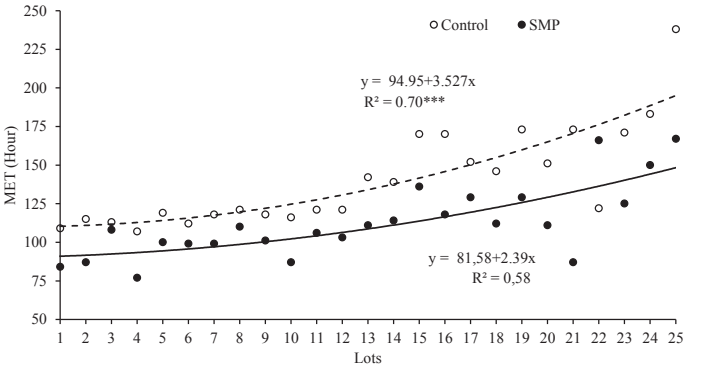


Figure 4- Changes in the mean emergence time (MET) of SMP (●) and control (○) cabbage seed lots

Şekil 4- Lahana tohum partilerinde KMP (●) ile kontrol (○) arasında ortalama çıkış hızı arasındaki değişim

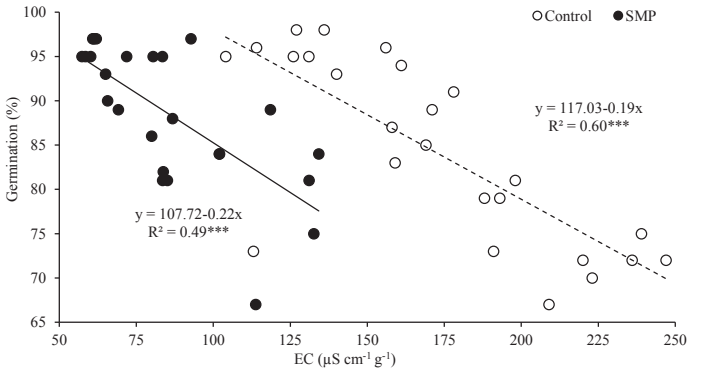


Figure 5- The relationship between EC and SMP (●) and control (○) cabbage seed lots

Şekil 5- Lahana tohum partilerinde KMP (●) ile kontrol (○) arasında elektriksel iletkenlik bakımından ilişki

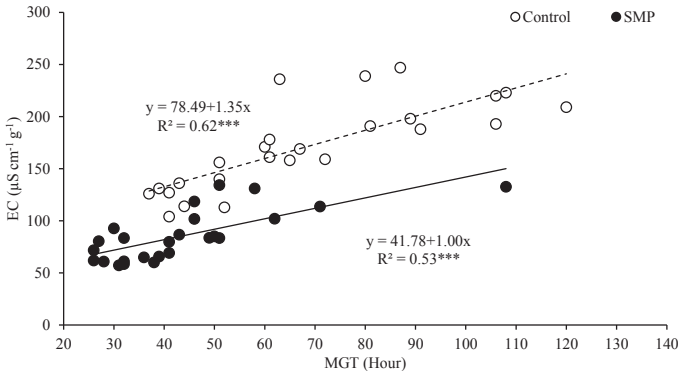


Figure 6- The relationship between mean germination time (MGT) and EC of SMP (●) and control (○) cabbage seed lots

Şekil 6- Lahana tohum partilerinde ortalama çimlenme zamanı (OÇZ) ile elektriksel iletkenlik bakımından KMP (●) ile kontrol (○) grubu arasındaki ilişki

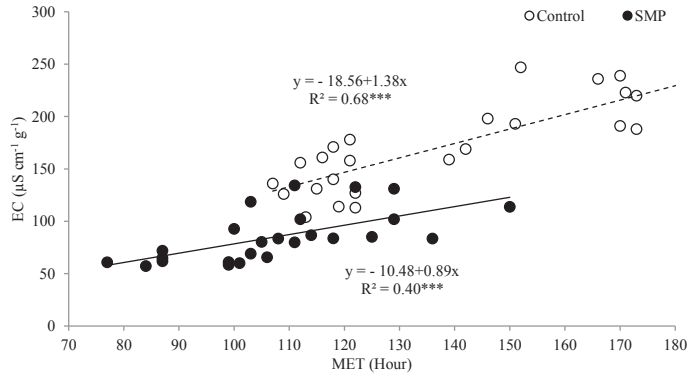


Figure 7- The relationship between mean emergence time (MET) and EC of SMP (●) and control (○) cabbage seed lots

Şekil 7- Lahana tohum partilerinde ortalama çıkış zamanı (OÇEZ) ile elektriksel iletkenlik bakımından KMP (●) ile kontrol (○) grubu arasındaki ilişki

Pandita et al 2010). Our results support the findings of previous studies of different crops regarding the earlier emergence of SMP treated lots. Our work reveals two important findings: 1) solid matrix priming can be used to repair ageing in left over and 2) can also be used for fast and well-developed transplant productions in cabbage seed lots.

In most seed companies, the seeds used in the production year may not necessarily be produced that same year. In some cases, seeds are left over from earlier production years, and may be earmarked for transplanting production season. In such cases, seed ageing may occur at different rates, depending on storage conditions (McDonald 1999), and seed quality may decline. This seed ageing process can be more severe in certain seeds, like cabbage. However, if this ageing level goes down to below 70%, it appears that matrix priming treatment may not be effective. This can be seen in control seeds in Figure 1.

This study revealed that seed quality, indicated by germination percentage, and emergence percentage and rates can be enhanced by SMP. SMP can be also used to repair ageing and make left-over seed lots usable. No hybrid seeds were included in the study, although improvements in seed quality or the repair of ageing can be more valuable in hybrid seeds due to their high cost. One of the basic problems of solid matrix priming is separating seeds from matrix material after the treatment. Normally researchers do this through sieving the material (Taylor et al 1988). In our work we separated seeds by hand. However, large amount of seeds can be primed in plastic perforated bags which let the seeds to take water but not get out. So seeds after the treatment can be easily taken out from the medium.

Fast germination and emergence following SMP will lead to larger seedlings and well-developed transplants in the modules (Mavi et al 2010). A large number of studies have indicated that earlier emergence results in larger transplants in either greenhouse or field conditions (Mavi et al 2010; Matthews et al 2012). One reason for fast emergence can be the completion of the first two imbibitions phases in the seed germination stages.

The repair mechanism of SMP or other priming treatments may involve various biochemical mechanisms, such as enzymes (Powell et al 2000; Sung & Chiu 2001), hormonal activity (Kepczynska et al 2007) or cell wall rejuvenation (McDonald 1999). In the present study, it was found that SMP reduced EC levels in treated seeds when compared to the control, which indicates that the treatment helped to rejuvenate cell structure and reduce leakage from the cell. Solid leakage is an indicator of a compact cell structure and is used as a seed vigor test (Matthews et al 2012).

High leakage (EC) is negatively correlated with seedling emergence and germination percentages. Moreover, a shorter time to germination (MGT and MET) is well correlated with high germination and emergence percentages. This indicates that treated seeds germinate faster and produce larger seedlings and such treatments repair cell wall structures that have deteriorated as a result of ageing, resulting in better performances in terms of germination and emergence.

SMP was found to be effective regarding the use of O_2, cheap and easy to apply in vegetable seeds (Khan 1992). The method is also considered as optimum for both larger seed species, such as beans (Parera & Cantliffe 1991), and smaller ones (Jett et al 1996). SMP has been used extensively to enhance emergence, however the present work makes a new approach, using SMP for the activation of the repair mechanism which can be used for the recovery of cabbage seed that has suffered rapid seed quality deterioration due to adverse conditions. Moreover, it can be considered a valuable approach for transplanting production companies, allowing them to save left over seeds. The effect of SMP is based on reducing seed leakage from the cell walls, among other physiological changes (Kepczynska et al 2007).

In conclusion, SMP treatments can be used enhance stored and relatively low-quality cabbage seed lots in terms of germination and emergence percentages, and can be considered a valuable method in ensuring strong, fast and high-quality cabbage transplants. Enhancing seed quality in aged seeds can also be used for saving left-over seeds in the production system which can be economically advantageous for companies.

References

Carlos A P, Ping Q & Daniel J D (1993). Enhanced celery germination at stress temperature via solid matrix priming. *HortScience* **28**(1): 20-22

Hacısalihoglu G (2007). Germination characteristics of three warm-season turfgrasses subjected to matriconditioning and aging. *HortTechnology* **17**(4): 480-485

Heydecker W & Coolbear P (1977). Seed treatments for improved performance, survey and attempted prognosis. *Seed Science and Technology* **5**: 353-425

ISTA (2008). International Rules for Seed Testing. International. Seed Testing Association, Bassersdorf, Switzerland

Jett L W, Welbaum G E & Ronald D M (1996). Effects of matric and osmotic priming treatments on broccoli seed germination. *Journal of the American Society for Horticultural Science* **121**(3): 423-429

Kepczynska E, Piekna-Grochala J & Kepczynski J (2007). Seed germination of two tomato cultivars following matrix-conditioning under optimal and stress temperatures. *Seed Science and Technology* **35**: 749-753

Khan A A (1992). Preplant physiological seed conditioning. *Horticultural Reviews* **13**: 131-181

Khan A A, Maguire J D, Abawi G S & Ilyas S (1992). Matriconditioning of vegetable seeds to improve stand establishment in early field plantings. *Journal of the American Society for Horticultural Science* **117(**1): 41-47

Kubik K K, Eastin J A, Eastin J D & Eskridge K M (1988). Solid matrix priming of tomato and pepper. Proc. Int. Conf. Stand Estab. Hortic Crops. Lancaster pp. 86-96

Matthews S, Demir I, Celikkol T, Kenanoglu B B & Mavi K (2009). Vigour tests for cabbage seeds using electrical conductivity and controlled deterioration to estimate relative emergence in transplant modules. *Seed Science and Technology* **37**: 736-746

Matthews S, Noli E, Demir I, Khajeh-Hosseini M & Wagner M H (2012). Evaluation of seed quality: from physiology to international standardization. *Seed Science Research* **22**: 69-73

Mavi K, Demir I & Matthews S (2010). Mean germination time estimates the relative emergence of seed lots of three cucurbit crops under stress conditions. *Seed Science and Technology* **38**: 14-25

McDonald M B (1999). Seed deterioration: Physiology, repair and assessment. *Seed Science and Technology* **27**: 177-237

Pandita V K, Anand A, Nagarajan S, Seth R & Sinha S N (2010). Solid matrix priming improves seed emergence and crop performance in okra. *Seed Science and Technology* **38**: 665-674

Parera C A & Cantliffe D J (1991). Improved germination and modified imbibitions of *shrunken2* sweet corn by seed disinfestations and solid matrix priming. *Journal of the American Society for Horticultural Science* **116**: 942-945

Parera C A & Cantliffe D J (1992). Enhanced emergence seedling vigor in shrunken-2 sweet corn via seed disienfection and solid matrix priming. *Journal of the American for Horticultural Science* **117**: 400-403

Pill G W & Kilian A E (2000). Germination and emergence of parsley in response to osmotic or matric seed priming and treatment with gibberellins. *HortScience* **35**(7): 907-909

Powell A A, Yule L J, Jingh H C, Groots P C S, Bino R J & Pritchard W H (2000). The influence of aerated hydration seed treatment on seed longevity as assessed by the viability equation. *Journal of Experimental Botany* **51**(353): 2031-2043

Sung J M & Chiu K Y (2001). Solid matrix priming can partially reverse the deterioration of sweet corn induced by 2.2. azobis hydrochloride generated free radicals. *Seed Science and Technology* **29**: 287-298

Taylor A G, Klein D E & Whitlow T H (1988). Solid matrix priming of seeds. *Scientia Horticulturae* **37**: 1-11

Wang H Y, Chen C L & Sung J M (2003). Both warm water soaking matriconditioning treatments enhance anti-oxidation of bitter gourd seeds germinated at sub-optimal temperature. *Seed Science and Technology* **31**: 47-56

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Chlorella vulgaris Üretimi ve Sera Organik Domates Yetiştiriciliğinde Biyogübre Olarak Kullanımının Etkileri

Sena ÖZDEMİR[a], Atakan SUKATAR[a], Gölgen Bahar ÖZTEKİN[b]

[a]*Ege Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, 35100, İzmir, TÜRKİYE*

[b]*Ege Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, 35100, İzmir, TÜRKİYE*

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Atakan SUKATAR, E-posta: atakan.sukatar@ege.edu.tr, Tel: +90 (232) 311 24 47
Geliş Tarihi: 26 Şubat 2015, Düzeltmelerin Gelişi: 01 Eylül 2015, Kabul: 04 Eylül 2015

ÖZET

Chlorella vulgaris mikroalginin üretilmesi ve biyogübre olarak kullanımının domates bitkisi üzerinde bitki gelişimi, verim ve meyve kalitesine etkilerinin araştırılması amacıyla yürütülen çalışmada, C. *vulgaris* tübüler fotobiyoreaktörde üretilmiş ve serada organik domates (cv. Şimşek) yetiştiriciliğinde 3 farklı formda [toprağa toz alg uygulaması (2.5 g fide^{-1}), toprağa sıvı alg uygulaması (250 mL fide^{-1}), yaprağa sıvı alg spreylenmesi] denemeye alınmıştır. Alg uygulanmayan bitkiler kontrol grubunu oluşturmuştur. Deneme tesadüf parseli deneme desenine uygun olarak kurulmuş; üretim 2014 yılı Mart-Haziran aylarında gerçekleştirilmiştir. Elde edilen sonuçlar *C. vulgaris*'in biyogübre olarak kullanımının bitki gelişimi, verim ve bazı meyve kalite parametrelerini (kuru ağırlık, toplam suda çözünür kuru madde, titre edilebilir asit ve vitamin C) artırdığını; kullanılan uygulamalar içerisinde özellikle toprağa kuru alg uygulamasının daha iyi sonuçlar verdiğini; doğa dostu bir gübre olarak *C. vulgaris*'in organik tarımda kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Mikroalg; Organik tarım; Bitki gelişimi; Verim; Kalite

Production of *Chlorella vulgaris* and Its Effects on Plant Growth, Yield and Fruit Quality of Organic Tomato Grown in Greenhouse as Biofertilizer

ARTICLE INFO
Research Article
Corresponding Author: Atakan SUKATAR, E-mail: atakan.sukatar@ege.edu.tr, Tel: +90 (232) 311 24 47
Received: 26 February 2015, Received in Revised Form: 01 September 2015, Accepted: 04 September 2015

ABSTRACT

This study was carried out to examine the production of *Chlorella vulgaris* and its effects on growth, yield, and fruit quality of organically grown tomato production in greenhouses, *C. vulgaris* was cultured in a tubular photobioreactor system and was used in three different forms [dry algae application to soil (2.5 g seedling^{-1}), liquid algae application to soil (250 mL seedling^{-1}), foliar spray] used as biofertilizer on tomato production (cv. Simsek). Plants with no algae application were used as control. The experiment was designed according to randomized parcel and production was

performed between March and June of 2014. Obtained results showed that using *C. vulgaris* as a biofertilizer increased plant growth, yield and some fruit quality (dry weight, total soluble solids, titratable acidity and vitamin C); among the used treatments, applications to soil -especially dry algae- showed better performance; *C. vulgaris* may be used as a nature-friendly fertilizer in organic farming.

