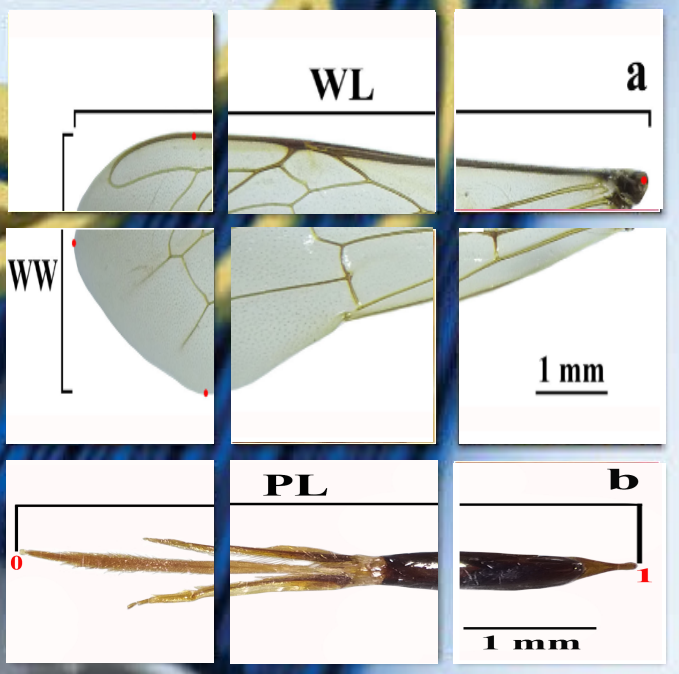
tbd

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

e-ISSN: 2148-9297



Yıl/Year 20
Sayı/Number 1
Cilt / Volume 26

KAPAK FOTOĞRAFI (COVER PHOTO) :
Wing (a), proboscis and (b), leg of honey bee.
Photo: Meral KEKEÇOĞLU

Ankara Üniversitesi
ZİRAAT FAKÜLTESİ

Ankara University
Faculty of Agriculture

TARIM BİLİMLERİ DERGİSİ

Journal of Agricultural Sciences

ISSN **2148-9297** (Online)

Cilt Volume	**26**	Sayı Number	**1**	**2020**

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

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 Local, taxonomy and case related studies, ordinary and out-of-date research articles are out of scope of journal.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 **26** Sayı Number **1** **2020**

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. Ergin DURSUN
Sorumlu Yazı İşleri Müdürü Editor-in-Chief	Prof. Dr. Ayten NAMLI
Yayın Ünitesi Sorumlusu In Charge of Publication Unit	Zir. Müh. Asım GÖKKAYA
Dergi Yönetimi Journal Administrator	Öğr. Gör. Mücahit ŞAR Salih ÖZAYDIN
Web Sayfası Koordinatörü Web Page Coordinator	Öğr. Gör. Murat KÖSEÇAVUŞ
Grafik Tasarım Graphic Design	Salih ÖZAYDIN
Basım Tarihi Date of Publication	04/03/2020
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

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

Antibacterial Effect of Bacteriocinogenic Enterococci from Different Sources on *Listeria monocytogenes*

Emel UNAL TURHAN[a], Zerrin ERGINKAYA[b], Alin BAYRAM TUREMIS[b], Mevhibe TERKURAN[b]

[a]*Osmaniye Korkut Ata University, Kadirli Applied Sciences School, Department of Food Technology, Osmaniye, TURKEY*

[b]*University of Cukurova, Faculty of Agriculture, Department of Food Engineering, Adana, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Emel UNAL TURHAN, E-mail: emelunalturhan@gmail.com, Tel: +90 (328) 888 00 90
Received: 27 July 2018, Received in Revised Form: 17 September 2018, Accepted: 17 December 2018

AUTHORS ORCID ID
(Emel UNAL TURHAN: 0000-0002-0284-574X), (Zerrin ERGINKAYA: 0000-0001-6208-2927), (Alin BAYRAM TUREMIS: 0000-0002-8014-6012), (Mevhibe TERKURAN: 0000-0002-3150-459X)

ABSTRACT
In this study, antimicrobial activity of partially purified enterocins and crude bacteriocins from *Enterococcus* isolates with different sources was investigated against *Listeria monocytogenes* by disk diffusion assay. Totally 70% of enterococcal isolates (*Enterococcus faecalis* and *Enterococcus faecium* from food and clinical sources) were found as potential bacteriocinogenic strains. Both of food and clinical enterococcal isolates also exhibited antimicrobial properties against *L. monocytogenes*. Additionally, the present study detected that inhibitory activity was strain-specific. Both crude bacteriocins and partially purified enterocins from *E. faecium* isolates showed lower antimicrobial activity against *L. monocytogenes* than *E. faecalis* isolates. The inhibition diameters obtained with crude enterocins and partially purified enterocins were respectively ranging from 12.33 mm to 13.25 mm and from 8.66 mm to 9.25 mm. Crude bacteriocins retained antibacterial activity after heat treatment except 120 ºC and also remained functional at pH values between 3 and 11. As a result, it was considered that enterocins could be benefit in heated and acidic or basic food products as biopreservative.

Keywords: Bacteriocin; Enterocin; *Enterococcus* spp.; *Listeria monocytogenes*

1. Introduction

Enterocin is novel bacteriocin produced by *Enterococcus* spp. and active against various pathogenic or food spoilage bacteria such as *Listeria* spp., *Clostridium* spp., *Staphylococcus* spp., *Bacillus* spp., *Campylobacter* spp. and *Escherichia coli* (Moreno et al 2003; Campos et al 2006; Javed et al 2010; Anandani & Khan 2014; Nami et al 2015). Bacteriocinogenic Enterococci strains, mostly *E. faecalis* and *E. faecium*, are isolated from different sources including vegetables, mostly fermented foods (cheese, sausages and other meat products), gastrointestinal system and various clinical specimens like urine, skin swab, pus and blood (De Vuyst et al 2003; Theppangna et al 2007; Ogaki et al 2016).

In recent years, there has been an increased tendency to use and study natural additives, such as natural antimicrobials and antioxidants. For this reason, bacteriocins have attracted more and more great attention (Savadago et al 2004; Yıldırım et al 2014; Ogaki et al 2016). The use of bacteriocins or bacteriocinogenic

DOI: 10.15832/ankutbd.448475

cultures seen as a useful biocontrol method in food preservation to decrease the growth of spoilage or pathogenic microorganisms (Nascimento et al 2010; Ogaki et al 2016). In general, many researchers focused on the effect of temperature, medium composition and pH in bacteriocin production, but there are insufficient information deal with effect of these factors on inhibitory activity of bacteriocins (Aymerich et al 2000; Meera & Devi 2012). Also, while most of the papers on enterocins have related to bacteriocinogenic enterococci from food sources, less attention has been given to isolates from clinical origins. The isolation of novel bacteriocins will be beneficial (Ogaki et al 2016). *L. monocytogenes* was most sensitive indicator to enterocins among pathogenic bacteria (Aymerich et al 2000; Nascimento et al 2010; Nami et al 2015). *Listeria monocytogenes* is one of the most important foodborne pathogens and resistant to adverse conditions including a wide range of temperatures and pH, high NaCl, sodium nitrite and various disinfectants. The prevention of *L. monocytogenes* growth in foods is highly difficult due to its resistance (Aymerich et al 2000). Investigators have come up with this problem from *L. monocytogenes* by using bacteriocins and described a large number of antilisterial bacteriocins (Ennahar & Deschamps 2000). Bacteriocins were mostly tested in meat and dairy product to inhibit *L. monocytogenes*. For example; enterocin from *E. faecium* DPC1146 had inhibitory effect on *L. monocytogenes* in milk. A drop in viable cell counts of *L. monocytogenes* in enterocin AS-48 added meat sausages was observed (Galvez et al 2008).

The aim of the present study was to isolate bacteriocinogenic enterococci from clinical and food sources and to evaluate the effect of temperature and pH on antibacterial activity of crude bacteriocins supernatant against *L. monocytogenes*.

2. Material and Methods

2.1. Samples and bacterial strains

In this study, enterococci strains identified before by VITEK-2 automated identification system in the University central laboratory were used. As seen in Table 1, a total of 20 enterococcal isolates were selected from clinical cases (5 of *E. faecalis* and 5 of *E. faecium*) and from foods (5 of *E. faecalis* and 5 of *E. faecium*). Enterococci strains isolated from food samples (white cheese, Tulum cheese, raw chicken meat, fermented sausages) were provided by Cukurova University, Food Engineering Department. Enterococcal isolates provided various clinical sources were collected from the Central Laboratory of Balcalı Hospital, Adana-Turkey during 2010-2011. All enterococcal isolates (food and clinical) were stored in Brain Hearth Infusion Broth (BHI-Fluka, Germany) including 10% sheep blood and 10% glycerol (v/v) at -20 °C. All enterococcal strains were subcultured twice prior to the experiments. Enterococci were grown in De Man, Rogosa and Sharpe broth (MRS broth; Merck, Darmstadt, Germany). *Listeria monocytogenes* ATCC7644 (Remel-USA) was used as indicator organism. *L. monocytogenes* was growth in BHI broth and stored at -20 °C in BHI broth supplemented with 20% (v/v) glycerol (Ogaki et al 2016).

2.2. Antibacterial spectrum of partially purified bacteriocins from enterococcal isolates

Enterocins were partially purified from food and clinically isolates of *E. faecium* and *E. faecalis* according to modificated method of Anandani & Khan (2014), Moreno et al (2003), Savadago et al (2004) and Yıldırım et al (2014). The enterococcal isolates were incubated for 48 h at 37 °C, in 250 mL MRS broth. After incubation, cells were removed by centrifugation (10000 g at 4 °C, 20 min), and pH of the cell free culture supernatant (CFS) was adjusted to pH 6.5 by the addition of 10 N NaOH to exclude antimicrobial effect of organic acid. Then, CFS was filter-sterilized (0.45 µm membrane-Millipore, Carrigtwohill, Ireland). The final concentration of sterile suspension was adjusted to 40% saturation of ammonium sulphate by slowly adding, and shaken overnight at 4 °C. The mixture was centrifuged (13000 g at 4 °C, 45 min) and after harvesting of the surface specimens and bottom pellets were performed resuspension in 10 mL sodium phosphate buffer (10 mM, pH 7). One volume of this suspension was mixed with 15 volumes of a methanol-chloroform (1:2, v/v) and then extraction of this mixture was performed at 4 °C for 1 h. The sample was centrifuged (15500 g, 4 °C, 30 min), the supernatant fraction decanted and the pellet air-dried. After resuspension of the pellet with 10 mL of ultrapure water (MilliQ; Millipore N.V., Brussels, Belgium) partially purified bacteriocin was obtained and was stored at -20 °C.

After purification, the antibacterial activity of enterocin was analyzed on Mueller Hinton Agar by disk diffusion assay against *L. monocytogenes* as a target (indicator) strain with a bit modification of previous reports (Yamato et al 2003; Savadago et al 2004; Campos et al 2006; Zheng et al 2015; Khalkhali & Mojgani 2017). Disk diffusion assay was used for detection of bacteriocin activity in enterococcal isolates. *L. monocytogenes* indicator strain at a 10^6 cfu mL^{-1} concentration was spread on Mueller-Hinton agar (Oxoid, England). Then, 100 µL portions of samples from enterococci were placed on paper disks (thick, 6 mm, Oxoid, England), which had previously been placed on the agar plates. The plates were incubated at 37 °C, for 24 h and translucent halos in the bacterial lawn surrounding the disks showed antibacterial activity. Diameters of inhibition zone around the disks were measured in millimeters.

Table 1- Inhibition zones from CB and partially purified enterocins obtained from each enterococcal isolate (as mm)

Code of samples	*Origin of samples*	*Species of isolate*	*Partially purified enterocins*	*CB*
F1	Urfa cheese	*E. faecalis*	8.00	12.00
F2	Fermented sausages	*E. faecalis*	10.00	12.00
F3	Raw chicken meat	*E. faecalis*	9.00	16.00
F4	Raw chicken meat	*E. faecalis*	0.00	0.00
F5	Fermented sausages	*E. faecalis*	10.00	12.00
F6	Antep cheese	*E. faecium*	10.00	14.00
F7	Erzincan Tulum cheese	*E. faecium*	0.00	0.00
F8	Hatay cow cheese	*E. faecium*	9.00	13.00
F9	Kasseri cheese	*E. faecium*	0.00	0.00
F10	Fermented sausages	*E. faecium*	8.00	10.00
C1	Clinical origin	*E. faecalis*	0.00	0.00
C2	Clinical origin	*E. faecalis*	11.00	12.00
C3	Clinical origin	*E. faecalis*	8.50	15.00
C4	Clinical origin	*E. faecalis*	8.00	12.00
C5	Clinical origin	*E. faecalis*	9.00	14.00
C6	Clinical origin	*E. faecium*	0.00	0.00
C7	Clinical origin	*E. faecium*	8.00	15.00
C8	Clinical origin	*E. faecium*	10.00	12.00
C9	Clinical origin	*E. faecium*	0.00	0.00
C10	Clinical origin	*E. faecium*	8.00	11.00

CB: crude bacteriocins (sterile cell free supernatant at pH 7)

2.3. Antibacterial spectrum of crude bacteriocins from bacteriocinogenic enterococcal isolates at different pH and temperature

The overnight bacteriocinogenic cultures were centrifuged at 10000 g for 30 min at 4 ºC. Cell free supernatants (CFS) were adjusted to pH 6.5 by the addition of 10 N NaOH and sterilized by filtration through a 0.45 µm membrane (Millipore, Carrigtwohill, Ireland). CFS was resuspended in 10 mM sodium phosphate buffer (pH 7) (Nascimento et al 2010; Nami et al 2015; Khalkhali & Mojgani 2017). This resuspended CFS (pH 7) was used as crude bacteriocins (CB) to detect antimicrobial spectrum of bacteriocinogenic cultures (Cintas et al 1998). The inhibitory spectrum of CB in different pH and temperature was studied by determining the antagonistic action of CB, against indicator organism (10^6 cfu mL^{-1}) by disk diffusion assay as mentioned above. The antibacterial activity was detected by measuring the clear zones around the disks containing CB. The clear inhibition zones were given in mm. Thermal stability of bacteriocinogenic enterococci was determined by incubation of CB at 60 °C, 70 °C, 80 °C, 90 °C, 110 °C, and 121 °C for 15 minutes. After incubation, bacteriocin samples were cooled to +4 °C (Campos et al 2006; Javed et al 2010). CB exposured to heat treatment was tested for antibacterial activity as described above. The pH stability of bacteriocinogenic enterococci was assayed at pH values 3, 5, 7, 9, and 11. pH level was adjusted with addition of 4 N HCl or 4 N NaOH to CB. For each test, 50 mL of CB was mixed with 2 mL of sodium phosphate buffer (10 mM) at each pH, and samples were incubated at room temperature (25 °C) for 2 h (Franz et al 1997; Javed et al 2010). The antibacterial activity in each sample was determined as described above.

3. Results and Discussion

Nowadays, there is a trend to detect novel enterocins from different origins due to their antimicrobial activity (Moreno et al 2003). Especially, it was focused on antilisterial activity of enterocins from *E. faecium* from various food such as meat products, fermented sausages, cheese (Ennahar & Deschamps 2000; Aymerich et al 2000; Marekova et al 2003; Vimont et al 2017). In our study, enterocins from different origins were compared in view of their antibacterial activity against *L. monocytogenes* strain. Both clinical and foodborne enterococci that are used in this paper may be candidate strains for practical use. However there is a needed more information for distinction among enterocins (Moreno et al 2003). Therefore, bacteriocinogenic enterococcal strains should be carefully and individually assessed for their safety and associated risk factors (Khalkhali & Mojgani 2017).

In this study, 20 strains of enterococci (10 *E. faecalis* and 10 *E. faecium*) from different sources were collected. Especially, *E. faecalis* and *E. faecium* were selected because pervious researchers reported that bacteriocinogenic strains are mostly belong to *E. faecium* and *E. faecalis* (De Vuyst et al 2003; Theppangna et al 2007; Özdemir et al 2011; Vimont et al 2017; Vijayakumar & Muriana 2017). As observed in Table 1, eight of *E. faecalis* strains and six of *E. faecium* (totally 70% of strains) were bacteriocinogenic and the rest of strains (totally 20% of strains) did not produce any bacteriocin.

3.1. Antibacterial activity of CB and partially purified bacteriocins from bacteriocinogenic enterococci

As seen in Table 2, both CB and partially purified bacteriocins had antibacterial effect on *L. monocytogenes*. Inhibition zones from CB varied from 12.33 mm to 13.25 mm whereas inhibition zones from enterocins were found between 8.66 mm and 9.25 mm. When enterococcal strains were compared, CB and partially purified enterocins from *E. faecalis* had highest inhibition effect. Inhibition zones by CB and enterocin from *E. faecalis* were measured respectively as 13.12 mm and 9.18 mm in diameter. *E. faecalis* accounted for greater percentage (57.14%) of antibacterial activity from the samples than *E. faecium* (42.85%) as reported in Anandani & Khan (2014). Similarly; De vuyst et al (2003) found that 58.7% of the *E. faecium* strains and 68.3% of the *E. faecalis* were bacteriocinogenic.

Table 2- The average of inhibition zones from CB and partially purified enterocins obtained from bacteriocinogenic enterococci (as mm)

Bacteriocinogenic enterococcal isolates	*CB*	*Partially purified enterocins*
Food isolates	12.71	9.14
Clinical isolates	13.00	8.92
E. faecalis	13.12	9.18
E. faecium	12.50	8.83
E. faecalis from food isolates	13.00	9.25
E. faecium from food isolates	12.33	9.00
E. faecalis from clinical isolates	13.25	9.12
E. faecium from clinical isolates	12.66	8.66

CB, crude bacteriocins

Antimicrobial effect of CB was found higher than partially purified enterocins. It was considered that presence of other inhibitory substances in CB caused additional antimicrobial activity (Zheng et al 2015). CB from clinical isolates led to higher inhibition than from food origin, while the opposite was observed for enterocin. As for isolate species, there are differences between antibacterial activity of isolate species (*E. faecalis* or *E. faecium*) and the effectiveness of the antibacterial activity of bacteriocinogenic enterococci is mostly relevant to the species. Klibi et al (2008) confirmed in our results that *E. faecalis* had higher antibacterial effect than *E. faecium* on *L. monocytogenes*. Generally, antimicrobial potential of enterococci was heterogeneous and strain-specific (Campos et al 2006; Nascimento et al 2010; Gómez et al 2012).

3.2. The effect of pH and temperature on antimicrobial activity of CB from bacteriocinogenic enterococci

Antibacterial activity of CB at different pH and temperature were presented in Table 3. CB exhibited a broader pH and temperature range of activity against *L. monocytogenes*. The activity of CB against *L. monocytogenes* was maintained in all pH range (3-11) and temperature grades except 120 °C, depending on enterococci strains. Antibacterial activity of CB reached maximum levels at pH 7 and 60 °C. CB from *E. faecalis* was found more resistant to pH and temperature deviations than *E. faecium*. Reduction in activity of CB from *E. faecalis* and *E. faecium* at 110 °C were found respectively at level of 26.30% and 20.97%. Especially, bacteriocins have lost their activity after the exposure to thermal stress at 120 °C and any inhibition zones did not observed on plates.

Antibacterial activity of the enterocins depends on the pH and temperature (Moreno et al 2003). Antibacterial effects of CB from our isolates were investigated at various pH and thermal conditions. Significant differences were recorded in the pH and thermal stability of the studied enterocins in accordance with Khalkhali & Mojgani (2017). As the temperature grades subjected to bacteriocins was increased, the inhibition zone was decreased in diameter. These results confirmed previous reports referring that the inhibitory action of the bacteriocinogenic enterococci reduced as temperature grade increased (Moreno et al 2003; Yamato et al 2003; Zhou et al 2014; Khalkhali & Mojgani 2017). These bacteriocinogenic enterococci may be applied as biopreservatives for various food products subjected to heat treatment such as pasteurization, cooking, sterilization (Campos et al 2006). Ghrairi et al (2008) detected that enterocin MMT21 exposured to heat treatment at 100 °C for 15 minutes did not exhibit any inhibition effect against *L. monocytogenes*. Bilgin (2008) reported that heat treatment at 90 °C for 30 minutes retains activity of enterocin HZ whereas as temperature increased to 110 °C and 121 °C for 15 minutes, a 50% and a 100% reduction in activity, respectively was observed. As seen our results, CB from *E. faecalis* strains were more resistant to increase in temperature grade than *E. faecium*. CB from clinical isolates was more sensitive to heat treatment than food isolates. Our results were confirmed by Uymaz (2009). Enterocins have mostly maintained inhibition effect both acidic and basic pH (Uymaz 2009). In general, they maintain their activity at diverse pH values between pH 4 and pH 8 (Ennnahar et al 1998). Ghrair et al (2008), enterocin produced by *E. faecium* MMT21 isolated from Tunisian Rigouta cheese had inhibitor effect on *L. monocytogenes* at pH between 2-10. Similarly, in our study, CB has continued its activity at pH ranging from 3 to 11. However, level of antibacterial activity of CB changed according to pH and highest inhibition effect was observed at pH 7. Antibacterial activity of bacteriocins gradually subsided as the pH values became more and more acidic or basic (Ennahar & Deschamps 2000; Javed et al 2010; Zhou et al 2014). As a matter of fact, the present study detected that there are a drop in activity of CB at high and low pH. Similar results were reported by various researchers: Bilgin (2008) stated that enterocin HZ by *E. faecium* obtained from local white cheese protect its activity in the range of pH 2-9, while half of its activity at pH 10, the majority of its activity at pH 11 and totally of inhibition activity at pH 12 was lost. Line et al (2008) reported that enterocin was active between pH 5.0 and 8.7 except pH 3.0 and above pH 9.5.

Table 3- The average of inhibition zones from crude bacteriocin (CB) obtained from bacteriocinogenic enterococci at different pH and temperature (as mm)

	Temperatures (°C)						*Acidity*				
Bacteriocinogenic enterococcal isolates	*60*	*70*	*80*	*90*	*110*	*120*	*pH 3*	*pH 5*	*pH 7*	*pH 9*	*pH 11*
Food isolates	12.54	12.25	11.54	11.17	10.04	0.00	9.88	10.46	12.71	9.25	9.13
Clinical isolates	11.96	11.42	10.92	9.96	9.50	0.00	9.50	9.75	13.00	11.42	10.38
E. faecalis	12.29	11.75	11.42	10.79	9.67	0.00	10.00	10.33	13.12	10.25	9.88
E. faecium	12.21	11.92	11.04	10.33	9.88	0.00	9.38	9.88	12.50	10.42	9.63
E. faecalis from food isolates	12.33	12.00	11.33	11.33	9.33	0.00	10.00	10.67	13.00	9.00	9.00
E. faecium from food isolates	12.75	12.50	11.75	11.00	10.75	0.00	9.75	10.25	12.33	9.50	9.25
E. faecalis from clinical isolates	12.25	11.50	11.50	10.25	10.00	0.00	10.00	10.00	13.25	11.50	10.75
E. faecium from clinical isolates	11.67	11.33	10.33	9.67	9.00	0.00	9.00	9.50	12.66	11.33	10.00

4. Conclusions

The present work detected that food and clinical enterococcal isolates had ability to form bacteriocinogenic affect against *L. monocytogenes*. This research clearly suggests the potential usefulness of the bacteriocins obtained from *E. faecalis* and *E. faecium* at broad pH and temperature range. Nonetheless, the inhibitory action of the bacteriocinogenic enterococci reduced as temperature grade increased. Enterocins has mostly maintained inhibition effect at both acidic and basic pH values. Their activity is the highest at neutral pH levels. Enterocins from food and clinical sources have potential to use in food industry as biopreservatives against *L. monocytogenes*. However, the relationship between bacteriocin production, hemolysis, antibiotic resistance and the presence of virulence factors should be individually evaluated to control the safety and risk factors of bacteriocins from food and clinical sources. As a result, enterocin producer enterococcal strains that are safe and their enterocins may be used for food preservation.

References

Anandani J H & Khan Z H (2014). Isolation, partial purification and biochemical characterization of enterocin producing enterococci. *International Journal of Advanced Research* 4(2): 18-20

Aymerich T, Garriga M, Ylla J, Vallier J, Monfort J M & Hugas M (2000). Application of enterocins as biopreservatives against *Listeria innocua* in meat products. *Journal of Food Protection* 63(6): 721-726

Bilgin H (2008). Antibacterial activity of a bacteriocinogenic bacterium isolated from a fermented milk product. Master thesis, Gaziosmanpaşa University (Published), Graduate School of Natural and Applied Sciences, 53 pages, Tokat, Turkey

Campos C A, Rodriguez O, Calo-Mata P, Prado M & Barros-Velazquez J (2006). Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (Psettamaxima). *Food Research International* 39: 356-364

Cintas L M, Casaus P, Fernandez M F & Hernandez H E (1998). Comparative antimicrobial activity of enterocin L50, pediocin PA-1, nisin A and lactocin S against spoilage and foodborne pathogenic bacteria. *Food Microbiology* 15: 289-298

De Vuyst L, Morenoa M R F & Revets H (2003). Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. *International Journal of Food Microbiology* 84: 299-318

Ennahar S & Deschamps N (2000). Anti-Listeria effect of enterocin A, produced by cheese-isolated *Enterococcus faecium* EFM01, relative to other bacteriocins from lactic acid bacteria. *Journal of Applied Microbiology* 88: 449-457

Franz C M A P, Toit M D, Holy A, Schillinger U & Holzapfel W H (1997). Production of nisin-like bacteriocins by *Lactococcus lactis* strains isolated from vegetables. *Journal of Basic Microbiology* 37(3): 187-196

Galvez A, Lopez R L & Abriouel H (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical Reviews in Biotechnology* 28: 125-152

Ghrairi T, Free J, Berjeaud J M & Manai M (2008). Purification and characterization of bacteriocin produced by *Enterococcus faecium* from Tunisian Rigouta Cheese. *Food Control* 19: 162-169

Gómez N C, Abriouel H, Grande M J, Pulido R P & Gálvez A (2012). Effect of enterocin AS-8 in combination with biocides on planktonic and sessile *Listeria monocytogenes*. *Food Microbiology* 30: 51-58

Javed I, Ahmed S, Manam S, Riaz M, Ahmad B, Ali M I, Hameed A & Chaudry G J (2010). Production, characterization, and antibacterial activity of a bacteriocin from newly isolated *Enterococcus faecium* IJ-31. *Journal of Food Protection* 73(1): 44-52

Khalkhali S & Mojgani N (2017). Bacteriocinogenic potential and virulence traits of *Enterococcus faecium* and *E. faecalis* isolated from human milk. *International Journal of Microbiology* 9(4): 224-233

Klibi N, Jouini A, Rojo-Bezares B, Masmoudi A, Ruiz-Larrea F, Boudabous A & Torres C (2008). Phenotypic and genotypic characterization of bacteriocins in clinical enterococcal isolates of Tunisia. *World Journal of Microbiology and Biotechnology* 24: 653-657

Line J E, Svetoch E A, Eruslanov B V, Perelygin V V, Mitsevich E V, Mitsevich I P, Levchuk V P, Svetoch O E, Seal B S, Siragusa G R & Stern N J (2008). Isolation and purification of enterocin e-760 with broad antibacterial activity against gram-positive and gram-negative bacteria. *Antibacterial Agents Chromatography* 52(3): 1094-1100

Marekova M, Laukova A, De Vuyst L, Skaugen M & Nes I F (2003). Partial characterization of bacteriocins produced by environmental strain *Enterococcus faecium* EK13. *Journal of Applied Microbiology* 94: 523-530

Meera N S & Devi M C (2012). Partial characterization and optimization of parameters for bacteriocin production by probiotic lactic acid bacteria. *Journal of Microbiology and Biotechnology Research* 2(2): 357-365

Moreno M R F, Callewaert R, Devreese B, Van Beeumen J & De Vuyst L (2003). Isolation and biochemical characterization of enterocins produced by enterococci from different sources. *Journal of Applied Microbiology* 94: 214-229

Nami Y, Haghshenas B, Haghshenas M & Khosroushahi A Y (2015). Antibacterial activity and the presence of virulence factors and bacteriocin structural genes in *Enterococcus faecium* CM33 isolated from ewe colostrum. Frontier Microbiology 6(782): 1-10

Nascimento M S, Moreno I & Kuaye A Y (2010). Antibacterial activity of *Enterococcus faecium* fair-E 198 against Gram-Positive pathogens. *Brazilian Journal of Microbiology* 41: 74-81

Ogaki M B, Rocha K R, Terra M R, Furlaneto M C & Maia L F (2016). Screening of the enterocin-encoding genes and antibacterial activity in Enterococcus species. *Journal of Microbiology and Biotechnology* 26(6): 1026-1034

Özdemir G B, Oryaşın E, Bıyık H H, Özteber M & Bozdoğan B (2011). Phenotypic and genotypic characterization of bacteriocins in enterococcal isolates of different sources. *Indian Journal of Microbiology* 51(2): 182-187

Savadago A, Ouattara C A T, Bassole I H N & Traore A S (2004). Antibacterial activities of lactic acid bacteria strains isolated from Burkinofaso fermented milk. *Pakistan Journal of Nutrition* 3(3): 174-179

Theppangna W, Murase T, Tokumaru N, Chikumi H, Shimizu E & Otsuki K (2007). Screening of the enterocin genes and antibacterial activity against pathogenic bacteria in enterococcus strains obtained from different origins. *Journal of Veterinary Medicine Science* 69(12): 1235-1239

Uymaz B (2009). Isolation and identification of probiotic lactobacilli originated from food and human and characterization of the ability of bacteriocin production. . PhD Thesis, Ankara Üniversitesi (Published), Graduate School of Natural and Applied Sciences, 146 pp, Ankara, Turkey

Vijayakumar P P & Muriana P M (2017). Inhibition of *Listeria monocytogenes* on ready-to-eatmeats using bacteriocin mixtures based on mode-of-action. *Foods* 6(22): 1-13

Vimont A, Fernandez B, Hammami R, Ababsa A, Daba H & Fliss I (2017). Bacteriocin-producing *Enterococcus faecium* lcw44: a high potential probiotic candidate from raw camel milk. *Frontier Microbiology* 8(865): 1-8

Yamato M, Ozaki K & Ota F (2003). Partial purification and characterization of the bacteriocin produced by *Lactobacillus acidophilus* YIT0154. *Microbiology Research* 158: 169-172

Yildirim Z, İlk Y, Yildirim M, Tokatli K & Öncül N (2014). Inhibitory effect of enterocin KP in combination with sublethal factors on *Escherichia coli* O157:H7 or *Salmonella* Typhimurium in BHI broth and UHT milk. *Turkish Journal Biology* 38: 412-419

Zhou F, Zhao H, Bai F, Dziugan P, Liu Y & Zhang B (2014). Purification and characterization of the bacteriocin produced by *Lactobacillus plantarum*, isolated from Chinese pickle. *Czech Journal Food Science* 32(5): 430-436

Zheng W, Zhang Y, Lu H M, Li D T, Zhang Z L, Tang Z X & Shi L E (2015). Antibacterial activity and safety evaluation of KQ2.6 isolated from Peacock feces. *BMC Biotechnology* 15(30): 1-8

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 Genetic Characterization of *DGAT1* Gene in Donkey Populations Reared in Thrace Region of Turkey

Fulya ÖZDIL[a]

[a]*Namık Kemal University, Faculty of Agriculture, Department of Agricultural Biotechnology, 59030 Tekirdağ, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Fulya ÖZDIL, E-mail: fozdil@nku.edu.tr, Tel: +90 (282) 250 2233
Received: 05 August 2018, Received in Revised Form: 07 November 2018, Accepted: 20 November 2018

AUTHORS ORCID ID
(Fulya ÖZDIL: 0000-0002-5298-6997)

ABSTRACT
AcylCoA: diacylglycerol acyltransferase (*DGAT1*) gene has a considerable effect on milk content and yield in cattle with a substitution of lysine by alanine in the exon 8 of the gene. Moreover there are many other researches comprising the *DGAT1* gene on different farm animals, such as buffalo, sheep and goat but there is no information about the *DGAT1* gene in donkeys. In this study, the polymorphism of *DGAT1* gene in donkey populations reared in Thrace region of Turkey has been investigated by restriction fragment length polymorphism (RFLP) via *Eae*I (*Cfr*I) restriction enzyme. *Eae*I restriction site was found in cattle breeds which resulted after K232A substitution, Lysine (AAG) to Alanine (GCG) variant but this restriction site was not found in donkey populations. A novel single-nucleotide polymorphism (G→A substitution) in the *DGAT1* gene at position 10,435 lacks this restriction site which results only Alanine variant (GCA) instead of Lysine variant. This novel single-nucleotide polymorphism in the *DGAT1* gene was found in the studied donkey breeds.