Keywords: Microalgae; Organic agriculture; Planth growth; Yield; Quality

1. Giriş

Dünyada artan nüfus ile birlikte tarım yapılabilecek arazilerin de giderek azalmasıyla daha fazla gıda üretimine ihtiyaç duyulmuş ve birim alandan daha fazla ürün elde etmek zorunda kalınmıştır. Bu amaçla da kimyasal gübre kullanımı oldukça artmıştır. Tarım alanlarındaki bu yoğun kimyasal kullanımı, verim ve üretimi artırmış fakat sürdürülebilir toprak verimliliği ile birlikte doğal dengenin bozularak tehlikeye girmesine sebep olmuştur. Sonuçta da başta gelir düzeyi yüksek ülkelerde olmak üzere birçok ülkede üretici ve tüketiciler örgütlenerek insanlarda toksik etki yaratmayan ve doğayı tahrip etmeyen yöntemlerle üretilen tarımsal ürünleri tercih etmeye başlamışlardır. Bu amaçla insan ve çevreye dost üretim sistemlerini içeren, kimyasal gübre ve ilaçların kullanımını yasaklayan, organik gübreleme ile ekim nöbeti uygulamayı, parazit ve predatörler gibi doğal kaynaklardan yararlanmayı tavsiye eden ve üretimde ürünün kalitesinin yükselmesini amaçlayan bir üretim şekli olan "organik tarım'' ortaya konmuştur (Hekimoğlu & Altındeğer 2006).

Organik tarımda sınırlı olan gübreler konusunda yapılan alternatif arayışlar, alglerin gübre olarak kullanılabileceğini göstermiştir (Abetz 1980; Şimşek 1995). İlk defa Norveç, İrlanda, Fransa ve Amerika gibi denize kıyısı olan ve denizsel alglerin bol bulunduğu ülkelerde mevcut algleri değerlendirmek için bu alglerden yararlanma yolları aranmış ve verimsiz toprakların değerlendirilmesinde alglerin gübre olarak kullanımıyla ilgili ilk adımlar atılmıştır (Whapham et al 1994; Güner & Aysel 1996; Kumbul 2000). Şimdiye kadar yapılan biyogübre çalışmalarında ağırlıklı olarak denizsel algler (kelpler) ve Cyanophyta üyesi filamentöz algler kullanılmış, mikroalglerin kullanımı ise geri planda kalmıştır (Sivasankari et al 2006; Selvam & Sivakumar 2014) .

Biyoteknolojik uygulanabilirliği en yüksek olan mikroalg türlerinden *C. vulgaris* geniş çapta ticarileştirilmiş olup, insanlar tarafından gıda takviyesi ve hayvanlar için yem katkı maddesi olarak kullanılmaktadır. Chlorophyta üyesi olan bu alg, % 42-58 gibi yüksek protein içeriğine sahip olması nedeniyle alternatif protein kaynağı olarak görülmekte ve birçok ülke tarafından çeşitli amaçlar için kültüre alınmaktadır (Safi et al 2014). Tarımda genellikle azot kaynağı olarak kullanılan kimyasal gübrelerin yerine, *C. vulgaris* gibi yüksek protein içeriğine sahip olan alglerin kullanımı, daha ucuz ve çevre sağlığına zarar vermeyen bir uygulama olacaktır. Ancak gerek dünyada ve gerekse ülkemizde mikroalglerin biyogübre olarak kullanılması ile ilgili yapılan araştırmalar sınırlı sayıda ve dar kapsamlıdır.

Yürütülen bu çalışmada, yeşil alglerden zengin protein içeriğine sahip *C. vulgaris'*in tübüler fotobiyoreaktörle geniş ölçekli üretimi yapılmış ve elde edilen biyogübre ülkemizin birçok kesiminde yetiştirilen ve büyük bir tarımsal öneme sahip domates bitkisinde denenmiş, biyogübre uygulamasının; bitki gelişimi, verim ve meyve kalite parametreleri üzerine etkileri incelenmiştir. Böylelikle *C. vulgaris*'in özellikle organik tarımda kimyasal gübrelere alternatif olarak kullanılabilecek bir biyogübre olup olmayacağı araştırılmıştır.

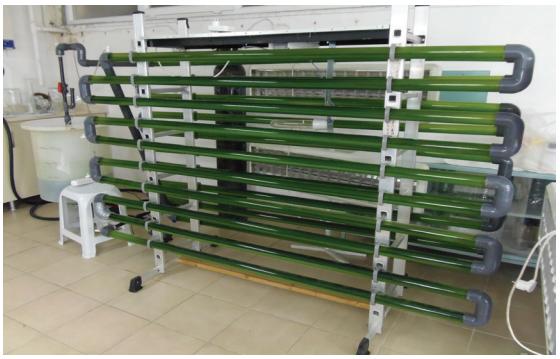
2. Materyal ve Yöntem

Ekim 2013-Temmuz 2014 döneminde yürütülen bu araştırma, (I) *C. vulgaris* mikro alginin üretilmesi ve (II) elde edilen mikroalgin serada organik domates yetiştiriciliğinde biyogübre olarak kullanılması şeklinde iki aşamada gerçekleştirilmiştir.

2.1. Chlorella vulgaris mikroalginin üretilmesi

Kullanılan *C. vulgaris* türü Ege Üniversitesi (E.Ü.), Mikroalg Kültür Koleksiyonu'ndan (EGE-MACC) temin edilmiş ve E.Ü. Fen Fakültesi, Biyoloji Bölümü'nde tübüler fotobiyoreaktörde, Bold Basal Medium (BBM) (pH: 6.8) kültürü kullanılarak üretilmiştir (Guillard 1973; Sukatar 2002). Üretimin ilk aşaması olan aşı kültüründe mikroalgler BBM ortamı içeren 70 mL tüp içerisinde 1 hafta inkübe edilmiş, süre sonunda 15 günlük aralıklarla 250 mL, 500 mL ve 5 L'lik hacimlere (25 °C, 12 saat karanlık-12 saat aydınlık, 20 µmol foton m^{-2} s^{-1} ışık şiddeti altında) aktarılmıştır. Başlangıçta elle çalkalama şeklinde yapılan alg karıştırma işlemi, hacim büyüdükçe besleme sistemine hava üflemesi ile gerçekleştirilmiştir. 150 L'lik tübüler fotobiyoreaktöre aktarıma uygun olup olmadığını belirlemek için de 2 gün ara ile Neubauer lamı (Marienfield, 0.0025 m^2) kullanılarak mikroskopta hücre sayımı yapılmıştır. Kültür hacminin artırılması sırasında trinokuler mikroskoba bağlı kamera (CX31RTSF-5, Olympus) ile hücre boyutu, rengi ve şekline bakılmış; alglerin hücre morfolojileri incelenmiştir.

C. vulgaris'in büyük çaplı üretiminde toplam üretim kapasitesi 150 L olan, ortasında fotoperiyodu ayarlanabilir fotosentez lambası bulunan, her biri 195 cm uzunluk ve 5 cm çapa sahip yatay olarak yerleştirilmiş 20 adet akrilik borudan ve 100 L'lik toplama tankından oluşan tübüler fotobiyoreaktör kullanılmıştır (Şekil 1). Toplama tankındaki alg kültürünün sirkülasyonunu sağlamak için pompa (New Jet 3000 model, 55W, 3000 L kapasiteli, max. su yüksekliği: 2.90 m) kullanılmıştır. Fotobiyoreaktörde sirküle olan kültürün akış hızı saniyede 20 cm yer değiştirme şeklinde olmuştur.



Şekil 1- Tübüler fotobiyoreaktörün genel görünümü

Figure 1- General view of tubuler photobioreactor

Tübüler fotobiyoreaktörde üretim arka arkaya 2 tekrarlı olarak gerçekleştirilmiştir. Her tekrarda, toplama tankı ve biyoreaktörün sterilizasyonu çeşme suyu ve ardından % 16'lık sodyum hipoklorit ilaveli su ile sağlanmıştır. Her tekrarda 150 L'lik biyoreaktöre 5 L'lik *C. vulgaris* kültürü aşılanmış ve üretim 12:12 saat (aydınlık:karanlık) döngüsünde, 25 gün sürdürülmüştür. Her iki üretimde de toplama tankından günlük örnekler alınarak pH ve sıcaklık ölçümleri yapılmış, mikroskop altında hücre sayısı, şekil ve yapısı belirlenmiştir. Üretim süreci sonunda sistem içerisindeki 100 L'lik *C. vulgaris* kültürü şişelere aktarılmış ve -20 °C'de saklanmıştır (Cirik & Gökpınar 1993).

Elde edilen sıvı kültür, büyük ölçekli separatörde (CTC-1-06-107, Westfalia GmbH) 10000 rpm'de santrifüjlenmiştir. Separatörden alınan alg pastası 45 °C sıcaklığa ayarlı etüvde kurutumuş, kuru haldeki *C. vulgaris* sıvı azot ile havanda 10 dakika parçalanmıştır (Zheng et al 2011). *C. vulgaris* kuru ekstraktının toplam N miktarı, modifiye Kjeldahl Metodu ile; diğer elementlerin (K, Ca, Mg) analizleri ise nitrik:perklorik asit karışımında yaş yakılmış örneklerle yapılmıştır. Potasyum (K) alev fotometresinde; Ca ve Mg ise absorbsiyon spektrofotometresinde tayin edilmiştir (Kacar 1972).

2.2. Sera denemesi

Sera denemesi 2014 yılı ilkbahar döneminde E.Ü. Ziraat Fakültesi Bahçe Bitkileri Bölümü'nde organik tarım çalışmalarının yapıldığı, PE örtülü-yay çaltılı 520 m^2'lik seranın bir kısmında gerçekleştirilmiştir. Araştırmanın yürütüldüğü sera topraklı (kumlu killi, hafif alkali, kireçli; tuzluluğu düşük, organik maddece zengin) olup, kurulduğu andan bu yana sadece organik sebze üretim ve araştırmalarında kullanılmıştır.

Araştırmada bitkisel materyal olarak seralarda en fazla üretilen tür olan domates (*Solanum lycopersicum)*; çeşit olarak ise ilkbahar yetiştiriciliğine uygun bir salkım domates çeşidi olan Şimşek F_1 (Bircan Tohum, Antalya) kullanılmıştır. Domates fideleri hazır fide firmasından (Antalya Fide A.Ş, Antalya) temin edilmiş ve seraya 04.03.2014 tarihinde 90x50x50 cm mesafeler ile çift sıralı olarak dikilmiştir.

Araştırmada *C. vulgaris*'in 3 farklı uygulama şekli [I: Toprağa toz alg uygulaması (2.5 g fide^{-1}), II: Toprağa sıvı alg uygulaması (250 mL fide^{-1}), III: Yaprağa sıvı alg spreylenmesi (biyoreaktörden çıkan sıvı algin direkt kullanımı ve tüm yaprakların yıkanması şeklinde)] denenmiş ve alg uygulanmayan bitkiler IV: Kontrol grubunu oluşturmuştur. Deneme tesadüf parselleri deneme desenine göre 3 tekrarlı kurulmuş ve her tekrarda 12 bitkiye yer verilmiştir. Toprağa kuru alg uygulaması, dikim öncesi açılan dikim çukurlarına belirtilen miktarın dökülmesi ve üzerine fide dikilmesi şeklinde; sıvı alg ise fide dikiminden sonra fide köklerine belirtilen dozun dökülmesi şeklinde olmuştur. Toprağa sıvı alg uygulamasında fidelere dikim sonrası ayrıca can suyu verilmemiştir. Yapraktan spreyleme ile alg uygulamasında ise tübüler fotobiyoreaktörden çıkan alg direkt kullanılmış, alg tüm yapraklar yıkanacak şekilde fidelere püskürtülmüştür.

Bitkilerin beslenmesinde sadece sera toprağı işlenirken, organik tarım yönetmeliğine uygun olarak taban gübrelemesi (50 kg da^{-1} Biofarm, Çamlı Yem Besicilik, Işıkkent-İzmir) yapılmış ve üretim dönemi boyunca başka gübre kullanılmamıştır. Bitkilerin sulanmasında damla sulama yöntemi kullanılmış (2 L h^1 debiye sahip boru içine entegre damlatıcılı); sulama miktarının belirlenmesinde ise sera içerisine yerleştirilmiş Class A Pan'dan yararlanılmıştır.

Üretimde organik tarım esaslarına bağlı kalınmış; bitki bakım işleri (sardırma, koltuk alma, çapalama, budama, hastalık ve zararlı mücadelesi, hasat vs) Sevgican (2002)'a göre yürütülmüştür. Tozlaşmaya yardımcı olmak amacı ile vibratör kullanılmış; haftada 2 gün sabah erken saatlerde çiçekler sarsılmıştır. Bitki büyümesi 7 salkım üzerinden büyüme ucunun alınması şeklinde durdurulmuştur.

Yetiştirme periyodu boyunca (15.05.2014-30.06.2014) toplam 9 hasat yapılmış, her hasatta konusuna göre elde edilen meyvelerin ağırlıkları alınarak toplam verim (kg m^{-2}), toplanan meyvelerin sayıları alınarak toplam meyve sayısı (adet m^{-2}) belirlenmiştir. Ortalama meyve ağırlıkları (g meyve^{-1}) toplam ağırlık/meyve sayısı ile hesaplanmıştır. Hasat edilen meyveler boylama halkalarından geçirilerek 4 farklı boya (Ø<3.5, 3.5-4.5, 4.5-5.5, Ø>5.5) ayrılmış ve her sınıfa dahil meyvelerin oranı % olarak verilmiştir.

03.06.2014 tarihinde 3. salkımdaki kızaran meyveler hasat edildikten sonra uygulamalara ait her tekerrürden 10 adet homojen meyve seçilerek kalite analizleri yapılmıştır. Meyvelerin sertliği (N) Effegi uçlu FT011 penetrometre (Fruit Tester, Alfonsine, Italy) yardımıyla ölçülmüş; bu meyvelerin yaş ağırlıkları alınarak, 65 ºC sıcaklığa ayarlı etüvde kurutulup tartılmış ve kuru ağırlıkları [KA (g)] belirlenmiştir (Kacar 1972). Toplam suda çözünebilir kuru madde miktarı'nın [TSÇKM (%)] belirlenmesi için parçalayıcı ile parçalanan meyve, kaba filtre kağıdından süzüldükten sonra 1-2 damla örnek, dijital el reflaktoremetresi (Euromex RD 645, The Netherlands) ile okunmuş, yine süzükten alınan örneğin; 0.1 N NaOH çözeltisi ile pH metre (MP220, Mettler Toledo, Schwerzenbach, Switzerland) yardımıyla titrasyonu yapılmış ve titre edilebilir asit miktarı [TA (mval 100 mL^{-1})] harcanan NaOH miktarı üzerinden hesaplanmıştır (Karaçalı 1993). Süzüğe batırılan el tipi EC (Mettler Toledo-MC-126, Schwerzenbach, Switzerland) metre ve masa tipi pH metre (Mettler Toledo-MP220, Schwerzenbach, Switzerland) probu yardımı ile meyve suyu elektriksel iletkenlik [EC (dS m^{-1})] ve pH değerleri belirlenmiştir. Meyve vitamin C içeriği (mg 100 mL^{-1}) oksalik asit ile stabilize edilmiş örneklerin, 2-6 diklorofenlindefenol boya maddesi ile renklendirilmesi esasına göre spektrofotometrik (Varian Cary 100 UV-Visible spektrofotometre; Varian, Inc., Polo Alto, California, USA) yöntemle belirlenmiştir (Pearson 1970). Seçilen meyvelerin rengi renkölçerle (Minolta CR-300, Japan) L

[parlaklık (L)], a (pozitif a kırmızı, negatif a yeşil) ve b (pozitif b sarı, negatif b mavi) üzerinden belirlenmiştir (McGuire 1992).