Keywords: *DGAT1* Gene; RFLP; *Equus asinus*; Donkey; Turkey

1. Introduction

Triglycerides are mainly synthesized from diglycerides that *DGAT1* is an enzyme that catalyzes a crucial role in mammalian triglyceride synthesis. *DGAT1* enzyme acts an important role in lipogenesis pathway in many tissues (Cases et al 1998) so the *DGAT1* gene, encoding this enyzme has been found relevant in milk production. In many of the studies, associations between *DGAT1* gene polymorphism and milk composition and production traits have been investigated (Spelman et al 2002; Weller et al 2002; Thaller et al 2003; Gautier et al 2007). Fat is an important component of mammalian milk. *DGAT1* gene is found as a potential candidate gene for milk fat yield in cattle (Schennink et al 2007). Moreover *DGAT1* gene, is also a candidate gene, because it has been found at the centromere region of the bovine 14th chromosome and includes 17 exons of variable sizes encoding a 489 amino acid protein that spans a quantitative trait locus (QTL) for milk production traits (Coppieters et al 1998; Grisart et al 2002). In the 8th exon of *DGAT1* gene (10.433th and 10.434th bp), two single-nucleotide polymorphisms (SNPs) have been reported and generated to QTL (quantitative trait loci) variation. These polymorphisms caused the substitution of lysine to alanine (K232A) and consulted to considerably affect the milk fat composition in cattle (Coppieters et al 1998; Winter et al 2002; Grisart et al 2002; 2004; Kaupe et al 2004). In *DGAT1* gene, Alanine variant (A allele) and Lysine variant (K allele) were related with high milk yield and high milk fat yield in cattle, respectively (Coppieters et al 1998; Winter et al 2002; Grisart et al 2004).

DOI: 10.15832/ankutbd.451099

In many countries, donkey breeds which were used as pack animals in rural areas have become extinct or critically endangered. In the last years, donkey populations have declined dramatically in Turkey. All over the World, donkeys which are under threat of extinction, have to be characterized both morphologically and genetically in order to constitute conservation strategies. For the last years, donkey milk has been used as curative, reformative, nutritive substance as well as cosmetics. Due to its rich content, the scientific interest to donkey milk has been increased recently. Cow's milk allergy is an important problem in infants and many researches show exciting findings on equid (horse and donkey) milk tolerability (Salimei et al 2004). So the candidate gene, *DGAT1*, which is found in association with milk production traits, should be investigated in donkeys in order to identify the gene regulation of donkey milk genetic parameters. So the aim of this study was to search the *DGAT1* gene in donkey populations reared in Thrace region of Turkey and introduce the variation in this gene region. Also the RFLP characterization of *DGAT1* gene in donkeys was conducted for the first time in Turkey.

2. Material and Methods

In this study, 61 blood samples were collected from Thrace region of Turkey, Kırklareli Province. 41 samples were collected from a donkey farm in Koruköy Village and 10 samples each were collected from Üsküp and Kuzulu Villages. Blood samples were collected from the vena jugulars of the donkeys and used for the DNA extraction.

All DNA isolations were done according to phenol chloroform extraction method with slight modifications (Sambrook et al 1989). PCR reactions and cycling conditions were carried out as reported in Kaupe et al (2004) in a 25 µL volume using 50 ng of genomic DNA

437 bp of *DGAT1* gene were amplified by Polymerase Chain Reaction (PCR) using the primers given in Kaupe et al (2004). To check whether the allelic variation that were reported in cattle, (10.433^{th}-10.434^{th} bp of the *DGAT1* gene, Genbank Accession no. JF894305) was also found in donkeys; *DGAT1* gene region amplification was digested with *Eae*I restriction enzyme (NEW England Biolabs Inc). The digested fragments were separated using 2% agarose gels, stained with SYBRSafe DNA gel stain (Thermo Fisher Scientific) and visualized with Vilber Lourmat gel imaging system.

The *DGAT1* genes of two samples were sequenced on an Applied Biosystems 3500XL Genetic Analyzer System (Applied Biosystems, USA) in order to verify the sequence variations of the *Eae*I restriction site.

3. Results and Discussion

The 437 bp of PCR products (including the primers) were amplified and digested with *Eae*I restriction enzyme. In all of the studied DNA samples uncut single band of 437 bp were obtained (Figure 1).

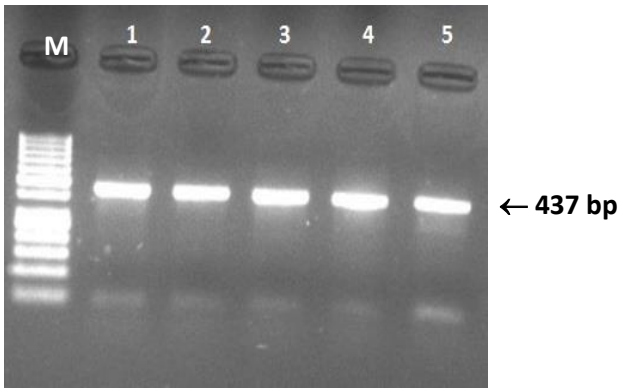


Figure 1- Undigested PCR products of *DGAT1* gene (437 bp) via *Eae*I restriction enzyme in donkeys. M: 50 bp DNA ladder (Invitrogen 10416014)

The sequencing of this region revealed polymorphisms when compared to cattle and buffalo *DGAT1* gene sequences. In the 8th exon of the gene, 10.433th-10.434th bp of the *DGAT1* gene, two SNPs were reported in cattle which revealed two different alleles, Lysine (A A G) and Alanine (G C G) alleles (Table 1). A→G and A→C base substitutions revealed *Eae*I recognition site and Alanine allele was obtained. Genbank records of these alleles are given in Table 1. The DNA sequence of this gene region in donkey revealed again Alanine allele, A→G and A→C base substitutions at position 10,433 and 10,434 were again determined but also a novel polymorphism at 10.435th of the *DGAT1* gene, G→A transition which also revealed Alanine (GCA) allele was obtained but this substitution resulted the loss of *Eae*I restriction site. As a result, no *Eae*I restriction in *DGAT1* gene was obtained in donkey populations.

Table 1- Allelic variation of *Eae*I restriction site in *DGAT1* gene in cattle, buffalo and donkeys

Nucleotide positions (Genbank no: AJ318490)	*10433*	*10434*	*10435*	*10436*	*10437*	*10438*	*10439*	*Allele*	*Genbank accession no*	*EaeI restriction*
Cattle	G	C	G	G	C	C	A	Alanine (A)	EU348567	+
Cattle	A	A	G	G	C	C	A	Lysine (K)	EU077528	-
Buffalo	A	A	G	G	C	C	A	Lysine (K)	JQ627609	-
Donkey	G	C	A	G	C	C	A	Alanine (A)	NW_014638167	-

*Eae*I (*Cfr*I) restriction Site: (T/C)GGCC(A/G) 10434 to 10439

4. Conclusions

Around the middle of the 20th century, as a consequence of industrialization in agriculture and the spreading several highly selected breeds, many animal populations have become extinct or are declining and endangered. In many countries, donkey breeds which were used as pack animals in rural areas have become extinct or critically endangered. In the last years, donkey populations have declined dramatically in Turkey (Anonymous 2018). So both morphological and genetic studies have to be conducted on Turkish native donkey breeds.

In this study, we used PCR-RFLP method by *Eae*I restriction enzyme of *DGAT1* gene to introduce the genetic polymorphism in donkey populations from Thrace region of Turkey. The 437 bp of *DGAT1* gene were amplified and digested with *Eae*I restriction enzyme and no restriction site was obtained in Turkish donkey populations as well as Anatolian buffalo populations (Özdil & İlhan 2012).

The sequencing of this region revealed polymorphisms when compared to cattle and buffalo *DGAT1* gene sequences. In the 8th exon of the gene, 10.433th-10.434th bp of the *DGAT1* gene, two SNPs were reported in cattle which revealed two different alleles, Lysine and Alanine alleles (Table 1). The DNA sequence of this gene segment in donkeys revealed Alanine allele, G and C at position 10.433 and 10.434, respectively, but also produced a novel polymorphism at position 10.435, G→A which also revealed Alanine allele but without *Eae*I digestion.

In many of the studies, *DGAT1* gene is indicated as a functional candidate gene that has a substitution of lysine by alanine (K232A) allele generating a fundamental effect on milk fat composition and yield (Coppieters et al 1998; Smith et al 2000; Winter et al 2002; Grisart et al 2002; 2004). In *DGAT1* gene while Lysine (AAG) variant (K allele) was related with high fat percentage of milk, Alanine variant (A allele) of this gene was related with high milk yield (Winter et al 2002; Grisart et al 2002; 2004). Also K allele reported to be the wild type allele (Coppieters et al 1998; Grisart et al 2002; Kaupe et al 2004). In this study only Alanine allele which is responsible for high milk yield in cattle, is found in donkeys. Also a novel polymorphism (G →A) at position 10.435 bp of the *DGAT1* gene is reported in donkey populations. This study provides an insight to donkey genetics and indirect evidence that all of the Thrace donkey populations in Turkey have fixed allele with respect to *DGAT1* Alanine allele.

References

Anonymous (2018). Turkish Statistical Institude. Animal statistics database. Retrieved in May, 10, 2018 fromhttps://biruni.tuik.gov.tr/hayvancilikapp/hayvancilik.zul

Cases S, Smith S J, Zheng Y W, Myers H M, Lear S R, Sande E, Novak S, Collins C, Welch C B, Lusis A J, Erickson S K & Farese Jr R V (1998). Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proceedings of the National Academy of Sciences of the United States of America USA* 95: 13018-13023

Coppieters W, Riquet J, Arranz J J, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Nezer C, Simon P, Vanmanshoven P, Wagenaar D & Georges M (1998). A QTL with major effect on milk yield and composition maps to bovine chromosome 14. *Mammalian Genome* 9: 540-544

Gautier M, Capitan A, Fritz S, Eggen A, Boichard D & Druet T (2007). Characterization of the DGAT1 K232A and variable number of tandem repeat polymorphisms in French dairy cattle. *Journal of Dairy Science* 90: 2980-2988

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M & Snell R (2002). Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* 12: 222-231

Grisart B, Farnir F, Karim L, Cambisano N, Kim J J, Kvasz A, Mni M, Simon P, Frère J M, Coppieters W & Georges M (2004). Genetic and functional confirmation of the causality of the *DGAT1 K232A* quantitative trait nucleotide in affecting milk yield and composition. *Proceedings of the National Academy of Sciences of the United States of America* 101: 2398-2403

Kaupe B, Winter A, Fries R & Erhardt G (2004). *DGAT1* polymorphism in *Bos indicus* and *Bos Taurus* cattle breeds. *Journal of Dairy Research* 71: 182-187

Özdil F & İlhan F (2012). *DGAT1*-exon 8 polymorphism in Anatolian buffalo. *Livestock Science* 149: 83-87.

Salimei E, Fantuz F, Coppola R, Chiofalo B, Polidori P & Varisco G (2004). Composition and characteristics of ass's milk. *Animal Research* 53: 67-78

Sambrook J, Fritsch E F & Maniatis T (1989). Molecular cloning: A laboratory Manual, vol. 3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y

Schennink A, Stoop W M, Visker M H P W, Heck J M L, Bovenhuis H, van der Poel J J, van Valenberg H J F & van Arendonk J A M (2007). *DGAT1* underlies large genetic variation in milk-fat composition of dairy cows. *Animal Genetics* 38: 467-473

Smith S J, Cases S, Jensen D R, Chen H C, Sande E, Tow B, Sanan D A, Raber J, Eckel R H & Farese R V.Jr (2000). Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nature Genetics* 25: 87-90

Spelman R J, Ford C A, McElhinney P, Gregory G C & Snell R G (2002). Characterization of the DGAT1 gene in the New Zealand dairy population. *Journal of Dairy Science* 85: 3514-3517

Thaller G, Krämer W, Winter A, Kaupe B, Erhardt G & Fries R (2003). Effects of DGAT1 variants on milk production traits in German cattle breeds *Journal of Animal Science* 81: 1911-1918

Weller J I, Golik M, Seroussi E, Ezra E & Ron M (2002). Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. *Journal of Dairy Science* 86: 2219-2227

Winter A, Kramer W, Werner F A O, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack J E, Thaller G & Fries R (2002). Association of a lysine-232⁄ alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. *Proceedings of the National Academy of Sciences of the United States of America* 99: 9300-9305

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

Members' Willingness to Invest Capital in the Agricultural Producer Unions: A Case of Samsun Province in Turkey

Bakiye KILIÇ TOPUZ[a]

[a]*Igdir University, Faculty of Agriculture, Department of Agricultural Economics, Igdir, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Bakiye KILIÇ TOPUZ, E-mail: bakiyekilic@hotmail.com, Tel: +90 (476) 223 00 10
Received: 20 May 2018, Received in Revised Form: 11 October 2018, Accepted: 27 November 2018

AUTHORS ORCID ID
(Bakiye KILIÇ TOPUZ: 0000-0002-3607-4306)

ABSTRACT
The research aimed to reveal the members' willingness to invest capital in the Agricultural Producer Union (APU) and the factors influencing their decisions. The main data of the study obtained through the surveys from 420 members of the APU. Members' willingness to invest capital in their unions and effective factors were determined using the Contingent Valuation Method and the Random Effects Tobit model, respectively. The research revealed that 44% of the union members were willing to make an average contribution of 355 TL to their unions. Random Effects Tobit model results revealed that member's trust, membership fee, participation in trainings, memberships of livestock union, apple and beekeeping, export crops through the union, attending the general assembly, level of education and agricultural experience positively affected the willingness to invest capital, while membership of the organic hazelnut and vegetables union, gender, management experience, age and total income negative affected the willingness to invest capital. In order to increase members' willingness to invest capital to their unions, members' trusts and participations in the training activities and administration of the unions should be increased.

Keywords: Willingness to invest capital; Agricultural producer union; Random effects Tobit model; Samsun

1. Introduction

Producers in the agricultural sector become members of agricultural organizations to meet their economic expectations such as providing agricultural inputs at a cheaper price, marketing their products more easily and at a higher price and increasing income and profitability (Karantininis & Zago 2001; Hansen et al 2002; Österberg & Nilsson 2009; Kılıç 2011; Kılıç Topuz 2017). Encouraging farmers to organize under farmer organizations and developing farmer organizations is stated as one of the prior issues and targets of agriculture policies in the main policy documents as 10th Development Plan, Agricultural Law No. 5488 and the 2013-2017 Strategic Plan of Ministry of Food, Agriculture and Livestock (RG 2006; GTHB 2013; RG 2013). In Turkey, producers come together under the economic, social and professional organizations. While producers are organized within agricultural cooperatives, APU, grower unions, irrigation unions and agricultural foundations for economic and social purposes, they come together within the agricultural chambers and farmer association for professional purposes. A total of 11,480,690 farmers were members of 15,222 agricultural organizations in Turkey. The share of APU in agricultural organizations was 6% (TRGM 2017). While the number of farmer organizations has increased in Turkey, they are not able to fulfill the functions expected from them (Özdemir et al 2010; Mülayim 2010). Capital insufficiency was stated as one of the main problems for the farmer organizations to provide sufficient and effective services

DOI: 10.15832/ankutbd.425327

to their members (Kaygusuzoğlu 2002; Şafaklı 2003; Rehber 2006; IRFO 2008; Mülayim 2010; Kılıç 2011; GTB 2012). Therefore, the farmer organization should have enough capital to provide sufficient services to their members. Membership share and fee constituted the most important legal income sources for agricultural producer organizations in Turkey (Kılıç Topuz et al 2017).

Reasons such as insufficiency of agricultural cooperatives in fulfilling the functions expected of them, problems of Agricultural Sales Cooperatives and their imposing a burden on the state budget, need for harmonization to the European Union's Common Agricultural Policy and lack of organizations for some crops or products have led to the emergence of APUs as a new organizational model in Turkey. APUs are legal entities in Turkey established voluntarily at the provincial or district level on the basis of crop or crop group a with the Law No 5200. APUs could get their income from membership fees, the share of services received from the sale price of the marketed products, wages from consultancy services provided to members, domestic and foreign donations, funds and aids, rents or revenues obtained from real estate, advertising, promotion and publications (RG 2004). APUs could not get enough capital through the mentioned income items and they could not provide sufficient services to their members. Therefore, it is of great importance that the members invest sufficient capital in the agricultural organizations.

The literature review revealed that only one research has been conducted about the willingness to invest capital in agricultural organizations (Newbery et al 2013). The study found that 87% of the members of the unions in England were willing to invest capital in their agricultural organizations and the willingness to invest capital was higher in the unions where the number of members and trust was higher. The Logit Regression model was used to determine the influential factors on investing capital. On the other hand, in Turkey, no study has been carried out on the willingness of the farmers to invest capital in their organizations. Therefore, this study aimed to reveal the members' willingness to invest capital in their unions and the factors influencing their investment decisions.

2. Material and Methods

2.1. Material

The main data was obtained from the surveys conducted April 2014-January 2015 with the members of 14 APUs in Samsun province, Turkey. As the sampling criterion, land property for the vegetable farms, animal assets for the livestock farms and the number of beehive for the beekeeping farm were used. Simple random sampling method was used (Equation 1) to determine the number of sample from the producer unions of organic hazelnut, vegetable, apple and beekeeping for which sampling criterion showed normal distribution. Stratified sampling method (Equation 2) was used to determine the number of sample from the producer unions of livestock and dairy cattle for which sampling criterion did not show normal distribution (Yamane 1967).

$$n = \frac{N(zC)^2}{Nd^2+(zC)^2} \quad (1)$$

Where; N refers to the number of farmers, z is standard normal distribution value corresponding to desired trust level (1.645), C is variation coefficient (standard deviation/average), d is the error margin accepted in the research ($\pm\%10$), n is the number of samples required.

$$n = \frac{N.\sum(N_h S_h^2)}{N^2D^2+\sum(N_h S_h^2)} \quad (2)$$

Where; n refers to the sample size, N is the number of units in the population, N_h is the number of units in the h^{th} layer, S_h^2 is the variation of the h^{th} layer. D^2 equals (d^2/Z^2), d is the difference between the maximum amount of error or sample mean that can be accepted by the researcher and population while z refers to the z value in the standard normal distribution table according to the error margin. The study was based on 10% error margin and 95% confidence level and the number of samples was calculated as 420. The adequacy of the sample number was tested by the Kaiser-Meyer-Olkin (KMO) sampling adequacy

measure and the Bartlett test. The KMO value was found to be 0.957 and the sample was determined to be high enough.

2.2. Method

Survey data were checked and then entered into the SAS 9.0 program to be analyzed. To determine the members' willingness to invest capital to their unions, Contingent Valuation Method (CVM) was used. CVM was widely used to determine willingness to pay for a specific feature (Mitchell & Carson 1989; Garrod & Willis 1990). The reliability of the survey was tested by reliability analysis, the members' response consistency to the survey questions was measured by the Cronbach Alpha approach and the research was found to be highly reliable (0.724). The members were asked that when the union increase net return of the farm at different amount (e.i. 0 TL, 1000 TL, 2000 TL, …, 10,000 TL), how much capital would you willing to invest to your union in each situation. With CVM, the level of respondents' willingness to pay based on assumption can be higher than what they pay in real life. Therefore, the respondents were also asked how sure they were on willingness to invest capital to the union (1: Not sure at all, … 10: definitely sure). Subsequently, it was accepted that the members were willing to invest capital to the union when the sureness level of member was 8 and above. The NLOGIT 5.0 package program was used to estimate the model of the study. Panel data was used in the study. There are union members in the horizontal section while in the time dimension, there are the amounts of the willingness to pay for the increment in the offer amount of each thousand $ for the amount of 11 proposals presented to each member (0, 1000, 2000, 3000, ..., 10,000 TL).

In the following, the base Tobit model proposed by Tobin (1958) will be adopted as the starting point to study the left-censored crash rate data. Under panel data formation, repeated observations are given for each group. As discussed above, correlations may exist among these repeated observations. A Tobit model with random effects is therefore proposed as it is capable of accounting for both censoring effects and serial correlations. The random effects Tobit model is applied to account for such correlations across observations in addition to unobserved heterogeneity. In the present study, random effects Tobit model is developed based on a typical left-censored Tobit model (with a lower limit of zero). A baseline structure for a left-censored Tobit model with panel data can be described as follows:

$$Y_{it}^{*} = X_{it}\beta + \varepsilon_{it}, i = 1, \dots, N, t = 1, \dots, T_i \text{ and} \quad (3)$$

$$Y_{it} = Y_{it}^{*}\ if\ Y_{it}^{*} > 0 \quad (4a)$$

$$Y_{it} = 0\ if\ Y_{it}^{*} \leq 0 \quad (4b)$$

Where; N is the number of members, t is the number of the repeated offer amount (0, 1000, 2000, 3000, ..., 10,000 TL), Y_{it} is the dependent variable (willingness to invest capital, WTI) and Y_{it}^{*} is the latent variable observed only when positive. X_{it} is a vector of explanatory variables, β is a vector of estimable coefficients, and ε_{it} is the error term.

The essential assumption for the random effects Tobit model to distinguish it from its fixed effects counterpart is that the heterogeneity (i.e. random effects) is assumed to be uncorrelated with the independent variables X_{it}. Thus, the corresponding log-likelihood function for the random effects Tobit model is derived by obtaining the unconditional density through integrating the random effects μ_i out of the conditional density (Greene 2012):

$$f(Y_{it}IX_{it}, \mu_i; \theta) = \prod_{Y_{it}>0} \sigma_v \frac{1}{\sigma_v} \varphi\left(\frac{Y_{it}X_{it}\beta - \mu_i}{\sigma_v}\right) \prod_{Y_{it}=0} \phi\left(\frac{-X_{it}\beta - \mu_i}{\sigma_v}\right) \quad (5a)$$

The panel level likelihood l_i is given by

$$l_i = \int_{-\infty}^{\infty} \frac{e^{-\frac{v_i^2}{2\sigma_v^2}}}{\sqrt{2\pi\sigma v}} \{\prod_{t=1}^{n_i} F(Y_{it}^*, X_{it}\beta + v_i)\} dv_i \quad (5b)$$

$$\equiv \int_{-\infty}^{\infty} g(Y_{it}^*, X_{it}, v_i) dv_i$$

Where; $\varphi(*)$ is standard normal density function; $\varphi(*)$ is standard normal distribution function.

Both Gauss-Hermite quadrature and simulation-based maximum likelihood estimations can be adopted to get the maximum of the log-likelihood function of random effect models (Greene 2012). The first approach gives approximated estimation in which the estimation accuracy is partly determined by the integration points used. Random effects Tobit model can be viewed as a special case of random parameter Tobit model, in which only the constant term is treated as a random parameter (Anastasopoulos & Mannering 2009; Lord & Mannering 2010) and simulation-based maximum likelihood estimation can be exploited to solve the log-likelihood function.

From the data described in the previous section, the final random-effects Tobit model estimated in this study was shown by the Equation 6:

$$WTI = ß_0 + ß_1 TRUST_{it} + ß_2 STOCK_{it} + ß_3 APPLE_{it} + ß_4 BEEKPNG_{it} + ß_5 VEGETAB_{it} + ß_6 ORGHAZEL_{it} + ß_7 CAPITAL_{it} + ß_8 JOINEDU_{it} + ß_9 EXPORT_{it} + ß_{10} GENMEET_{it} + ß_{11} DISTANC_{it} + ß_{12} SUPINPUT_{it} + ß_{13} MARKETNG_{it} + ß_{14} MANAGEXP_{it} + ß_{15} VISITFRE_{it} + ß_{16} PERFORMC_{it} + ß_{17} FRS_{it} + ß_{18} NUMORG_{it} + ß_{19} INSURANC_{it} + ß_{20} HSIZE_{it} + ß_{21} INCOME_{it} + ß_{22} MARSTAT_{it} + ß_{23} SOCSEQUR_{it} + ß_{24} MAINPROF_{it} + ß_{25} EDUCAT_{it} + ß_{26} EXPERIEN_{it} + ß_{27} GENDER_{it} + ß_{28} AGE_{it} + \varepsilon_{it} \quad (6)$$

In research, the variables that were thought to be effective on members' willingness to invest capital were selected. For example, if members' income change, members' willingness to invest capital can change. Similarly, product marketing or supply input through union can cause members' willingness to invest capital.

3. Results and Discussion

3.1. Descriptive results

The average capital of APUs was 196 thousand TL ($89.4 thousand[1]). APUs obtained 40.7% of their capital from membership fees. The other main income sources were agricultural support deductions (25.8%), consultancy services (23%), marketing of the crops or products (6.8%), real estate sales or rents (1.5%) and donations, funds and subsidies (1.4%). Dairy cattle and livestock producer unions have the highest equity capital due to the deductions from animal husbandry subsidies. About 86% of the unions had a capital insufficiency problem. APUs had averagely 612 members.

Descriptive statistics was given in Table 1. Research results revealed that the members were willing to invest capital an average of 154 TL ($70.3) in their union. Of the farmers interviewed 36% were from dairy cattle, 22% from organic hazelnut, 16% from livestock, 14% from vegetables, 10% from beekeeping and 2% from apple agricultural producer unions. Ninety-eight percent of the union members were male and the average age of the members was 50. Average education period was 6 years and 68% and 13% of the members graduated from primary and secondary school, respectively. Ninety-four percent of the members stated their main profession as farmer and their agricultural experience was 27 years. The average number of households was 4.88 persons.

[1] 2.19 Turkish Liras=$1 (average exchange rate of dollar in 2014)

The average performance index of the unions was 43.5%. The average trust level of the members to the union, executive board and other members was 3.17. Only 21% and 15% of the members marketed and exported their crops through the unions, respectively. The respondents were averagely members of two farmer organizations and majority of them (85%) paid fully their entrance fees to the unions. While only 5% of members provide input from their unions, and 32% of them participated in the trainings of the union. Research conducted in the South Eastern Anatolia Project by Karlı & Çelik (2003) revealed that 40.7% of the members provided inputs from agricultural organizations, 28% and 15.6% of them marketed their products and participated in training activities. The average distance between the members' farms and the unions was 13 km and the members visited their unions 51 times in a year. The VIF values of the independent variables used in the model are less than 20 and this indicates that there were no multiple correlations between the independent variables.

Table 1- Descriptive statistics of the variables of the models

Variables	*Definition of the variables*	*N*	*Mean*	*Std. dev.*	*VIF*
Dependent variable					
WTIC	Willingness to invest capital (TL)	4,620	154.86	313.03	-
Independent variables					
Union variables					
TRUST	Trust index for the union, executive board and other members (1: The lowest, …, 5: The highest)	4,620	3.17	1.21	1.72
STOCK	= 1 if the respondent is a member of livestock; 0 otherwise	4,620	0.16	0.37	1.81
APPU	= 1 if the respondent is a member of apple; 0 otherwise	4,620	0.02	0.16	1.34
BEEKU	= 1 if the respondent is a member of beekeeping; 0 otherwise	4,620	0.10	0.29	1.64
VEGU	= 1 if the respondent is a member of vegetables; 0 otherwise	4,620	0.14	0.34	2.20
OHAZU	= 1 if the respondent is a member of organic hazelnut; 0 otherwise	4,620	0.22	0.41	3.25
CAPITAL	= 1 if the respondent paid entrance capital to union; 0 otherwise	4,620	0.85	0.36	1.16
ATTEXT	= 1 if the respondent participated in the trainings; 0 otherwise	4,620	0.32	0.47	1.72
EXPORT	= 1 if the respondent exported through union; 0 otherwise	4,620	0.15	0.35	2.98
GENAS	= 1 if the respondent attended general assembly; 0 otherwise	4,620	0.60	0.49	1.66
DISTAN	Distance between farm and union (km)	4,620	13.30	9.88	1.41
SUPPINP	= 1 if the respondent supplied input from union; 0 otherwise	4,620	0.05	0.21	1.38
MARKETNG	= 1 if the respondent marketed product through union; 0 otherwise	4,620	0.20	0.40	2.08
EXPER	= 1 if the respondent had experience of union-management; 0 otherwise	4,620	0.08	0.28	1.23
VISIT	Frequency of visiting union (times year^{-1})	4,620	51.52	98.37	1.42
PERFOR	Performance index of the union (%)	4,620	43.55	8.08	1.67
Farm variables					
FRS	=1 if the member is registered to the Farmer Registration System; 0 no; 2 no land	4,620	1.08	0.41	1.22
NUMORG	The number of agricultural organization membership (unit)	4,620	2.13	0.97	1.46
INSUR	= 1 if the respondent had agriculture insurance; 0 otherwise	4,620	0.18	0.39	1.40
HSIZE	Household size (person)	4,620	4.88	2.46	1.29
INCOME	Total income of member (TL years^{-1})	4,620	91.45	119.26	1.45
Members variables					
MARITS	= 1 if the respondent is married; 0 single	4,620	0.95	0.22	1.14
SOCSEC	= 1 if the respondent had social security; 0 no	4,620	0.91	0.28	1.15
PROFES	= 1 if the respondent's main profession is agriculture; 0 otherwise	4,620	0.94	0.24	1.21
EDUC	Education status of the member (years)	4,620	6.25	2.96	1.39
EXPER	Agricultural experience of the member (years)	4,620	27.55	13.51	2.45
GENDER	= 1 if male; 0 female	4,620	0.98	0.14	1.11
AGE	Age of member (years)	4,620	50.14	11.63	2.57

In Turkey, farmers are obliged to pay the designated entry fee to become a member of APUs. Members are also obliged to pay annual dues for each year. Membership entrance fees cannot be less than 10% and more than half of the gross amount of the monthly minimum wage (RG 2004). The members' willingness to invest capital in their unions was given in Table 2. The results show that 44% of the members were willing to provide a high level of capital contribution to their unions if the unions increase net income of the farms. Newbery et al (2013) determined that 87% of the members in England were willing to invest capital for the survival of their union.

It was determined that if the unions do not make any additional contribution to the farm's income, the members were willing to invest an average capital of 116 TL ($52.9). The members were willing to invest capital of 151.5 TL ($69) (15.1% of the income increase provided), in return for the union's provision of an increment of 1,000 TL ($456) in the farm's income. If the income increment is 10,000 TL ($4,566), the members were willing to invest capital of 600 TL ($274) (6% of the income increase provided) to their union.

Table 2- The members' willingness to invest capital

	The increment provided by the union to the members' net profit (TL)											
	0	*1,000*	*2,000*	*3,000*	*4,000*	*5,000*	*6,000*	*7,000*	*8,000*	*9,000*	*10,000*	*Mean*
Frequency of WTIC (%)	32.9	44.0	44.5	44.8	46.4	46.7	44.5	42.9	42.9	42.9	42.9	44.2
Average WTIC (TL)	116.0	151.5	204.1	256.3	310.9	362.3	400.9	453.3	502.4	546.2	600.1	354.94

Depending on the increase of 1,000 TL ($456) that the union will provide to the income of the farm, the capital that the members were willing to invest in their union changed between 35.5 TL ($16.2) and 54.6 TL ($24.9) (average 48.4 TL). Considering the fact that APUs had an average of 612 members and a capital of 196 thousand TL ($90 thousand), it can be understood that for each increment of 1,000 TL ($456) that the union will provide to their members, a total of 40 thousand TL ($18 thousand) (20.4%) will be added to the total equity of the union; 103 thousand TL ($47 thousand) (52.6%) for an increment of 5,000 TL ($2,283) and 158 thousand TL ($72 thousand) (80.6%) for an increment of 10,000 TL ($4,566). These results imply that there may be an increase of 20.4% to 80.6% in the equity of the unions in return for the incomes that they provide to their members. In addition, the increased equity capital in the unions will also facilitate the possibility of external financing

3.2. Random effects Tobit model results

The estimation results of the Random Effects Tobit model was given in Table 3. Estimated Tobit model results indicated that there was a statistically significant positive relationship between the variables of trust, entrance fee payment, participation in trainings, being a member of the livestock union, marital status, being a member of the apple agricultural producer union, exporting through union, social security, attending the general assembly, being a farmer, the number of farmer organization membership, being a member of the beekeeping union, distance to the union, educational status and agricultural experience and the members' willingness to invest capital. On the other hand, there was a statistically negative relationship between the variables of input supply, membership to the vegetable and organic hazelnut unions, gender, management experience, insurance, marketing crops tough the union, household size, age, total income, frequency of visiting the union and the members' willingness to invest capital in their unions.

The Tobit model results show that the members can increase their capital contribution to their unions by 340 TL ($155) with a 1 unit increase in trust index. That is, as the members' trust to their unions, union executive board and other members increase, their capital contribution to their unions would be increased. Şafaklı (2003) showed that less trust in the organizations caused capital shortage. Newbery et al (2013) found also that there was a positive relationship between the members' trust and the members' willingness to invest capital to their organizations. On the contrary, Kovacic et al (2000) found that there was no statistically significant effect between the trust and the farmers' willingness to cooperate. Capital insufficiency and distrust were determined as the most important problems in agricultural organizations. In

order to increase the amount of capital which is one of the most important elements in the success of the unions, the members' trust to their unions, executive board and other members should be established and increased.

The members' willingness to invest capital to the livestock union was 390 TL ($178) higher than the dairy cattle union. The members' willingness to invest capital in the apple agricultural producer union was 247 TL ($113) than the dairy cattle producer union. The members' willingness to invest capital in the beekeeping producer union was 57 TL ($26) higher than the dairy cattle producer union. On the other hand, the members' willingness to invest capital in the vegetable producer union was 286 TL ($130) less than the dairy cattle producer union while the members' willingness to invest capital to the organic hazelnut producer union was 426 TL ($195) less than the dairy cattle producer union.

The member participated in the general assembly of unions were willing to invest 153 TL ($70) more to their unions when compared to their counterparts. Participation levels of the members to the unions was not common. Therefore, the unions should increase their participation in general assembly meetings. Phillipson et al (2006) stated also that unions typically suffer from poor attendance at meetings and weak participation in other activities of the union.

Table 3- Random Effects Tobit model of willingness to invest capital

Independent variables		*Tobit model coefficient*	*Marginal effects*		
			Coefficient	*Elasticities*	*z-value*
Constant		-2842.71***	-	-	-
Constant		686.472***	-	-	-
Union variables	TRUST	339.684***	115.285***	5.09	2.85
	STOCK	390.244***	132.445***	0.29	2.76
	APPU	247.358***	83.9509***	0.03	2.66
	BEEKU	57.0525**	19.3630**	0.02	1.63
	VEGU	-286.339***	-97.1807***	-0.18	-2.75
	OHAZU	-426.474***	-144.741***	-0.44	-2.85
	CAPITAL	709.968***	240.956***	2.84	2.89
	ATTEXT	422.519***	143.399***	0.64	2.78
	EXPORT	211.701***	71.8491***	0.14	2.64
	GENAS	153.715***	52.1692***	0.43	2.62
	DISTAN	32.0513***	10.8779***	2.01	2.79
	SUPPINP	-590.982***	-200.573***	-0.13	-2.75
	MARKETNG	-82.7980***	-28.1008**	-0.07	-2.34
	EXPER	-142.228***	-48.2706***	-0.05	-2.60
	VISIT	-0.22209***	-0.07537**	-0.05	-2.11
	PERFOR	0.19670	0.06676	0.04	0.21
Farm variables	FRS	208.646	7.08124	0.10	1.23
	NUMORG	93.7519***	31.8184***	0.94	2.78
	INSUR	-99.5203***	-33.7762**	-0.08	-2.47
	HSIZE	-20.2047***	-6.85728**	-0.46	-2.42
	INCOME	-0.55725***	-0.18913***	-0.24	-2.65
Member variables	MARITS	364.809***	123.813***	1.63	2.93
	SOCSEC	155.926***	52.9198***	0.67	2.78
	PROFES	100.248***	34.0230**	0.44	2.50
	EDUC	31.5024***	10.6916***	0.93	2.94
	EXPER	4.81106***	1.63283***	0.62	2.60
	GENDER	-288.318***	-97.8522**	-1.33	-2.31
	AGE	-4.35089***	-1.47665**	-1.03	-2.18
Log-Likelihood		-15460.71078			
N		4.620			

** and *** indicates statistically significant at the level of 5% and 1%, respectively

Members who paid full entrance fee were willing to invest 709 TL ($324) more capital to their unions when compared to those who did not pay. Members who participated in the trainings of the unions were willing to invest 422 TL ($192) more capital to their unions when compared to those who did not participate. Members who had one year higher education were willing to invest 31 TL ($14) more capital to their unions when compared to their counterparts. Members who exported their crops through the union were willing to invest 211 TL ($96) more capital to their unions when compared to their counterparts. Female and one year younger members were willing to invest 288 TL ($131) and 4 TL ($1.8) higher capital, respectively when compared to their counterparts.

When the marginal effects of the explanatory variables in the Tobit model are evaluated, 1% increase in trust level of the members would lead to a 5.09% increase in willingness to invest capital. The members who totally paid entrance fee were willing to invest capital was 2.84% higher than their counterparts. The members who participated in the trainings of the union was 0.64% higher than their counterparts. The female and one year younger members were willing to invest 1.33% and 1.03% higher capital than their counterparts.

4. Conclusions

In this study, levels of willingness to invest capital were found as moderate level. There was a positive relationship between the variables of trust, participation in trainings and general assembly, paying entrance fees and exporting crops through the union and the members' willingness to invest capital to the unions.

When the unions increase the income of their members, the members would provide 40-158 thousand TL ($18.2-72.1 thousand) to their unions. In order to increase the willingness of the members to invest capital in the unions; the unions should increase the incomes of their members. This can also increase the members' willingness to provide capital to their unions. Trust had very high impacts on the members' willingness to invest capital to the unions. In order to increase the capital of the unions, it is of great importance that the members' trust the unions should be increased. In order to increase the trust of the members, the producer unions should take an active role in marketing products or crops and supply input. Increasing the participation of members in the trainings organized by the unions could help to increase capital investment of the members. Therefore, it is of great importance that the unions should organize training programs according to the needs of members and each member should be encouraged to participate in these training programs. If the unions sell their members' crops or products to the foreign markets, this will encourage member merchant cooperativeness. Exporting members' crops or products to foreign markets would increase capital accumulation in the unions by the members. As the members older, they were willing to provide less capital to their unions. Therefore, raising awareness of older members on the benefits of capital accumulation in the unions and encouraging young farmers on being a member of the unions would increase capital accumulation in the unions. Moreover, the members should actively participate in the governance of the unions to increase capital of the unions. This study concluded also that this study should expand to other farmer unions in the regional or country levels.

Acknowledgements

I would like to thank the members and executive boards of Agricultural Producer Unions in Samsun province for their valuable contributions to this research.

References

Anastasopoulos P C & Mannering F L (2009). A note on modeling vehicle accident frequencies with random-parameters count models. *Accident Analysis & Prevention* 41(1): 153-159

Garrod G & Willis K G (1990). Contingent valuation techniques: a review of their unbiasedness, efficiency and consistency. Countryside Change Initiative Working Paper 10. Newcastle: University of Newcastle upon Tyne

Greene W (2012). Limdep, Version 10.0. Econometric Software, Inc., Plainview, NY

GTB (2012). Gümrük ve Ticaret Bakanlığı Kooperatifçilik Genel Müdürlüğü, Türkiye kooperatifçilik stratejisi ve eylem planı 2012-2016, Ankara

GTHB (2013). Gıda, Tarım ve Hayvancılık Bakanlığı 2013-2017 stratejik planı, Ankara

Hansen M, Morrow J & Batista J C (2002). The impact of trust on cooperative membership retention, performance and satisfaction: an exploratory study. *The International Food and Agribusiness Management Review* 5(1): 41-59

IRFO (2008). Çiftçi örgütlerinin kurumsal güçlendirilmesi projesi, Uygulama ve tamamlama raporu, Türkiye ormancılık kooperatifleri merkez birliği

Karantininis K & Zago A M (2001). Endogenous membership in mixed duopsonies. *American Journal of Agricultural Economic* 83(5): 1266-1272

Karlı B & Çelik Y (2003). GAP alanındaki tarım kooperatifleri ve diğer çiftçi örgütlerinin bölge kalkınmasındaki etkinliği. *Tarımsal Ekonomi Araştırma Enstitüsü*, Yayın No: 97, ISBN: 975-407-122-5

Kaygusuzoğlu M (2002). Ekonomik krizden çıkışta mooperatifçilik, Karınca kooperatif postası, Türk kooperatifçilik kurumu, Ankara

Kılıç B (2011). Samsun ilindeki tarımsal kalkınma kooperatiflerinde ortak-kooperatif ilişkilerinin analizi. Yüksek lisans tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun (Yayınlanmış)

Kılıç Topuz B (2017). Samsun ili tarımsal üretici birliklerinde güven ve performansa etkili faktörler. Doktora tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Samsun

Kılıç Topuz B, Bozoğlu M & Başer U (2017). Güncel gelişmeler kapsamında Türkiye'deki çiftçi örgütlerine yönelik mevzuatın değerlendirilmesi. *Üçüncü Sektör Sosyal Ekonomi Dergisi*, 52(Özel sayı): 140-161

Kovacic D, Juracak J & Zutinic D (2000). Willingness of farmers to cooperate field study results in the Zagreb rural area. *Portal Znanstventh Casopisa Republike Hrvatskb* 6(56): 1119-1129

Lord D & Mannering F (2010). The statistical analysis of crash-frequency data: a review and assessment of methodological alternatives. *Transportation Research Part A: Policy Practice* 44(5): 291-305

Mitchell R C & Carson R T (1989). Using Surveys to Value Public Goods: The Contingent Valuation Method. Resources for the Future, Washington DC

Mülayim Z G (2010). Kooperatifçilik. Yetkin Yayınları, Ankara

Newbery R, Sauer J, Gorton M, Phillipson J & Atterton J (2013). Determinants of the performance of business associations in rural settlements in the United Kingdom: an analysis of members' satisfaction and willingness-to-pay for association survival, *Environment and Planning* 45: 967-985

Österberg P & Nilsson J (2009). Members' perception of their participation in the governance of cooperatives: the key to trust and commitment in agricultural cooperatives. *Agribusiness* 25(2): 181-197

Özdemir G, Keskin G & Özüdoğru H (2010). Türkiye'de ekonomik krizlere alternatif bir çözüm önerisi: tarımda güçlü ve etkin üretici örgütlenme modeli. *IX. Tarım Ekonomisi Kongresi*, 22-24 Eylül, Şanlıurfa, s. 518-525

Phillipson J, Gorton M & Laschewski L (2006). Local business co-operation and the dilemmas of collective action: rural micro-business networks in the north of England. *Sociologia Ruralis* 46: 40-60

Rehber E (2006). Tarımda yeni nesil kooperatifler. Tarım ve Köy İşleri Bakanlığı, *Türktarım Dergisi* 171: 40-43

RG (2004). 29.06.2004 tarih 25514 Sayılı Resmi Gazete, Tarımsal Üretici Birlikleri Kanunu. http://www.resmigazete.gov.tr/eskiler/2004/07/20040706.htm#2 (Erişim tarihi: 11.01.2018)

RG (2006). Tarım Kanunu, 18.04.2006 tarih 5488 Sayılı Kanun, http://www.resmigazete.gov.tr/eskiler/2006/04/20060425-1.htm (Erişim tarihi: 10.01.2018)

RG (2013). 06.07.2013 tarih 28699 Sayılı Kanun, 10. Kalkınma Planı, http://www.resmigazete.gov.tr/eskiler/2013/07/20130706M1-1.htm (Erişim tarihi: 02.02.2018)

Şafaklı O (2003). KKTC'de kooperatiflerin finansmanı üzerine bir çalışma. *Yönetim ve Ekonomi* 10(2): 175-189

TRGM (2017). Tarım Reformu Genel Müdürlüğü, Tarımsal örgütlenme tablosu. http://www.tarim.gov.tr/TRGM/Belgeler/Tarimsal Örgütlenme Tablosu (Erişim tarihi: 18.12.2017)

Tobin J (1958). Estimation of relationships for limited dependent variables. *Econometrica* 26(1): 24-26

Yamane T (1967). Elemantary Sampling Theory. Prentice Hall Inc., Englewoods, New Jersey

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

Comparison of Bread Wheat Genotypes for Leaf Rust Resistance Genes

İsmet BAŞER[a], Behiye Banu BİLGEN[b], Alpay BALKAN[a], Zahit Kayıhan KORKUT[a], Oğuz BİLGİN[a], Eylem GÜLFİDAN[a]

[a]*Tekirdağ Namık Kemal University Agricultural Faculty Department of Field Crops 59030 Tekirdağ, TURKEY*

[b]*Tekirdağ Namık Kemal University Agricultural Faculty Department of Agricultural Biotechnology 59030 Tekirdağ, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: İsmet BAŞER, E-mail: ibaser@nku.edu.tr, Tel: +90 (282) 250 20 02
Received: 25 July 2018, Received in Revised Form: 19 November 2018, Accepted: 21 November 2018

AUTHORS ORCID ID
(İsmet BAŞER: 0000-0001-6748-375), (Behiye Banu BİLGEN: 0000-0001-8323-2509), (Alpay BALKAN: 0000-0002-9203-6144), (Zahit Kayıhan KORKUT: 0000-0002-2336-2791), (Oğuz BİLGİN: 0000-0002-4338-9912), (Eylem GÜLFİDAN: 0000-0002-2173-6354)

ABSTRACT
Leaf rust caused by *Pucinia recondita tritici* is one of the most important diseases of bread wheat worldwide. It is considered that the most environmentally sound; low cost method of controlling leaf rust is to breed and grow genetically resistant wheat varieties. In the research, twenty-four bread wheat varieties grown intensively were used as genetic material in Trakya Region where the North-West Part of Turkey. To create artificial leaf rust epidemic in field conditions, two sensitive varieties (Morrocco and Cumhuriyet 75) was sown after each ten genotypes, and the reactions of the varieties to leaf rust were investigated in field conditions. Isogenic lines carrying the genes *Lr*9, *Lr*14, *Lr*19, *Lr*24 and *Lr*47 from CIMMYT were used as control genotypes in molecular analysis.

In the field conditions, although Pehlivan, Selimiye, Sagittario, Tina, Anapo, Montchill and Saraybosna were the most sensitive genotypes, Nota, Kate A1, Prostor and Sana were the most resistant bread wheat varieties to leaf rust. It was determined that Sana, Pehlivan, Golia, Falmura 85, Saroz 95, Renan, Sirena, Kate A1, Selimiye, Bezostoja 1, Saraybosna, Nina and Tina varieties have *Lr*9 gene with SSR analysis. It has been observed that all bread wheat varieties carry *Lr*14, *Lr*19, *Lr*24 and *Lr*47 (except Krasunia, Aldane and Gelibolu varieties) genes.

It is revealed that *Lr*9 and *Lr*47 genes should be taken into consideration in the studies to be performed in the region and these genes will be useful to examine together with a larger number of leaf rust genes for more successful results in breeding studies.

Keywords: Bread wheat; Leaf rust; SSR; STR; Molecular characterization

1. Introduction

Bread wheat (*Triticum aestivum* L.) was one of the earliest domesticated food crops and, for 8,000 years, is a basic nutrient of about 35% of the world's population, provides 20% of the calories from food of the major civilizations (CGIAR 2012). It can be said that the most important reasons for this are the richness of the variety, the widespread use of the industry and its wide adaptability. Undoubtedly, wheat will be among the first few products to meet this nutritional need. Despite the use of many methods to protect plants from damage caused by diseases, crop losses can reach very high levels. It is believed that product

DOI: 10.15832/ankutbd.447752

losses resulting from diseases are 12% of the total product in the world (Agrios 1997; Boyraz & Delen 2005). Diseases not only reduce the quantity of the products but also the quality of the products.

As in the world, wheat is produced in a wider area than other cultivated plants in our country, and Trakya Region is one of the important wheat production regions. Abiotic and biotic stress factors in the region lead to quantitative and qualitative losses in different levels. The most important biotic stress factors in the Trakya region are leaf rust and the yield losses due to this disease changes depending on the severity of the disease and the susceptibility of the wheat genotypes. In other words, the severity or infection type of leaf rust is determined by the interaction between the resistance genes of the bread wheat genotype and the aggressive genes of the rust disease. The brown rust (10-75%) and yellow rust (20-60%) in wheat were reported to cause significant yield losses (Aktaş 2001). Leaf rust disease is the oldest disease known in wheat (Kolmer 2013) and causes significant loss of grain number and grain weight (Marasas et al 2004; Kolmer et al 2005; Huerta-Espino et al 2011; Draz et al 2015). It can cause product losses ranging from 2% to 90% worldwide (Bajwa et al 1986; Aktaş 2001; Singh et al 2004). The loss of crops is different according to the sensitivities of the varieties, the environmental conditions and the race of the disease

Few studies have been carried out on wheat varieties' resistance to rust diseases in Trakya region. The morphological and molecular identification of the leaf rust resistance is important in bread wheat varieties. When these features are revealed in genotypes, the experience and knowledge required for wheat breeding trials will be ensured and crop losses of producers will be reduced.

In many cases resistance genes can only be identified using molecular markers (Melchinger 1990). Over the last 15 years many efficient markers for leaf rust resistance genes have been described. The molecular markers most closely linked to *Lr* genes are based on the PCR technique, as the majority of these can be applied relatively easily in wheat breeding programmers (Imbaby et al 2014). SSR (Simple Sequence Repeat) is one of the PCR based DNA markers that provides determination of polymorphism in repeat motifs in a microsatellite locus (Jones et al 1997). SSR and other molecular markers have been used for determination of resistant genes for rust diseases (Gupta et al 2010). Seyfarth et al (2000) used the SSR markers in two different wheat populations to identify the *Lr*13 gene. Greganova et al (2003) investigated the *Lr*13 gene in Slovak winter wheat cultivars with SSR markers. In Blaszczyk et al (2005), Nearly Isogenic Lines (NIL) marker are used as materials for the first time for *Lr*13 gene. Polymorphism determined at the DNA level between two close isogenic lines was found to be very likely to be in a linkage with the target gene (Masojc 2002). Thatcher isogenic lines are very useful in detecting pathogen virulent combinations as well as in identifying resistance genes (Winzeler et al 2000).

In recent years, the use of leaf rust resistant genes in winter wheat breeding has reduced the losses caused by leaf rust. But, there are many races of leaf rust and varieties are not resistant to all races. Every few years, new disease races are observed and durable varieties can be sensitive. The duration of a leaf rust resistance of wheat varieties can vary from 2 to 4 years. Wheat breeding programs should be continued by transferring new resistance genes to new varieties (Lipps 2006). This disease is widespread in our country, especially Trakya, in all coastal regions like Aegean, Marmara and Black Sea (Altay 1980). The diseases resistance is provided by the durability in the adult plant period (McIntosh et al 1995; Seyfarth et al 2000).

Disease damage is seen in different levels every year in the Trakya region. Despite the intense chemical application for rust diseases, the product losses caused by the diseases increase every year. One of the most effective and accepted approaches to prevent disease is the development of cultivars resistance. If the brown basal reactions of common wheat genotypes can be determined, resistant genotypes can be used as a source of resistance in the breeding programs. Thus, the effectiveness of breeding programs can be improved by selecting appropriate parents. In addition, the use of resistant genotypes will provide significant contributions to both the region and the country's economy.

The aim of this study was to identify *Lr* resistance genes present in a collection of bread wheat cultivars grown in Trakya Region where the North-West Part of Turkey and to determine the level of adult plant resistance to leaf rust in these cultivars.

2. Material and Methods

2.1. Material

The study was carried out in the Department of Field Crops, Agricultural Faculty, Tekirdağ Namık Kemal University during 2015 growing period. 24 bread wheat varieties were used genetic material (Table 1). The isogenic lines RL6010, RL6013, RL6009, and RL6010 from the CIMMTY were used to determine whether the *Lr*9, *Lr*14a, *Lr*19, *Lr*24 and *Lr*47 genes were present in the wheat genotypes.

Table 1- Wheat genotypes tested for leaf rust resistance

Genotypes and their abbreviations	
Kate A-1 (Ka)	Esperia (Es)
Pehlivan (Pe)	Sagittaria (Sag)
Prostor (Pr)	Krasunia (Kr)
Saroz 95 (Sar)	Sirena (Si)
Selimiye (Se)	Anapo (An)
Saraybosna (Sa)	Bereket (Be)
Gelibolu (Ge)	Falmura 85 (Fa)
Tekirdağ (Te)	Nota (No)
Bezostoja 1 (Be)	Golia (Go)
Pamukova 97 (Pa)	Sana (San)
Montchill (Mo)	Tina (Ti)
Rumeli (Ru)	Nina (Ni)

2.2. Methods

Adult plant resistance to leaf rust was investigated on the experimental area of Department of Field Crops, Agricultural Faculty, Tekirdağ Namık Kemal University. The resistance to leaf rust disease provides resistance in the adult plant period (McIntosh et al 1995; Seyfarth et al 2000). Seeds of all bread wheat genotypes were sown in November 11, 2015. The tested varieties were sown in experimental units (plots) containing three rows with 6 m long and 20 cm with 500 seeds per square meter. The experiment was designed in a complete randomized block design with three replicates. Two sensitive genotypes were sown after each ten genotypes. Morocco bread wheat variety which is sensitive to leaf rust was sown around the parcels. All cultural practices such as fertilization, irrigation and other management were applied according to standard procedures in the region. In the study, the variance analysis for decare grain yield was analyzed according to the randomized block design. In order to determine the difference between genotypes, Duncan test was applied.

2.2.1. Disease observations

When rust symptoms were fully developed nearly at the early dough stage (Large 1954), the leaf rust data of adult plant reaction were scored as plant response and rust severity are combined together. Plant response was expressed in five infection types according to Johnston & Browder (1966) i.e. Immune (0), no uredia or other macroscopic sign of infection, Resistant (R), small uredia surrounded by necrosis, Moderately Resistant (MR), small to medium uredia surrounded by chlorosis or necrosis, Moderately Susceptible (MS), medium-sized uredia that may be associated with chlorosis and Susceptible (S), large uredia without chlorosis or necrosis. Rust severity was expressed as percentage coverage of leaves with rust pustules following Cobb's scale modified by Peterson et al (1948).

2.2.2. DNA isolation

For the DNA isolation, the fresh leaves belonging to individuals of each genotype were used. Each sample was ground with a ball mill (Retsch® MM400). Total genomic DNA was isolated by using modified CTAB

method (Doyle & Doyle 1990). The DNA in samples was quantified with Qubit® 2.0 Fluorometer and also controlled by electrophoresis on 1% agarose gels with RedSafe Nucleic Acid Staining Solution in 1X TBE buffer at 80 V constant in 30 minute and visualized under UV light (Gel Imaging System Vilber Lourmat Quantum ST5). The extracted DNA samples was diluted to 25 ng μL^{-1} for PCR analysis and stored at - 20 ºC for further use.

2.2.3. Molecular marker analysis

Five primers were used in the analysis of five different leaf rust resistance genes. The characteristics of the used primers are shown in Table 2.

Table 2- Characteristics of the markers used in the leaf rust resistance genes

Lr Gene	*Primer name*	*5' →3' primer sequences*	*Product size (bp)*	*Reference*
Lr9	J13/1 J13/2	TCCTTTTATTCCGCACGCCGG CCACATACCCCAAAGAGACG	1110	Schachermayr et al (1994)
Lr14a	Xgwm146-F Xgwm146-R	CCAAAAAAACTGCCTGCATG CTCTGGCATTGCTCCTTGG	174	Röder et al (1998)
Lr19	GbF GbR	CATCCTTGGGGACCTC CCAGCTCGCATACATCCA	130	Prins et al (2001)
Lr24	J09/1 J09/2	TCTAGTCTGTACATGGGGGC TGGCACATGAACTCCATACG	350	Schachermayr et al (1994)
Lr47	PS10L PS10L2	TCTTCATGCCCGGTCGGGT GGGCAGGCGTTTATTCCAG	224	Helguera et al (2000)

Molecular marker analysis was performed at the experimental field area and laboratories of Department of Field Crops and Department of Agricultural Biotechnology, Agricultural Faculty, University of Tekirdağ Namık Kemal. The DNA amplifications were carried out by using the Applied Biosystems® Veriti® Thermal Cycler, Applied Biosystems® ProFlex™ PCR System Thermal Cycler, with the following PCR profile shown in Table 3.

Table 3- Amplification parameters for all studied primer pairs

	PCR cycle condition
J13/1 J13/2	95 °C for 5 min., 35 cycles of (95 °C 1 min., 62 °C 1 min., 72 °C 1 min.), 72 °C 10 min
Xgwm146-F Xgwm146-R	95 °C for 5 min., 35 cycles of (95 °C 1 min., 56 °C 1 min., 72 °C 1 min.), 72 °C 10 min
GbF GbR	95 °C for 5 min., 35 cycles of (95 °C 1 min., 56 °C 1 min., 72 °C 1 min.), 72 °C 10 min
J09/1 J09/2	95 °C for 5 min., 35 cycles of (95 °C 1 min., 56 °C 1 min., 72 °C 1 min.), 72 °C 10 min
PS10L PS10L2	95 °C for 5 min., 35 cycles of (95 °C 1 min., 62 °C 1 min., 72 °C 1 min.), 72 °C 10 min

The volume of the reaction mixture was 10 µL, containing 1X reaction buffer, 2.5 mM $MgCl_2$, dNTPs (each 0.2 mM), 0.5 µM of each primer and 1.5 U of *Taq* polymerase. The template for PCR amplification consisted of 50 ng of genomic DNA. PCR products were visualized on 1.7% agarose gels with RedSafe Nucleic Acid Staining Solution in 1X TBE buffer and using a 100 bp DNA ladder at 80 V constant in 1

hour and visualized under UV light (Gel Imaging System Vilber Lourmat Quantum ST5) to determine the size of amplified fragments.

3. Results and Discussion

The cultivated wheat varieties have suffered from sudden epidemics during the last decades from the perspective of change in weather conditions in relation to the genetic makeup of both host and parasite. Currently, intensive chemical spraying is applied to wheat production areas against rust diseases. In Turkey, several genes, including leaf rust-resistant as *Lr*9, *Lr*19, *Lr*24 and *Lr*28, have been found to induce disease resistance.

3.1. Disease evaluation

Observations on leaf rust resistance levels of bread wheat varieties under field conditions indicate that the varieties show different responses to leaf rust (Table 4). The Nota with 5 MR value, and Kate A1, Prostor and Sana varieties with 10 MR values showed the highest resistance. Flamura 85, Golia, Krasunia, Rumeli, Pamukova and Tekirdağ varieties were followed by these varieties with 20 MS value. The most sensitive genotypes for leaf rust among the 24 wheat genotypes was determined in Pehlivan and Bereket varieties with values of 40 S value. Bread wheat varieties Selimiye and Sagittario have followed these varieties with 40 MS values. Montchill with 30 MS value, Saraybosna, Saroz 95, Sirena, Anapo and Tina varieties with 20 MS value were later ranked. Bereket and Pehlivan bread wheat varieties which were observed the highest leaf rust values gave the lowest grain yields. The other wheat varieties with high leaf rust values were also in the lower order of grain yield. The highest grain yield was obtained from Rumeli, Krasunia, Tekirdağ and Tina (MR) genotypes.

Table 4- Leaf rust values in bread wheat varieties

Varieties	*Leaf rust values*	*Grain yield (kg da^{-1})*
Kate A-1	10 MR	646.667 cd
Pehlivan	40 S	538.000 fgh
Prostor	10 MR	457.667 k
Saroz 95	20 MS	568.667 ef
Selimiye	40 MS	608.000 de
Saraybosna	20 MS	515.667 ghi
Gelibolu	30 MR	481.333 ik
Tekirdağ	20 MR	691.333 abc
Bezostoja 1	30 MR	502.667 ghi
Pamukova 97	20 MR	358.000 l
Montchill	30 MS	663.667 bc
Rumeli	20 MR	742.333 a
Esperia	40 MR	657.000 bc
Sagittaria	40 MS	503.667 hik
Krasunia	20 MR	699.667 ab
Sirena	20 MS	544.667 fg
Anapo	20 MS	602.667 e
Bereket	40 S	378.667 l
Falmura 85	20 MR	560.667 ef
Nota	5 MR	577.667 ef
Golia	20 MR	461.667 k
Sana	10 MR	492.333 ik
Tina	20 MR	734.000 a
Nina	40 MR	731.333 a
HKO		616.894

3.2. Molecular evaluation

Isogenic lines carrying the *Lr* genes provided in CIMMTY (Mexico) were used as materials to determine whether the 24 wheat cultivars carry the *Lr*9, *Lr*14, *Lr*19, *Lr*24, and *Lr*47 genes. The data obtained using 5

different molecular markers (SSR primers) were given in Table 5 and Figure 1.