Üretim dönemi sonunda 23.06.2014 tarihinde her uygulamadan seçilen 2 bitkide bitki gelişim ve biyomas değerlerine bakılmıştır. Bitkiler sökülmeden toprak yüzeyinden büyüme ucuna kadar olan gövdede şerit metre yardımı ile bitki boyu (cm); dijital kumpas ile gövdenin orta yerinden gövde çapı (mm) ölçülmüş; daha sonra sökülen bitkilerin kök boyu ölçülmüş ve bitki kök, gövde, yaprak, salkım ve meyvelerine ayrılarak tartılıp yaş ağırlıkları, 65 °C sıcaklığa ayarlı etüvde kurutulup tartılarak da kuru ağırlıkları (g) belirlenmiştir (Kacar 1972).

Hasat döneminde her tekerrürden alınan genç yaprakların renkölçerle L, a ve b değerleri ile a ve b değeri üzerinden hesaplanan Hue ve Kroma renk değerleri belirlenmiş, aynı yaprakların % 80'lik aseton ile homojenize edildikten sonra spektrofotometrik yöntem ile klorofil a, klorofil b ve toplam klorofil değerleri (mg L^{-1}) belirlenmiştir (Arnon 1949).

Araştırmadan elde edilen verilere, bir SPSS programı olan PAWS Statics 18 istatistiksel analiz paket programı kullanılarak varyans analizi uygulanmış ve ortalamalar arasındaki farklılıkları belirlemek için % 5 önem düzeyinde Duncan testi yapılmıştır. Tablolarda olasılık (*P*) değerleri verilmiştir.

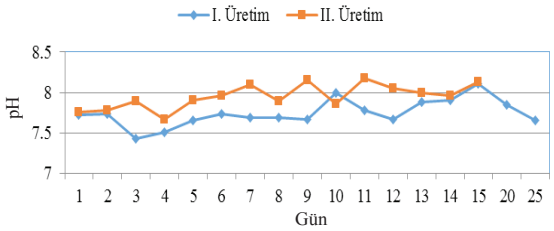
3. Bulgular ve Tartışma

3.1. Chlorella vulgaris üretimi ile ilgili bulgular

Yapılan her iki üretim sonucunda tübüler fotobiyoreaktörde sıvı olarak toplam 200 L alg; toplam miktarın santrifüjlenmesi ile 240.2 g pasta kıvamında alg biyoması ve pastanın kurutulması ile de 58.42 g kuru alg elde edilmiştir.

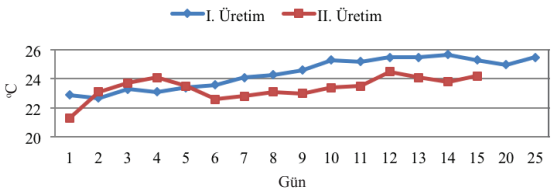
Çoğaltılarak elde edilen *C. vulgaris*'in yapılan element analizinde içeriğinin % 5.49 N, % 0.72 K, % 5.39 Ca ve % 0.69 Mg olduğu belirlenmiştir.

C. vulgaris alginin tübüler fotobiyoreaktörde üretimi sırasında elde edilen pH 7.2 ile 8.1 arasında değişim göstermiştir (Şekil 2). pH'ın üretim sürecinde giderek arttığı gözlenmiş ve sisteme saf CO_2 verilerek pH istenilen aralıkta tutulmaya çalışılmıştır. Sistemde sıcaklık iki dönemde de 21.3 ile 25.7 °C arasında değişm göstermiştir. Üretimin sonuna doğru hücre sayısında artmalara bağlı olarak sıcaklığın da arttığı gözlenmiştir (Şekil 3). Üretim boyunca her gün yapılan hücre sayımları, *C.vulgaris* hücresinin I. üretimde 464 ile 2380; II. üretimde 311 ile 897x10^4 hücre mL^{-1} arasında değiştiğini göstermiştir (Şekil 4).



Şekil 2- Tübüler fotobiyoreaktörde *C. vulgaris*'in üretiminde pH'nın değişimi

Figure 2- pH changes during the production of C. vulgaris in tubuler photobioreactor

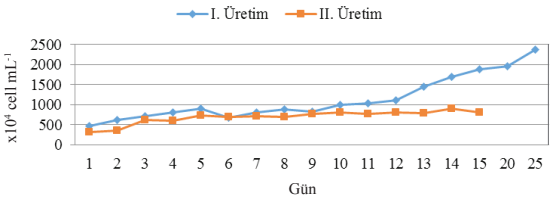


Şekil 3- Tübüler fotobiyoreaktörde *C. vulgaris*'in üretiminde sıcaklığın değişimi

Figure 3- Temperature changes during the production of C. vulgaris in tubuler photobioreactor

Yürütülen bu çalışmada *C. vulgaris*'ten biyogübre elde etmek üzere bir tasarım olarak oluşturulan tübüler fotobiyoreaktörün avantajları yanında akrilik borularda belirli bir günden sonra meydana gelen alg yapışmaları ve bunların temizliğindeki zorluklar gibi büyük dezavantajları da olmuştur (Naz & Gökçek 2004). Bu amaçla, ilk olarak HCl çözeltisiyle borulardaki yapışma yok edilmeye çalışılmış; başarılı olamayınca % 16'lık sodyum hipoklorit ile yapışma temizlenmiştir. Fakat hem kullanılan maddenin

kimyasal olması hem de akrilik borularda aşınma ve matlaşma yapması ayrıca matlaşma nedeni ile üretilen alg hücrelerinin ışığı eşit oranda alamaması ihtimali ile başka temizleme yöntemleri denenmiş ve en etkin yöntemin borularla aynı çapta süngerlerin sistemde döndürülerek yapılan mekanik temizleme şekli olduğu görülmüştür. Mekanik temizlemede sisteme atılan süngerin borularda ilerleyebilmesi için pompanın gücünün de bu süngeri döndürebilecek kapasitede olması ve sistemdeki boruların süngeri toplama tankına düşürebilecek şekilde tasarlanması gerekmektedir.



Şekil 4- Tübüler fotobiyoreaktörde *C. vulgaris*'in üretiminde hücre sayısının değişimi

Figure 4- Cell number changes during the production of C. vulgaris in tubuler photobioreactor

3.2. Sera denemesine ait bulgular

C. vulgaris'in farklı uygulamaları kontrole göre bitki boyu, gövde çapı ve kök boyunu artırmış ancak bu etki istatistiksel olarak (P>0.05) önemsiz bulunmuştur (Çizelge 1). En uzun bitki boyu ve en kalın gövde çapı toprağa sıvı alg uygulamasından; en uzun kök boyu yapraktan alg spreylenmesi uygulamasından elde edilmiştir (Çizelge 1).

Çizelge 1- Bitki boyu, gövde çapı ve kök boyu üzerine uygulamaların etkisi

Table 1- Effects of treatments on plant height, stem diameter and root lenght

Uygulama	*Bitki boyu (cm)*	*Gövde çapı (mm)*	*Kök boyu (cm)*
Toprağa sıvı	189.7	15.8	24.9
Toprağa kuru	189.2	15.1	27.1
Yaprağa sprey	186.9	14.9	28.2
Kontrol	181.8	12.4	22.2
P	0.251	0.221	0.780

Üretim dönemi sonunda sökülen bitki organlarına ait yaş ve kuru ağırlıklara farklı alg uygulamalarının etkileri Çizelge 2'de verilmiştir. Uygulamaların yaprak yaş ağırlığı (P= 0.024), meyve yaş (P= 0.004) ve kuru (P= 0.000) ağırlığı üzerine etkisi istatistiksel olarak önemli bulunmuştur. Kontrol uygulaması ile karşılaştırıldığında toprağa kuru alg uygulaması en başarılı sonucu vermiş ve toplam bitki yaş ağırlığını % 32.7, toplam bitki kuru ağırlığını % 32.5 oranında artırmıştır. Dört ay süren yetiştiricilik süresi sonunda önceki çalışmalara benzer şekilde, uygulama şekli fark etmeksizin tüm bitkilerin gelişiminin kontrol bitkilerinden daha fazla olduğu belirlenmiştir (Kumbul 2000).

Uygulamaların toplam verim, toplam meyve adedi ve ortalama meyve ağırlığı üzerine etkisi önemli bulunurken, meyve sınıflandırması üzerine etkisi önemsiz bulunmuştur. Alg uygulamaları verim değerlerini artırmış; toplam verim değerleri 15.11-10.22 kg m^{-2} arasında değişmiş ve en yüksek toplam verim toprağa kuru alg uygulamasından elde edilmiştir (Çizelge 3). Kontrol uygulaması ile

Çizelge 2- Bitki organlarına ait biyomas değerleri (g) üzerine uygulamaların etkisi

Table 2- Effects of treatments on biomass of plant tissue (g)

Uygulama	*Yaprak*		*Gövde*		*Salkım*		*Meyve*		*Kök*	
	YA	*KA*	*YA*	*KA*	*YA*	*KA*	*YA*	*KA*	*YA*	*KA*
Toprağa sıvı	733.5 a	148.1	291.4	56.4	44.7	11.8	3670.7 b	256.7 b	25.5	6.2
Toprağa kuru	781.0 a	161.9	357.9	65.8	66.9	15.8	4231.9 a	291.5 a	34.8	8.6
Yaprağa sprey	680.5 a	134.5	255.8	50.4	47.3	11.9	3494.6 b	261.9 b	34.3	8.3
Kontrol	501.2 b	111.8	254.2	49.7	42.0	9.9	2863.6 c	189.2 c	23.6	6.5
P	0.024	0.139	0.326	0.430	0.132	0.118	0.004	0.000	0.406	0.398

YA, yaş ağırlık; KA, kuru ağırlık

karşılaştırıldığında toplam verim toprağa sıvı, toprağa kuru ve yaprağa sprey uygulamalarında sırasıyla % 21.9, 32.4, 18.1 oranında artış göstermiştir. Toplam meyve adedi 32.4 ile 24.2 adet m^{-2} arasında; ortalama meyve ağırlığı 135.2 ile 118.9 g arasında değişm göstermiş; en yüksek değerler alg uygulamalarından elde edilmiştir. Toprağa sıvı alg uygulamasında ortalama meyve ağırlığının düşük ancak toplam meyve adedinin yüksek olduğu görülmüştür. Meyve sınıflandırılmasında çapı 5.5 cm'den büyük 1. boy meyveler en fazla % 78.4 ve % 77.4 ile toprağa sıvı ve kuru alg uygulaması yapılan bitkilerden, çapı 3.5 cm'den küçük olan 4. boy meyveler ise en fazla % 4.5 ile kontrol grubundaki bitkilerden; en az ise % 0.6 ile toprağa kuru alg uygulaması yapılan bitkilerden alınmıştır (Çizelge 3).

Verim değerleri kullanılan çeşide, iklim koşullarına, üretim sistemine, yetiştirme periyodu gibi etkenlere bağlı olarak değişebilmektedir. Genel olarak serada konvansiyonel çift ürün (kısa dönem) yetiştiriciliğinde domates veriminin 8 ile 13 kg m^{-2} arasında değiştiği belirtilmiştir (Greer & Diver 2000; Sevgican 2002). Yürütülen bu çalışmada verim değerleri 10.22 (kontrol) ile 15.11 (topraktan kuru alg uygulaması) kg m^{-2} arasında değişmiş ve serada organik tarım esaslarına göre yürütülen çalışmalardan elde edilen verim değerlerine benzer sonuçlar vermiştir (Tüzel et al 2001; 2003). Özellikle alg kullanılan uygulamalarda verim değerleri hem kontrol uygulamasına göre hem de olması gereken sınır değerlerden yüksek bulunmuş; ayrıca birinci sınıf meyve adedi fazlalaşmıştır. Ayrıca ilk hasatta kontrol grubuna ait bitkilerden sadece 1 adet meyve hasat edilirken; alg uygulamaların yapıldığı bitkilerde toplam 14 adet kızarmış meyve hasat edilmiştir. Bu da *C. vulgaris*'in biyogübre olarak uygulanmasının domates bitkilerinde meyve oluşumunda erkencilik de gösterdiğini ortaya koymuştur. Elde ettiğimiz bu sonuçlar sağlıklı ve bilinçli beslenme yanında çevreci yaklaşımların önemsendiği günümüz koşullarında konvansiyonel üretime yakın ve/veya fazla verim elde edilmesini sağlayan alg kullanımının kimyasal gübrelemeye alternatif olarak kullanılabileceğini göstermektedir.

Mikroalgler salgıladıkları şelatlar sayesinde ortamdaki besin maddelerinden bitkinin daha iyi faydalanmasına; ürettikleri organik maddeler veya ölen alglerin ayrışarak sağladığı inorganik maddelerle yine bitkilerin beslenmesine artı katkıda bulunmaktadır. Ayrıca mikroalglerin ürettikleri bir takım enzimler ve hormonların bitki büyüme ve gelişmesi ile verimi arttırmada etkili olduğu bilinmektedir (Ergün et al 2010). Yürütülen çalışmada alg uygulaması ile kontrol bitkilerine göre daha yüksek verim ve bitki gelişimi elde edilmiştir. Benzer sonuçlar *C. vulgaris*'in biyogübre olarak kullanıldığı marulda (Article 2008) ve üzümde (Abd & Moniem 2008) yapılan çalışmalarda da belirtilmiştir. Elde ettiğimiz bu sonuçların *C. vulgaris*'in sahip olduğu ve yetiştirilen bitkiye aktardığı yüksek protein içeriğinden kaynaklandığı düşünülmektedir. Farklı formlarda uygulanan *C. vulgaris*'den verim ve bitki gelişimi açısından en iyi sonuç toprağa yapılan kuru alg uygulamasından elde edilmiştir. Kuru alg uygulaması dikim çukurlarına doğrudan algin verilmesi şeklinde yapılmış; algin uygulanan su ile zamana bağlı olarak çözünmesi ve toprakta yavaş salınımına bağlı olarak çözünen

Çizelge 3- Uygulamalarının verim değerleri üzerine etkileri

Table 3- Effects of treatments on plant yield parameters

Uygulama	*Toplam verim (kg m^{-2})*	*Toplam meyve adedi (adet m^{-2})*	*Ortalama meyve ağırlığı (g)*	*Meyve sınıflandırması (%)*			
				<3.5 cm	*3.5-4.5 cm*	*4.5-5.5 cm*	*>5.5 cm*
Toprağa sıvı	13.10 b	32.4 a	113.7 b	2.2	2.7	16.7	78.5
Toprağa kuru	15.11 a	31.3 a	135.2 a	0.6	5.6	16.3	77.5
Yaprağa sprey	12.48 b	27.5 ab	127.1 ab	2.9	6.5	15.5	75.0
Kontrol	10.22 c	24.2 b	118.9 ab	4.5	9.8	15.4	70.3
P	0.004	0.049	0.042	0.165	0.309	0.990	0.564

bileşiklerinin bitki tarafından daha fazla absorbe edilmesi nedeni ile bitki gelişimi ve verimi arttırdığı düşünülmektedir.