Assessment among varieties based on the results of molecular marker analysis, it has been determined that Esperia, Sagittario, Rumeli, Prostor, Golia, Bereket, Montchill, Aldane, Pamuova, Tekirdağ and Krasunia bread wheat varieties carry the *Lr*9 gene, the other 13 bread wheat varieties do not carry this gene. Although all bread wheat varieties possess the *Lr*19, *Lr*14a and *Lr*24 genes, 21 bread wheat varieties of them possess *Lr*47 gene. Only Krasunia, Golia and Aldane bread wheat varieties do not carry the *Lr*47 gene. Bread wheat varieties Nota, Sana, Prostor showed the most resistant to leaf rust in the field conditions. Then, Tekirdağ, Pamukova 9, Rumeli, Krasunia, Flamura 85, Golia and Tina varieties were also most resistant other varieties. The most sensitive varieties in terms of leaf rust resistance were Pehlivan, Bereket, Selimiye Sagittario and Montchill varieties, and Saraybosna, Sirena Anapo and Tina varieties were followed them.

Table 5- Presence of *Lr* resistance genes in the wheat genotypes [(+) presence ofgene, (-) absence of gene]

Genotypes	*Lr9*	*Lr14a*	*Lr19*	*Lr24*	*Lr 47*
Sana	+	+	+	+	+
Pehlivan	+	+	+	+	+
Golia	+	+	+	+	+
F-85	+	+	+	+	+
Krasunia	-	+	+	+	-
Saroz-95	+	+	+	+	+
Aldane	-	+	+	+	-
Pamukova	-	+	+	+	+
Tekirdağ	-	+	+	+	+
Montchill	-	+	+	+	+
Renan	+	+	+	+	+
Syrena	+	+	+	+	+
Kate A-1	+	+	+	+	+
Selimiye	+	+	+	+	+
Bezostaja 1	+	+	+	+	+
Saraybosna	+	+	+	+	+
Nina	+	+	+	+	+
Gelibolu	-	+	+	+	-
Bereket	-	+	+	+	+
Tina	+	+	+	+	+
Esperia	-	+	+	+	+
Sagittario	-	+	+	+	+
Rumeli	-	+	+	+	+
Prostor	-	+	+	+	+
Positive control	+	+	+	+	+

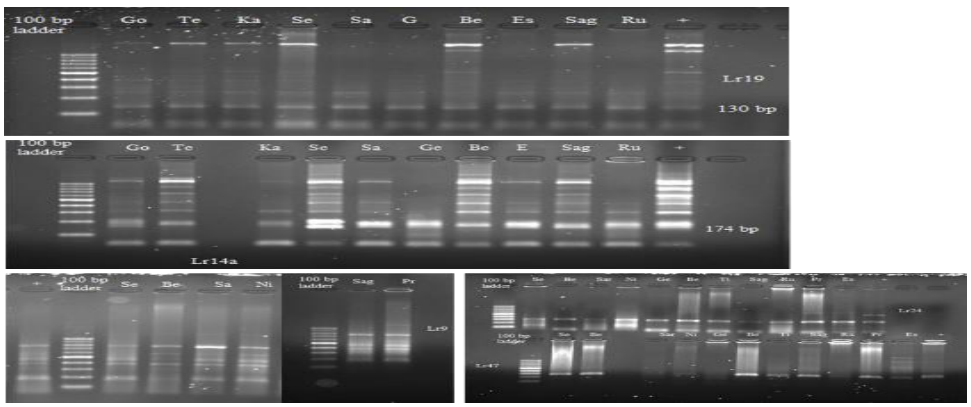


Figure 1- PCR amplification of some genotypes for A) Lr19 B) Lr14 c)….. genes using specific molecular markers

4. Conclusions

The detection of resistant genotypes with more stable and easier molecular markers replaced the older methods such as inoculation by pathogens or more complicated markers with poor repeatability. Various marker types are available for marker assisted selection of resistant genotypes for leaf rust such as ISSR, SCAR, AFLP, STS and SSR (Gold et al 1999; Prins et al 2001; Gupta et al 2006; Li et al 2010; Zhang et al 2011; Zhou et al 2012; Zhou et al 2013). According to the results obtained in uncontrolled conditions, the bread wheat genotypes were affected at different levels of leaf rust. While Pehlivan, Gelibolu, Bereket and Pamukova and Golia varieties which are sensitive to leaf rust were located in the lower ranks due to yielding grain, Nina, Tina, Krasunia, Rumeli and Tekirdağ varieties which are more resistant to leaf rust have higher values in terms of grain yield. The obtained data do not show that selection may be successful for rust disease in field conditions.

In this study, the molecular genetic markers of *Lr*9, *Lr*14, *Lr*19, *Lr*24, and *Lr*47 in bread wheat cultivars were used to identify the 23 wheat cultivars. Esperia, Sagittario, Rumeli, Prostor, Gelibolu, Bereket, Montchill, Aldane, Pamuova, Tekirdag and Krasunia did not carry the *Lr*9 gene and the remaining 13 bread wheat genotypes carried the *Lr*9 gene. *Lr*14a, *Lr*19 and *Lr*24 genes were found in all of the 24 tested bread wheat cultivars. Tonk & Yüce (2007) studied SSR markers for *Lr*13 gene in 41 Thatcher near isogenic lines, they reported that the F_1 individuals (İzmir 85 x resistant near isogenic line) might have *Lr*13 gene. Kolmer et al (2012) studied various varieties in Samsun, İzmir and Sakarya at 2009-2011 and reported presence of *Lr*34 in 2 varieties, *Lr*37 in 3 varieties. Khurana et al (2004), *Lr*1, *Lr*3, *Lr*10, *Lr*13, *Lr*23 and *Lr*26 gene was reported in 37 wheat lines from Turkey. Studies by different researchers with different materials in different years and areas have shown that leaf rust is different in terms of resistance genes (Moldovan et al 2004; Xu et al 2005; Elyasi-Gomari & Lesovaya 2009; Huerta-Espino et al 2011; Gorash et al 2014; Zaman et al 2017).

Imbaby et al (2014) studied *Lr* genes in fifteen wheat cultivars from Egypt. They reported ten genes, *Lr*13, *Lr*19, *Lr*24, *Lr*26, *Lr*34, *Lr*35 *Lr*36, *Lr*37, *Lr*39, and *Lr*46, in fifteen wheat cultivars using various molecular markers. *Lr*13, *Lr*24, *Lr*34, and *Lr*36 genes were the most frequently occurred in fifteen Egyptian wheat cultivars (100%). The percentage of other genes were as *Lr*26 and *Lr*35 (93%), *Lr*39 (66%), *Lr*37 (53%), *Lr*19 (33.3%) and *Lr*46 (26.6%). Wang et al (2014) reported that wheat line 5R618, F_2 plants and $F_{2:3}$ families from a cross between 5R618 and Zhengzhou5389 (susceptible) may contain *Lr*9, *Lr*24, *Lr*19, *Lr*28, *Lr*39, *Lr*42, *Lr*47, *Lr*51, and *Lr*53 genes using molecular markers. Vanzetti et al (2011) studied 66 wheat cultivars from Argentina to identify *Lr* genes that condition leaf rust resistance. Vanzetti et al (2011) determined presence of *Lr*9, *Lr*10, *Lr*19, *Lr*20, *Lr*21, *Lr*24, *Lr*25, *Lr*26, *Lr*29, *Lr*34, *Lr*35, *Lr*37, *Lr*47 and *Lr*51 by molecular markers. They conclude that combinations including seedling resistance genes like *Lr*16, *Lr*47, *Lr*19, *Lr*41, *Lr*21, *Lr*25 and *Lr*29, with adult plant resistance genes like *Lr*34, *SV2* and *Lr*46 might provide durable and effective resistance to leaf rust in their studied region. According to the obtained data in our study, it is seen that there are some deviations in molecular and morphological data on leaf rust. This suggests that especially morphological studies should be done for a few years. It is revealed that *Lr9* gene and *Lr*47 genes should be taken into consideration in the studies to be performed in the region, and also it would be useful to examine a larger number of leaf rust genes for successful results in plant breeding.

Marker assisted selection (MAS) studies which researchers or breeders use special molecular markers linked to *Lr* genes, provides the pyramiding of several effective resistance genes. Detection of resistance genes via molecular markers is easy, cheap and time effective way to detect resistance in wheat varieties of unknown parentage. According to our results, in order to design crossing program for breeding strategies obtained information from MAS might be used.

Acknowledgements

This work was supported by Tekirdağ Namık Kemal University, Scientific Research Projects Unit (Project No. NKUBAP.00.24.AR.14.01).

References

Agrios G N (1997). Plant Pathology, Fourth Edition ed., Academic Press, San Diego, pp. 635

Aktaş H (2001). Önemli hububat hastalıkları ve survey yöntemleri. Tarım ve Köyişleri Bakanlığı. Bitki Sağlığı Araştırmaları Daire Başkanlığı, Ankara, 74 s

Altay F (1980). Kahverengi pasa mukavemet kaynakları ve ıslahı. *Bitki Islahı Sempozyumu*, 22-25 Mayıs 1979, Menemen-İzmir, Ege Tarımsal Araştırma Enstitüsü Yayınları, No: 17/41, s. 217-240

Bajwa M A, Aqil K A & Khan N I (1986). Effect of leaf rust on yield and kernel weight of spring wheat. *RACHIS* 5: 25-28

Blaszczyk L, Tyrka M & Chelkowski J (2005). Pst1AFLP based markers for leaf rust resistance genes in common wheat. *Journal Applied Genetics* 46(4): 357-364

Boyraz N & Delen S (2005). Bitkilerin hastalıklara karşı dayanıklılığında konukçu enzimlerin rolü. S.Ü. *Ziraat Fakültesi Dergisi* 19(35): 51-59

CGIAR (2013). Wheat 2012 annual report. Agricultural research for development ımproves food security, CIMMYT, Mexico, pp. 32

Doyle J J & Doyle J L (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15

Draz I S, Abou-Elseoud M S, Kamara A M, Alaa-Eldein O A & El-Bebany A F (2015). Screening of wheat genotypes for leaf rust resistance along with grain yield. *Annals of Agricultural Sciences* 60(1): 29-39

Elyasi-Gomari S & Lesovaya G M (2009). Harmfulness of wheat leaf rust in the eastern part of forest-steppe of Ukraine. *Archives of Phytopathology and Plant Protection* 42: 659-665

Gold J, Harder D, Townley-Smith F, Aung T & Procunier J (1999). Development of a molecular marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines. *Electronic Journal of Biotechnology* 2: 1-2

Gorash A, Galaev A, Babayants O & Babayants L (2014). Leaf rust resistance of bread wheat (*Triticum aestivum* L.) lines derived from interspecific crosses. *Zemdirbyste-Agriculture* 101(3): 295-302

Greganova Z, Kraic J & Galova Z (2003). Diagnostic of wheat leaf rust resistance genes by DNA application in MAS. *Czech Journal of Genetics and Plant Breeding* 39(4): 127-129

Gupta P K, Landridge P & Mir R R (2010). Marker-assisted wheat breeding: present status and future possibilities. *Molecular Breeding* 26: 145-161

Gupta S K, Charpe A, Koul S, Haque Q & Prabhu K (2006). Development and validation of SCAR markers co-segregating with an Agropyron elongatum derived leaf rust resistance gene *Lr24* in wheat. *Euphytica* 150: 233-240

Helguera M, Khan I A & Dubcovsky J (2000). Development of PCR markers for the wheat leaf rust resistance gene Lr47. *Theoretical and Applied Genetics* 100: 1137-1143

Huerta-Espino J, Singh R P, German S, McCallum B D, Park R F & Chen W Q (2011). Global status of wheat leaf rust caused by *P. triticina*. *Euphytica* 179: 143-160

Imbaby I A, Mahmoud M A, Hassan M E M & Abd-El-Aziz A R M (2014). Identification of leaf rust resistance genes in selected egyptian wheat cultivars by molecular markers. *The Scientific World Journal* pp. 1-7

Johnston C O & Browder L E (1966). Seventh revision of the international register of physiologic races of *Puccinia recondita* f. sp. *tritici*. *Plant Disease Report* 50: 756-760

Jones N, Ougham H & Thomas H (1997). Markers and mapping: We are all geneticists now. *New Phytologist* 137: 165-177

Khurana R, Nayar S K & Lakhanpal T N (2004). Brown rust resistance in wheat lines from Turkey. *Plant Disease Research (Ludhiana)* 19: 20-24

Kolmer J A (2013). Leaf rust of wheat: Pathogen biology, variation and host resistance. *Forests* 4: 70-84

Kolmer J A, Long D L & Hughes M E (2005). Physiological specialization of *Puccinia triticina* on wheat in the United States in 2003. *Plant Disease* 89: 1201-1206

Kolmer J A, Hanzalova A, Goyeau H, Bayles R & Morgounov A (2012). Genetic differentiation of the wheat leaf rust fungusPuccinia triticinain Europe. *Plant Pathology*: 1-11

Large E C (1954). Growth stages in cereals. Illustration of the Feekes scale. *Plant Pathology* 3: 128-129

Li X, Li Z F, Li Y N, Zhao Z Q, Liu D Q, Wang C F, Gao L J & Sun D J (2010). Genetic analysis and molecular mapping of leaf rust resistance gene in wheat line Xinong 1163-4. *Scientia Agricultura Sinica* 43: 2397-2402

Lipps P E (2006). Ohio State University, Extension Fact Sheet, Plant Pathology, http://ohioline.osu.edu./ac-fact/0006.html

Marasas C N, Smale M & Singh R P (2004). The Economic impact in developing countries of leaf rust resistance breeding in CIMMYT-related spring bread wheat. International Maize and Wheat Improvement Center, Mexico, DF, pp. 33

Masojc P (2002). The application of molecular markers in the process of selection. *Cellular & Molecular Biology Letters* 7: 499-509

McIntosh R A, Wellings C R & Park R F (1995). Wheat rusts: An atlas of resistance genes. Kluwer Academic Publishers, Dordrecht

Melchinger A E (1990). Use of molecular markers in breeding for oligogenic disease resistance. *Plant Breeding* 104: 1-19

Moldovan M, Moldovan V & Kadar R (2004). Characterization of wheat rust and powdery mildew populations in Transylvania and implications in breeding for resistance. *Romanian Agricultural Research* 21: 1-11

Peterson R F, Campbell A B & Hannah A E (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal Research* 60: 496-500

Prins R, Groenewald J Z, Marais G F., Snape J W & Koebner R M D (2001). AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat. *Theoretical and Applied Genetics* 103: 618-624

Röder M S, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P & Ganal M (1998). A microsatellite map of wheat. *Genetics* 149: 2007-2023

Seyfarth R, Feuillet C, Schachermayr G, Messmer M, Winzeler M & Keller B (2000). Molecular mapping of the adult-plant leaf rust resistance gene *Lr13* in wheat (*Triticum aestivum* L.). *Journal of Genetics Breeding* 54: 193-198

Singh R P, Huerta-Espino J, Pfeiffer W & Figueroa-Lopez P (2004). Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. *Plant Disease* 88: 703-708

Tonk F A & Yüce S (2007). Ekmeklik buğday İzmir 85 çeşidinde ve Thatcher yakın izogenik hatlarında kahverengi pas dayanıklılık geni *Lr*13'ün SSR markörleriyle incelenmesi. *Ege Üniversitesi Ziraat Fakültesi Dergisi* 44: 13-25

Vanzetti L S, Campos P, Demichelis M, Lombardo L A, Aurelia P R, Vaschetto L M, Bainotti C T & Helguera M (2011). Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. *Electronic Journal of Biotechnology* 14(3): 1-17

Wang J, Shi L, Zhu L, Li X & Liu D (2014). Genetic analysis and molecular mapping of leaf rust resist. genes in the wheat line 5R618. *Czech J. Genet. Plant Breed.* 50: 262-267

Winzeler M, Mesterházy A, Park R F, Bartos M, Csösz H, Goyeau M, Ittu E, Jones F, Löschenberger K, Manninger M, Pasquini K, Richter D, Rubiales G, Schachermayr A, Strzembicka M, Trottet O, Unger G & Vida Walther U (2000). Resistance of European winter wheat germplasm to leaf rust. *Agronomie* 20: 783-792

Xu X, Bai G, Carver B F, Shaner G E, Hunger R M (2005). Molecular characterization of slow leaf-rusting resistance in wheat. *Crop Science* 45: 758-765

Zaman A U, Khatun T, Hanefi M & Sahebi M (2017). Genetic analysis of rust resistance genes in global wheat. Cultivars. *Agriculture and Environmental Biotechnology* pp. 431-445

Zhang H, Xia X C, He Z H, Li X, Li Z F & Li D Q (2011). Moleculer mapping of leaf rust resistance gene LrBi16 in Chinese wheat cultivar Bimail16. *Moleculer Breeding* 28: 527-534

Zhou Y, Wu Y, Li X, Li Z F & Li D Q (2012). Genetic analyses and moleculer mapping of leaf rust genes in two Chinese wheat lines. *Scientia Agricultura Sinica* 45: 3273-3380

Zhou Y, Xia X C, He Z H, Li X, Li Z F & Li D Q (2013). Fine mapping of leaf rust resistance gene LrZH84 using expressed sequence tag and sequence-tagged site markers and allelism with other genes on wheat chromosome 1B. *Phytopathology* 103: 169-174

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

Intermittent Microwave Drying of Apple Slices: Drying Kinetics, Modeling, Rehydration Ratio and Effective Moisture Diffusivity

Nazmi İZLİ[a], Ahmet POLAT[a]

[a]*Bursa Uludag University, Faculty of Agriculture, Department of Biosystems Engineering, Gorukle Campus, 16059, Bursa, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Nazmi İZLİ, E-mail: nazmiizli@gmail.com, Tel: +90 (224) 294 16 04
Received: 27 September 2018, Received in Revised Form: 20 November 2018, Accepted: 22 November 2018

AUTHORS ORCID ID
(Nazmi İZLİ: 0000-0002-2084-4660), (Ahmet POLAT: 0000-0003-1673-7165)

ABSTRACT
In this research, thin layer drying characteristics, rehydration ratio and effective moisture diffusivity of apple were investigated using microwave dryer which has intermittent and continuous modes. Drying time varied between 25 and 215 minutes and they declined with the rise in microwave power and reduction in pulsing ratio. In an attempt to pick the optimum thin layer models for the drying applications, 8 mathematical models suited to the experimental results. On the grounds of the statistical tests evaluation, Midilli et al model which represent drying characteristics are optimally suited than other models. The highest rehydration ratio was recorded for the samples dried at 100W continuous mode and the lowest ratio at 300W continuous application. Effective moisture diffusivity values were computed by the 2nd law of Fick and changing between 3.04×10^{-9} and 2.53×10^{-8} $m^2 s^{-1}$. Consequently, the intermittent microwave method could be used as a favorable drying method for obtaining high-quality fruit slices or processing valuable material and continuous microwave drying can be taken as another drying approach for apple samples.

Keywords: Apple; Drying characteristics; Rehydration ratio; Effective moisture diffusivity

1. Introduction

Apple is a fruit that exists all year long, low-calorie and rich in various vitamins, minerals, dietary fibers, and it is a plentiful source of dietary polyphenols. For this reason, it is favorable for normal growth, development and consequentially for the whole well-being. It is noted that consuming apple helps human to refrain and protect from several diseases such as the risk of certain cancer types, ischemic heart diseases, thrombotic stroke, type 2 diabetes, and asthma (Saxena et al 2016). Apples are consumed as both fresh fruits and other processed products such as jams, juices, marmalades and other dehydrated products (An et al 2015). Food drying has been extensively used throughout the world history and it has provided elasticity in the utilization of foods in their offseason. Now, the industry on the dehydration of foods holds down a crucial position among other food industries across the globe. During drying, several modifications may occur at inner section of the foods, and these physicochemical and structural modifications have an impact on the attributes of the product: composition, color, and texture. However, those attributes connected with the transfer of heat and mass. In the literature, it is seen that transference of moisture, physical structures, and chemical configuration generally varies in foods (Cruz et al 2015). Microwave drying method is an

DOI: 10.15832/ankutbd.464715

alternative procedure so as to save time and energy. In this method, a microwave field induces volumetric heating of the wet solid, causing a water vapor pressure gradient between the surface and inner section of the material and accelerating the transfer of moisture (Junqueira et al 2017). However, the main disadvantage of using microwave method is the non-uniform distribution of moisture and temperature, that finding in cold and hot spots on the heated product. Intermittent application of microwave is one of the applicable remedies to curtail non-uniformity (Gunasekaran & Yang 2007). In literature, different variety of apple have been dried with different drying methods such as hot air (Sturm et al 2014), explosion puffing (Yi et al 2016) and freeze drying (Djekic et al 2018) by many researchers. However, there are limited studies intermittent and continuous microwave on drying apple (Aghilinategh et al 2015). In addition, the intermittent microwave has successfully been applied on a limited number of agricultural products, for instance, pistachio nuts (Kermani et al 2017) and quince (Dehghannya et al 2018). The targets of this work were to (1) settle the effect of different intermittent microwave conditions on thin layer drying kinetics of apple samples, (2) pick the most favorable drying models and (3) evaluate the quality of dried apples by analyzing the effective moisture diffusivity and rehydration ratio parameters.

2. Material and Methods

2.1. Drying equipment

Fresh 'Granny Smith' variety of apple samples disposed of in the experiments were bought from a local market in Bursa province of Turkey and kept at 4±0.5 °C temperature until completion of the experiments. In advance of the drying processes, the apple samples were peeled and sliced into 5 mm thickness by using a dicer (Börner, Wingene, Belgium) and dried in this form. The initial moisture level of these samples was computed to be 4.76 (g water g dry matter^{-1}) on a dry basis (d.b.) by use of forced air convection oven (ED115 Binder, Germany) drying at 105 °C for 24 hours period. The drying experiments were performed in a modified laboratory microwave oven. In this oven, microwave power between 80 and 900W can be applied either in pulsed or continuous mode. Our experimental conditions were: three levels of microwave power (100, 200 and 300 W), continuous and two pulsed operating modes. The microwave operating mode was assigned a pulsing ratio (PR), which is computed using Equation 1 where t_{on} corresponds to magnetron power on-time and t_{off} corresponds magnetron off-time (Gunasekaran & Yang 2007).

$$\mathrm{PR} = \frac{(t_{on} + t_{off})}{(t_{on})} \tag{1}$$

For the continuous mode PR= 1 (t_{on} = 60 s and t_{off} = 0 s) and for pulsed mode PR= 2 (t_{on} = 30 s and t_{off} = 30 s) and PR= 3 (t_{on} = 20 s and t_{off} = 40 s). The loss of moisture was recorded by taking the apple sample from the oven at 5-min intervals and weighing it on a digital balance (Shimadzu, Japan) that has a precision level of 0.01 g; the sample was returned to the oven within 20 s for continued drying (Kayisoglu & Ertekin 2011).

2.2. Mathematical modeling

The results data on moisture ratio (*MR*) were fitted with 8 commonly used drying equations (Table 1). The *MR* and drying rate (DR) are determined as follows (Kipcak 2017):

$$MR = \frac{M_t - M_e}{M_o - M_e} \tag{2}$$

$$DR = \frac{M_{t+dt} - M_t}{dt} \tag{3}$$

In the foregoing formula M_o corresponds the moisture content level at the beginning, M_t corresponds the moisture content level at a given time, M_e corresponds the equilibrium level of moisture content, M_{t+dt} is the moisture content at $t + dt$ and t is the drying time (min). After analyzing the formula, the values of M_e are rather small concerning M_t or M_o. Ultimately as proposed by some of the researchers, the moisture ratio formula was shortened in this way:

$$MR = \frac{M_t}{M_o} \tag{4}$$

Table 1- Thin layer drying models used for the apple drying kinetics

No	*Model name*	*Model*	*References*
1	Henderson and Pabis	$MR = a\exp(-kt)$	Doymaz et al (2015)
2	Newton	$MR = \exp(-kt)$	Horuz et al (2018)
3	Page	$MR = \exp(-kt^n)$	Coradi et al (2017)
4	Logarithmic	$MR = a\exp(-kt) + c$	Kayran & Doymaz (2017)
5	Two-term model	$MR = a\exp(-k_0 t) + b\exp(-k_1 t)$	Murthy & Manohar (2014)
6	Wang and Singh	$MR = 1 + at + bt^2$	Doymaz et al (2015)
7	Diffusion Approach	$MR = a\exp(-kt) + (1-a)\exp(-kbt)$	Murthy & Manohar (2014)
8	Midilli et al	$MR = a\exp(-kt^n) + bt$	Kayran & Doymaz (2017)

2.3. Rehydration ratio measurement

Prior to the execution of the analysis of the quality features, slices of the dried apple (10.0±0.1 g) were placed inside distilled water at 20 °C for 14 h, by being adhered to 1:50 solid to liquid ratio. (Vega-Gálvez et al 2009) The apple slices were then taken out, drained for 30 s, and weighed out using an electronic digital balance (Shimadzu, Japan) having ± 0.001 g accuracy. For each application, this procedure was repeated in triplicate. Eventually, rehydration ratio (*R*) was computed using Equation 5 as (Sunjka et al 2008) where, M_1 and M_2 are sample weights (g) before and after rehydration, respectively.

$$R = \frac{M_2 - M_1}{M_1} \tag{5}$$

2.4. Effective moisture diffusivity

Drying of agricultural products in a falling rate period is embedded into a mass-diffusion equation in accordance with the second law of Fick on diffusion is presented in Equation (6) below:

$$\frac{\partial M}{\partial t} = \nabla M\left[D_{eff}(\nabla M)\right] \tag{6}$$

The Equation (6) that explains the 2nd law of Fick on unsteady state diffusion can be utilized to figure out the moisture ratio calculated in Equation (7). The diffusion equation for infinite slab proposed by Kayran & Doymaz (2017), and assumed uniform moisture distribution at the beginning, negligible shrinkage and external resistance, and constant diffusivity, is presented in the equation below:

$$MR = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)^2\pi^2 D_{eff}\,t}{4L^2}\right) \quad (7)$$

Above; D_{eff} corresponds to the effective moisture diffusivity ($m^2\ s^{-1}$), L corresponds to the half-thickness of samples (m), t corresponds to the time (s), and n corresponds to a positive integer. With regard to longer periods of drying, the foregoing equation can be shortened to an only first term of series, without having a significant impact on the accuracy of the assumption.

$$MR = \frac{8}{\pi^2}\exp\left(-\frac{\pi^2 D_{eff}\,t}{4L^2}\right) \quad (8)$$

Typically, the effective moisture diffusivity (D_{eff}) was also computed by means of the slope of Equation (8). Namely, a straight line with a slope of K was derived when ln (MR) was plotted versus time:

$$K = \frac{\pi^2 D_{eff}}{4L^2} \quad (9)$$

With the help of the slope value (Equation 9), the effective moisture diffusivity could be settled.

2.5. Statistical analysis

The study was realized by the aid of randomized plots factorial design. In the course of calculation of the inspected items, three replicates were utilized. While interpreting the outcomes, JMP (SAS Institute Inc., USA) and MATLAB (MathWorks Inc., MA) software technologies were employed. Significance levels of mean differences were tested and the least significant difference (LSD) test resulted in a 5% significance level. It has been determined that the most convenient model that expresses the drying attributes of lime samples in a thin layer is the one that has lowest reduced chi-squared (χ^2) value, lowest root mean square error ($RMSE$) value and the highest coefficient of determination (R^2). The mentioned statistical values are described as below:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(MR_{pre,i} - MR_{\exp,i})}{N}} \quad (10)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{\exp,i} - MR_{pre,i})^2}{N - z} \quad (11)$$

Here; $MR_{\exp,i}$ means the experimental MR, $MR_{pre,i}$, means the predicted MR, N means the observation number and z stands for the number of constants.

3. Results and Discussion

3.1. Drying kinetics of apple

The effects of continuous and intermittent microwave power on moisture content with drying period and drying rate versus drying period are presented in Figures 1 and 2, respectively. As it is presented in Figure 1, the level of the microwave power had a remarkable impact on the moisture content of the apple samples,

expectedly. The findings indicated that drying period got shorter substantially depending on increasing level of microwave power. Similar result has been found by Çelen et al (2011) with a 'Granny Smith' apple variety. For samples, the drying period required to attain the final moisture content (0.1% d.b) was 80, 150 and 215 minutes for 100W-PR= 1, PR= 2 and PR= 3; 40, 75 and 125 min for 200W-PR= 1, PR= 2 and PR= 3 and 25, 45 and 75 min for the 300W-PR= 1, PR= 2 and PR= 3, respectively. As the findings for the continuous mode, the sample at dried 300W has a less drying period. At the greater microwave power levels, quicker transference of mass was realized because of the rise in heat production in the sample and hence the drying time became shorter (Kumar et al 2016). The findings indicate that all each microwave power levels of the PR= 1 application had the minimum drying period. Therefore, the total drying period is shorter in the continuous mode than in the intermittent mode (Gunasekaran & Yang 2007). At the outset of both drying processes, the drying rates were greater. It could be explained by a falling in each working cycle (t_o + t_{off}) of the microwave for the intermittent microwave drying application. Seeing that microwave power off-time yields a rest period for the redistribution of temperature and moisture inside the food and by the way, microwave power on-time allows the period for warming-up (Beaudry et al 2003). The drying rates of each sample dried at continuous mode was higher than intermittent mode at first stages because of the giving constant microwave energy. Since the amount of moisture that apple slices contain was greater at the first stage of the drying, based upon the greater diffusion of moisture, absorption of microwave power and rates of drying were found to be more. As the drying process goes on, moisture loss of the product culminated in a fall in the absorption of microwave power and rates of drying (Soysal et al 2009). Identical findings were gathered with apple (Aghilinategh et al 2015), rice (Xu et al 2017) and mussels (Kipcak 2017).

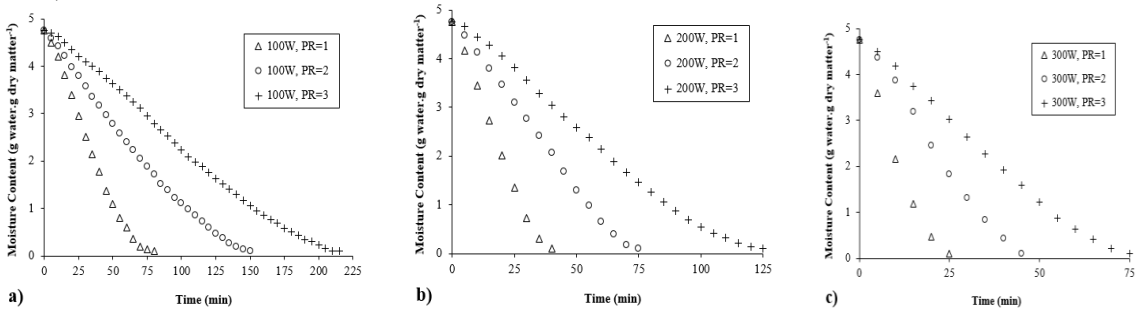


Figure 1- The moisture content of apple vs. time during pulsed microwave drying at different conditions

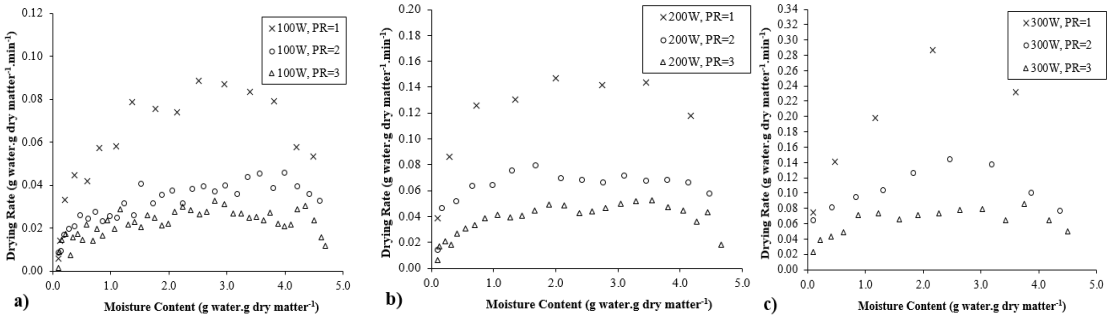


Figure 2- The drying rate vs. moisture content of apple at drying conditions

3.2. Modeling of drying curves

Table 2 shows the findings of the statistical analyses of the 8 distinct thin layer drying models, inclusive of drying model coefficients and the comparison criteria for assessing the quality of the model fit (R^2, *RMSE* and χ^2). R^2 values were greater than 0.906, and RMSE and χ^2 values were smaller than 0.102 and 94.386×10^{-4}, respectively revealing good fit findings in all cases. Based on these findings, the Midilli et al model was the optimum of the evaluated models at explaining the variations in moisture ratios found in all tests. In addition, in all cases of the Midilli et al model R^2 values were more than 0.998; and the *RMSE* and χ^2 values were less than 0.014 and 1.647×10^{-4}, in return. According to these computed findings, Midilli et al model

can be considered as presenting the thin-layer drying behavior of the apple samples. The difference between the predicted and experimental moisture ratio using the most relevant models with drying period at picked temperatures and microwave power levels for dried apple is presented in Figure 3. Obviously, the findings obtained from the Midilli et al model are quite close to the experimental values. In addition, the Midilli et al model could adequately describe the drying curves of apple under the picked experimental conditions. The findings of this study are in agreement with former findings proclaimed in the drying of pulped coffee (Coradi et al 2017), guava pulp (Maciel et al 2017), and apricot halves (Kayran & Doymaz 2017) for Midilli et al model.

Table 2- Forecasted data of statistical analyses obtained from thin layer drying models

No	*100W, PR=1*			
	Model coefficients	*R^2*	*RMSE*	*$\chi^2(10^{-4})$*
1	a= 1.126; k= 0.02965	0.9498	0.0768	48.7908
2	k= 0.02635	0.9331	0.0886	79.6781
3	k= 0.002217; n= 1.669	0.9975	0.0170	2.6731
4	a= 1.75; k= 0.01212; c= -0.6973	0.9914	0.0318	6.6619
5	a= 43.38; k_o= 0.05617; b= -42.37; k_1= 0.05754	0.9904	0.0336	11.0080
6	a= -0.01797; b= 0.0000635	0.9902	0.0340	11.5937
7	a= 1.239; k= 0.02604; b= 0.9515	0.9237	0.0947	84.8834
8	a= 0.9867; k= 0.002567; n= 1.597; b= -0.00069	0.9994	0.0071	1.0405
No	*100W, PR=2*			
	Model coefficients	*R^2*	*RMSE*	*$\chi^2(10^{-4})$*
1	a= 1.108; k= 0.01498	0.9651	0.0583	29.4053
2	k= 0.01344	0.9506	0.0693	48.2401
3	k= 0.001684; n= 1.478	0.9946	0.0229	5.5703
4	a= 1.738; k= 0.006021; c= -0.7104	0.9986	0.0115	0.8235
5	a= 16.34; k_o= 0.02637; b= -15.32; k_1= 0.02787	0.9907	0.0301	9.4359
6	a= -0.009356; b= 0.0000177	0.9984	0.0124	1.3022
7	a= -0.3939; k= 0.01454; b= 0.9452	0.9473	0.0716	49.7150
8	a= 0.9867; k= 0.002566; n= 1.266; b= 0.00069	0.9997	0.0052	0.2621
No	*100W, PR=3*			
	Model coefficients	R^2	RMSE	$\chi^2(10^{-4})$
1	a= 1.142; k= 0.009964	0.9495	0.0714	42.6928
2	k= 0.008636	0.9243	0.0874	75.6288
3	k= 0.000368; n= 1.665	0.9947	0.0231	5.1703
4	a= 2.518; k= 0.002557; c= -1.474	0.9969	0.0176	2.1284
5	a= 27.07; k_o= 0.01854; b= -26.05; k_1= 0.01925	0.9864	0.0370	13.1762
6	a= -0.005568; b= 0.00000363	0.9950	0.0225	4.3567
7	a= 0.0000392; k= 0.01439; b= 0.5998	0.9206	0.0896	77.4281
8	a= 0.9909; k= 0.0006739; n= 1.477; b= -0.00069	0.9995	0.0069	0.3965
No	*200W, PR=1*			
	Model coefficients	*R^2*	*RMSE*	*$\chi^2(10^{-4})$*
1	a= 1.092; k= 0.05347	0.9387	0.0890	65.1837
2	k= 0.04898	0.9336	0.0927	83.5024
3	k= 0.006074; n= 1.68	0.9941	0.0277	7.6681
4	a= 2.24; k= 0.01576; c= -1.212	0.9928	0.0305	5.2148
5	a= 56.74; k_o= 0.09428; b= -55.72; k_1= 0.09573	0.9733	0.0588	32.3902
6	a= -0.03218; b= 0.0001681	0.9932	0.0297	6.3986
7	a= -0.9022; k= 0.05104; b= 0.9784	0.9115	0.1001	95.2979
8	a= 0.9929; k= 0.008585; n= 1.492; b= -0.00292	0.9981	0.0156	2.2205
No	*200W, PR=2*			
	Model coefficients	*R^2*	*RMSE*	*$\chi^2(10^{-4})$*
1	a= 1.114; k= 0.02691	0.9338	0.0855	63.0934
2	k= 0.02396	0.9189	0.0946	88.3009
3	k= 0.001702; n= 1.709	0.9895	0.0340	11.8914
4	a= 4.225; k= 0.00379; c= -3.202	0.9964	0.0199	2.2661
5	a= 41.86; k_o= 0.05235; b= -40.82; k_1= 0.05364	0.9733	0.0543	28.6440
6	a= -0.01482; b= 0.000015	0.9960	0.0211	2.8826
7	a= -0.5643; k= 0.02561; b= 0.9582	0.9067	0.1015	94.3856
8	a= 0.9875; k= 0.002866; n= 1.449; b= -0.00301	0.9983	0.0136	1.6468

Table 2 (Continue)- Forecasted data of statistical analyses obtained from thin layer drying models

No	*200W, PR=3*			
	Model coefficients	R^2	*RMSE*	$\chi^2(10^{-4})$
1	a= 1.14; k= 0.01756	0.9485	0.0747	46.2486
2	k= 0.0153	0.9251	0.0900	81.4717
3	k= 0.0008704; n= 1.683	0.9959	0.0211	4.4787
4	a= 2.234; k= 0.005249; c= -1.184	0.9953	0.0226	3.1747
5	a= 26; k_o= 0.03326; b= -24.99; k_1= 0.03465	0.9882	0.0357	12.8191
6	a= -0.00997; b= 0.000014	0.9928	0.0279	6.7776
7	a= 1.968; k= 0.01472; b= 0.961	0.9189	0.0936	84.4770
8	a= 0.9938; k= 0.001395; n= 1.519; b= -0.00090	0.9994	0.0083	0.5989
No	*300W, PR=1*			
	Model coefficients	R^2	*RMSE*	$\chi^2(10^{-4})$
1	a= 1.055; k= 0.09247	0.9571	0.0800	53.4380
2	k= 0.08809	0.9607	0.0765	55.6568
3	k= 0.02202; n= 1.547	0.9987	0.0137	1.8168
4	a= 1.497; k= 0.04614; c= -0.4759	0.9906	0.0375	11.7476
5	a= 8.206; k_o= 0.1767; b= -7.208; k_1= 0.2022	0.9939	0.0302	8.3524
6	a= -0.06244; b= 0.0009068	0.9946	0.0283	7.5788
7	a= 1.063; k= 0.08767; b= 0.925	0.9346	0.0987	69.5035
8	a= 1.001; k= 0.02614; n= 1.444; b= -0.00182	0.9997	0.0064	0.6431
No	*300W, PR=2*			
	Model coefficients	R^2	*RMSE*	$\chi^2(10^{-4})$
1	a= 1.111; k= 0.0441	0.9328	0.0912	68.1232
2	k= 0.03947	0.9211	0.0294	88.0011
3	k= 0.00339; n= 1.761	0.9965	0.0208	4.0195
4	a= 3.298; k= 0.008451; c= -2.26	0.9930	0.0294	6.6326
5	a= 21.46; k_o= 0.08827; b= -20.45; k_1= 0.09305	0.9851	0.0429	18.1082
6	a= -0.0246; b= 0.000048	0.9916	0.0322	10.0784
7	a= 25.28; k= 0.02298; b= 0.9754	0.9278	0.0945	78.2707
8	a= 0.9987; k= 0.004916; n= 1.576; b= -0.00254	0.9996	0.0074	0.4178
No	*300W, PR=3*			
	Model coefficients	R^2	*RMSE*	$\chi^2(10^{-4})$
1	a= 1.119; k= 0.02798	0.9451	0.0782	51.5717
2	k= 0.02484	0.9285	0.0892	79.3566
3	k= 0.0021; n= 1.667	0.9946	0.0245	6.7456
4	a= 2.514; k= 0.007219; c= -1.478	0.9959	0.0213	2.5684
5	a= 35.16; k_o= 0.05173; b= -34.12; k_1= 0.05314	0.9821	0.0446	19.5241
6	a= -0.01608; b= 0.000033	0.9946	0.0245	4.5823
7	a= 5.073; k= 0.01659; b= 0.8993	0.9369	0.0839	65.0382
8	a= 0.9945; k= 0.003396; n= 1.462; b= -0.00192	0.9993	0.0091	0.8938

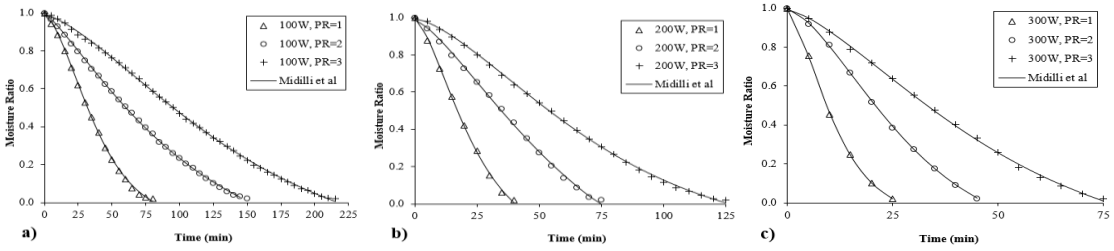


Figure 3- A comparison of the appropriate model to experimental moisture ratios at specific drying times

3.3. Rehydration ratio

Rehydration is a sophisticated operation and it points out the physical and chemical modifications triggered by drying applications. It has been detected from Figure 4 that the highest rehydration ratio has computed at drying 100W-PR= 1 microwave application and lowest value has been seen drying at 300W-PR= 1 mode.

The finding pointed out that the increase in microwave power supported the decrease in rehydration capacity. To make it clear, during drying at high microwave powers, permanent cellular rupture and dislocation happens, introduces tissue integrity loss and produces a dense structure of collapsed, substantially shrunken capillaries with reduced hydrophilic attributes. The reduced hydrophilic attributes came up with lower rehydration capacity values, prevented imbibition of water, and left unfilled pores behind (Horuz et al 2018). The findings showed that the rehydration ratio of PR= 3 has the highest level to PR= 1 and PR= 2 for 200W and 300W microwave power levels. A similar observation has been seen by Aghilinategh et al (2015) with apple.

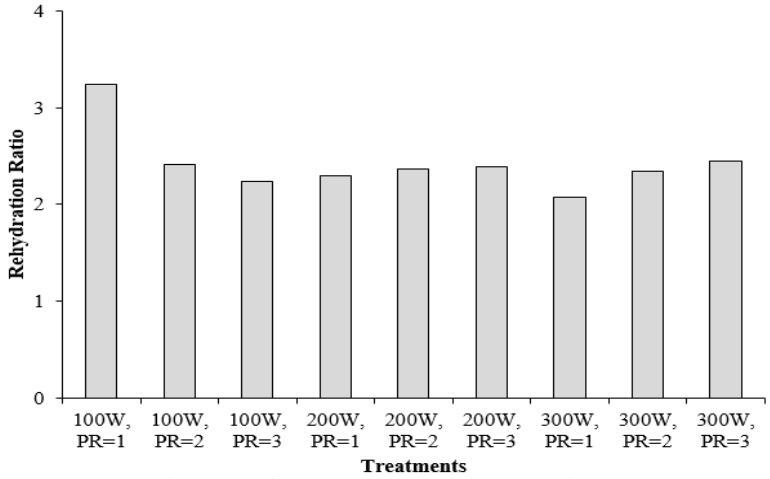


Figure 4- The rehydration ratio results of apple under drying conditions

3.4. Effective moisture diffusivity

The effective diffusivity values of 100W-PR= 1, PR= 2 and PR= 3; 200W-PR= 1, PR= 2 and PR= 3 and 300W-PR= 1, PR= 2 and PR= 3, drying conditions ranged from 8.11×10^{-9}, 4.05×10^{-9}, 3.04×10^{-9}; 1.52×10^{-8}, 8.11×10^{-9}, 5.07×10^{-9} and 2.53×10^{-8}, 1.22×10^{-8} and 8.11×10^{-9} m^2 s^{-1}, respectively. The values of effective moisture diffusivities in continuous and intermittent microwave dried apple (Red delicious variety) has been given by Aghilinategh et al (2015). There have been differences between with our presented values. This differences may occur using different varieties of apples. It is easy to observe that D_{eff} values upsurge substantially with the rise in microwave power. The reason for this may be the rise in microwave power led to a sudden rise in the sample temperature that consecutively boosted the vapor pressure. The effective moisture diffusivity value has been found higher at drying applications run at continuous microwave drying mode concerning the ones run at the intermittent mode. Because the continuous mode of microwave evokes the rise in temperature and correspondingly the water vapor pressure more than that of the intermittent mode, that assists the moisture diffusion on the product surface. As the PR number increases, effective moisture diffusivity decreases. This may be because of sample cooling as a result of power-off-times at high pulsing ratios. Similar observations were recorded by Sharifian et al (2015) with fig fruit. Doymaz et al (2015) reported that moisture diffusivity of dried bean ranged from 1.387×10^{-8} and 3.724×10^{-8} m^2 s^{-1} at 180-800 W microwave power. Puangsuwan et al (2015) also found that the D_{eff} of palm fruit increases with microwave power. The values of moisture diffusivity values found in our research almost in line with the values reported in the literature, whereas there exist some variations. These variations may arise from the geometric shape and type, moisture content at the beginning and in the end and also chemical and physical attributes of products, dryer type and pre-applications (Horuz et al 2018).

4. Conclusions

In this research, the effect of different continuous and intermittent microwave drying applications on the drying kinetics, rehydration ratio and effective moisture diffusivity attributes of apple samples were explored. The findings verified that continuous microwave drying provides more positive effect drying period and drying rate than the intermittent mode. Midilli et al is the model that optimally represents the

drying curves of apple slices at all microwave power levels and pulsing modes. Highest values of rehydration and are obtained for apple dried at 100W-PR= 1 application. Effective moisture diffusivity of dried apple, ranged from $3.04x10^{-9}$ and $2.53x10^{-8}$ m^2 s^{-1} at microwave power of 100W-PR= 3 and 300W-PR= 1 mode, respectively. The findings of the current study pointed out that intermittent and continuous modes of microwave drying are an appropriate alternative for drying apple.

Abbreviations and Symbols	
M_0	Initial moisture content
M_t	The moisture content at a particular time
M_e	Equilibrium moisture content
$MR_{exp,i}$	Experimental moisture ratio at the test number i,
$MR_{pre,i}$	Predicted moisture ratio at the test number i,
N	Observation number
z	Total count of constant
$RMSE$	Root mean square error
R^2	Coefficient of determination
χ^2	Reduced chi-square
a,b,c,n, k_o,k_1	Model constants
D_{eff}	Effective moisture diffusivity

References

Aghilinategh N, Rafiee S, Gholikhani A, Hosseinpur S, Omid M, Mohtasebi S S & Maleki N (2015). A comparative study of dried apple using hot air, intermittent and continuous microwave: evaluation of kinetic parameters and physicochemical quality attributes. *Food Science & Nutrition* 3(6): 519-526

An F, Qiu D, Song H, Wu X, Tong J & Guo R (2015). Effects of instant pressure drop puffing with super-heated vapor on the physical properties of Granny Smith apple chips. *Journal of Food Process Engineering* 38(2): 174-182

Beaudry C, Raghavan G S V & Rennie T J (2003). Microwave finish drying of osmotically dehydrated cranberries. *Drying Technology* 21(9): 1797-1810

Çelen S, Kahveci K & Akyol U (2011). Single layer drying behavior of apple slices in a microwave dryer. *In Defect and Diffusion Forum* 312: 842-847

Coradi P C, Borém F M & Reinato C H (2017). Mathematical modeling of drying the pulped coffee (Coffea arabica L.) at different air conditions. *Revista Brasileira de Tecnologia Agroindustrial* 11(2): 2397-2419

Cruz A C, Guiné R P & Gonçalves J C (2015). Drying kinetics and product quality for convective drying of apples (cvs. Golden Delicious and Granny Smith). *International Journal of Fruit Science* 15(1): 54-78

Dehghannya J, Hosseinlar S H & Heshmati M K (2018). Multi-stage continuous and intermittent microwave drying of quince fruit coupled with osmotic dehydration and low temperature hot air drying. *Innovative Food Science & Emerging Technologies* 45: 132-151

Djekic I, Tomic N, Bourdoux S, Spilimbergo S, Smigic N, Udovicki B, Hofland G, Devlieghere F, Rajkovic A & Rajkovic A (2018). Comparison of three types of drying (supercritical CO2, air and freeze) on the quality of dried apple-Quality index approach. *LWT* 94: 64-72

Doymaz I, Kipcak A S & Piskin S (2015). Microwave drying of green bean slices: Drying kinetics and physical quality. *Czech Journal of Food Science* 33(4): 367-376

Gunasekaran S & Yang H W (2007). Optimization of pulsed microwave heating. *Journal of Food Engineering* 78(4): 1457-1462

Horuz E, Bozkurt H, Karataş H & Maskan M (2018). Simultaneous application of microwave energy and hot air to whole drying process of apple slices: Drying kinetics, modeling, temperature profile and energy aspect. *Heat and Mass Transfer* 54(2): 425-436

Junqueira J R D J, Corrêa J L G & Ernesto D B (2017). Microwave, convective, and intermittent microwave–convective drying of pulsed vacuum osmodehydrated pumpkin slices. *Journal of Food Processing and Preservation* 41(6): 1-8

Kayisoglu S & Ertekin C (2011). Vacuum drying kinetics of Barbunya bean (Phaseolus vulgaris L. elipticus Mart.). *The Philippine Agricultural Scientist* 94(3): 285-291

Kayran S & Doymaz İ (2017). Infrared drying and effective moisture diffusivity of apricot halves: Influence of pretreatment and infrared power. *Journal of Food Processing and Preservation* 41(2): 1-8

Kermani A M, Khashehchi M, Kouravand S & Sadeghi A (2017). Effects of intermittent microwave drying on quality characteristics of pistachio nuts. *Drying Technology* 35(9): 1108-1116

Kipcak A S (2017). Microwave drying kinetics of mussels (Mytilus edulis). *Research on Chemical Intermediates* 43(3): 1429-1445

Kumar V, Sharma H K & Singh K (2016). Mathematical modeling of thin layer microwave drying of taro slices. *Journal of the Institution of Engineers (India): Series A* 97(1): 53-61

Maciel R M, Afonso M R, Costa J, Severo L S & Lima N D D (2017). Mathematical modeling of the foam-mat drying curves of guava pulp. *Revista Brasileira de Engenharia Agrícola e Ambiental* 21(10): 721-725

Murthy T P K & Manohar B (2014). Hot air drying characteristics of mango ginger: Prediction of drying kinetics by mathematical modeling and artificial neural network. *Journal of Food Science and Technology* 51(12): 3712-3721

Puangsuwan K, Chongcheawchamnan M & Tongura C (2015). Effective moisture diffusivity, activation energy and dielectric model for palm fruit using a microwave heating. *Journal of Microwave Power and Electromagnetic Energy* 49(2): 100-111

Saxena S, Verma J & Gautam S (2016). Potential prophylactic properties of apple and characterization of potent bioactive from cv. "Granny Smith" displaying strong antimutagenicity in models including human lymphoblast TK6+/− cell line. *Journal of Food Science* 81(2): 508-518

Sharifian F, Nikbakht A M, Arefi A & Modarres Motlagh A (2015). Experimental assessment of energy and mass transfer in microwave drying of fig fruit. *Journal of Agricultural Science and Technology* 17: 1695-1705

Soysal Y, Ayhan Z, Eştürk O & Arıkan M F (2009). Intermittent microwave–convective drying of red pepper: Drying kinetics, physical (colour and texture) and sensory quality. *Biosystems Engineering* 103(4): 455-463

Sturm B, Vega A M N & Hofacker W C (2014). Influence of process control strategies on drying kinetics, colour and shrinkage of air dried apples. *Applied Thermal Engineering* 62(2): 455-460

Sunjka P S, Orsat V & Raghavan G S V (2008). Microwave/vacuum drying of cranberries (Vacccinium macrocarpon). *American Journal of Food Technology* 3(2): 100-108

Vega-Gálvez A, Di Scala K, Rodríguez K, Lemus-Mondaca R, Miranda M, López J & Perez-Won M (2009). Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (Capsicum annuum, L. var. Hungarian). *Food Chemistry* 117(4): 647-653

Xu F, Chen Z, Huang M, Li C & Zhou W (2017). Effect of intermittent microwave drying on biophysical characteristics of rice. *Journal of Food Process Engineering* 40(6): e12590. https://doi.org/10.1111/jfpe.12590

Yi J Y, Zhou L Y, Bi J F, Wang P, Liu X & Wu X Y (2016). Influence of number of puffing times on physicochemical, color, texture, and microstructure of explosion puffing dried apple chips. *Drying Technology* 34(7): 773-782

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 Soil Quality Index for Tea Cultivated Soils in Ortaçay Micro Catchment in Black Sea Region

Orhan DENGİZ[a], Serkan İÇ[b], Fikret SAYGIN[b], Ali İMAMOĞLU[c]

[a]*Ondokuz Mayıs University, Agricultural Faculty, Plant Nutrition and Soil Science Department, Samsun, TURKEY*

[b]*Republic of Turkey Ministry of Food Agriculture and Livestock, Black Sea Agricultural Research Institute, Samsun, TURKEY*

[c]*Nevşehir Hacıbektaş Veli University, Faculty Art and Science, Geography Department, Nevşehir, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Orhan DENGİZ, E-mail: odengiz@omu.edu.tr, Tel: +90 (0505) 438 33 53
Received: 10 October 2018, Received in Revised Form: 26 November 2018, Accepted: 28 November 2018

AUTHORS ORCID ID
(Orhan DENGİZ: 0000-0002-0458-6016), (Serkan İÇ: 0000-0001-6976-762X), (Fikret SAYGIN: 0000-0001-7771-806X), (Ali İMAMOĞLU: 0000-0001-9197-1029)

ABSTRACT
The objective of this research was to determine soil quality by taking into consideration the integrated Soil Quality Index (SQI_w) model on tea plantations located in Ortaçay Micro Catchment of Rize. In the SQI_w model, soil indicators were weighted by means of the Analytical Hierarchy Process (AHP). Various indicator units were normalized by a Standard Scoring Function. A total of 22 soil quality indicators were included in the SQI_w model by grouping into 4 criteria which are; i-soil physical properties, ii- soil chemical properties, iii-macronutrient elements, iv-micronutrient elements. Twenty eight soil samples were collected from tea cultivated gardens including dominantly Leptosol and Alisol-Acrisol great soil groups based on FAO/WRB classification. The results indicated that 25% of the soil samples studied had weak quality level, whereas 75% were in moderate SQI_w class in terms of tea requirements of the soil quality.

Keywords: Soil quality index; Analytical Hierarchy Process; Tea plant; Soil indicators

1. Introduction

Turkey is one of the most significant and unique countries in the World for producing tea plant (*Camellia sinensis L.*) and it is ranked as the fifth largest producer among the World's tea production (FAO 2009). Tea production is generally located in about 35 km narrow strip along a coast in the north-eastern Black Sea Region spanning roughly 180 km from Hopa near the border of Georgia to Araklı township of Trabzon (Müftüoğlu 1987; Özyazıcı et al 2011). Approximately 76000 ha of soils are involved in tea cultivation, in provinces Artvin (11%), Rize (65%), Trabzon (21%), Giresun and Ordu (3%) (Müftüoğlu et al 2010). However, west part of the Araklı (Trabzon) located on poor soil quality area is not suitable for tea production due to economic feasibility (Müftüoğlu 1987). Therefore, in order to get optimum growth conditions for tea planting and good yields, well permeable deep soils are required; with organic matter content >2% and soil reaction from 4.5 to 5.5. Groundwater level should be deeper than 90 cm. Soil compaction in subsoil affects root development of the tea plants and causes their susceptibility to

DOI: 10.15832/ankutbd.468900

draught/waterlogging in dry/wet periods. Thus hard pans in the subsoil should be absent down to 2 m (Özcan et al 2017).

Originally, soil quality was defined as the capacity of a particular soil to sustainably function within particular ecosystem, either natural or managed, and its assessment is a tool in order to support plant growth while maintaining environmental quality and productivity (Doran & Parkin 1994; Karlen et al 2001). In several studies, soil quality indicators used for assessment of the soil quality are various physico-chemical and biological soil properties, sensitive to disturbance (Gülser 2004; Candemir & Gülser 2011; Gülser et al 2015; Demir & Gülser 2015).

These heterogeneous properties are utilized using numerical quality indices. Multiplicative, additive or weighted mean procedures are employed in integration of unitless parameters (gained by normalization) into quality indices, such as the integrated Soil Quality Index, SQI_w (Doran & Parkin 1994; Andrews et al 2002; Qi et al 2009). The SQI_w synthesizes the weights (equal for each indicator) of all selected indicators into the resulting index in a formula which utilizes a simple scoring system. Objective of this study was to determine soil quality by using the integrated Soil Quality Index model on tea plantations in micro catchment located in Rize province of the Black Sea Region.

2. Material and Methods

2.1. The study field

The research area covering about 170 km^2 is located in Ortaçay Catchment, which extends from 4527000 to 4545000 N and from 633000 to 645000 E (UTM, 37 Zone m) in eastern highland Black Sea Region of Turkey (Figure 1).

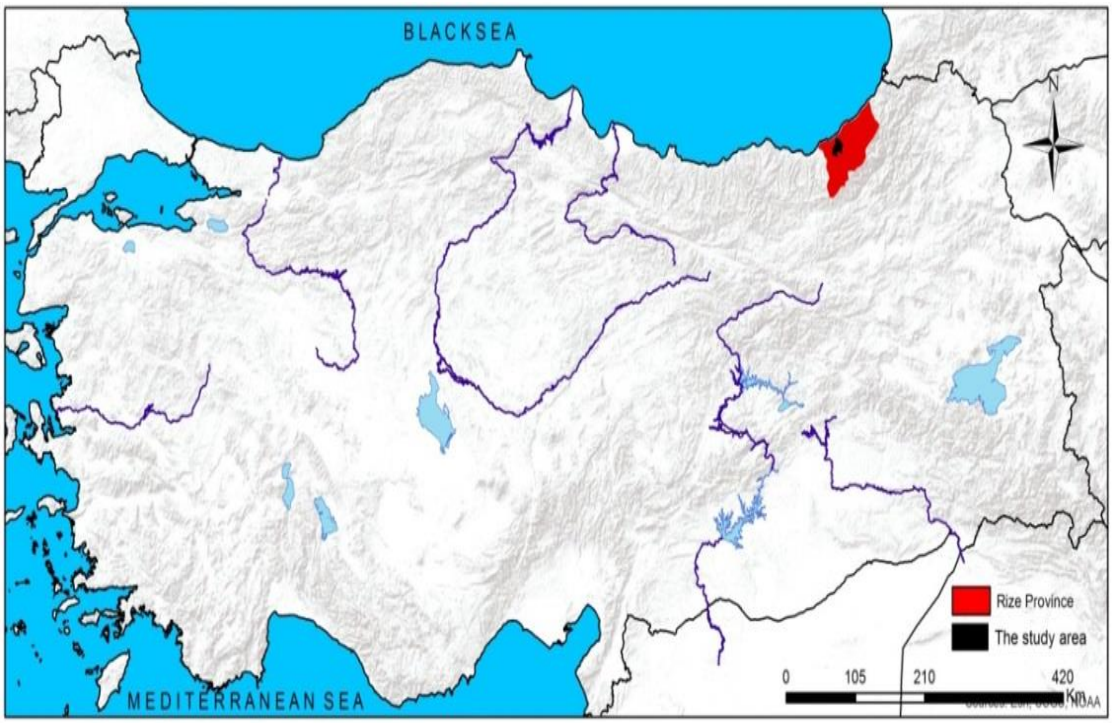


Figure 1- Location of the study area

The catchment lies at an elevation above the sea level from 70 to 1972 m. The study area has different topographic features such as hilly, rolling, flat, etc. Only 7.2% of the total area is almost flat (Figure 2). Most of the total area corresponding with 11978 ha has more than 15% slope.

In addition, as for aspect of the study area, in general the southerly (south-easterly, south-westerly) and northerly (north easterly, north westerly) aspects prevail. In the region, the current climate can be called as semi-humid based on the meteorological data covering the period between years 1981-2011. In addition, average annual precipitation and temperature of the study area are 2304.1 mm and 14.3 ºC, respectively.