Yapılan üç farklı alg uygulamasının KA, sertlik, meyve suyu EC ve pH değeri, TSÇKM, TA, renk ile Vitamin C içeriği üzerinde kısmi artış göstermesine rağmen bu artış istatistiksel olarak önemsiz bulunmuştur (Çizelge 4).

Farklı alg uygulamalarının genç domates yapraklarında hue renk değeri hariç diğer renk parametreleri üzerine etkisi ($P \leq 0.05$) ve aynı yapraklarda belirlenen klorofil a, klorofil b ve toplam klorofil değerleri üzerine etkisi ($P = 0.000$) önemli bulunmuştur. Yüksek L, b ve chroma; düşük a ve hue değeri ile yaprağa spreylenen alg uygulaması diğer uygulama ve kontrol bitkilerine göre en parlak ve en koyu doygun yeşil renge sahip olmuştur. Benzer şekilde en yüksek klorofil a, b ve toplam klorofil içeriği ile yaprağa spreylenen alg uygulamasına sahip bitkilerden elde edilmiştir. Bunu toprağa sıvı alg uygulaması izlemiş, kontrol ve toprağa kuru alg uygulaması aynı istatistiksel grupta yer almakla birlikte en düşük klorofil içeriklerine sahip olmuşlardır (Çizelge 5).

Fotosentez metabolizması üzerinde etkili bir pigment olan klorofilin en yüksek miktarı yapraktan alg spreylemesi uygulamasından elde edilmiş; kontrol grubuna oranla klorofil a miktarı % 59.5, klorofil b miktarı % 86.7 ve toplam klorofil miktarı % 74.8 oranında artış göstermiştir. Elde edilen bu sonuçlar yapraklara uygulanan *C. vulgaris* alginin bitki yapraklarında fotosentezi arttırdığının bir göstergesi olmuştur. Whapham et al (1993) tarafından yapılan benzer bir çalışmada da en koyu yeşil yaprağa sahip bitkilerin yapraktan alg uygulanan bitkiler olduğu belirtilmiştir.

4. Sonuçlar

Elde edilen sonuçlar genel olarak değerlendirildiğinde; üretilen *C. vulgaris* alginin bitki gelişimi ve verimi teşvik ettiği, bazı meyve kalite parametrelerini de arttırdığı görülmüştür. Çalışmada kullanılan

Çizelge 4- Uygulamaların bazı meyve kalite parametreleri üzerine etkisi

Table 4- Effects of treatments on some fruit quality parameters

Uygulama	KA (%)	Sertlik (N)	EC (dS m^{-1})	pH	TSÇKM (%)	TA (mval 100 mL^{-1})	RENK L	RENK a	RENK b	Vitamin C (mg 100 mL^{-1})
Toprağa sıvı	6.53	40.2	4.6	4.5	5.3	6.0	40.48	22.69	26.93	14.0
Toprağa kuru	6.01	39.9	5.0	4.6	4.5	5.6	40.84	21.75	26.55	15.0
Yaprağa sprey	6.53	43.0	5.1	4.6	5.2	5.6	39.32	23.14	25.96	14.2
Kontrol	5.94	40.1	4.9	4.6	4.8	5.3	40.83	22.10	26.72	12.0
P	0.113	0.137	0.350	0.240	0.366	0.089	0.431	0.382	0.815	0.499

Çizelge 5- Yaprak renk değerleri ile klorofil (a, b ve toplam) içeriği üzerine uygulamaların etkisi

Table 5- Effects of treatments on leaf color and chlorophyll (a, b and total) content

Uygulama	Renk					Klorofil (mg L^{-1})		
	L	a	b	Hue	Chroma	a	b	Toplam
Toprağa sıvı	40.83 c	-10.46 a	15.17 c	124.59	18.43 a	7.76 b	2.56 b	10.31 b
Toprağa kuru	44.08 bc	-12.83 b	19.94 b	122.76	23.71 b	5.01 c	1.81 b	6.82 c
Yaprağa sprey	47.75 a	-15.38 c	25.79 a	120.82	30.03 c	12.61 a	16.02 a	28.62 a
Kontrol	44.75 ab	-13.82 bc	21.30 b	123.07	25.39 b	5.11 c	2.10 b	7.21 c
P	0.022	0.006	0.011	0.197	0.008	0.000	0.000	0.000

biyogübrenin domates dışında diğer türlerde de kullanıldığında olumlu sonuçlar elde edileceği beklenmektedir.

Denemeye alınan uygulamalar içerisinde toprağa yapılan uygulamaların (özellikle kuru alg uygulamasının) daha olumlu sonuçlar verdiği görülmüştür. Elde edilen umutvar sonuçlar dahilinde farklı doz ve uygulama şekli ile araştırmaların devam ettirilmesi gerekmektedir.

Üretim boyunca yaşanan sorunlar tübüler sistemlerin modifiye edilerek daha iyi tasarımla kullanılmasını ve bununla beraber üretimde daha fazla artış sağlanabileceğini göstermektedir.

Tarımda fazlaca kullanılan bir çok kimyasal içerikli gübre yeraltı ve yer üstü su kaynaklarında kirliliğe ve toprakta tuzlanmaya sebep olarak tarımsal üretimi kısıtlamaktadır. Yürütülen bu çalışmada kullanılan mikroalg tamamen biyolojik olup, çevreye zarar vermeden biyogübre olarak kullanılabilmektedir. Organik gübre çeşitliliğinin sınırlı olduğu organik tarımda da alternatif bir gübre olarak kullanımı önerilmektedir.

Teşekkür

Bu çalışma Ege Üniversitesi Bilimsel Araştırma Projeleri "2014 FEN 005" no'lu proje kapsamında desteklenmiştir.

Kaynaklar

Abd E A & Moniem E (2008). Effect of green alga cells extract as foliar spray on vegetative growth, yield and berries quality of superior grapevines. *American-Eurasian Journal of Agricultural & Environmental Sciences* **4**(4): 427-433

Abetz P (1980). Seaweed extracts: Have they a place in Australian agriculture or horticulture? *Journal of the Australian Institute of Agricultural Science* **46**: 23-29

Arnon D I (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24**(1): 1-15

Article F L (2008). Effect of *Chlorella vulgaris* as biofertilizer on growth parameters and metabolic aspects of lettuce plant. *Journal of Agriculture & Social Sciences* **4**: 165-169

Cirik S & Gökpınar Ş (1993). Plankton Bilgisi ve Kültürü. Ege Üniversitesi Su Ürünleri Fakültesi Yayınları: 47, İzmir

Ergün O, Daşgan Y H & Işık O (2010). Su kültüründe yetiştirilen kıvırcık marul bitkisinde mikroalg (*Chlorella vulgaris*) uygulamasının etkileri. *9. Ulusal Sebze Tarımı Sempozyumu*, *Bildiriler*: 12-14 Eylül 2012, Konya, s. 330-334

Greer L & Diver S (2000). Organic Greenhouse Vegetable Production: Horticulture Production Guide. Fayetteville: Appropriate Technology Transfer for Rural Areas (ATTRA), pp. 31, AR, USA

Guillard R R L (1973). Division rates. In: Stein, J.R. (Ed.), Handbook of Phycological Methods–Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, 289-311 pp

Güner H & Aysel V (1996). Marine Benthic Vegetation, Chapter 18. Ecological studies Vol. 123, Schramm/ Nienhuis (eds) Springer-Verlag Berlin Heidelberg, 421-432 pp.

Hekimoğlu B & Altındeğer M (2006). Organik Tarım ve Bitki Koruma Açısından Organik Tarımda Kullanılacak Yöntemler, Samsun Valiliği Gıda Tarım ve Hayvancılık İl Müdürlüğü, Samsun, 200 s

Kacar B (1972). Bitki ve Toprağın Kimyasal Analizleri. II. Bitki Analizleri. Ankara Üniversitesi Ziraat Fakültesi Yayınları, 642 s, Ankara

Karaçalı İ (1993). Bahçe Ürünlerinin Muhafaza ve Pazarlanması. Ege Üniversitesi Ziraat Fakültesi Yayınları: 494, İzmir

Kumbul B (2000). Deniz yosunlarının bahçe bitkilerinde kullanım alanları. Yüksek lisans tezi, Akdeniz Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Antalya

McGuire G R (1992). Reporting of objective color measurements. *HortScience* **27**(12): 1254-1255

Naz M & Gökçek K (2004). Fotobiyoreaktörler: Fototropik mikroorganizmalar için alternatif üretim sistemleri. *Ulusal Su Günleri*: 6-8 Ekim, İzmir, s. 545-551

Pearson D (1970). The Chemical Analysis of Food. Auxil, London

Safi C, Zebib B, Merah O, Pontalier P Y & Vaca-Garcia C (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable and Sustainable Energy Reviews* **35**: 265-278

Selvam G G & Sivakumar K (2014). Influence of seaweed extract as an organic fertilizer on the growth and yield of *Arachis hypogea L*. and their elemental composition using SEM-energy dispersive spectroscopic analysis. *Asian Pacific Journal of Reproduction* **3**(1): 18-22

Sevgican A (2002). Örtüaltı Sebzeciliği. Cilt I (Topraklı Tarım). Ege Üniversitesi Ziraat Fakültesi Yayınları: 528, İzmir

Sivasankari S, Venkatesalu V, Anantharaj M & Chandrasekaran M (2006). Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresource Technology* **97**(14): 1745-1751

Sukatar A (2002). Alg Kültür Yöntemleri. Ege Üniversitesi Fen Fakültesi Yayınları: 184, İzmir

Şimşek Z (1995). Klemantin mandarinde bilezik alma, demir bileşikleri ve deniz yosunu özü uygulamalarının verim ve kalite üzerine etkileri. Yüksek lisans tezi, Akdeniz Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Antalya

Tüzel Y, Tuncay Ö, Anaç D & Tüzel İ H (2001). Effects of different organic fertilizers and irrigation levels on yield and quality of organically grown greenhouse tomatoes. *Organic Agriculture in the Mediterranean Basin* 285-298 pp

Tüzel Y, Yağmur B & Gümüş M (2003). Organic tomato production under greenhouse conditions. *Acta Horticulture* **614**: 775-780

Whapham C A, Blunden G, Jenkins T & Hankins S D (1993). Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *Journal of Applied Phycology* **5**: 231-234

Whapham C A, Jenkins T, Blunden G & Hankins S D (1994). The role of seaweed extracts, *Ascophyllum nodusum*, in the reduction in fecundity of *Meloidogyne javanica*. *Fundamental and Applied Nematology* **17**(2): 181-183

Zheng H, Yin J, Gao Z, Huang H, Ji X & Dou C (2011). Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: A comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Applied Biochemistry and Biotechnology* **164**(7): 1215-1224

Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Changes in Nutrient Concentrations of Maize (*Zea mays* var. *intendata*) Leaves under Potassium and Magnesium Applications in Central Anatolia

Hakan ERTİFTİK[a], Mehmet ZENGİN[b]

[a]*Doger Chemistry Erkin Agriculture Corporation, 42040 Konya, TURKEY*

[b]*Selcuk University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 42075, Konya, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Mehmet ZENGİN, E-mail: mzengin@selcuk.edu.tr, Tel: +90 (532) 563 64 33
Received: 27 February 2014, Received in Revised Form: 29 September 2015, Accepted: 29 September 2015

ABSTRACT

This research was carried out to determine the effects of potassium (0, 40, 80, 120 kg K_2O ha^{-1}; as potassium sulfate; 50% K_2O) and magnesium (0, 20, 40, 60 kg MgO ha^{-1}; as magnesium sulfate; 16% MgO) applied to the soil, either separately or in various combinations, on some nutrients (N, P, K, Ca, Mg, Fe, Zn) in maize leaves grown under field conditions in semi-arid Central Anatolia in Turkey in 2009 and 2010. The study was designed as a factorial arrangement in randomized block design with four replications. After soil analysis of the study areas, K and Mg-fertilizers were applied at sowing. The results showed that the K applications alone could increase the nutrient concentrations of the leaves, and synergic relations were found between K and P, Fe, or Zn. Synergic relations were also found between Mg and P or Fe. Generally, combined applications of K and Mg resulted in higher nutrient concentrations in the leaves by ameliorating the antagonistic effect of poor soil K-Ca-Mg ionic balances. The leaf nutrient concentrations were generally higher in the first year (2009) than that of the experiment than in the second year (2010).

Keywords: Maize; Leaf; Potassium; Magnesium; Nutrients

İç Anadolu Bölgesi'nde Potasyum ve Magnezyum Uygulanan Mısırın (*Zea mays* var. *intendata*) Yaprak Besin Elementi İçeriklerindeki Değişimler

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Mehmet ZENGİN, E-posta: mzengin@selcuk.edu.tr, Tel: +90 (532) 563 64 33
Geliş Tarihi: 27 Şubat 2014, Düzeltmelerin Gelişi: 29 Eylül 2015, Kabul: 29 Eylül 2015

ÖZET

Bu araştırma, toprağa ayrı ayrı ve belli kombinasyonlarda uygulanan potasyum (0, 40, 80, 120 kg K_2O ha^{-1}; potasyum sülfat; % 50 K_2O) ve magnezyum (0, 20, 40, 60 kg MgO ha^{-1}; magnezyum sülfat; % 16 MgO)'un İç Anadolu'da yarı

kurak iklim koşullarında 2009 ve 2010 yıllarında yetiştirilen mısırın yapraklarındaki bazı besin elementlerine (N, P, K, Ca, Mg, Fe, Zn) etkilerini belirlemek amacıyla yapılmıştır. Denemeler tesadüf bloklarında faktöriyel deneme deseninde dört tekerrürlü olarak planlanmıştır. Toprak analizleri yapılan deneme alanlarına K ve Mg'lu gübreler ekimde tek seferde tabana verilmiştir. Araştırma sonuçlarına göre, tek başına verilen K yaprağın besin elementlerini artırmış, K ile P, Fe ve Zn arasında sinerjik ilişkiler belirlenmiştir. Diğer taraftan, Mg ile P ve Fe arasında sinerjik etkileşimler saptanmıştır. Genellikle K ile Mg'un birlikte uygulanması topraktaki antagonistik ilişkileri azaltarak yaprakta daha yüksek besin elementlerinin bulunmasını sağlamıştır. Denemenin ilk yılında yaprağın besin element içerikleri ikinci yıldakilerden daha yüksek bulunmuştur.