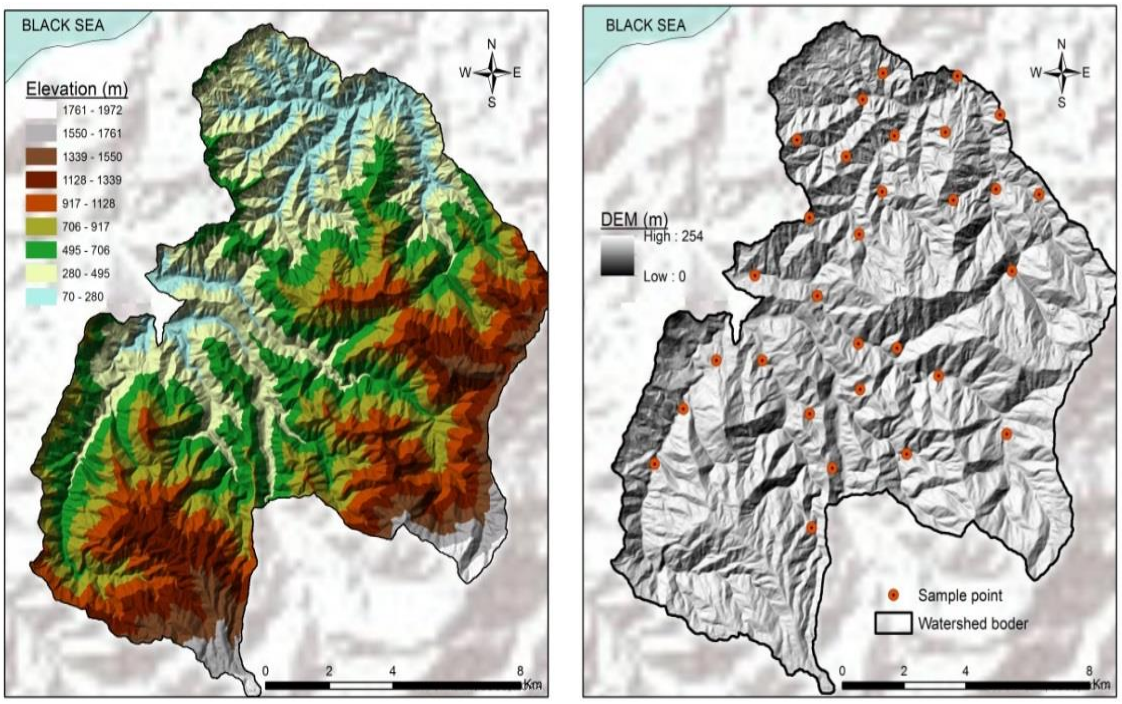


Figure 2- Elevation and hillshade maps of the study area

2.2. Sampling and indicator scoring

Field study was conducted in 2017. In total 28 soil samples from Leptosol and Alisols-Acrisols soil units were taken on tea gardens of the Ortaçay Catchment. The sampling was carried out after harvest in the autumn between two cropping seasons in order to reduce the influence of agricultural practices during the growing season, i.e. fertilization. Soil samples were taken from soil surface layer (0-20 cm) and their coordinates were recorded using GPS device (Figure 2). A total of 22 soil quality indicators were determined and included in the SQI_w model by grouping into 4 criteria which are; i- soil physical properties (aggregate stability - AS, erodibility ratio - ER, structure stability ratio - SSR, clay ratio - CR, percentage of sand, silt and clay), ii- soil chemical properties (soil reaction - pH, electrical conductivity - EC, organic matter - OM and lime content - $CaCO_3$), iii- macronutrient elements (total nitrogen - TN, available phosphorus - AvP, exchangeable potassium - exK, exchangeable calcium - exCa, exchangeable magnesium - exMg and exchangeable sodium - exNa), iv- micronutrient elements (available iron - AvFe, available manganese - AvMn, available zinc - AvZn, available copper - AvCu). Table 1 shows the selected analytical protocols.

In this study, due to variation of units of the indicators, a standard scoring function (SSF) (Andrews et al 2002) was used and scores ranging from 0 to 1 were attributed. Three types of indicators were separated according to their affiliation to soil quality, where the most desired soil functionality was associated with low, intermediate or high values (Liebig et al 2001): (1) “More is better” function (MB) was affiliated to $CaCO_3$ content, clay content, clay ratio (CR), aggregate stability (AS) and structure stability index (SSI) considering structural stability, resistance to soil erosion, available water capacity and then organic matter OM content, macro- and micronutrient elements for their parts in soil fertility as their high content is favourable for sustainable tea cultivation. (2) “Less is better” function (LB) was affiliated to Na content, erodibility ratio (ER), dispersion ratio (DR), sand and silt content for their part in degradation of soils. (3)

"Optimal range" function (OR) was affiliated to pH where scores were distributed using the both previous function types depending on whether the value of this indicator was lower or higher than the optimal range. The SSF equations (Andrews et al 2002) for the indicators were given in Table 2.

Table 1- Protocol measurements for indicators selected in the study

Parameters	*Unit*	*Protocol*	*Reference*
Aggregate stability (AS)	%	Wet sieving	Kemper & Rosenau (1986)
Dispersion ratio (DR)	%	DR= (a/b)* 100	Lal & Elliot (1994)
Erodibility ratio (ER)	%	ER= (a/b)*(A/c)*100	Lal & Elliot (1994)
Structure stability index (SSI)	%	SSI=∑b-∑b	Lal & Elliot (1994)
Clay ratio (CR)	%	CR=(100-c)/c	Bouyoucos (1935)
Texture (Clay, Silt and Sand)	%	hydrometer method	Bouyoucos (1951)
OM	%	Walkley-Black wet digestion	Nelson & Sommers (1982)
pH	1:2.5	(w:v) soil-water suspension	Soil Survey Laboratory (1992)
EC	dS m^{-1}	(w:v) soil-water suspension	Soil Survey Laboratory (1992)
$CaCO_3$	%	Scheibler calcimeter	Soil Survey Staff (1993)
$NaHCO_3$-P	mg kg^{-1}	Bray and Kurtz	Kacar (1994)
Total N	%	Kjeldahl	Bremner & Mulvaney (1982)
NH4OAC-K, Ca, M, Na	mg kg^{-1}	Ammonium acetate extraction, flame spectrometry detection	Soil Survey Laboratory (1992)
DTPA-Cu, Fe, Mn, Zn	mg kg^{-1}	DTPA extraction, AAS detection	Lindsay & Norvell (1978)

a is the percentage of silt plus clay in suspension, b is the percentage of silt plus clay dispersed with chemical agent, A is the field capacity, c is the percentage of clay dispersed with chemical agent

Table 2- Standard scoring functions and parameters for soil indicators

Parametres	*FT**	*L*	*U*	*SSF Equation***
ER	LB	8.50	85.28	$f(x)=\begin{cases}0.1 & x\le L\\ 1-0.9\times\frac{x-L}{U-L}+0.1 & L\le x\le U\\ 1 & x\ge U\end{cases}$
DR	LB	3.85	18.14	
Sand	LB	35.37	76.31	
Silt	LB	15.34	53.90	
EC	LB	0.025	0.614	
Na	LB	0.00	1.06	
$CaCO_3$	MB	0.00	1.58	$f(x)=\begin{cases}0.1 & x\le L\\ 0.9\times\frac{x-L}{U-L}+0.1 & L\le x\le U\\ 1 & x\ge U\end{cases}$
Clay	MB	4.12	23.41	
AS	MB	55.52	92.02	
SSI	MB	22.73	59.31	
CR	MB	3.27	23.27	
OM	MB	1.17	11.5	
P	MB	4.55	128.18	
N	MB	0.13	0.63	
Ca	MB	0.08	24.96	
K	MB	0.06	1.18	
Mg	MB	0.05	3.09	
Fe	MB	39.5	281.37	
Cu	MB	0.47	22.97	
Zn	MB	0.14	26.04	
Mn	MB	1.71	60.61	
pH	OR	L1	U1	$f(x)=\begin{cases}0.1 & x\le L\ or x\ge U\\ 0.9\times\frac{x-L1}{L2-L1}+0.1 & L\le x\le L2\\ 1\end{cases}$
		3.38	7.37	
		L2	U2	
		3.38	6.00	$f(x)=\begin{cases}0.1 & L2\le x\le U1\\ 0.9\times\frac{x-U1}{U2-U1}+0.1 & U1\le x\le U2\\ 1\end{cases}$

*, FT means function type; MB means more is better; LB, means low is better; OR, means optimal range; **SSF, means standard scoring function; in these three equations, x is the monitoring value of the indicator, f(x) is the score of indicators ranged between 0.1 and 1, and L and U are the lower and the upper threshold value, respectively

2.3. Soil quality Index and weight assignment by Analytical Hierarchy Process

Successful land cultivation and farming, generating diverse kinds of land utilization, is determined by environmental conditions, which can be described by set of soil and land quality indicators. Consequently, land mapping units can be described by a set of land characteristics, which are land and soil attributes affecting their suitability for certain land utilization types (Van Diepen et al 1991). Land utilization type in the present research is tea production. Soil requirements for tea cultivation including soil physical and chemical properties were determined based on literature (Kacar 1984; Özyazıcı et al 2010 and 2013; Saygın et al 2017). Soil characteristic indicators and weighting rates commonly used in tea growing soil quality assessment were applied to compile information on the study area; they are listed as follows: aggregate stability, erodibility ratio, structure stability ratio, clay ratio, percentage of sand, silt and clay, soil reaction, electrical conductivity, organic matter and lime content, total nitrogen, phosphorus, potassium, calcium, magnesium and sodium, iron, zinc, copper, and manganese. All indicators were scored and weighted, then soil quality indices were estimated for each soil sample using the following formula (1) (Doran & Parkin, 1994);

$$\mathrm{SQI}_w = \sum_{i=1}^{n} (W_i . X_i) \quad (1)$$

Where; SQI_w is tea soil quality index, W_i is weighting of indictor i, X_i is score of indictor i obtained by SSF, n is number of indicator.

Land use suitability for the particular land use type is directly proportional to the SQI_w (Table 3). In this table, Class VI is the most suitable or excellent for tea plant, while classes I and II are not appropriate for tea cultivation in terms of soil quality (Da Silva et al 2015; Nabiollahi et al 2017).

Table 3- Soil quality index classes (Da Silva et al 2015; Nabiollahi et al 2017)

Class	*Definition*	*Index value*
I	Very poor	< 0.0
II	Poor	0.0-0.19
III	Weak	0.20-0.39
IV	Moderate	0.39-0.59
V	Strong/ Suitable	0.60-0.79
VI	Excellent/The most suitable	0.80-1.00

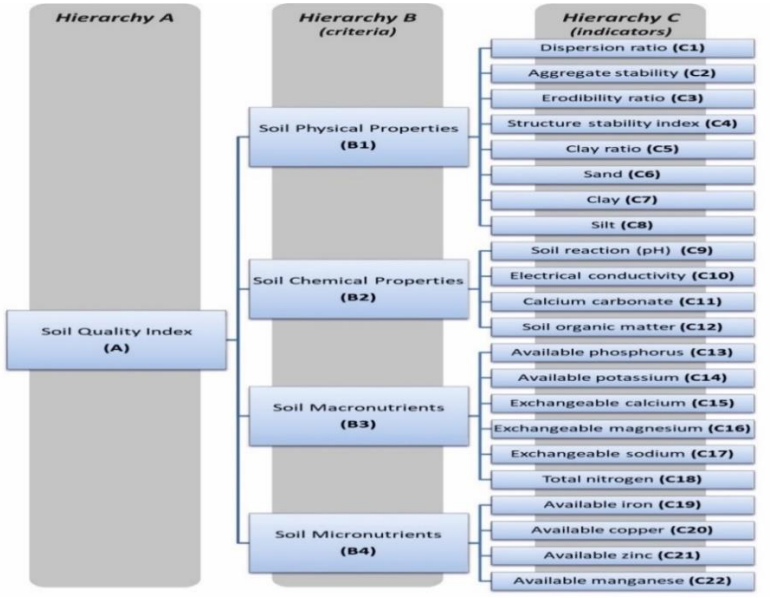


Figure 3- Hierarchical structure for the parameters' weight assignments

A total of 22 soil quality indicators were grouped into 4 criteria: physical, chemical, macronutrients and micronutrients, which means A, B, and C matrices in the hierarchy were logically designed (Figure 3).

Each indicator (hierarchy C) has an importance level that variously affects the land suitability for tea plant. The weighting process has to be carried out for both, hierarchy B and C, in order to learn also the importance level of criteria in Hierarchy B (Özyazıcı et al 2013).

In order to assign weights of indicators and criteria, Analytical Hierarchy Process according to Saaty (1980) was employed due to its capability to handle heterogeneous factors on multi-criteria decision level (Jiuquan et al 2015). The hierarchical structure makes possible to assess contribution of particular criteria at lower levels to higher-level criteria. Analytical Hierarchy Process (AHP) weighting utilizes the pairwise comparison matrix instead of taking expert opinions into consideration directly. Indicator weights (W_i) were determined by judging two criteria against each other and assigning values from the scale between 9 and 1/9 as described by Saaty (1980) and Table 4. Some researchers such as Rezaei-Moghaddam & Karami (2008) and Dengiz et al (2015) stated that the pairwise comparison simplifies the decision making process by independent assessment of the contribution of each criterion.

Table 4- The comparison scale in AHP (Saaty, 1980)

Intensity of importance	*Definition*	*Explanation*
1	Equal importance	Two activities contribute equally to the objective
3	Weak importance of one over another	Experience and judgment slightly favour one activity over another
5	Essential or strong importance	Experience and judgment strongly favour one activity over another
7	Demonstrated importance	An activity is strongly favoured and its dominance is demonstrated in practice
9	Absolute importance	The evidence favouring one activity over another is of the highest possible order of affirmation
2,4,6,8	Intermediate values between the two adjacent judgments	When compromise is needed
Reciprocals of above nonzero values	If activity *i* has one of the above nonzero numbers assigned to it when compared with activity *j*, then j has the reciprocal value when compared with *i*	

A square matrix was constructed from the pairwise comparisons of the indicators, normalized and weighted with respect to the indicators (details in Bhushan & Rai 2004; Şener et al 2010; Dengiz et al 2015). After that, assessment of the matrix consistency was carried out. The consistency index, CI, was estimated as (2):

$$CI = (\lambda_{max}\text{-}n)/(n\text{-}1) \quad (2)$$

Where; CI, means the consistency index; λ_{max}, means the highest principal eigenvalue of the matrix, and *n* means the order of the matrix. Consistency ratio was then calculated (3):

$$CR = CI/RI \quad (3)$$

Where; CR is the consistency ratio and RI, means the random index (see Table 5). Revision of the judgements is needed if CI failed to reach a threshold level. In general, a consistent matrix should have CR ≤ 0.1.

Table 5- Values of Random index (RI) (Saaty, 1980)

n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RI	0.00	0.00	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49	1.51	1.48	1.56	1.57	1.59

3. Results and Discussion

3.1. Soil physico-chemical properties

The physico-chemical characteristics of the 28 soil samples taken from the tea gardens in Ortaçay micro catchment of Rize province showed changefulness as a result of dynamic interactions among natural environmental factors, including the degree of soil formation, leaching process, and agricultural activities such as tillage systems or fertilization (Başkan et al 2017).Their descriptive statistical parameters are given in Table 6. According to Table 6, AS, DR, SSI, AvFe, sand and silt content showed normal distribution, whereas other parameters were found in unsymmetrical position called as skewness. Variability of the properties in terms of coefficient of variation (CV) was classified as low (<15%), medium (15-35%) and moderate (>35%) (Mallants et al 1996). In this case, AvP and AvFe showed very high variation (more than 100%). Total N concentrations varied between 0.13% and 0.63% with the average of 0.30%. The mean values of organic matter and $CaCO_3$ content (%) were 0.15 and 5.39, respectively. Soil texture class slightly varied from sandy loam to loam and sandy clay loam. Clay content was between 4.12% and 23.41% and content of sand varied between 35.37% and 76.31%. In addition, Table 6 shows also statistical distribution of micronutrient elements concentration. According to limit values of AvZn for tea plant reported in Lindsay & Norvell (1978), FAO (2008) and Özyazıcı et al *(*2011), level of AvZn was found insufficient in most of the soil samples and its mean values was 3.53 mg kg^{-1}. On the other hand, other micronutrient elements' concentrations were determined as sufficient. Finally, minimum and maximum values of SQI_w changed between 0.29 and 0.53.

Table 6- Descriptive statistical analysis of physical and chemical properties of soil samples

Parameters	*Mean*	*SD*	**CV*	*Variance*	*Min.*	*Max.*	***Skewness*	*Kurtosis*
AS (%)	70.73	8.75	36.50	76.69	55.52	92.02	0.39	0.14
DR (%)	10.31	4.19	14.29	17.63	3.85	18.14	0.25	-0.89
ER (%)	29.86	18.28	76.78	334.16	8.50	85.28	1.64	3.02
SSI (%)	38.53	8.17	36.58	66.88	22.73	59.31	0.22	0.33
CR (%)	9.63	5.09	20.00	25.94	3.27	23.27	1.02	0.54
Sand (%)	56.89	9.34	40.94	87.30	35.37	76.31	0.13	0.00
Clay (%)	11.50	5.14	19.29	26.47	4.12	23.41	0.69	-0.01
Silt (%)	31.60	7.97	38.56	63.55	15.34	53.90	0.47	1.19
pH (1:2.5)	4.34	0.94	3.99	0.89	3.38	7.37	1.69	3.02
EC (dS m^{-1})	0.29	0.15	0.59	0.02	0.03	0.61	0.64	-0.59
$CaCO_3$ (%)	0.15	0.28	1.49	0.07	0.10	1.59	5.29	28.00
OM (%)	5.39	2.29	10.33	5.28	1.17	11.50	0.55	0.49
AvP (mg kg^{-1})	48.23	35.71	123.63	1275.77	4.55	128.18	0.79	-0.45
exK (mg kg^{-1})	0.35	0.28	1.12	0.08	0.06	1.18	1.36	1.48
exCa (mg kg^{-1})	4.13	6.71	24.88	45.09	0.08	24.96	2.08	3.80
exMg (mg kg^{-1})	0.87	0.90	3.04	0.82	0.05	3.09	1.36	0.82
exNa (mg kg^{-1})	0.19	0.28	1.06	0.07	0.00	1.06	1.82	2.72
TN (%)	0.30	0.11	0.50	0.01	0.13	0.63	0.67	0.99
AvFe (mg kg^{-1})	146.36	63.86	241.87	4078.56	39.50	281.37	0.07	-0.78
AvCu (mg kg^{-1})	2.91	4.41	22.50	19.46	0.47	22.97	3.96	17.00
AvZn (mg kg^{-1})	3.53	5.52	25.90	30.53	0.14	26.04	3.21	10.95
AvMn (mg kg^{-1})	17.05	13.94	58.90	194.35	1.71	60.61	1.31	1.94
SQI_w	0.42	0.06	0.24	0.00	0.29	0.53	-0.70	0.06

SD, Standard deviation; Min., Minimum; Max., Maximum; n, sample number; *CV, (Coefficient of Variation**)**: < 15 = Low variation; 15-35 = Moderate variation; >35 = High variation; **skewness:< $\left|\mp 0.5\right|$ = Normal distribution; 0.5-1.0 = Application of character changing for dataset, and > 1,0 → application of Logarithmic change

3.2. Computation of soil quality index

According to the approach of Doran & Jones (1996), in order to start the calculation of a SQI_w firstly soil quality indicators were defined as the processes and features of the soil which are sensitive to variability induced by both natural and artificial indicators. Therefore, soil quality indicators can be divided as either inherent or dynamic. The inherent indicators are for example particle size distribution or mineral

composition, while the dynamic ones reflect soil conditions resulting from current agrotechnology. In this case Wienhold et al (2004) pointed out that dynamic indicators are used to evaluate how soil management decisions affect soil properties. This approach established in total 22 soil quality indicators enabling to reflect main effects as a result of agriculture management practices and inherent characters of soil for the tea plant.

Weightings were assigned to each soil sample as follows. Firstly, AHP approach was performed to determine eigenvector values. In this step the consistency ratio was determined far below the highest value at which the weighting could be called consistent, which is 0.1. Success of the AHP succeeded in weighting was reported also by Wali et al (2016). Contribution weights of soil indicators to the SQI_w estimated by the AHP were given in Table 7. The highest value (0.369) was determined for hierarchy B1 (soil physical indicators) whereas, the lowest value (0.126) was found for hierarchy B4 (soil micronutrient elements concentration). In addition, the highest values of indicators for each hierarchy B1, B2, B3 and B4 were calculated for AS (0.315), OM (0.400), TN (0.405) and AvFe (0.053), respectively.

Table 7- Contribution weight of soil indicators to soil quality calculated by the AHP

Hierarchy A					
Hierarchy C	*Hierarchy B*				*Combined weight* $\sum B_i \times C_i$
	B1	B2	B3	B4	
	0.369	0.299	0.206	0.126	
DR (%)	0.076				0.028
AS (%)	0.315				0.116
ER (%)	0.129				0.048
SSI (%)	0.109				0.040
CR (%)	0.095				0.035
Sand (%)	0.066				0.024
Clay (%)	0.125				0.046
Silt (%)	0.085				0.031
pH (1:2.5)		0.207			0.062
EC (dS m^{-1})		0.071			0.021
$CaCO_3$ (%)		0.322			0.096
OM (%)		0.400			0.120
AvP (mg kg^{-1})			0.252		0.052
AvK (mg kg^{-1})			0.154		0.032
exCa (mg kg^{-1})			0.082		0.017
exMg (mg kg^{-1})			0.076		0.016
exNa (mg kg^{-1})			0.031		0.006
TN (%)			0.405		0.083
AvFe (mg kg^{-1})				0.421	0.053
AvCu (mg kg^{-1})				0.099	0.012
AvZn (mg kg^{-1})				0.359	0.045
AvMn (mg kg^{-1})				0.121	0.015
Total	1	1	1	1	1

These results can be called consistent and the highest value of hierarchy B1 can be explained. Most of the tea plantations in this catchment have been located on steep hillsides. In addition, this area receives more than 2300 mm annual precipitations. Therefore, these areas are under potentially high risk in terms of soil erosion, particularly in tea cultivation or management period. For that reason, soil erodibility factors or erosion sensitivity parameters such as aggregate stability, dispersion ratio and others, which show soil resistance to erosion, were determined. Moreover, soil texture selected as physical parameter is also significant in terms of soil physical, chemical and biological effects on tea plant growth. On the other hand, other indicators can be arranged by management practices such as pH regulation by adding lime to supply tea plant's requirements onto soil reaction and elimination of insufficient macro- or micronutrient elements by fertilization.

In hierarchy B2, OM obtained the highest value due to its inevitable importancy as well as for its effect on biological and physico-chemical soil properties. This indicator is at the same time contained in lowering

of erosion risks, storage and supply of nutrient elements, overall improvement of soil fertility and affects cation exchange capacity, too. On the other hand, this indicator can be affected by soil and tea crop management practices. The tea plant cannot grow in strong acid conditions such as pH < 4 (Saygın et al 2017), but reaction of some of the soil samples was lower than 4. According to values of EC, all soil samples were described as nonsaline due to high leaching process and found as the lowest weight value in B2 hierarchy.

As for hierarchy B3, the highest weight values were found for the main macronutrient elements total nitrogen (0.405) and available phosphorus (0.252). Although soil fertility and yields were significantly improved by intensification of management practices, unfavourable environmental impacts can be observed in the catchment, such as soil acidification induced by enormous application of mineral fertilizers, especially nitrogen, and decreased use of organic fertilizers. Particularly, Acrisol-Alisol great soil group which has low base capacity has pH < 5. For that reason, nitrogen fertilizers such as calcium ammonium nitrate should be used. Finally, the lowest weight value belongs to hierarchy B4. Sufficient amount of available micronutrient elements was determined in all soils except AvZn which was found low in soil samples No. 4, 8, 9, 13, 18, 22, and 25.

Secondly, score values of all indicators were determined by using the best soil functionality and were joined with high, low or moderate (optimal range) values ranging between 0 and 1 based on their function on soil quality. Finally, after assigning the eigenvector for each indicator and determining the scoring values, weighted linear combination technique was employed to estimate the SQI_w values for individual soil samples.

The assessment of results, taking into consideration the six SQI_w classes (Table 3), showed that mostly moderate quality soils (Class IV) were dominant with 75% of the total soil samples in the catchment, whereas 25% of soil samples were found weak (Class II) in terms of soil quality. Samples with excellent or strong (Class VI and Class V) and poor or very poor quality (Class II and IV) did not match the established criteria (Table 8).

In this respect, samples can be separated into two various soil quality classes due to soil heterogeneity. (1) The sandy loam and loamy sand, weak quality soils, which closely correspond with the Leptosol were found on the steep slope land. Quality of such soils in the study area is significantly limited by low OM, water retention and too low soil pH for tea cultivation; (2) the loamy clay and sandy clay loam, moderate quality soil located on generally Alisol-Acrisol great soil groups. Although some soils (samples 9 and 23) have higher content of OM, soil quality was classified as weak due to low resistance capacity for soil erosion and insufficient nutrient elements.

Table 8- Soil quality index values of each soil sample for tea plant

Sample	*Coordinate*		*Land*	*Soil quality*		*Sample*	*Coordinate*		*Land*	*Soil quality*	
no	*East*	*North*	*use*	*Index*	*Class*	*no*	*East*	*North*	*use*	*Index*	*Class*
1	633392	4533243	tea	0.465	4	15	641558	4533520	tea	0.436	4
2	638413	4534658	tea	0.295	3	16	641158	4542516	tea	0.501	4
3	636883	4536169	tea	0.296	3	17	643199	4544209	tea	0.506	4
4	635391	4536169	tea	0.428	4	18	640129	4543548	tea	0.449	4
5	639141	4533119	tea	0.483	4	19	638000	4542408	tea	0.287	3
6	638473	4531436	tea	0.433	4	20	639589	4541933	tea	0.423	4
7	640047	4535351	tea	0.470	4	21	640784	4544289	tea	0.503	4
8	636645	4538572	tea	0.359	3	22	644450	4541011	tea	0.402	4
9	638403	4540207	tea	0.434	4	23	645845	4540864	tea	0.365	3
10	638661	4537992	tea	0.422	4	24	644965	4538697	tea	0.369	3
11	639987	4536643	tea	0.461	4	25	643063	4540704	tea	0.367	3
12	641242	4536520	tea	0.459	4	26	644580	4543115	tea	0.524	4
13	642587	4535734	tea	0.464	4	27	642805	4542625	tea	0.428	4
14	644799	4534084	tea	0.405	4	28	640760	4540941	tea	0.429	4

4. Conclusions

Soil quality evaluation presents a useful tool for agriculture managers and policy makers to obtain a better understanding of the influence of different agricultural systems onto soil resources. Because, the used model collected all related soil indicators into consideration and reflected the most consistent and logical results. Soil quality of Ortaçay Catchment in Rize province, a typical tea (*Camellia sinensis* L.) growing area located in east part of Black Sea Region, was assessed using soil quality index (SQI_w) method. Twenty two indicators were grouped into 4 criteria (soil physical, chemical properties, micro- and macronutrient status of soils) by taking into consideration their effects on tea plant after taking 28 representative soil samples from the study area. According to soil quality assessment results, poor, very poor, strong and excellent soil quality classes for tea plant were not detected in the study area. Most of the soil samples' quality showed moderate level and rest of them have weak quality due to a propensity for soil erosion or other problems such as course texture or insufficient nutrient elements. For that reason, some biophysical measures to increase soil quality level by creating optimum tea plant growing medium should be taken such as liming, application of suitable fertilization program, increasing of resistance to soil erodibility. Moreover, the present monitoring of soil quality gives future opportunity to evaluate the system of land management for tea cultivation in humid and sub-humid terrestrial ecosystem. For further monitoring of soil quality in similar areas, some land properties such as soil depth and slope should be considered as well.