Anahtar Kelimeler**:** Mısır; Yaprak; Potasyum; Magnezyum; Besin elementleri

1. Introduction

Maize, which is the third most important agricultural product after wheat and barley in terms of its production area in Turkey, has a mean yield of about 7,190 kg ha^{-1} and a total yield of 4.25 million tons produced from a cultivated area of 592,000 ha. Maize is also the eighth most important crop after wheat, barley, sugar beet, chickpea, sunflower, rye and beans in the province of Konya, where the mean yield is about 7,930 kg ha^{-1} and the total yield is 104,129 tons produced from an area of 13,138 ha (Anonymous 2009). Generally, while corn has a high yield potential in semi-humid and semi-arid regions, their yields can be relatively low or limited due to inadequate soil and crop macro- and micro-nutrient management practices, climate conditions, and soil properties (Wang et al 2006; Kovačević et al 2008; 2009; Zengin et al 2008; Cela et al 2010; Stingu et al 2011). Since soils in the Konya region are highly calcareous ($CaCO_3$> 15%), the exchangeable and available Ca levels are high, which results in cultivated plants having visible or hidden K and Mg deficiencies even if there are adequate levels of K and Mg in the soils. Consequently, potato and sugar beet plants have exhibited positive responses to additional fertilization with K and Mg that was applied because of previous failures to obtain adequate levels of K and Mg in the plants due to the high levels of Ca in the excessively calcareous soils around Konya and in semi arid Central Anatolia (Zengin et al 2008; 2009). However, neither in the province of Konya nor elsewhere in Turkey, any sufficient number of studies on the effects of K and Mg fertilizers on the levels of maize leaf nutrients has been conducted.

Karaman et al (1999), who investigated the effect of fertilization with K and Mg on maize growth and on K and Mg absorption, stated that K-fertilization should be carried out in proportion to Mg levels, especially in Mg-deficient soils. Spear et al (2003), who studied the effects of growing maize and sunflower under eight different concentrations of K for 27 days, reported that the amount of K in the plant decreased with increasing K concentrations and observed Mg deficiencies. Plant species, soil, climate and growing conditions all affected the K uptake by the plants (Turan & Horuz 2012). Kanyanjua et al (2006) reported that the optimal K dose for maize in Kenya was 30 kg K_2O ha^{-1}. Izsaki (2006) examined the effects of N-P-K fertilization applied in different doses on the levels of nutrient elements in maize leaves in the Szarvas region of Hungary and concluded that the doses of 206 to 232 mg K_2O kg^{-1} increased the levels of other nutrients, except for those of Cu, in maize leaves to a greater extent than higher doses of 321 to 465 mg K_2O kg^{-1}. Tan et al (2007), who investigated the effects of K fertilizers on maize yield in China for 13 years, found that K accumulated at significant levels in the soil with an annual dose of 225 kg K_2O ha^{-1} and proposed that the most appropriate annual dose of K was just 112.5 kg K_2O ha^{-1}. Tomov et al (2008) investigated the effects of a various N-P-K doses and of manure on nutrient uptake and maize yield in the Plovdiv region of Bulgaria. They reported that there was a yield increase of between 35.2% and 21.7% depending on the types and doses of the fertilizer when compared with the control and that 2.0 to 2.7 kg N, 0.6 to 1.2 kg P_2O_5 and 2.0 to 2.9 kg K_2O was required to produce 100 kg of corn.

In this study, the effects of various doses of K and Mg and their interactions on some nutrients concentrations of maize leaves were investigated in Kadınhanı District of Konya Province, semi-arid Central Anatolia.

2. Material and Methods

Field trials by using a hybrid maize cultivar (*Zea mays* var. *indentata*) Pioneer-3394 brand were conducted in the Kadınhanı District of Konya Province in the Central Anatolia Region of Turkey in 2009 and 2010. The experimental area was situated at an altitude of 1100 m above the sea level. Climate in this district is characterized by hot and dry summers and cold and rainy winters with a long-term (1975 to 2010 years) annual mean precipitation of about 327 mm. Total precipitation during the maize growth period in the experimental areas (i.e., between May and October) was 128.8 mm in 2009 and 117.8 mm in 2010 (DMI 2011) (Table 1).

Some physical and chemical properties of the soil samples taken from the experimental areas are given in Table 2. Soils were slightly alkaline, non-saline, low in organic matter, high in lime, and clay loam

Table 1- Some climate parameters of the study years and of a long-time period in Kadınhanı District

Çizelge 1- Çalşmanın yürütüldüğü yıllarda ve uzun yıllar ortalaması olarak Kadınhanı'nın kimi iklim verileri

Climate parameters		*Months*							
	Year	*May*	*June*	*July*	*August*	*September*	*October*	*Mean/ Total*	*Long-term (1975-2010)*
Mean temperature (°C)	2009	12.90	18.25	20.60	19.00	15.32	14.31	16.73	11.10
	2010	14.40	18.22	22.86	24.58	19.29	17.32	19.45	
Total precipitation (mm)	2009	37.4	24.0	8.0	0.2	19.4	21.4	128.8	327.1
	2010	14.6	59.0	0.2	0.8	0.4	26.0	117.8	
Mean air humidity (%)	2009	69	57	55	42	56	59	56.3	61.2

Table 2- Soil analysis results of maize field

Çizelge 2- Mısır tarlası toprak analiz sonuçları

Parameters	*2009*	*2010*	*Method*
pH (1:2.5 s:w)	7.52	7.32	pH meter
EC (1:5 s:w; µS cm^{-1})	141	180	EC meter
Organic matter (%)	1.52	1.93	Walkley-Black
Lime (%)	6.31	16.11	Scheibler calcimeter
Clay (%)	30.4	30.1	Bouyoucos (1951)
Silt (%)	26.0	34.6	Bouyoucos (1951)
Sand (%)	43.6	35.3	Bouyoucos (1951)
Texture	Clay loam	Loam	-
NH_4-N + NO_3-N (mg kg^{-1})	15.3	15.7	Extraction with 2 N KCl
P (mg kg^{-1})	3.76	13.25	Extraction with $NaHCO_3$
K (mg kg^{-1})	603	430	Extraction with NH_4OAc
Ca (mg kg^{-1})	6401	7631	Extraction with NH_4OAc
Mg (mg kg^{-1})	285	316	Extraction with NH_4OAc
K (me 100 g^{-1})	1.54	1.10	Ca:K 20.78 34.68 12 (ideal)
Ca (me 100 g^{-1})	32.00	38.15	Ca:Mg 13.50 14.50 6 (ideal)
Mg (me 100 g^{-1})	2.37	2.63	Mg:K 1.54 2.39 2 (ideal)
Na (mg kg^{-1})	45	143	Flame-photometer
Fe (mg kg^{-1})	1.65	0.16	Soltanpour & Workman (1981)
Zn (mg kg^{-1})	0.55	0.42	Soltanpour & Workman (1981)
Mn (mg kg^{-1})	2.37	2.04	Soltanpour & Workman (1981)
B (mg kg^{-1})	0.90	2.69	Kacar (1994)
Cu (mg kg^{-1})	0.79	1.03	Soltanpour & Workman (1981)

s:w, soil water ratio

and loam in texture. At the two locations, N was low, extractable K and Ca were very high while Mg was sufficient, Fe and Zn were low while Mn, B and Cu were adequate. In addition, while the P level was low in the first area, it was moderate in the second area (Ülgen & Yurtsever 1974; Sillanpaa 1990).

In terms of soil fertility, the most suitable balances between exchangeable Ca, K and Mg are supposed to be Ca:K= 12, Ca:Mg= 6 and Mg:K= 2 (Jokinen 1981); whereas, they were determined to be Ca:K= 20.78, Ca:Mg= 13.50 and Mg:K= 1.54 in the fields for the first year, and Ca:K= 34.68, Ca:Mg= 14.50 and Mg:K= 2.39 in the second year. Therefore, there were imbalances between these cations and Ca was disproportionally high (Table 2). Thus, even if K and Mg were present in the soil in adequate amounts, the plants could not uptake sufficient amounts, and hidden or visible deficiency symptoms could occur.

The basal fertilizers used in the experiments were DAP (18% N, 46% P_2O_5), urea (46% N) and ammonium nitrate (33% N). The fertilizers to be tested using different doses (0, 40, 80, 120 kg K_2O ha^{-1}) were potassium sulfate (50% K_2O, soluble in water) and magnesium sulfate (16% MgO, soluble in water) at the rates of 0, 20, 40, 60 kg MgO ha^{-1}, which were applied during sowing in the both study years.

Based on the results of the soil analysis, K and Mg-fertilizers with the fertilizers to rectify the soil nutrient deficiencies (100 kg P_2O_5 ha^{-1}, 5 kg Fe ha^{-1} and 5 kg Zn ha^{-1}) were applied at the sowing depth to all the plots at once. The 200 kg N ha^{-1} was applied manually to the soil as a top fertilizer, using urea (46% N) during the first hoeing and ammonium nitrate (33% N) during the second hoeing.

Field experiments were conducted according to a completely randomized block design in factorial arrangement. In two years, at each of the locations that were near to each other, 64 plots were established with four replications of each treatment. Plots had dimensions of 4.2 m x 5.0 m (21 m^2). The row intervals were 70 cm at sowing and planting intervals along the rows were set at 22 cm during seed drilling. The separation between the plots was 1 m.

Soil samples were collected from the experiment areas, from the 0 to 30 cm soil layer, before fertilizing and sowing. They were analyzed for; pH in a 1:2.5 soil:water solution ratio with pH-meter; electrical conductivity (EC) in a 1:5 soil:water solution by EC-meter (Jackson 1962); organic matter content using the Smith & Weldon (1941) method; lime content by the Scheibler Calcimeter (Çağlar 1949); texture by Bouyoucos Hydrometer (Bouyoucos 1951); inorganic N (NH_4-N+NO_3-N) in a 2 N KCl extract by Kjeldahl distillation; available P using the Olsen method with a spectrophotometer (Bayraklı 1987); exchangeable cations (K, Ca, Mg and Na) in 1 N NH_4OAc solution (Bayraklı 1987) by the ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer; Varian, Vista Axiel Simultaneous) (Soltanpour & Workman 1981); extractable Fe, Zn, Mn, Cu in the 0.05 M DTPA+0.01 M $CaCl_2$+0.1 M TEA (pH= 7.3) solution (Lindsay & Norvell 1978) using the ICP-AES; and available B in a $CaCl_2$+mannitol solution (Kacar 1972) using the ICP-AES.

In all of the experiments, fully expanded leaves of corncob were sampled at the time of corncob formation and were immediately transported in paper bags inside a cooling bag to laboratory for analysis of the total mineral nutrients. After washing with tap water, they were washed once more with distilled water, then with 0.1 N HCl solution, then further twice with distilled water and once more again with deionized distilled water (Bayraklı 1987). Following drying at 70 ^oC, leaf samples were ground and subjected to digestion by 15 mL HNO_3+5 mL $HClO_4$ using a microwave system (CEM, Mars 5) (Soltanpour & Workman 1981). The amounts of total P, K, Ca, Mg, Fe, and Zn in the extracts were determined by the ICP-AES as described by Soltanpour & Workman (1981). One blank and one certified reference material (1547 Peach Leaves, NIST, National Institute of Standards and Technology, Gaithersburg-USA) were added into the microwave set of 40 cells to confirm the reliability of the leaf analysis (Soltanpour & Workman 1981). Total N concentrations in the leaf samples were determined by micro Kjeldahl method (Bayraklı 1987) after digesting in H_2SO_4+H_2O_2.

Irrigation water was provided on four occasions for a period of eight hours by sprinkler irrigation. The sprinkler irrigation was carried out on the dates specified in Table 3, taking into account the periods when the plants needed water.

Table 3- Time-table for experimental procedure

Çizelge 3- İş-zaman takvimi

Experimental procedure	*2009*	*2010*
Forming of plots and sowing	13.05.2009	10.05.2010
Top fertilizing and 1. hoeing	09.06.2009	17.06.2010
Top fertilizing and 2. hoeing	03.07.2009	13.07.2010
1. Irrigation	11.06.2009	18.06.2010
2. Irrigation	04.07.2009	14.07.2010
3. Irrigation	05.08.2009	12.08.2010
4. Irrigation	01.09.2009	09.09.2010
Leaf sampling	27.07.2009	02.08.2010

The statistical analysis of the data was performed by Minitab for analysis of variance and Mstat for separating the treatments means by LSD and Duncan's Multiple Range Test (Düzgüneş et al 1983).

3. Results and Discussion

3.1. Nitrogen

The KxMg interaction effect was statistically significant ($P<0.01$) on the total N concentration of the maize leaves for both the first and the second years (Table 4).

In the first year, the total N concentrations in the leaves ranged between 2.28% (K_8Mg_6) and 2.59% (K_4Mg_6), while in the second year they were between 2.27% (K_0Mg_0) and 2.62% ($K_{12}Mg_4$; Table 5). In the first year, the N concentration was increased by 2.0%, compared to the control (N, P), by the K_4Mg_6

Table 4- Analysis of variance results for the effects of K and Mg on some nutrient concentrations of maize leaves in 2009 and 2010

Çizege 4- Mısır yapraklarının bazı besin elementi konsantrasyonlarına K ve Mg'un etkisine ilişkin 2009 ve 2010 yılı varyans analizi sonuçları

		2009				
Source of variation	*Df*	*Mean of squares*				
		N	*P*	*K*	*Ca*	*Mg*
K	3	0.01990	0.00013542	0.037803**	0.0029299*	0.00005556
Mg	3	0.02695	0.00007431	0.045025**	0.0014465	0.00012778
KxMg	9	0.03696**	0.00017801**	0.028153**	0.0009225	0.00015000*
Error	32	0.01221	0.00005625	0.008185	0.0007083	0.00006458
		Fe	*Zn*			
K	3	158.51*	2.086			
Mg	3	106.10	5.194			
KxMg	9	73.35	7.355*			
Error	32	39.37	3.376			
		2010				
Source of variation	*Df*	*Mean of squares*				
		N	*P*	*K*	*Ca*	*Mg*
K	3	0.064208**	0.0005722	0.00894	0.000367	0.0006944
Mg	3	0.059952**	0.0004278	0.03000	0.002789	0.0031722**
KxMg	9	0.038876**	0.0006667**	0.03780*	0.006319	0.0021593**
Error	32	0.009485	0.0002146	0.01521	0.006173	0.0006146
		Fe	*Zn*			
K	3	1495.0**	5.289			
Mg	3	265.4	1.828			
KxMg	9	629.0**	9.430			
Error	32	147.0	7.597			

**: $P<0.01$; *: $P<0.05$; Df, degrees of freedom

treatment, while in the second year it did so by 15.4% in the case of $K_{12}Mg_4$. The measured total N values were considered to be slightly deficient according to the reported optimal range of total N values (2.7% to 4.0%) for maize leaves (Jones et al 1991). Under the current conditions, the amount of N applied (200 kg N ha^{-1}) was probably too low for growing maize. In the first year of the experiments, the measured total N concentration of the leaves was less than the one in the second year (Table 5). The reason for this could be differences in the weather conditions (Table 1), studied soils (Table 2), and the timing of the leaf sampling (Table 3). In both years, the N concentrations of the leaves were slightly higher with the KxMg applications than with applications of either K or Mg alone. This observation may result from the better plant growth in terms of better utilizing the N as a result of the improvement of the initially poor K-Ca-Mg balance in the experimental soils by the combination of KxMg fertilizers. Samui et al (1987), Izsaki (2006) and Szulc (2010) reported similar results.