References

Andrews S S, Karlen D L & Mitchell J P (2002). A comparison of soil quality indices methods for vegetable production system in northern California. *Agriculture, Ecosystems and& Environment* 90: 25-45

Bhushan N & Rai K (2004). Strategic Decision Making: Applying the Analytic Hierarchy Process. Springer-Verlag, New York, pp. 172

Bouyoucos G J (1935). The clay ratio as a criterion of soils to erosion. *Journal of the American Society of Agronomy* 27: 738-751

Bouyoucos G J (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. *Agronomy Journal* 43: 435-438

Baskan O, Dengiz O & Demirag İ T (2017). The land productivity dynamics trend as a tool for land degradation assessment in a dryland ecosystem. Environmental Monitoring and Assessment 189 (212): 3-21

Candemir F & Gülser C (2011). Effects of different agricultural wastes on some soil quality indexes at clay and loamy sand fields. *Communications in Soil Science and Plant Analysis* 42: 13-28

Da Silva A F, Barbosa A P, Zimback C R L, Landim P M B & Soares A (2015). Estimation of croplands using indicator kriging and fuzzy classification. *Computers and Electronics in Agriculture* 111: 1-11

Dengiz O, Özyazıcı M A & Sağlam M (2015). Multi-Criteria assessment and geostatistical approach for determination of rice growing suitability sites in Gokirmak catchment. *Paddy Water Environment* 13: 1-10. DOI 10.1007/s10333-013-0400-4

Demir Z & Gülser C (2015). Effects of rice husk compost application on soil quality parameters in greenhouse conditions. *Eurasian Journal of Soil Science* 4(3):185-190

Doran J W & Parkin B T (1994). Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (Eds.), Defining Soil Quality for a Sustainable Environment. *Soil Science Society of America* Inc., Madison, WI, USA, pp. 3-21 (SSSA Special Publication No. 35)

Doran J W & Jones A J (1996). Methods for Assessing Soil Quality. SSSA Special Publication No. 49. *Soil Science Society of America*, Madison, Wisc

FAO (2008). Guide to laboratory establishment for plant nutrient analysis. FAO Fertilizer and Plant Nutrition Bulletin, No: 19, FAO: Rome, Italy

FAO (2009). FAOSTAT-Tea Production 2009; FAO: Rome, Italy

Gülser C (2004). A Comparison of some physical and chemical soil quality indicators influenced by different crop species. *Pakistan Journal of Biological Sciences* 7: 905-911

Gülser C, Kızılkaya R, Aşkın T & Ekberli İ (2015). Changes in Soil Quality by Compost and Hazelnut Husk Applications in a Hazelnut Orchard. *Compost Science and Utilization* 23(3): 135-141

Jiuquan Z, Su Y, Wu J & Liang H (2015). GIS based land suitability assessment for tobacco production using AHP and fuzzy set in Shandong province of China. *Computers and Electronics in Agriculture* 114: 202-211

Kacar B (1984). Çayın gübrelenmesi. Çay İşletmeleri Genel Müdürlüğü, Çay-Kur Yayını No: 4, Ankara, pp. 356

Kacar B (1994). Chemical Analysis of Soil and Plant (III), Ankara pp. 229-233

Karlen D L, Andrews S S & Doran J W (2001). Soil quality: Current concepts and applications. *Advances in Agronomy* 74: 1-40

Kemper W D & Rosenau R C (1986). Aggregate stability and size distribution. Editor: Klute, A Methods of Soil Analysis, Part I-Physical and Mineralogical Methods, 2nd ed., *Soil Science Society of America* Book Series No: 5, SSA and ASA, Madison, Wisconsin, pp. 425-442

Lal R & Elliot W (1994). Erodibility and erosivity. In R. Lal (Ed.), *Soil Erosion Research Methods* (2nd ed.), Delray Beach: St. Lucie Press, pp. 181- 210

Liebig M A, Varvel G E & Doran J W (2001). A simple performance-based index for assessing multiple agroecosystem functions. *Agronomy Journal* 93: 313-318

Lindsay W L & Norvell W A (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* 42: 421-428

Mallants D, Mohanty B P, Jacques D & Feyen J (1996). Spatial variability of hydraulic properties in a multi-layered soil profile. *Soil Science* 161: 167-181

Müftüoğlu N M (1987). Determination of Acidification of Tea Soils by various periods and methods and Evaluation of the Data Obtained. General Directorate of Tea Enterprises. Head of the Institute of Tea, Study Report of 1986, Rize, Turkey. 178-190 (in Turkish)

Müftüoğlu N M, Yüce E, Turna T, Kabaoğlu A, Özer S P & Tanyel G (2010). The evaluation of some properties of tea soils in the Eastern Black Sea Region. *Journal of Ege University Faculty of Agriculture*. 5th Plant Nutrition and Fertilization Cong. 3-7 June 2010. Proc. pp. 63-67

Nabiollahi K, Taghizadeh-Mehrjardi R, Kerry R & Moradian S (2017). Assessment of soil quality indices for salt-affected agricultural land in Kurdistan Province, Iran. *Ecological Indicators* 83: 482-494

Nelson D W & Sommers L E (1982). Total carbon, organic carbon and organic matter. In L. A. Page, R. H. Miller, & D. R. Keeney (Eds.), Methods of soil analysis, part 2. Chemical and microbiological methods (2nd ed.,), *American Society of Agronomy*, Madison, Wisconsin, pp. 539-579

Özcan H, Dengiz O & Erşahin S (2017). Soils of Turkey (ed: Kapur, S., Akça, E., Günal, H). Alisols-Acrisols. World Soils Book Series, Springer, pp. 207-215

Özyazıcı G, Özyazıcı M A, Özdemir O & Sürücü A (2010). Some physical and chemical properties of tea grown soils in Rize and Artvin provinces. *Anadolu Journal of Agricultural Sciences* 25: 94-99

Özyazıcı M A, Özyazıcı G & Dengiz O (2011). Determination of micronutrients in tea plantations in the eastern Black Sea Region, Turkey. *African Journal of Agricultural Research* 6: 5174-5180

Özyazıcı M A, Dengiz O & Aydoğan M (2013). Çay yetiştirilen tarım topraklarının reaksiyon değişimleri ve alansal dağılımları. *Journal of Soil and Water* 2: 23-29

Qi Y, Darilek Huang J L, Zhao B, Sun Y & Gu Z (2009). Evaluating soil quality indices in an agricultural region of Jiangsu Province, China. *Geoderma* 149: 325-334

Rezaei-Moghaddam K & Karami E (2008). A multiple criteria evaluation of sustainable agricultural development models using AHP. *Environment, Development and Sustainability* 10: 407-426

Saaty T (1980). The Analytic Hierarchy Process. McGraw-Hill, New York, USA. 287

Saygın F, Gürsoy F E, Demirağ Turan İ & Dengiz O (2017). Çataklı çayı havzası doğu yakasında çay tarımı yapılan toprakların fiziksel, kimyasal ve verimlilik özelliklerinin belirlenmesi. *Türkiye Tarımsal Araştırmalar Dergisi* 4: 143-154

Şener Ş, Şener E, Nas B & Karagüzel R (2010). Combining AHP with GIS for landfill site selection: A case study in the Lake Beyşehir catchment area (Konya, Turkey). *Waste Management* 30: 2037-2046

Soil Survey Laboratory (1992). Procedures for collecting soil samples and methods of analysis for soil survey. Soil Surv. Invest. Rep. I. U.S. Gov. Print. Office, Washington D.C. USA

Soil Survey Staff (1993). Soil Survey Manuel. USDA Handbook. No: 18, Washington D.C. USA

Van Diepen C A, van Keulen H, Wolf J & Berkhout J A A (1991). Land evaluation: from intuition to quantification. In: Advances in Soil Science (Ed. BA Stewart). Springer, New York, USA, pp. 139-204

Wali E, Datta A, Shrestha R P & Shrestha S (2016). Development of a land suitability model for saffron (*Crocus sativus L.*) cultivation in Khost Province of Afghanistan using GIS and AHP techniques. *Archives of Agronomy and Soil Science* 62: 921-934

Wienhold B J, Andrews S S & Karlen D L (2004). Soil Quality: A Review of the Science and Experiences in the USA. *Journal of Environmental Geochemistry and Health* 26: 89-95

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

Alleviation of Toxic Effects of Untreated Wastewater on Selective Vegetables Using Soil Organic Amendments

Zaffar MALIK[a], Ghulam Hassan ABBASI[a], Sardar Alam CHEEMA[b], Muhammad DAWOOD[d], Muhammad Babar JAVED[a], Muhammad RAFAY[e], Malik Noman MANSHA[a], Muhammad Imran KHAN[c]

[a]*Department of Soil Science, University College of Agriculture and Environmental Sciences, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur (63100), PAKISTAN*

[b]*Department of Agronomy, University of Agriculture Faisalabad (38040), PAKISTAN*

[c]*Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (38040), PAKISTAN*

[d]*Department of Environmental Sciences, Bahauddin Zakariya University, Multan, PAKISTAN*

[e]*Department of Forestry, University College of Agriculture and Environmental Sciences, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, PAKISTAN*

ARTICLE INFO
Research Article
Corresponding Author: Muhammad Imran KHAN, E-mail: khanimran1173@yahoo.com, Tel: +92 (41) 920 12 20
Received: 01 August 2018, Received in Revised Form: 30 November 2018, Accepted: 02 January 2019

AUTHORS ORCID ID
(Zaffar MALIK: 0000-0002-4343-3733), (Ghulam Hassan ABBASI: 0000-0002-0166-6264), (Sardar Alam CHEEMA: 0000-0001-8600-9046), (Muhammad DAWOOD: 0000-0002-8642-2735), (Muhammad Babar JAVED: 0000-0002-6453-6694), (Muhammad RAFAY: 0000-0003-2839-6986), (Malik Noman MANSHA: 0000-0002-2158-2826), (Muhammad Imran KHAN: 0000-0002-3822-6916)

ABSTRACT
In this work, a pot experiment was conducted to evaluate the effect of organic amendments in alleviation of toxicity of untreated domestic wastewater to okra (*Abelmoschus esculentus*) and purslane (*Portulaca oleracea). Three organic amendments including* farm yard manure (FYM), poultry manure (PM) and bagasse ash (BGA) at the rate of 1% and 3% were applied to the soil to evaluate their positive impact on vegetable plants. Results showed that untreated wastewater impeded the growth of both vegetables and increased uptake of heavy metals (HMs) (i.e. Cu, Ni, Pb and Zn) by plants whereas organic amendments significantly improved the growth of plants and decreased the uptake of HMs. Shoot and root lengths and dry weights of plants were boosted by the FYM and PM, while chlorophyll a and b and carotenoids were enhanced by PM and BGA amendments. Interestingly, reduced HMs uptake was found where 3% of BGA or FYM were applied. Overall the results of this study showed that all three organic amendments were useful for improving growth of vegetables and alleviating the phytotoxicity of untreated wastewater and uptake of HMs by plants. Our findings suggest that these organic materials could be useful for improving agricultural productivity in untreated wastewater irrigated areas.

Keywords: Domestic wastewater; Farm yard manure; Poultry manure; Bagasse ash; Heavy metals

1. Introduction

Amount of wastewater produced by industrial, commercial and domestic sources has been increased significantly with increase in population growth, urbanization and industrialization to cope with the necessities of life. The wastewater is widely used to irrigate agricultural land due to scarcity of water (Zhao

DOI: 10.15832/ankutbd.449876

et al 2018). Both positive and negative impact has been observed for using wastewater in agriculture. Domestic wastewater contains high amount of nutrients essential for crop development compared to fresh water which might decrease the dependency on chemical fertilizers and can also reduce the discharge into water bodies (Urbano et al 2017). However, many negative impacts have been found to be associated with application of untreated wastewater such as deterioration of soil quality, reduction in crop yield, and heavy metals (HMs) pollution (Mukherjee et al 2013; Becerra-Castro et al 2015).

Many countries of the world are facing water crisis due to changing environmental condition, global warming and other associated phenomena (Rekik et al 2017). Lack of basic infrastructure to treat wastewater and water shortage have compelled under developed countries to use untreated wastewater to grow crops (Contreras et al 2017). Pakistan is also facing severe water shortage and utilization of wastewaters for irrigation is now becoming widespread practice especially in urban areas for growing vegetables. Long term irrigation of soil with untreated wastewater was reported to degrade soil quality and reduce crop yield due its toxic ingredients (Ullah et al 2012). In addition, wastewater irrigation can add non-essential potentially toxic metals such as lead (Pb), nickel (Ni), zinc (Zn) and copper (Cu) to soil and these metals remain available to plants for uptake and enhances the chances of HMs uptake and accumulation in plants, and eventually can transfer to higher trophic levels through food-chain (Singh et al 2010; Roy et al 2013; Stanojkovic-Sebic et al 2015).

Therefore, it is imperative to explore different strategies to mitigate toxic effect of untreated wastewater to crop plants. Among different options, organic amendment is considered as a most appropriate approach to minimize negative impacts of untreated wastewater on crop growth and to alleviate the toxic effects of wastewater pollutants (i.e. HMs). These amendments are easily available locally and have enormous potential for providing nutrients, improving soil structure and restricting non-essential elements in food chain. Organic muck contains significant amount of nitrogen (N), phosphorus (P), potassium (K) and micro-nutrients which are essential for normal plant growth (Okoli & Nweke 2015). In addition, organic manure such as farm yard manure (FYM), poultry manure (PM) and bagasse ash (BGA) are reported to have significant influences on plant growth, yield and physiological features of plants (Gebrtsadkan & Assefa 2015; Bhushan et al 2016; Adekiya & Agbede 2017). Heavy metals can also be sequestered in soil using organic amendments through sorption or precipitation that reduces the availability of metals for plant uptake or accumulation (Achiba et al 2016).

Studies revealed that PM has been very effective in enhancing crop growth because of its high N content. The role of PM in increasing crop growth and decreasing the salt stress in wheat has also been reported (Rady et al 2016). The positive impact on carrot growth by the application of PM was also reported (Sylvestre et al 2015). Similarly, the application of BGA is shown to have profound effects on crop production and is known to improve the soil water use efficiency (Bhushan et al 2016). Since organic amendments are used historically to improve the crop growth, to the best of our knowledge, their comparative effects along with the application of untreated domestic wastewater has not been investigated. In this study, a pot experiment was conducted to examine the effects of domestic untreated wastewater on different morphological and physiological features of okra (*Abelmoschus esculentus*) and purslane (*Portulaca oleracea*) crops in the presence of different soil organic amendments (i.e. FYM, PM and BGA).

2. Material and Methods

2.1. Experimental setup

A pot experiment was conducted to evaluate the effect of organic amendments in alleviation of toxicity of untreated domestic wastewater to okra and purslane. Seeds of hybrid varieties of okra and purslane were obtained from the local seed market. Soil, domestic wastewater and tap-water were collected from the farm area near Bahawalpur. The BGA was collected from Ashraf Sugar Mill on the Ahmad Pur Road, the FYM was obtained from Jafferi farm house Bahawalpur and the PM was obtained from Slamat poultry farm, Shadab Colony, Bahawalpur.

Pots were filled with 3 kg of the air-dried soil (< 2 mm) along with 1% and 3% of each of different

amendments (i.e. BGA, FYM, and PM). Overall, there were eight treatments: T1: Tap water, T2: Domestic wastewater, T3: Domestic wastewater + 1% FYM, T4: Domestic wastewater + 3% FYM, T5: Domestic wastewater +1% PM, T6: Domestic wastewater + 3% PM, T7: Domestic wastewater + 1% BGA, T8: Domestic wastewater + 3% BGA. All the treatments were applied in triplicates. The treatment pots were placed following complete randomized design. After filling the pots with soil and amendments, they were brought to the field capacity with tap water one day before sowing of okra and purslane. Both the crops were periodically irrigated with untreated wastewater.

2.2. Water soil and plant analyses

Domestic wastewater (DW) and tap water (TW) were collected in 2-L polythene bottles and transported immediately to the laboratory. After filtration through Whatman No. 42 filter paper, the basic physico-chemical properties such as electrical conductivity (EC), soil reaction (pH), soluble and total phosphorous, total dissolved solids (TDS), carbonates (CO_3), bicarbonates (HCO_3), sodium (Na), magnesium (Mg), and calcium (Ca) were determined according to the standard methods (APHA 1998). The filtrates were also analyzed for Cu, Ni, Pb and Zn using atomic absorption spectrophotometer (AAS).

For initial characterization, the soil samples were air dried, ground with pestle and mortar and sieved through 2 mm sieve. The soil samples were analyzed for pH, EC, exchangeable cations (K, Ca, Mg and Na), SO_4 and Cu, Ni, Pb and Zn. Total nitrogen was measured by macro-Kjeldahl method (Bremner & Mulvaney 1982) and available phosphorus was determined by Bray P1 method (Olsen & Sommers 1982). To determine the total metals (Cu, Ni, Pb and Zn) concentrations, soil samples were digested following the Hossner method (Hossner 1996) and the concentrations of Pb, Cu, Ni and Zn were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES). Both the AAS and ICP-AES methods were independently checked to make sure the reliability of the analytical methods.

Vegetable leaves, stem and root samples were manually harvested. All the collected samples of the vegetables were washed with distilled water to remove airborne dust. All the samples were then oven-dried in a hot air oven at 70-80 ºC for 24 h, to remove all moisture. Dried samples were powdered using a mortar and pestle and sieved. The samples were digested using dry ash method (Hseu 2004) to determine the HMs concentration in plant parts.

2.3. Statistical analysis

To test the statistical significance of the mean values, one-way analysis of variance (ANOVA) was employed. After significant difference, Duncan's Multiple Range (DMR) was used for the pair wise comparisons. SAS 9.4 was used for the statistical analysis.

3. Results

3.1. Physico-chemical properties of soil and domestic wastewater

The basic physico-chemical properties of the soil and domestic wastewater are given in Table 1. Briefly, the pH, EC and total soluble salts (TSS) of soil were 7.9, 1.5 dS m^{-1} and 15 meq L^{-1}, respectively, and of wastewater were 7.8, 1.14 dS m^{-1} and 230 meq L^{-1}, respectively.

Results showed that in general the application of domestic wastewater with soil amendments significantly improved the shoot-root length, shoot-root fresh and dry weights of okra and purslane (Figure 1). Maximum increase in the shoot length of okra and purslane was recorded in T3 (1% FYM) and/or T5 (1% PM) (Figure 1). Also, maximum increase in shoot fresh and dry weight of okra were recorded in T6 (3% PM) and of purslane were recorded in T5 (1% PM) (Figure 1).

3.2. Growth parameters of vegetables

Root results showed that a significant increase in the root length was found in all three treatments where

they were applied at 3% (T4, T6 and T8) (Figure 1). Similarly, a huge increase in the root dry weight, particularly of okra, was found where FYM and PM were applied at 3% (Figure 1). Overall, the application of FYM and PM showed significant growth increase as compared to the control (T2).

Table 1- Physico-chemical properties of soil and untreated domestic wastewater used in this study

Parameters	*Soil*	*Domestic wastewater*
pH	7.9	7.8
EC	1.5 dS m^{-1}	1.4 dS m^{-1}
TSS	15 meq L^{-1}	230 meq L^{-1}
Saturation percentage	34	-
Sulphates	11.24 µg g^{-1}	-
Chlorides	3.2 µg g^{-1}	-
Carbonates	-	-
Bicarbonates	0.56 µg g^{-1}	-
Organic matter	0.48%	-
Textural class	Loam	-
Cu	-	0.02 µg g^{-1}
Zn	-	0.05 µg g^{-1}
Pb	-	3.21 µg g^{-1}
Ni	-	0.18 µg g^{-1}
TDS	-	610 µg g^{-1}
BOD	-	710 µg g^{-1}
COD	-	989 µg g^{-1}
N	0.02%	-
P	14.7 µg g^{-1}	-
K	0.91%	-

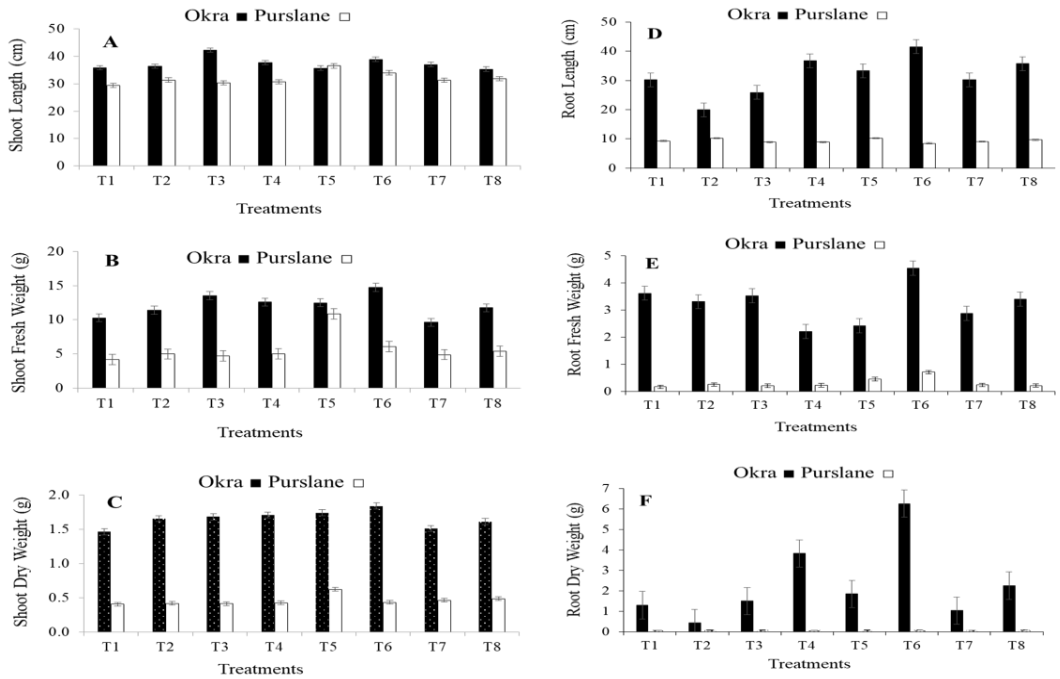


Figure 1- Effect of untreated wastewater and organic amendments on shoot length (A), shoot fresh weight (B), shoot dry weight(C), root length (D), root fresh weight (E) and root dry weight (F) of okra and purslane. Bars in the graphs show the average values of triplicate samples measured separately and error bars are the standard deviations. T1: Tap water, T2: Domestic wastewater, T3: Domestic wastewater + 1% Farm yard manure (FYM), T4: Domestic wastewater + 3% FYM, T5: Domestic wastewater + 1% Poultry manure (PM), T6: Domestic wastewater + 3% PM, T7: Domestic wastewater + 1% Bagasse ash (BGA), T8: Domestic wastewater + 3% BGA+

3.3. Physiological parameter of vegetables

Physiological parameters including relative water content, chlorophyll a and b, and carotenoid content of both the okra and purslane were significantly ($P<0.05$) enhanced by different levels of organic amendments applied along with the domestic wastewater. Maximum relative water contents of okra and purslane were recorded in T6 with application of 3% PM. Chlorophyll contents were also significantly enhanced with the application of amendments. For example, maximum values of chlorophyll a in okra were found in T4 (3% FYM) and T8 (3% BGA), however for purslane crop, maximum values of chlorophyll a were found in T7 (1% BGA) and T8 (3% BGA). Maximum chlorophyll b contents of okra and purslane were found in T8 where 3% BGA was applied. Regarding carotenoid, the maximum value of both okra and purslane was recorded in T6 with application of 3% PM (Figure 2).

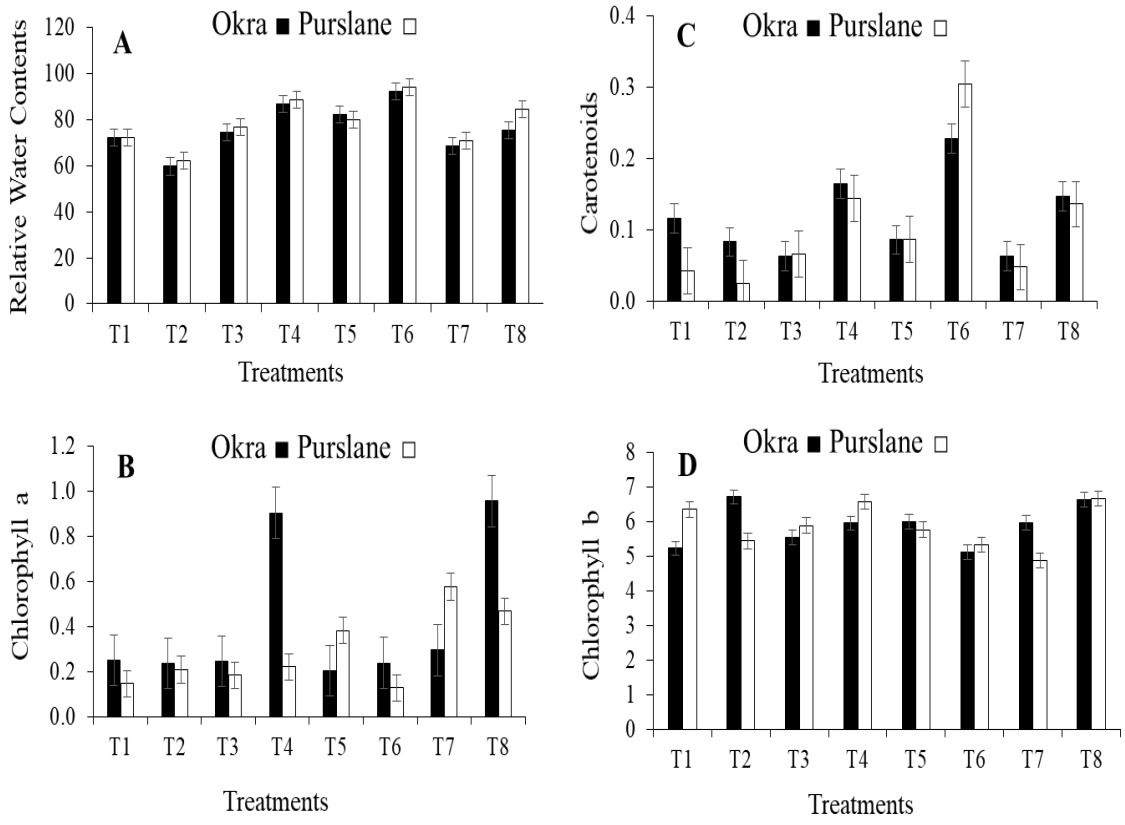


Figure 2- Effect of untreated wastewater and organic amendments on relative water content (A), carotenoid content (C), chlorophyll a (B), and chlorophyll b (D) of okra and purslane. T1: Tap water, T2: Domestic wastewater, T3: Domestic wastewater + 1% Farm yard manure (FYM), T4: Domestic wastewater + 3% FYM, T5: Domestic wastewater + 1% Poultry manure (PM), T6: Domestic wastewater + 3% PM, T7: Domestic wastewater + 1% Bagasse ash (BGA), T8: Domestic wastewater + 3% BGA

3.4. Heavy metal contents in vegetables

Overall, the application of organic amendments lowers the heavy metals (HMs) uptake by both vegetables (Figure 3 and 4). Greater HMs concentrations were found in the soil, as well as in the root and shoot of plants where only the wastewater was applied (Figure 3). However, by the application of organic amendments, a significant reduction was observed in the HMs concentrations. The application of FYM and PM at 3% level were found to be the most effective treatments to lower the HMs uptake by okra. For purslane, the application of BGA at 3% was found to be the most promising treatment in lowering the HMs uptake.

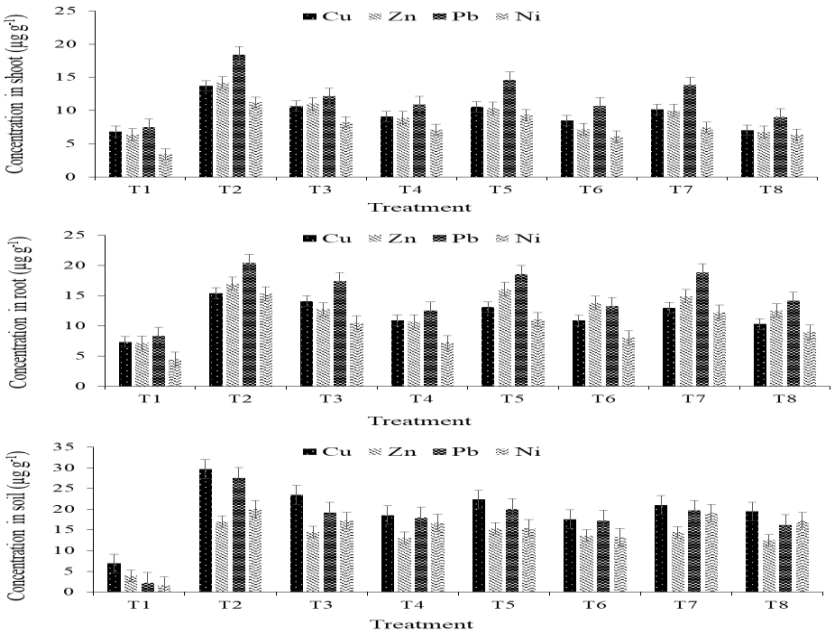


Figure 3- Effect of untreated wastewater and organic amendments on heavy metal contents in soil, root and shoot of okra. T1, Tap water; T2, Domestic wastewater; T3, Domestic wastewater + 1% Farm yard manure (FYM); T4, Domestic wastewater + 3% FYM; T5, Domestic wastewater + 1% Poultry manure (PM); T6, Domestic wastewater + 3% PM; T7, Domestic wastewater + 1% Bagasse ash (BGA); T8, Domestic wastewater + 3% BGA

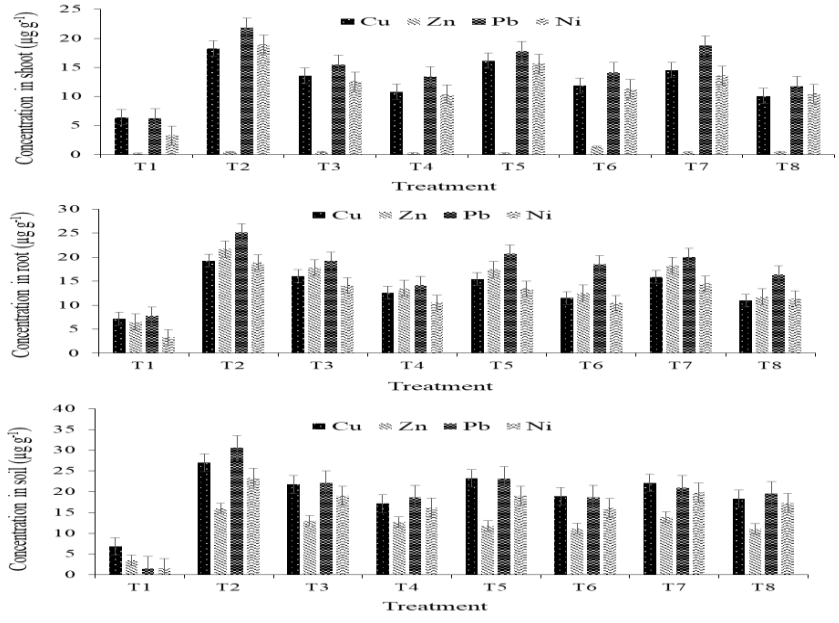


Figure 4- Effect of untreated wastewater and organic amendments on heavy metal contents in soil, root and shoot of purslane. T1, Tap water; T2, Domestic wastewater; T3, Domestic wastewater + 1% Farm yard manure (FYM); T4, Domestic wastewater + 3% FYM; T5, Domestic wastewater + 1% Poultry manure (PM); T6, Domestic wastewater + 3% PM; T7, Domestic wastewater + 1% Bagasse ash (BGA); T8, Domestic wastewater + 3% BGA

4. Discussion

The scarcity of freshwater and ever-increasing demand of irrigation water compel the farming community to use untreated wastewater particularly in the developing countries, to irrigate crops. Although reuse of wastewater is considered beneficial for environmental and economic view point, there are serious agricultural and health risks associated with this application: soil salinity, pathogenicity, reduced crop development and HMs contamination is one of the serious concerns (Contreras et al 2017; Urbano et al 2017).

In the current study, results of physiological features such as relative water contents, carotenoids and chlorophyll a and b of okra and purslane showed that they were drastically affected and reduced 5-20% by the application of untreated domestic wastewater as compared to the control (i.e. Tap water) (Figure 2). The probable reason of lowering the water content could be the reduction of the photosynthetic rate and CO_2 assimilation in the plants. Several studies indicated that high chemical stress (EC, BOD, COD and HMs) in untreated wastewater inhibits metabolic mechanism by disrupting photosynthesis, transpiration rate and stomatal conductance ultimately leading to growth inhibition (Abegunrin et al 2016; Akhkha et al 2017). Another reason for poor physiological features and growth inhibition of vegetables obtained in this study (Figure 1) could be due to the presence of toxic HMs in the untreated wastewater. HMs present in the soil are not phytoavailable due to poor solubility, however, HMs entry through anthropogenic means (domestic wastewater) increase their mobility in soil and pose threat to biota. This exogenous application of HMs, present in the wastewater, was able to inhibit the growth of both crops. Notably, high concentrations of HMs (Cu, Ni, Pb and Zn) in soil as well as in plant parts (okra and purslane) were found after untreated wastewater application.

Low relative water contents (i.e 10% lower than that of organic amended treatment) observed in plants without any soil organic amendment, could be due to the HMs toxicity. Numerous studies found similar results that HMs toxicity significantly decreases the water content in the crops. For instance, Pb toxicity was found to decrease amount of water in *Brassica juncea* (Zaier et al 2010). Inhibition of plant growth, particularly the root length, was reported by the excessive accumulation of Zn (Wang et al 2009). Excessive Cu in maize was reported to reduce the root length and plant height along with the substantial decrease in the overall plant growth (Ali et al 2002).

Interestingly, our results showed that the application of organic amendments significantly improved the physiological parameters and growth of both vegetables. Specifically, FYM and PM produced high carotenoids contents, while FYM and BGA produced more chlorophyll contents indicating that FYM was a better amendment as compared to PM or BGA to physiologically improve the growth of vegetable crops. Additionally, the application of organic amendments (i.e. FYM, PM and BGA) enhanced the immobilization of HMs and thus, did not allow them to transfer to the above ground biomass. Organic amendments through immobilization of HMs alleviated their phytotoxic effects and ultimately boosted the growth of plants (Figure 1). In general, FYM or PM applied at 3% level was found to be effective in enhancing the growth of both vegetables. Our results are in agreement with those of Meeinkuirt et al (2016), who reported that the growth of plants was significantly increased by the application of organic amendments on Cd contaminated soils.