3.2. Phosphorus

The KxMg interaction had a statistically significant ($P<0.01$) effect on the total P in maize leaves in both years (Table 4). In the first year, the total values of P ranged between 0.110% (K_8Mg_2) and 0.137% ($K_{12}Mg_4$), while in the second year they were between 0.070% (K_0Mg_0) and 0.117% (K_8Mg_6) (Table 5). In the first year, the P concentration of the leaf was increased as high as 14.2% comparing to the control

Table 5- Effects of K and Mg on total N, P and K concentrations of maize leaves

Çizelge 5- Mısır yapraklarının toplam N, P ve K konsantrasyonlarına K ve Mg'un etkisi

Fertilizer	*Doses (kg K_2O-MgO ha^{-1})*	*N (%)*		*P (%)*		*K (%)*	
		1. Year	*2. Year*	*1. Year*	*2. Year*	*1. Year*	*2. Year*
K	0	2.54	2.27	0.120	0.070	1.33	1.12
	K_4	2.52	2.52	0.133	0.093	1.41	1.05
	K_8	2.35	2.57	0.127	0.083	1.34	1.24
	K_{12}	2.39	2.29	0.133	0.117	1.21	1.33
	LSD (P<0.05)	-	-	-	-	-	-
Mg	0	2.54	2.27	0.120	0.070	1.33	1.12
	Mg_2	2.38	2.57	0.127	0.097	1.46	1.18
	Mg_4	2.48	2.57	0.130	0.083	1.51	1.30
	Mg_6	2.45	2.38	0.130	0.103	1.23	1.30
	LSD (P<0.05)	-	-	-	-	-	-
KxMg	K_0Mg_0	2.54 a*	2.27 d	0.120 ab	0.070 c	1.33 bc	1.12 cd
	K_4Mg_2	2.52 a	2.27 d	0.133 a	0.083 bc	1.44 ab	1.14 bcd
	K_4Mg_4	2.54 a	2.30 cd	0.123 ab	0.113 a	1.33 bc	1.37 a
	K_4Mg_6	2.59 a	2.45 bc	0.130 a	0.110 ab	1.32 bc	1.34 ab
	K_8Mg_2	2.32 b	2.46 abc	0.110 c	0.107 ab	1.24 c	1.16 bcd
	K_8Mg_4	2.57 a	2.42 bcd	0.133 a	0.087 bc	1.31 bc	1.16 bcd
	K_8Mg_6	2.28 b	2.44 bc	0.120 bc	0.117 a	1.54 a	1.25 abcd
	$K_{12}Mg_2$	2.31 b	2.52 ab	0.120 bc	0.103 ab	1.33 bc	1.27 abc
	$K_{12}Mg_4$	2.53 a	2.62 a	0.137 a	0.103 ab	1.31 bc	1.27 abc
	$K_{12}Mg_6$	2.52 a	2.40 bcd	0.123 ab	0.080 c	1.35 bc	1.05 d
	Lowest	2.28	2.27	0.110	0.070	1.21	1.05
	Highest	2.59	2.62	0.137	0.117	1.54	1.37
	LSD (P<0.05)	0.183	0.162	0.012	0.024	0.150	0.205

*, means shown with the same letters in the same column are not significant

for $K_{12}Mg_4$ treatment, while in the second year it showed tremendous increase by 67.1% in the K_8Mg_6 case. The measured values of P were well below the optimal P range (0.25% to 0.50%) for maize leaves (Jones et al 1991). The phosphorous fertilization (100 kg P_2O_5 ha^{-1}) in the current study was probably too low to meet the P requirement of maize. In the first year of the experiment, the P concentrations of the leaves were found to be greater than they were in the second year, as was the case for K and Zn (Table 5). The reason for this observation may be due to the differences in the weather conditions (Table 1), properties of the soils (Table 2) and the timing of leaf sampling (Table 3) in the two successive growing seasons. This occurred despite of smaller amounts of available P and higher alkaline pH value in the soil in the first year. In the second year, K and Mg applications resulted in decreases in the P concentration of the leaves. This could result from a relatively lower absorption of P in the plants that were growing better due to the improved Mg balance. In addition, P might not be taken up in adequate amounts by the plant due to the high pH and Ca concentration of the soil. Blasko (2006) and Izsaki (2006) reported similar results. Leaf P increased with the increases in Mg doses. Aktaş & Ateş (1998) also reported a synergic relation between Mg and P, while Szulc (2009) reported that P increased in corn leaves following an application of Mg.

3.3. Potassium

The KxMg interaction more significantly affected the total K concentration of the leaves ($P<0.01$) in the first year than in the second year ($P<0.05$) (Table 4). In the first year, the K values ranged between 1.21% (K_{12}) and 1.54% (K_8Mg_6), while in the second year they were between 1.05% (K_4) and 1.37% (K_4Mg_4; Table 5). The K concentration of the leaf was increased by 15.8 and 22.3% comparing to control in the first and second years, respectively. Results showed that the levels of both of the elements, K and Mg, need to be addressed in order to improve the K-Ca-Mg balance, which was initially poor due to the high Ca in the region's soils. Despite observation of no visual deficiency symptoms, the K concentrations were just below the deficiency threshold (1.7% to 3.0%) for maize leaves (Jones et al 1991). This situation can be affected by the maize variety, and differences in soil and climate factors. Comparatively higher K concentrations were observed in the first year. This could be attributed to lower exchangeable Ca was lower and higher exchangeable K resulting in a Ca:K ratio closer (20.78 and 34.68 in the first and second year respectively) to the ideal one (12) in the first years experimental field (Jokinen 1981). However, Zengin et al (2008; 2009) demonstrated that K and Mg fertilization was necessary for sugar beet under similar growth conditions in order to establish appropriate Ca-K and Ca-Mg balances in soils with excessive exchangeable Ca. Zengin et al (2008) reported similar results for potato cultivation in the region. Excessive Ca in the soil results in an antagonistic interaction among the three elements that hinders the plant uptake of K (Aktaş & Ateş 1998). The K and Mg treatments resulted in either increase or decrease in the leaf K concentration in the first growing season whereas there was a treatment induced increase in K concentration in the second year. In accordance with our results, Karaman et al (1999) emphasized that importance of proportional K and Mg treatments for a balanced Ca, Mg and K nutrition. Hermans et al (2004), Zengin et al (2008; 2009) and Szulc (2009) have noted the different responses of plants to K applications.

3.4. Calcium

The effect of only adding K on Ca concentration of the leaf was statistically significant ($P<0.05$) in the first year; however, none of the applications had a significant effect in the second year (Table 4). In the first year, the Ca values ranged between 0.283% (K_8Mg_6) and 0.360% (K_{12}), while they were between 0.460% (K_4) and 0.613% (Mg_4) in the second year (Table 6). The measured Ca concentrations were well in the optimal Ca range (0.21% to 1.00%) for maize leaves (Jones et al 1991). In both years, treatment induced-changes in the Ca concentrations of the leaves were related to the balance of K-Ca-Mg in the soil. According to Doll & Lucas (1973) the base saturation of a soil should be in the range of 3-5%

for K, 65-85% for Ca, and 6-12% for Mg in order to maintain ideal Ca, Mg and K nutrition of the plants. Similarly, the respective saturation rates were reported as 5%, 60% and about 10% for K, Ca, and Mg, respectively. The ideal ratios of the exchangeable cations should be Ca:K= 12, Ca:Mg= 6, and Mg:K= 2 to ensure adequate uptake of K, Ca and Mg from the soil (Jokinen 1981). However, in this study, the balances between these elements in either of the two experimental fields were not suitable for optimal K, Mg and Ca nutrition of plants.

3.5. Magnesium

The effects of the KxMg interaction on the total Mg concentration of the leaves were statistically significant in both growing seasons (Table 4). In the first year Mg concentrations range was 0.100% (K_4) and 0.117% (K_4Mg_2, K_8Mg_2, $K_{12}Mg_6$), while in the second year it was 0.143% (K_0Mg_0) and 0.230% (K_8Mg_4; Table 6). Leaf Mg concentrations were proportional to Mg treatments in both years whereas K treatments resulted in a decrease in the first year and an increase in the second year. Treatment induced increases in Mg concentrations were detrimental in the second growing season with as high as 60.8% in the K_8Mg_4 case. The leaf Mg concentrations were just about the deficiency threshold (0.2%) for maize leaves (Jones et al 1991). However, no Mg deficiency symptoms were evident in the plants.

Table 6- Effects of K and Mg on total Ca, Mg and Fe concentrations of maize leaves

Çizelge 6- Mısır yapraklarının toplam Ca, Mg ve Fe konsantrasyonlarına K ve Mg'un etkisi

Fertilizer	*Doses*	*Ca (%)*		*Mg (%)*		*Fe* (mg kg^{-1})	
	(kg K_2O-MgO da^{-1})	*1. Year*	*2. Year*	*1. Year*	*2. Year*	*1. Year*	*2. Year*
K	0	0.353 a*	0.486	0.113	0.143	73.19 b	111.90
	K_4	0.310 b	0.460	0.100	0.173	83.64 a	118.86
	K_8	0.320 ab	0.580	0.116	0.183	75.86 ab	102.73
	K_{12}	0.360 a	0.517	0.116	0.203	70.99 b	116.68
	LSD (P<0.05)	0.044	-	-	-	10.44	-
Mg	0	0.353	0.486	0.113	0.143	73.19	111.90
	Mg_2	0.357	0.493	0.114	0.203	81.63	115.16
	Mg_4	0.330	0.613	0.116	0.217	81.46	124.59
	Mg_6	0.350	0.517	0.115	0.213	84.94	129.73
	LSD (P<0.05)	-	-	-	-	-	-
KxMg	K_0Mg_0	0.353	0.486	0.113 a	0.143 d	73.19	111.90 bc
	K_4Mg_2	0.353	0.500	0.117 a	0.147 d	76.62	73.06 d
	K_4Mg_4	0.326	0.550	0.113 a	0.167 cd	75.84	112.12 bc
	K_4Mg_6	0.303	0.543	0.103 ab	0.173 cd	87.22	139.91 a
	K_8Mg_2	0.337	0.503	0.117 a	0.193 bc	79.58	137.87 a
	K_8Mg_4	0.333	0.493	0.107 ab	0.230 a	89.87	127.57 ab
	K_8Mg_6	0.283	0.553	0.103 ab	0.197 bc	69.81	107.09 c
	$K_{12}Mg_2$	0.310	0.500	0.100 b	0.150 d	74.81	115.49 bc
	$K_{12}Mg_4$	0.333	0.523	0.113 a	0.200 bc	77.14	110.83 bc
	$K_{12}Mg_6$	0.317	0.513	0.117 a	0.207 bc	78.18	122.31 abc
	Lowest	0.283	0.460	0.100	0.143	69.81	73.06
	Highest	0.360	0.613	0.117	0.230	89.87	139.91
	LSD (P<0.05)	-	-	0.013	0.041	-	20.16

*, means shown with the same letters in the same column are not significant

In the second year, the balances between exchangeable K-Ca-Mg in the study soils were worse than in the first year, which resulted in higher Mg contents in the leaves in the second year. Because Mg use improved bad ratio of Ca:Mg and Mg:K in the second year. Combinations of K and Mg were given in different doses that raised the Mg concentration of the leaves to higher levels than those in the control by reducing the antagonistic interaction. The concentrations of Mg in the leaves were increased as the doses of K increased. This situation is interested in Mg:K ratio in the soil. This ratio is high than normal value, so K applying was supported good plant growth and Mg absorption. However Aktaş & Ateş (1998), Sepehr et al (2002), Zengin et al (2008) and Szulc (2009) have all mentioned that increasing the doses of K applications reduced the Mg concentration of the leaves due to the antagonistic effect.

3.6. Iron

Both K and KxMg interaction had significant effects on Fe concentration of the maize leaves (Table 4). The Fe concentration ranges were 69.81 (K_8Mg_6), 89.87 mg kg^{-1} (K_8Mg_4) and 73.06 (K_4Mg_2), 139.91 mg kg^{-1} (K_4Mg_6) (Table 6) in first and second years, respectively. There was 22.8 (K_8Mg_4) and 25% (K_4Mg_6) treatment induced increase in Fe concentration in first and second years. The Fe concentrations were in the optimal range (21 to 250 mg kg^{-1}) for maize leaves (Jones et al 1991).

As the individual effects of K or Mg fertilizers considered, the total Fe concentration of the leaves was first increased and then decreased upon K fertilization, but it was proportionally increased due to Mg fertilization. This may be attributed to the synergistic relation of both K and Mg with Fe (Aktaş & Ateş 1998). In addition, the Fe concentrations were considerably lower in the first year. These changes in Fe concentrations can be related to differences in soil characteristics such as pH and organic matter (Table 2) and climate conditions (Table 1). As the pH value increased the available Fe concentration would be reduced (Aktaş 1991; Eyüpoğlu et al 1998). Izsaki (2006) reported that the Fe concentration of the leaves was increased by K fertilization to maize.

3.7. Zinc

The effect of the KxMg interaction on Zn concentration of the leaves was only statistically significant (P< of 0.05) in the first year (Table 4). The Zn concentration range were 15.44 (K_8Mg_6)-19.91 mg kg^{-1} (K_4) and 12.79 ($K_{12}Mg_4$)-18.44 mg kg^{-1} (K_8Mg_2) in the first and second years, respectively (Table 7). There was a general increase in Zn concentrations of the leaves depending on K and Mg treatments. However, the observed

Table 7- Effects of K and Mg on total Zn concentration of maize leaves

Çizelge 7- Mısır yapraklarının toplam Zn konsantrasyonuna K ve Mg'un etkisi

Fertilizer	*Doses (kg K_2O-MgO da^{-1})*	*Zn (mg kg^{-1})*	
		1. Year	*2. Year*
K	0	16.68	13.31
	K_4	19.91	13.55
	K_8	15.59	14.06
	K_{12}	18.82	16.74
	LSD (P<0.05)	-	-
Mg	0	16.68	13.31
	Mg_2	17.03	15.33
	Mg_4	16.37	12.88
	Mg_6	19.52	15.40
	LSD (P<0.05)	-	-
KxMg	K_0Mg_0	16.68 ab*	13.31
	K_4Mg_2	19.00 a	13.18
	K_4Mg_4	18.56 a	15.16
	K_4Mg_6	17.62 ab	14.39
	K_8Mg_2	18.10 ab	18.44
	K_8Mg_4	16.36 ab	14.68
	K_8Mg_6	15.44 b	16.24
	$K_{12}Mg_2$	17.56 ab	14.91
	$K_{12}Mg_4$	17.72 ab	12.79
	$K_{12}Mg_6$	19.33 a	13.86
	Lowest	15.44	12.79
	Highest	19.91	18.44
	LSD (P<0.05)	3.056	-

*, means shown with the same letters in the same column are not significant

Zn concentrations were below the optimal Zn concentration range (25 to 100 mg kg^{-1}) for maize leaves (Jones et al 1991). This could be attributed to insufficient basal Zn fertilization (5 kg ha^{-1}) and very high Zn adsorption capacity of calcareous soils. On the other hand, Aktaş & Ateş (1998) have reported that high lime contents and high pH hinder Zn uptake of plants.

Sepehr et al (2002) and Izsaki (2006) also reported that K increased the Zn concentration of the leaves, as in this study. This situation can be related to the increased plant growth induced Zn uptake from the soil.

As a conclusion; even if K and Mg concentrations were adequate in the experimental soils, the maize plants could not uptake sufficient amounts of K and Mg elements because of the excessive Ca saturation induced nutritional imbalances. Manipulations of the balances among Ca, Mg, and K resulted in enhancement in the nutritional status of maize through synergic relations between K or Mg and P, S, Fe, and Zn. In general, when the K and Mg were applied together to optimize the Ca:K, Ca:Mg and K:Mg ratios, the excessive Ca saturation related nutritional disorders in maize can be corrected to some extent in calcareous soils.

References

Aktaş M (1991). Plant Nutrition and Soil Fertility. Ankara University, Faculty of Agriculture, Publication No: 1202, Course Book No: 347, pp. 345, Ankara

Aktaş M & Ateş M (1998). Nutritional Disorders of Plants; causes, diagnosis and corrections. Engin Press, pp. 247, Ankara

Anonymous (2009). Records of Konya Province Agriculture Directorate. Retrieved in December, 26, 2009 from http://www.konyatarim.com.tr

Bayraklı F (1987). Soil and Plant Analysis. Ondokuz Mayıs University, Faculty of Agriculture, Publication No: 17, pp. 199, Samsun

Blasko L (2006). Acidification of a meadow chernozem (Luvic Pheozem) soil in the trans-Tisza Region. *Cereal Research Communications* **34**(1-2): 131-134

Bouyoucos G S (1951). A recalibration of the hydrometer methods for making mechanical analysis of soil. *Agronomy Journal* **54**: 464-465

Cela S, Berenguer P, Santiveri F & Loveras J (2010). Potential phosphorus, potassium, and magnesium surpluses in an irrigated maize monoculture fertilized with pig slurry. *Agronomy Journal* **102**: 96-102

Çağlar K Ö (1949). Soil Science. Publ. of Ankara Univ., Faculty of Agriculture, No: 10, pp. 270, Ankara

DMI (2011). Records of Government Meteorology Institute. http://dmi.gov.tr (Access Date: 21.01.2011)

Doll E C & Lucas R E (1973). Testing Soil for Potassium, Calcium and Magnesium. In: Soil Testing and Plant Analysis. Publ. Soil Sci. Soc. Amer. Madison, Wisc. USA, 133-151

Düzgüneş O, Kesici T & Gürbüz F (1983). Statistical Methods 1. Ankara University, Faculty of Agriculture, Publication No: 861, Course Book No: 229, pp. 218, Ankara

Eyüpoğlu F, Kurucu N & Talaz S (1998). General Situation of Turkish Soils with Respect to Some Available Micro-Nutrients (Fe, Cu, Mn, Zn). Soil and Fertilizer Research Institute Directorate Publication, pp. 72, Ankara

Hermans C, Johnson G N, Strasser, R J & Verbruggen N (2004). Physiological characterization of magnesium deficiency in sugar beet: Acclimation to low magnesium differentially affects photo-systems I and II. *Planta* **220**(2): 344-355

Izsaki Z (2006). Relationship between potassium supplies of the soil and the nutrient concentration of maize (*Zea mays* L.) leaves. *Cereal Research Communication* **34**(1-2): 501-504

Jackson M L (1962). Soil Chemical Analysis. Prentice-Hall, Inc. 183, New York, USA

Jokinen R (1981). The magnesium status of Finnish mineral soils and the requirement of the magnesium supply. *Magnesium-Bulletin* **3**(1a): 1-5

Jones J B, Wolf B & Mills H (1991). Plant Analysis Handbook. Micro-Macro Publishing Inc, Athens, GA, pp. 23-26, USA

Kacar B (1972). Chemical Analysis of Plant and Soil. Ankara University, Faculty of Agriculture, Publication No: 468, Ankara

Kacar B (1994). Chemical Analysis of Plant and Soil. III. Soil Analysis. Ankara University, Faculty of

Agriculture Education, Research and Developing Foundation No: 3, pp. 705, Ankara

Kanyanjua S M, Keter J K, Okalebo R J & Verchot L (2006). Identifying potassium-deficient soils in Kenya by mapping and analysis of selected sites. *Soil Science* **171**(8): 610-626

Karaman M R, Aksu A, Demirer T & Er F (1999). Effect of potassium and magnesium on the growth some nutrient status and K-Mg efficiency parameters of corn (*Zea mays* L.) grown on siltation soil. *Journal of University of Selcuk, Faculty of Agriculture* **13**(18): 107-117

Kovačević V, Rastija M, Šimić B, Andrić L & Kaučić D (2008). Phosphorus and potassium fertilization impacts on yield and nutritional status of maize. *Cereal Research Communication* **36**(1): 43-46

Kovačević V, Stojić B, Rastija M, Brkić I & Drezner G (2009). Response of maize, wheat and barley to phosphorus and potassium fertilization. *Cereal Research Communication* **37**: 129-132

Lindsay W L & Norwell W A (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**: 421-428

Samui R C, Bhattacharyya P & Roy A (1987). Effect of nutrients and spacing on oil yield and uptake of nutrients by Kharif Sunflower. *Journal of Indian Society of Soil Science* **35**(1): 9-73

Sepehr E, Malakouti M J & Rasouli M H (2002). The effect of K, Mg, S and micronutrients on the yield and quality of sunflower in Iran. *17th WCSS, 14-21 August 2002, Symposium* 4, pp. 2260, Thailand

Sillanpaa M (1990). Micronutrient assessment at the country level: An international study. Soil Bulletin 63, FAO, Rome, Italy

Smith H G & Weldon M D (1941). A comparison of some methods for the determination of soil organic matter. *Soil Science Society of America Proceeding* **5**: 177-182

Soltanpour P N & Workman S M (1981). Use of Inductively-Coupled Plasma Spectroscopy for the Simultaneous Determination of Macro and Micro Nutrients in NH_4HCO_3-DTPA Extracts of Soils. (Ed. Barnes R M). Developments in Atomic Plasma Analysis, USA, pp. 673-680

Spear S N, Asher C J & Edwards D G (2003). Response of Cassava, Sunflower and Maize to Potassium Concentration in Solution I. Growth and Plant Potassium Concentration. Dept. of Agric., Univ. of Queensland, St. Lucia, Brisbane, Qld 4067, pp.347-361, Australia

Stingu A, Stanescu I, Volf I & Popa V (2011). Hyperaccumulation of cadmium in maize plant (*Zea mays*). *Cellulose Chemistry Technology* **45**: 287-290

Szulc P (2009). Effect of nitrogen fertilization and methods of magnesium application on chlorophyll content, accumulation of mineral components and morphology of two maize hybrid types in the initial growth period. Part II. Uptake of mineral components. *Acta Scientiarum Polonorum-Agricultura* **8**(2): 51-62

Szulc P (2010). Response of maize hybrid (*Zea mays* L.), stay-green type to fertilization with nitrogen, sulfur, and magnesium. Part I. Yields and chemical composition. *Acta Scientiarum Polonorum-Agricultura* **9**(1): 29-40

Tan D, Jin J & Huang S W (2007). Effect of long-term application of K fertilizer on spring maize yield and soil K in Northeast China. *Scientia Agricola Sinica* **40**(10): 2234-2240

Tomov T, Manolov I & Tomova M (2008). Effect of different fertilizing systems on productivity and N, P & K taken up from maize hybrid Mikado. *Scientific Works of the Agrarian University* **53**: 119-124

Turan M & Horuz N (2012). Basic Principles of Plant Nutrition. In: Karaman M R (Ed), Plant Nutrition, 'Healthy Plant, Healthy Production'. 1. Press, Dumat Ofset, Ankara

Ülgen N & Yurtsever N. (1974). Guide for Fertilizer and Fertilization. Turkish Ministry of Agriculture, Soil Fertilizer Res. Inst. Publ. No: 28, pp. 115, Ankara

Zengin M, Gökmen F, Gezgin S & Çakmak İ (2008). Effects of different fertilizers with potassium and magnesium on the yield and quality of potato. *Asian Journal of Chemistry* **20**(1): 663-676

Zengin M, Gökmen F, Yazıcı M A & Gezgin S (2009). Effects of potassium, magnesium and sulphur containing fertilizers on yield and quality of sugar beets (*Beta vulgaris* L.). *Turkish Journal of Agriculture and Forestry* **33**: 495-502

TARIM BİLİMLERİ DERGİSİ-JOURNAL OF AGRICULTURAL SCIENCES

YAZIM KURALLARI

Genel

Tarım Bilimleri Dergisi, tarım bilimleri alanında ve yazım dili İngilizce olan özgün araştırma makaleleri yayınlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayanan çalışmalar derginin kapsamı dışındadır. Basılacak makalelerin daha önce hiçbir yerde yayınlanmamış olması ve yayın haklarının verilmemiş olması gerekir. Dergide yayımlanacak makalelerin her türlü sorumluluğu yazarına/yazarlarına aittir.

Yayımlanması için gönderilen eser, yayın ilkeleri doğrultusunda Dergi Editörler Kurulu tarafından ön incelemeye alınır. Dergi Editörler Kurulu, dergide yayınlanabilecek nitelikte bulmadığı makaleleri hakemlere göndermeden yazara/yazarlara iade kararı verme hakkına sahiptir. Ayrıca yazım kurallarına uymayan veya anlatım dili yetersiz olan makaleler, düzeltilmek üzere yazara/yazarlara iade edilir. Değerlendirmeye alınan makaleler, incelenmek üzere en az 2 hakeme gönderilir. Hakem değerlendirmesinden geçen makalelere ait düzeltmeler, düzeltmeler listesiyle birlikte en fazla 30 gün içerisinde sisteme yüklenerek gönderilmelidir. Bu süreden sonraki gönderimler kabul edilmez. Başeditör, hakem raporlarını ve/veya istenilen düzeltmelerin yeterli olup olmamasını dikkate alarak makalenin yayımlanıp yayımlanmamasına karar verir.

Makalede isimleri yer alan tüm yazarlar, yayım haklarını Tarım Bilimleri Dergisine verdiklerine dair **Makale Gönderme ve Telif Hakkı Devir Sözleşmesini** imzalamalıdır. Makalenin yayımlanması kabul edildikten sonra makale metninde, yazarlarında ve yazarların sıralamasında değişiklik yapılamaz. Makale yayıma kabul edildiğinde, sorumlu yazar Ankara Üniversitesi adına açılmış banka hesabına 300 TL yatırmalıdır. Makaleden sorumlu yazara banka hesap numarası, makalenin basıma kabul edilmesinden sonra bildirilir.

Makale Yükleme

Hazırlanan makaleler; sadece makaleden sorumlu yazar (makalenin yayım başlangıcından basım sonrasındaki her türlü yazışmalarda sorumluluğu bulunan) tarafından Tarım Bilimleri Dergisi web sayfasındaki çevrimiçi **Makale Gönderme ve Değerlendirme Sistemi** kullanılarak elektronik ortama yüklenmelidir. Makale yükleme bölümünün **"Başvuruyu Yükle"** bölümünde pdf formatındaki makale dosyasına ilave olarak "**Ek Dosyalar**" bölümüne aşağıdaki dosyaların da yüklenmesi gerekir.

- ✓ Makalenin Word (2003 veya daha üst versiyonları) formatındaki dosyası. Sisteme yüklenen makalenin hem pdf formatında ve hem de Word formatında iletişim, ad-soyad, kurum gibi yazarları tanıtıcı bilgiler bulunmamalıdır.
- ✓ Tüm yazarlar tarafından imzalanmış ve pdf formatında taranmış olan "Makale Gönderme ve Telif Hakkı Devir Sözleşmesi". Yayıma kabul edilmesi durumunda bu formların aslı da posta ile editöre gönderilmelidir.
- ✓ Yazar Makale Kontrol Listesi (pdf formatında),
- ✓ Yazarların ad-soyad, kurum ve iletişim bilgilerini içeren Word dosyası,
- ✓ Gerekiyorsa Etik Kurul Raporu eklenmelidir.

Derginin Kapsamı

Tarım Bilimleri Dergisi, tarım bilimleri alanında yapılan özgün araştırmaları ve yeni bulguları içeren makaleleri yayımlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayana çalışmalar derginin kapsamı dışındadır. Derleme makaleler, yayın komisyonunun çağrısı üzerine hazırlanmışsa normal inceleme ve değerlendirme sürecinden geçirilerek yayınlanır.

Makale Hazırlama

Makaleler, A4 boyutundaki kâğıdın tek yüzüne 12 punto Times New Roman yazı tipinde ve çift satır aralıklı yazılmalıdır. Sayfanın sağında, solunda, altında ve üstünde 3'er cm boşluk bırakılmalıdır. Makalenin her sayfası ve satırları numaralandırılmalıdır. Yazar ad(lar)ı açık olarak yazılmalı ve herhangi bir akademik unvan belirtilmemelidir. Editörler kurulu, anlatım dili yeterli olmayan makaleleri değerlendirme dışı tutabilir. Yazar(lar)ın makale göndermeden önce eseri dil yönünden bir dil bilimciye incelettirmesi tavsiye olunur. Sıralama olarak, İngilizce özet ve peşinden Türkçe özet verilir. Bu durum şekil ve çizelge başlıkları için de geçerlidir.

Makale; Türkçe Başlık, Türkçe Özet, Anahtar Kelimeler, İngilizce Başlık, İngilizce Özet, Keywords, 1.Giriş, 2.Materyal ve Yöntem, 3.Bulgular ve Tartışma, 4.Sonuçlar, Teşekkür (varsa), Kısaltmalar ve/veya Semboller (varsa), Kaynaklar bölümleri ile Şekil ve Çizelgelerden oluşmalıdır. Bölüm adları koyu yazılmalıdır.

Makale, "Kaynaklar" bölümü dâhil 16 sayfayı geçmemelidir. Yazar(lar), bu kısımların oluşturulmasında derginin web sayfasındaki **Makale Hazırlama Şablonunu** kullanmalıdır.

Başlık: Kısa ve açıklayıcı olmalı, 14 punto ve koyu, kelimelerin ilk harfi büyük olmalı, ortalanarak yazılmalı ve 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 13 punto ve koyu yazılmalıdır.

Özet ve Anahtar Kelimeler: Türkçe ve İngilizce özetlerin her biri 300 kelimeyi geçmemelidir. Türkçe ve İngilizce özetlerde sırasıyla "Özet" ve "Abstract" kelimeleri kullanılmalıdır. Özet, çalışmanın amacını, nasıl yapıldığını, sonuçları ve sonuçlar üzerine yazar(lar)ın yaptığı değerlendirmeleri içermelidir. Özetlerin 1 satır altına, her anahtar kelimenin ilk harfi büyük diğerleri küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi biçimde tanımlayacak ve aralarında noktalı virgül (;) olacak şekilde en fazla 6 anahtar kelime yazılmalıdır.

1. Giriş: Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir.

2. Materyal ve Yöntem: Kullanılan materyal ve yöntem aynı başlıkta verilmelidir. Alt başlık verilecekse bölüm numarası ile birlikte numaralandırılmalı (2.1. gibi) ve italik yazılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır.

3. Bulgular ve Tartışma: Elde edilen bulgular verilmeli, gerekirse çizelge, şekil ve grafiklerle desteklenerek bulgular açıklanmalıdır. Elde edilen bulgular tekrardan kaçınılması amacıyla ya çizelge ya da grafik olarak verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. İstatistiki analiz yönteminin doğru seçilmediği ve/ya analizin gereği gibi yapılmadığı durumlarda editörler kurulu makaleyi değerlendirme dışında tutabilir. Bulgular tartışılmalı ancak gereksiz tekrarlardan kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri açıklanmalıdır.

4. Sonuçlar: Elde edilen sonuçlar, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir. Giriş ile Bulgular ve Tartışma bölümünde verilen ifadeler bu kısımda aynı şekilde tekrar edilmemelidir.

Teşekkür: Gerekli ise mümkün olduğunca kısa olmalı ve yapılan katkı ifade edilerek verilmelidir.

Kısaltmalar ve/veya Semboller: Makalede kısaltmalardan mümkün olduğunca kaçınılmalıdır. Semboller Makale Hazırlama Şablonunda belirtildiği gibi verilmelidir. Kısaltma ve semboller metin içinde ilk kez kullanıldığı yerde açıklanmalıdır. Uluslararası geçerliliği olan ve yerleşik kısaltmalar tercih edilmelidir. Kısaltmalar makalenin başlığında kullanılmamalıdır. Semboller SI sistemine göre verilmelidir.

Kaynaklar: Eserde yararlanılan kaynaklara ilişkin atıf metin içinde "(Yazarın soyadı yıl)" yöntemine göre yapılmalıdır. Örnek: (Doymaz 2003), (Basunia & Abe 2001). Yazara atıf yapılırsa sadece yayının yılı parantez içine alınmalıdır. Örnek: Doymaz (2003)'e göre ya da Basunia & Abe (2001). Üç ya da daha fazla yazar için makale içindeki atıfta "et al" kullanılmalıdır. Örnek: (Lawrence et al 2001) veya Lawrence et al (2001)'e göre. Aynı yazarın aynı yıl içinde 1'den fazla yayını varsa, yıldan sonra küçük harfler verilmelidir. Örnek: (Akpınar et al 2003a). Aynı yazarın birden fazla yayınına atıf yapılacaksa yıldan sonra noktalı virgül (;) işareti ile ayırt edilmelidir. Örnek: (Akpınar 2007; 2009; 2013). Birden fazla atıf yapılırsa atıflar arasında noktalı virgül (;) kullanılmalı ve eskiden yeniye doğru yıl sırasına göre verilmelidir. Örnek: (Perl et al 1987; Bailly et al 1996; Copeland & McDonald 2001; Goel & Sheoran 2003). Eğer bilginin, kaynağın belirli bir sayfasından ya da sayfalarından alındığı belirtilmek istenirse (Hardeman & Jochemsen 2012, s 657-674; Naess 1991, s 34) biçiminde gösterilmelidir. Kaynaklarda Anonim ya da Anonymous şeklinde gösterim yapılmamalıdır.

Kaynaklar bölümünde metin içinde atıfı yapılan tüm kaynaklar alfabetik olarak (yazarların soyadlarına göre) ve orijinal dilinde verilir. Aynı yazara birden çok atıf yapılıyorsa önce tek isim, sonra iki isim ve sonra da üç ve daha fazla yazarlı kaynak sırasına göre hepsi kendi içinde eskiden yeniye yıl sırasına göre verilmelidir. İki veya daha fazla yazarlı eserlerin bildiriminde son yazardan önce "&" kullanılmalıdır. Örnek: Lawrence K C, Funk D B & Windham W R (2001). Dergi isimleri kısaltma yapılmadan tam adı ile ve italik yazılmalıdır. Kongre kitaplarında Türkçe ya da yabancı dilde özeti yayınlanmış çalışmalara atıf yapılamaz. Makaledeki yanlış atıf ve kaynak gösterimlerine ait sorumluluk yazar(lar)a aittir. Kaynaklar bölümündeki her bir kaynağın sonuna nokta (.) konmamalıdır.

Dergi:

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Bicer Y (2003a). Single layer drying behaviour of potato slices in a convective cyclone dryer and mathematical modelling. *Energy Conversion and Management* **44**(10): 1689-1705

Kitap:

Yıldırım O (1996). Bahçe Bitkileri Sulama Tekniği. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1438, Ders Kitabı: 420, Ankara

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Kitapta Bölüm:

Fıratlı Ç (1993). Arı yetiştirme. (Ed: M Ertuğrul), *Hayvan Yetiştirme*, Baran Ofset, Ankara, s. 30-34

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Yazarı Belirtilmeyen Kurum Yayınları:

TUİK (2012). Tarım İstatistikleri Özeti. Türkiye İstatistik Kurumu, Yayın No: 3877, Ankara

ASAE (2002). Standards S352.2, 2002, Moisture measurement-unground grain and seeds. ASAE, St. Joseph, MI

İnternetten Alınan Bilgi:

FAO (2013). Classifications and standards. http://www.fao.org/economic/ess/ess-standards/en/ (Erişim tarihi:10.02.2013)

Tez:

Koyuncu T (1992). Tarım arabalarında kullanılan çarpma etkili frenlerin araştırılması. Yüksek lisans tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Tam Metin Kongre/Sempozyum Kitabı:

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, Turkey, pp. 565–569

Kara Z & Beyoğlu N (1995). Konya ili Beyşehir yöresinde yetiştirilen üzüm çeşitlerinin göz verimliliklerinin belirlenmesi üzerine bir araştırma. *Türkiye II. Ulusal Bahçe Bitkileri Kongresi. Bildiriler (II)*: 3-6 Ekim, Adana, s. 524-528

Şekiller ve Çizelgeler: Şekil, grafik, fotoğraf ve benzerleri "Şekil", sayısal değerler ise "Çizelge" olarak belirtilmelidir. Tüm şekil ve çizelgeler makalenin sonuna yerleştirilmelidir. Şekil ve çizelgelerin boyu tek sayfa düzeninde en fazla 16x20 cm ve çift sütun düzeninde ise genişliği en fazla 8 cm olmalıdır. Şekil ve çizelgelerin boyutu baskıda çıkabilecek çözünürlükte olmalıdır. Araştırma sonuçlarını destekleyici nitelikteki resimler 600 dpi çözünürlüğünde "jpg" formatında olmalıdır. Renkli resimler yerine gri ya da siyah tonlu resimler tercih edilmelidir. Çizelgelerde düşey çizgi kullanılmamalı ve makale hazırlama şablonunda belirtildiği gibi hazırlanmalıdır. Her çizelge ve şekle metin içerisinde atıf yapılmalıdır. Tüm çizelge ve şekiller makale boyunca sırayla numaralandırılmalıdır (Çizelge 1 ve Şekil 1). Çizelge ve şekil başlıkları ve açıklamaları kısa ve öz olmalıdır. Çizelge ve şekillerin İngilizce başlıkları, Türkçe başlığın hemen altına italik olarak yazılmalı, ilk yazılan Türkçe başlık yazısı koyu olmalıdır. Şekil ve çizelge başlık yazıları 9.5 punto, şekil ve çizelgelerin içindeki yazılar 9 punto, çizelge altı yazılar 8 punto Times New Roman yazı karakterinde olmalıdır. Şekillerde yatay ve düşey kılavuz çizgiler ve rakamlar bulunmamalıdır. Ancak istatistiksel karşılaştırmalar yapılıyorsa küçük harfler bulunabilir. Çizelge ve şekillerde kısaltmalar kullanılmış ise hemen altına bu kısaltmalar açıklanmalıdır. Şekil ve çizelge başlıkları ile çizelge altı yazılarının sonuna nokta (.) konmamalıdır.

Birimler: Tüm makalelerde SI (Systeme International d'Units) ölçüm birimleri kullanılmalıdır. Ondalık kesir olarak nokta kullanılmalıdır (1,25 yerine 1.25 gibi). Birimlerde "/" kullanılmamalı ve birimler arasında bir boşluk verilmelidir (m/s yerine m s^{-1}, J/s yerine J s^{-1}, kg m/s^2 yerine kg m s^{-2} gibi). Sayı ile sembol arasında bir boşluk bırakılmalıdır (4 kg N ha^{-1}, 3 kg m^{-1} s^{-2}, 20 N m, , 1000 s^{-1}, 100 kPa, 22 °C ve % 29 gibi). Bu kuralın istisnaları düzlemsel açılar için kullanılan derece, dakika ve saniye sembolleridir (°, ′ ve ″). Bunlar sayıdan hemen sonra konmalıdır (10°, 45', 60" gibi). Litrenin kısaltması "l" değil "L" olarak belirtilmelidir. Cümle sonunda değillerse sembollerin sonuna nokta konulmamalıdır (kg. değil kg).

Formüller ve Eşitlikler: Formüller numaralandırılmalı ve formül numarası formülün yanına sağa dayalı olarak parantez içinde gösterilmelidir. Formüllerin yazılmasında Word programı matematik işlemcisi kullanılmalı, ana karakterler 12 punto, değişkenler italik, rakamlar ve matematiksel ifadeler düz olarak verilmelidir. Metin içerisinde atıf yapılacaksa "Eşitlik 1" biçiminde verilmelidir (…ilişkin model, Eşitlik 1' de verilmiştir).

JOURNAL OF AGRICULTURAL SCIENCES

Guide for Authors

Journal of Agricultural Sciences **is abstracted and/or indexed in:** Science Citation Index – Expanded, TUBITAK-ULAKBİM, CAB Abstracts, CAB International, FAO AGRIS/CARIS, and Directory of Open Access Journals (DOAJ).

Journal of Agricultural Sciences (JAS) is an international, double-blind peer-reviewed, open-access journal, published by the Faculty of Agriculture, Ankara University. The journal invites original research papers containing new insight into any aspect of Agricultural Sciences that are not published or not being considered for publication elsewhere. Preliminary, confirmatory or inconclusive research, review articles, case and local studies and works presenting taxonomy will not be published.

Before preparing papers for journal, authors should read through **Guide for Authors** and consult a current issue to make themselves familiar with general format.

The journal uses double-blind system for peer-review; both reviewers and authors' identities remain anonymous. The paper will be peer-reviewed by two reviewers from outside and one editor from the journal typically involve in reviewing a submission. Please note that authors are required to pay $ 100 for each manuscript published.

Manuscript Submission

Manuscripts should be submitted to journal's online submission system by the corresponding author. All submissions should include following documents:

- ✓ **Title page** with author names, titles, addresses and contact information (in Word format).
- ✓ **Manuscript** (in Word format version 2003 or above). All author names, titles and affiliations should be excluded.
- ✓ **Transfer of Copyright Form**. This form should be filled and signed by all authors and sent electronically as a scanned copy. Authors of the accepted papers should send the original version of this form.
- ✓ **Submission Check List** (in PDF format).
- ✓ **Ethics Committee Approval** (if needed).

Papers should be written with fluent English without any grammatical and typographical errors. Manuscripts with any of those errors will be rejected and sent to the authors for corrections before submission and review.

Manuscripts should be typed using Times New Roman font 12 pt. with numbered lines, in the left-hand margin and double spacing throughout, i.e. also for abstracts, footnotes and references. The pages of the manuscript, including the title page, abstract, references, tables, etc. should be numbered consecutively. Make the width at 3 cm for all margins. Place tables and figures with captions after the text. Each figure and table should be referred to in the text. Avoid excessive use of italics to emphasize part of the text.

Manuscripts should include the following sections; **Title** (short, specific and informative), **Keywords** (indexing terms, up to 6 items), **1. Introduction**, **2. Material and Methods**, **3. Results and Discussion**, **4. Conclusions**, **Acknowledgements** (if needed), **Abbreviations and Symbols** (if needed), **References**, **Figures and Tables** with captions not exceeding 16 pages (with references). All headings and titles should be written in Bold.

Acknowledgements

Acknowledgements should be a brief statement at the end of the text and may include source of financial support. The contract number should be provided.

References

Cite references in the text as author's family name should be followed by the year of the publication in parentheses (Peter 2010; Basunia & Abe 2001). Use et al after the first author's family name for citations with three or more authors (Lawrence et al 2001). For citations of the same authors published on the same year, use letters after the year (Dawson 2009a).

References cited in the text should be arranged chronologically. The references should be listed alphabetically on author's surnames, and chronological per author. Names of journals should be in full titles rather than the abbreviations. Avoid using citations of abstract proceedings. The following examples are for guidance.

Journal Articles

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Biçer Y (2003a). Single layer drying behavior of potato slices in a convective cyclone dryer and mathematical modeling. *Energy Conversion and Management* **44**(10): 1689-1705

Books

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Book Chapter

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds.), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Publications of Institutions / Standard Books

ASAE (2002). Standards S352.2, 2002, Moisture measurement - unground grain and seeds. ASAE, St. Joseph, MI

Internet Sources

FAO (2013). Classifications and standards. Retrieved in April, 12, 2011 from http://www.fao.org/economic/ess/ess-standards/en/

Thesis and Dissertations

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Conference Proceedings (Full papers)

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, pp. 565–569

Tables and Figures

Tables and Figures should be numbered consecutively and accompanied by a title at the top. All tables and figures should not exceed 16x20 cm size. Figures should have high resolution, minimum 600dpi in jpg format. For publication purposes use grayscale images. Avoid using vertical lines in tables.

Illustrations

Do not use figures that duplicate matter in tables. Figures can be supplied in digital format, or photographs and drawings, which can be suitable for reproduction. Label each figure with figure number consecutively.

Units

Units of measurement should all be in SI units. Use a period in decimal fractions (1.24 rather than 1,24). Avoid using "/". Include a space between the units (m s^{-1} rather than m/s,J s^{-1} rather than J/s, kg m s^{-2} rather thankg m/s^2). Units should have a single space between the number and the unit (4 kg N ha^{-1}, 3 kg m^{-1} s^{-2}, 20 N m, 1000 s^{-1}, 100 kPa, 22 °C). The only exceptions are for angular definitions, minutes, seconds and percentage; do not include a space (10°, 45›, 60», 29%). The abbreviation of liter is "L".

Formulas and Equations

Number each formula with the reference number placed in parentheses at the end. Use Word mathematical processor for formulas with 12pt., variances in Italics, numbers and mathematical definitions in plain text. If needed, refer as "Equation 1" in the text (….the model, as given in Equation 1).

Note: Title and Abstract, Tables and Figures Captions are translated into Turkish by editor for authors whose native language is not Turkish.