Furthermore, uptake and accumulation of HMs in different plant parts were significantly reduced in the presence of organic amendments. This accumulation of HMs was 5-30% lower than that of control (i.e. without organic amendment). Results of several studies support our findings where organic amendments were reported to decrease uptake and accumulation of HMs in different plant parts (Daniela et al 2015; Rady et al 2016; Meeinkuirt et al 2016). Likewise, FYM was observed more beneficial in lowering HMs uptake by plants (Rehman et al 2016; Achiba et al 2016). Although on different plant, the application of FYM and PM had previously been proven to improve the physiological parameters of bitter gourd (*Momordica charantia* L.) (Haq et al 2015). The possible explanation for this could be that organic amendments are rich sources of organic matter (OM) which on decomposition not only provides nutrients for plant growth but also reduces mobility and bioavailability of HMs in soil by complexation with humic substances to form organo-metal complexes (Moreno-Jiménez et al 2017). High OM contents in FYM and

PM could enhance plant growth by increasing biological activity, macro-nutrients, CEC and buffering capacity in soil (Paulose et al 2007). Another plausible reason for restricted entry of HMs to plant might be the pH as organic amendments increase soil pH which in turn decrease metals mobility in soil (Walker et al 2004; Moreno-Jiménez et al 2017).

BGA used as an absorbent for HMs in soil is another cost-effective technique reported in many studies. BGA has tremendous capacity for absorption of HMs (Ni, Cu, Zn, Pb and Cd) and this capacity could increase with increasing soil pH (Yu et al 2013; Malik et al 2017). Many functional groups such as hydroxyl, carbonyl and amine were reported to be involved in metal binding in cellulose rich material which could be used to treat wastewater (Noor et al 2017; Bhat et al 2017). In short, our findings suggest that organic amendments have potential not only to reduce toxic effects of untreated wastewater pollutants (i.e. HMs) but also provide nutrients for plant growth.

5. Conclusions

Application of organic amendments (i.e. FYM, PM, and BGA) greatly enhanced the growth of okra and purslane irrigated with untreated wastewater. Our findings suggest that application of soil organic amendments could greatly reduce the phytotoxicity of untreated wastewater. Our study results can be used to forecast adverse effects of chemical stresses (especially due to HMs) on other vegetables irrigated with industrial, commercial and other types of untreated wastewater. In addition, the results of the current study have important implications on the use of organic amendments for attenuation of HMs from contaminated soil and improving agricultural productivity in untreated wastewater irrigated areas.

References

Abegunrin T P, Awe G O, Idowu D O & Adejumobi M A (2016). Impact of wastewater irrigation on soil physico-chemical properties, growth and water use pattern of two indigenous vegetables in southwest Nigeria. *Catena* 139: 167-178

Achiba W B, Gabteni N, Laing G D, Verloo M, Jedidi N & Tahar G (2016). Heavy metal availability and uptake by wheat crops cultivated in Tunisian field plots amended during five years with municipal solid waste compost and farmyard manure. *Journal of Research in Environmental and Earth Sciences* 4: 146-154

Adekiya A O & Agbede T M (2017). Effect of methods and time of poultry manure application on soil and leaf nutrient concentrations, growth and fruit yield of tomato (*Lycopersicon esculentum* Mill). *Journal of the Saudi Society of Agricultural Sciences* 16(4): 383-388

Akhkha A, Boutraa T & Al-Shoaibi A K (2017). The influence of al-Madinah al-Munawwara treated and untreated domestic wastewater on growth and physiology of three tomato (*Lycopersicon esculentum* Mill.) genotypes. *Pakistan Journal of Botany* 49(3): 879-890

Ali N A, Bernal M P & Ater M (2002). Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant and Soil* 239(1): 103-111

APHA (1998). Standard methods for examination of water and wastewater. *American Public Health Association*, Washington, DC, USA

Becerra-Castro C, Lopes A R, Vaz-Moreira I, Silva E F, Manaia C M & Nunes O C (2015). Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environment International* 75: 117-135

Bhat S A, Singh J & Vig A P (2017). Amelioration and degradation of pressmud and bagasse wastes using vermitechnology. *Bioresource Technology* 243: 1097-1104

Bhushan G, Kumar S, Dwivedi S & Sharma S K (2016). Impact of bagasse ash amended Soil on growth and yield of *Pisum sativum*. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 7(1): 448-556

Bremner J M & Mulvaney C S (1982). Nitrogen -Total. In: Methods of soil analysis. Madison, WI: *American Society of Agronomy*, pp. 295-324

Contreras J D, Meza R, Siebe C, Rodríguez-Dozal S, López-Vidal Y A, Castillo-Rojas G & Vázquez-Salvador N (2017). Health risks from exposure to untreated wastewater used for irrigation in the Mezquital Valley, Mexico: A 25-year update. *Water Research* 123: 834-850

Daniela K, Jakub E & Lukas P (2015). Effect of compost amendment on heavy metals transport to plant. *Mendel Net* 249-254

Gebrtsadkan G & Assefa D (2015. Evaluating the effect of integrated use of farm yard manure and urea on the socio economic performance of Tomato (*Lycopersicon esculentum* Mill) at Tselemti Woreda, North western Tigray, Ethiopia. *Journal of Biology, Agriculture and Healthcare* 5(9): 158-164

Haq M A, Ahmad N, Farooq U, Zafar H & Ali M A (2015). Effect of different organic materials and chemical fertilizer on yield and quality of bitter gourd (*Momordica charantia* L.). *Soil & Environment* 34(2): 142-147

Hossner L R (1996). Dissolution for total elemental analysis. Methods of soil analysis part 3-*Chemical Methods* pp. 49-64

Hseu Z Y (2004). Evaluating heavy metal contents in nine composts using four digestion methods. *Bioresource Technology* 95: 53-59

Malik D S, Jain C K & Yadav A K (2017). Removal of heavy metals from emerging cellulosic low-cost adsorbents: A review. *Applied Water Science* 7(5): 2113-2136

Meeinkuirt W, Kruatrachue M, Pichtel J, Phusantisampan T & Saengwilai P (2016). Influence of organic amendments on phytostabilization of Cd-contaminated soil by *Eucalyptus camaldulensis*. *Science Asia* 42(2): 83-91

Moreno-Jiménez E, Sepúlveda R, Esteban E & Beesley L (2017). Efficiency of organic and mineral based amendments to reduce metal [loid] mobility and uptake (*Lolium perenne*) from a pyrite-waste contaminated soil. *Journal of Geochemical Exploration* 174: 46-52

Mukherjee V, Das A, Akhand A & Gupta G (2013). Toxicity and profitability of rice cultivation under wastewater irrigation: The case of the East Calcutta Wetlands. *Ecological Economics* 93: 292-300

Noor N M, Othman R, Mubarak N M & Abdullah E C (2017). Agricultural biomass-derived magnetic adsorbents: Preparation and application for heavy metals removal. *Journal of the Taiwan Institute of Chemical Engineers* 78: 168-177

Okoli P S O & Nweke I A (2015). Effect of poultry manure and mineral fertilizer on the growth performance and quality of cucumber fruits". *Journal of Experimental Biology and Agricultural Sciences* 3(4): 362-367

Olsen S R & Sommers L E (1982). Phosphorus 2nd ed. In: Methods of Soil Analysis. Agronomy Monograph No. 9. Madison, WI. *American Society of Agronomy*, pp. 403-430

Paulose B, Datta S P, Rattan R K & Chhonkar P K (2007). Effect of amendments on the extractability, retention and plant uptake of metals on a sewage-irrigated soil. *Environmental Pollution* 146(1): 19-24

Rady M M, Mounzer O, Alarcón J, Abdelhamid M & Howladar S (2016). Growth, heavy metal status and yield of salt-stressed wheat (*Triticum aestivum* L.) plants as affected by the integrated application of bio-, organic and inorganic nitrogen-fertilizers. *Journal of Applied Botany and Food Quality* 89: 21-28

Rehman M Z U, Rizwan M, Ali S, Fatima N, Yousaf B, Naeem A & Ok Y S (2016). Contrasting effects of biochar, compost and farm manure on alleviation of nickel toxicity in maize (*Zea mays* L.) in relation to plant growth, photosynthesis and metal uptake. *Ecotoxicology and Environmental Safety* 133: 218-225

Rekik I, Chaabane Z, Missaoui A, Bouket A C, Luptakova L, Elleuch A & Belbahri L (2017). Effects of untreated and treated wastewater at the morphological, physiological and biochemical levels on seed germination and development of sorghum (*Sorghum bicolor* (L.) Moench), alfalfa (*Medicago sativa* L.) and fescue (*Festuca arundinacea* Schreb.). *Journal of Hazardous Materials* 326: 165-176

Roy S, Banna L N, Mamun S A & Farukh M A (2013). Effects of industrial wastewater reuse for crop production: A case study in Tejgaon metropolitan area of Dhaka, Bangladesh. *Journal of the Bangladesh Agricultural University* 11(2): 183-188

Singh A, Sharma R K, Agrawal M & Marshall F M (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* 48(2): 611-619

Stanojkovic-Sebic A, Pivic R, Josic D, Dinic Z & Stanojkovic A (2015). Heavy metals content in selected medicinal plants commonly used as components for herbal formulations. *Journal of Agricultural Sciences* 21: 317-325

Sylvestre H, Constance M, Esdras N & Athanase N (2015). Effect of poultry manure and NPK (17-17-17) on growth and yield of carrot in Rulindo District, Rwanda. *International Journal of Novel Research in Life Sciences* 2: 42-48

Ullah H, Khan I & Ullah I (2012). Impact of sewage contaminated water on soil, vegetables, and underground water of peri-urban Peshawar, Pakistan. *Environmental Monitoring and Assessment* 184: 6411-6421

Urbano V R, Mendonça T G, Bastos R G & Souza C F (2017). Effects of treated wastewater irrigation on soil properties and lettuce yield. *Agricultural Water Management* 181: 108-115

Walker D J, Clemente R & Bernal M P (2004). Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste. *Chemosphere* 57: 215-224

Wang C, Zhang S H, Wang P F, Hou J, Zhang W J, Li W & Lin Z P (2009). The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere* 75: 1468-1476

Yu J X, Wang L Y, Chi R A, Zhang Y F, Xu Z G & Guo J (2013). Adsorption of Pb^{2+}, Cd^{2+} and Zn^{2+} from aqueous solution by modified sugarcane bagasse. *Research on Chemical Intermediates* 41: 1525-1541

Zaier H, Mudarra A, Kutscher D, de la Campa M R F, Abdelly C & Sanz-Medel A (2010). Induced lead binding phytochelatins in *Brassica juncea* and *Sesuvium portulacastrum* investigated by orthogonal chromatography inductively coupled plasma-mass spectrometry and matrix assisted laser desorption ionization-time of flight-mass spectrometry. *Analytica Chimica Acta* 671: 48-54

Zhao L, Xie J, Zhang H, Wang Z, Jiang H & Gao S (2018). Enzymatic activity and chlorophyll fluorescence imaging of maize seedlings (Zea mays L.) after exposure to low doses of chlorsulfuron and cadmium. *Journal of Integrative Agriculture* 17(4): 826-836

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

Insecticidal Efficacy and Repellency of Trans-Anethole Against Four Stored-Product Insect Pests

Mustafa ALKAN[a], Sait ERTÜRK[a]

[a]*Plant Protection Central Research Institute, Ankara, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Mustafa ALKAN, E-mail: alkan0101@gmail.com, Tel: +90 (312) 344 59 93
Received: 18 July 2018, Received in Revised Form: 26 December 2018, Accepted: 04 January 2019

AUTHORS ORCID ID
(Mustafa ALKAN: 0000-0001-7125-2270), (Sait ERTÜRK: 0000-0001-9961-3955)

ABSTRACT
In this study, it was investigated that repellency and insecticidal efficacy of trans-anethole of botanical origin on major stored product on pests species, namely *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* Jacquelin du Val (Coleoptera, Tenebrionidae) and *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). Repellency effect was evaluated by choice test in petri dishes, while the mortality effect was examined by exposure to wheat treated at different concentrations, such as 1, 2, 3, 4, and 8 µL trans anethole. In efficacy tests, after 72 hours exposure, the highest adult mortality was found on *T. castaneum* with a 60% mortality, while other test species showed no significant mortality. On the other hand, repellency tests revealed varying degree of repellency depending on the application dose of trans-anethole. It was determined that *S. granarius* belongs to repellent class III, while all of the other species fall under repellent class IV. F1 progeny decreased as trans-anethole concentration increased, and in this context 8 µL of trans-anethole is proved to be the optimal concentration causing maximum decrease in progeny production. Among the insect species tested, *T. confusum* was found to be the most sensitive to trans-anethole with 100% decrease in F1 progeny production. Our results indicate that trans-anethole can be used as a potential repellent for the control of major stored grain pests. Additionally, trans-anethole, by its contact efficacy, might be considered as a grain protectant against *S. granarius, S. oryzae, T. confusum* and *T. castaneum*.

Keywords: Apiaceae; Plant essential oil; Repellency; Progeny; Stored product insect

1. Introduction

Stored grains are often subjected to quality and quantity losses of varying magnitude during the storage (Boxall 2001; Ferizli & Emekci 2010). Grain deterioration which caused by several biotic and abiotic factors can be occurred in various ways, such as germination, clumping, self-heating, burning, baking quality, color and many others (Amruta et al 2015). Among the biotic factors in stored grain, insects stand as the most important cause of deterioration that result in significant economic losses (Boxall 2001). Post-harvest loss of grains caused by insect pests can be prevented or minimized through careful stored grain management. The most common and effective method against stored products pests is fumigation (Shaaya & Kostyukovsky 2011). Synthetic fumigants such as methyl bromide, aluminum phosphide, sulphuryl fluoride, carbonyl sulphide, ethane nitrile, and ethyl format are currently being used in pest control (Bond

DOI: 10.15832/ankutbd.445671

1984; Taylor 1994; Villers et al 2010; Mutungi et al 2014). These chemicals, however, can pose risks to environment and human health, as in the example of methyl bromide, which is currently prohibited by the Montreal Protocol due to its ozone depletion potential. European Pesticide Regulation [(EC) No. 1107/200] is another restrictive policy instrument in effect for the prevention of hazardous chemical use. Therefore, there is a big demand for novel pest control approaches using less toxic substances. Among the alternative approaches, secondary plant metabolites has gained much attention (Taiz & Zeiger 2002).

Secondary plant metabolites have behavioral and biological effects on insects are categorized in various classes (Güncan & Durmuşoğlu 2004). The most effective compounds against insect pests are alkaloids, glycosides, phenols, terpenoids, tannins, and saponins (Shanker & Solanki 2000). These compounds can play important roles in plant defense mechanisms against insects, such as toxicity, feeding cessation, repellency, locating prey or hosts by predators and parasitoids. Trans-anethole is a secondary metabolite which is synthesized from plants belonging to Apiaceae family, in particular. Bio-efficacy of plant essential oils and their major components against stored product insect pests are very well documented (Hikal et al 2017). Essential oils do not cause environmental pollution, do not leave residues unlike synthetic toxic chemicals, and are not dangerous to non-target organisms in nature (Regnault-Roger et al 2012). Moreover, fumigant activity of trans-anethole has been proven against major stored product insects in several studies (Shaaya et al 1991; Ho 2000; Mondal & Khalequzzaman 2010). For these reason essential oils can be promising materials in the development of alternative plant protection products against stored product pests (Shaaya et al 1991; Shaaya et al 1993; Ho 2000; Huang et al 2000; Wang et al 2001; Karakoç et al 2006; Mondal & Khalequzzaman 2010; Pimentel et al 2010).

In this study, insecticidal efficacy and repellency of trans-anethole against four major insect pests of stored products were investigated. In addition, inhibitory effect of trans-anethole on progeny production of the same insect species was also evaluated.

2. Material and Methods

2.1. Insect rearing

All of the test insects were obtained from laboratory colonies maintained continuously at the stored product pest laboratory of Directorate of Plant Protection Central Research Institute (Ankara, Turkey) since 2008. A rearing medium composed of a mixture of crushed soft wheat (*Tritucum aestivum* L.) and powdered dry yeast (*Saccharomyces cerevisiae* Hansen) (20:1 w/w) was used to rear *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) and *T. confusum* Jacquelin du Val (Coleoptera, Tenebrionidae). For rearing *Sitophilus granarius* and *S. oryzae* (L.) (Coleoptera: Curculionidae) whole soft wheat kernels were used (Ertürk et al 2017).

2.2. Contact toxicity of trans-anethole on grains

Experiments were conducted according to the standards given in Ndomo et al (2008). Five different doses of 1, 2, 3, 4, and 8 µL of trans-anethole (CAS number: 4180-23-8, 99%) were mixed with 1 mL of acetone and then each mixture was applied into 10 g wheat by using pipette and stirred with glass rod to ensure well mixture. In the control group, only 1 mL of acetone was applied to 10 g wheat. Treated wheat with trans-anethole solution at different doses was put under a fume cupboard for 5 minutes to evaporate the solvent and then transferred into jars, each of which contained 25 (one-day old) adult individuals of each species separately. The jars were kept in a climate chamber at 25±2 °C and 65% relative humidity (r.h.). The treatments were arranged randomized design with four replicates, each of which included the control group. After setting up the experiments, mortality counts for adults were made daily after treatment during following three days.

2.3. Effect of trans-anethole on F1 progeny

At the end of three days of contact toxicity tests, dead and live insects were removed from the treated wheat and the jars containing treated wheat only were retained at the same conditions for 60 days and at the end

of 60 days F1 progeny adults were counted.

Inhibition rate was calculated by following formula.

$$IR = \frac{Cn - Tn}{Cn} * 100$$

Where; %IR, inhibition rate; *C*n, number of newly emerged adults in untreated (control); *T*n, number of newly emerged insects in treated replicates.

2.4. Repellent effect of trans-anethole

McDonald et al (1970)'s procedure was followed for the repellent activity of trans-anethole. Accordingly, 9 cm diameter discs of Whatman No. 1 filter paper was used. One half of each disc was treated with 1, 2, 3, 4, or 8 µL of trans-anethole in 1 mL of acetone, while the other half treated by acetone only. After aerating filter paper discs for 5 minutes to evaporate solvent, 20 (one-day old) adult individuals of *T. castaneum or S. granarius* in each concentration were separately placed on filter paper discs in Petri dishes. The inner sides of Petri dishes were coated with Fluon®PTFE (AGC Chemicals Europe, Ltd) to prevent the insects escaping. The covers of the petri dishes were drilled 1 mm in diameter to ensure air circulation. Petri dishes then incubated for 2 h at 25±2 ºC in the dark. At the end of 2 h, Petri dishes were opened and insects were counted according to which half of the paper discs they were settled on.

Percentage repellency was calculated according to the formula below:

$$PR = \frac{Nc - Nt}{Nc + Nt} * 100$$

Where; PR, percentage repellency value; Nc, number of insects in untreated (control) group; Nt, number of insects in treated group

Average percentage repellency values were categorized according to 0-V scale of Juliana & Su (1983). [Class 0 (PR< 0.1%); Class I (PR= 0.1-20%); Class II (PR= 20-40%); Class III (PR= 40.1-60%); Class IV (PR= 60.1-80%); Class V (PR= 80.1-100%)]

2.5. Statistical analysis

Prior the statistical analyses, percent mortality data obtained from the toxicity and repellency tests were transformed using arcsine transformation. The transformed data were then subjected to analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($P<0.05$) using MINITAB® Release 16 package program.

3. Results and Discussion

3.1. Contact toxicity of trans-anethole on grains

Each species showed different levels of susceptibility to trans-anethole (Figure 1). *Tribolium castaneum* was the most sensitive species to essential oil with 60.16% mortality when exposed to trans-anethole at 8 µL for 72 h. Statistical analysis revealed that there were significant differences between doses ($F_{(4,15)}$= 307.87; $P<0.05$) Insecticidal efficacy of trans-anethole against *T. castaneum* increased with dose increases. The higher doses of trans anethole was not used for experiments, because of the odor residue problems on the treated product. The essential oil did not show any contact toxicity to *T. confusum* at any concentration rate tested throughout the exposure period. Toxicity effect on *S. oryzae* was also negligible with 8.67% mortality, at most, after 72 hours at the highest level of dosage ($F_{(4,15)}$= 21.96; $P<0.05$) (Figure 1).

Residual efficacy of plant essential oils as grain protectants was received less attention. In scientific arena, plant essential oils were rather used to evaluate their fumigant efficacy or contact toxicity by topical

applications. Therefore, in this study, residual toxicity of trans-anethole against major stored-product insects on treated wheat grain were studied. Although no significant effect was obtained against test species except *T. castaneum,* our findings are contradictory with the previous studies (Karakoç et al 2006; Alkan & Gökçe 2012).

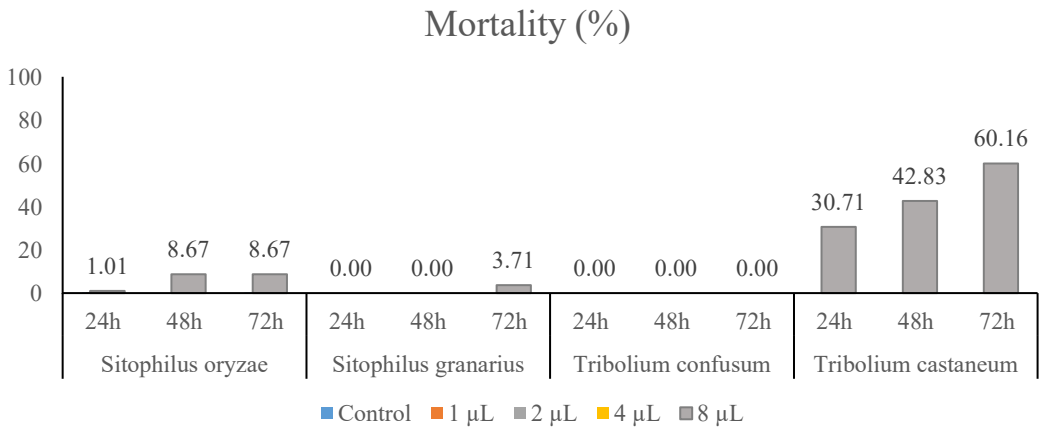


Figure 1- Mortality data of *S. granarius, S. oryzae, T. confusum* and *T. castaneum* exposed to wheat treated with trans-anethole at different rates (%)

3.2. F1 progeny assessment

F1 progeny suppression of test insects was increased with the application dose increased (Figure 2 and 3). The highest suppression rate was observed in *T. confusum* with a 100% decrease in F1 progeny at 8 µL of trans-anethole. Suppression rates in other species used in the experiments were as follows: *T. castaneum* (97.80%); *S. oryzae* (94.19%); and *S. granarius* (80%). Overall, the desired level of F1 progeny suppression activity was reached at the concentration of 8 µL. Tapondjou et al (2002) determined that, *S. granarius* was exposed for 48 h to 0.8% or 6.4% of the dry ground leaves *Chenepodium ambrosioides* L. (Chenepodiaceae) as grain protectant. Both low and high doses of *C. ambrosioides* completely suppressed in treated whole wheat against progeny production of *S. granarius*. Tapondjou et al (2005), showed that the F1 production of *S. zeamais* was completely suppressed on grains treated with *Eucalyptus saligna* and *Cupressus sempervirens* L. (Cupressaceae) crude oil extracts at the doses of 75 and 100 µL/40 g grain respectively. Contrary to other studies, it is believed that the 8 µL dose which provides 100% mortality in *T. confusum*, is due to the impurity of the crude essential oil composition used in other studies.

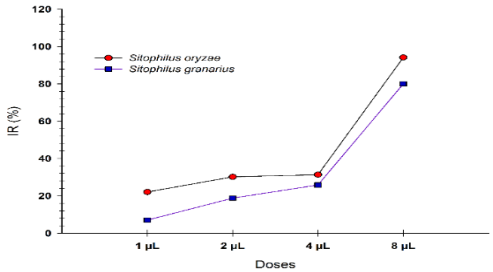


Figure 2- F1 progeny effect of Trans-anethole anetholeon *S. granarius* and *S. oryzae*

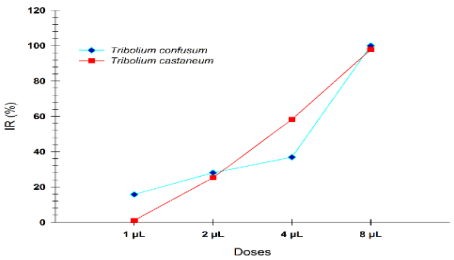


Figure 3- F1 progeny effect of Trans-on *T. confusum* and *T. castaneum*

3.3. Repellent effect of trans-anethole

Percentage repellency values increased as the concentration of trans-anethole increased (Table 1). PRs of four species at the lowest dose of 1 µL and at the highest dose of 8 µL of trans-anethole were, respectively, as follows: *S. granarius,* 12.5% and 97.5% ($F_{(3,15)}$= 175.0; P<0.05); *S. oryzae,* 47.5% and 95% ($F_{(3,15)}$= 26.9; P<0.05); *T. confusum,* 30% and 95% ($F_{(3,15)}$= 25.33; P<0.05); *T. castaneum,* 5% and 100% ($F_{(3,15)}$= 59.9; P<0.05).

Table 1- Repellency effects of Trans-anethole on test adults exposed to filter paper with four different concentrations for 2 h

	Repellency (%) ±SEM			
	Sitophilus granarius	*Sitophilus oryzae*	*Tribolium confusum*	*Tribolium castaneum*
1 µL	12.5±2.5c[1]	47.5±4.8c	30.0±5.8b	5.0±2.1b
2 µL	17.5±4.8c	57.5±2.5bc	80.0±8.2a	80.0±8.2a
4 µL	77.5±2.5b	65.0±2.9b	95.0±5.0a	90.0±5.8a
8 µL	97.5±2.5a	95.0±5.0a	95.0±5.0a	100.0±0.0a
Average	51.3±9.7	66.3±4.9	75.0±7.4	68.8±9.9
Repellency classes	III	IV	IV	IV

[1], Different letters in the same column indicate statistically different from each other (Anova P<0.05, Tukey test)

Essential oils and their compounds were previously reported having potent repellency activity on stored-product insects (Liu & Ho 1999; Isman 2000; Papachristos & Stamopoulos 2002; Garcia et al 2005). Amer & Mehlhorn (2006) emphasized the importance of studies with essential oils to unearth their repellency potential. In this regard, all of the four insect species we tested displayed a high sensitivity to trans-anethole and revealed high PRs. However, in terms of the lowest dose yielding the highest efficacy, *T. confusum* showed the highest sensitivity rate with 95% PR at 4 µL of trans-anethole. Our study should be considered as a preliminary attempt to reveal the potential of trans anethole to be used as a biorational pesticide. Moreover, further researches on such as slow release, encapsulation, and formulation of essential oils and their compounds are crucial to enhance their chance in the development of sustainable and cost-effective plant protection products.

4. Conclusions

The results of this study clearly demonstrate that trans-anethole with its efficacy in population suppression and its repellency, in particular, has a strong potential as a bio-pesticide against stored product insect pests. From environmental protection point of view it has also a high potential of use in organic farming, since trans-anethole is thought to be less harmful to environment. It can also be concluded that essential oils have broad spectrum pesticide activity due to the presence of several active ingredients that works with various mode of action than synthetic pesticides. Trans-anethole is found naturally in many plant species of Apiaceae, namely anise, fennel and star anise. There are not many alternatives to synthetic pesticides in controlling stored product pests. Therefore, trans-anethole, one of the few promising alternatives, is very important for controlling storage pests. However, in order to use Trans-anethole in field applications, additional studies on slow-release formulations and on residual remains on treated grains and on other surfaces should be undertaken.

Acknowledgements

The authors thanks to Prof. Dr. Mevlut EMEKCI for their valuable time and contribution to this study. This study was supported by the Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policy.

References

Alkan M & Gökçe A (2012). Toxic and behavioural effects of *Tanacetum abrotanifolium* L. DRUCE (Asteraceae) stem and flower extracts on *Sitophilus granarius* and *Sitophilus oryzae* (Col., Curculionidae). *Turkish Journal of Entomology* 36(3): 377-389

Amer A & Mehlorn H (2006). Larvicidal effects of varius essential oil against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitology Research* 99(4): 466-472

Amruta N, Sarika G, Umesha U, Maruthi J B & Basavaraju G V (2015). Effect of botanicals and insecticides seed treatment and containers on seed longevity of black gram under natural ageing conditions. *Journal of Applied and Natural Science* 7(1): 328-334

Bond E J (1984). Manual of fumigation for insect control. *FAO Plant Production and Protection Paper* 54

Boxall R A (2001). Post-harvest losses to insects - A world overview. *International Biodeterioration & Biodegradation* 48(1):137-152

Ertürk S, Yılmaz A, Akdeniz Fırat T & Alkan M (2017). Trans-anethole ve karbondioksit karışımının *Rhyzopertha dominica*, *Tribolium castaneum* ve *Sitophilus oryzae*'ye karşı fümigant etkisi. *Bitki Koruma Bülteni* 57(3): 391-400

Ferizli A G & Emekçi M (2010). Depolanmış ürün zararlılarıyla savaşım, sorunlar ve çözüm yolları. TMMOB Ziraat Mühendisleri Odası Ziraat Mühendisliği *VII. Teknik Kongresi Bildiriler Kitabı*, 11-15 Ocak 2010 Ankara, 2: 579-587

Garcia M, Donadel O J, Ardanaz C E, Tonn C E & Sosa M E (2005). Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum*. *Pest Management Science* 61(6): 612-618

Güncan A & Durmuşoğlu E (2004). Bitkisel kökenli doğal insektisitler üzerine bir değerlendirme. *Hasad Dergisi* 233: 26-32

Hikal W M, Baeshen R S & Said-Al Ahl H A H (2017). Botanical insecticide as simple extractives for pest control. *Cogent Biology* 3(1): 1-16

Ho S H (2000). Response of *Tribolium castaneum* and *Sitophilus zeamais* to potential fumigants derived from essential oils of spices. In: *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products*, 29 October - 3 November, Fresno, CA. pp. 119-124

Huang Y, Xing C S & Hung H S (2000). Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology* 93: 537-543

Isman M B (2000). Plant essential oils for pest and disease management. *Crop Protection* 19: 603-608

Juliana G & Su H C F (1983). Laboratory studies on several plant materials as insect repellents for protection of cereal grains, *Journal of Economic Entomology* 76: 154-157

Karakoç Ö C, Gökçe A & Telci İ (2006). Bazı bitki uçucu yağlarının *Sitophilus oryzae* L., *Sitophilus granarius* L. (Col.: Curculionidae) ve *Acanthoscelides obtectus* Say. (Col.: Bruchidae)'a karşı fumigant etkileri. *Türkiye Entomoloji Dergisi* 30(2): 123-135

Liu Z L & Ho S H (1999). Bioactivity of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas against the grain storage insects, *Sitophilus zeamais* Motsch. and *Tribolium castaneum* (Herbst). *Journal of Stored Product Research* 35(4): 317-328

McDonald L L, Guy R H & Speirs R D (1970). Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insect. Marketing research report no.882. Agricultural Research Service, US Deparment of Agriculture, Washington, pp.183

Mondal M & Khalequzzaman K (2010). Toxicity of naturally occurring compounds of plant essential oil against *Tribolium castaneum* (Herbst). *Journal of Biological Sciences* 10: 10-17

Mutungi C, Affognon H, Njoroge A, Baributsa D & Murdock L (2014). Storage of mung bean (*Vigna radiate* [L.] Wilczek) and pigeonpea grains (*Cajanus cajan* [L.] Millsp) in hermetic triple-layer bags stops losses caused by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Product Research* 58: 39-47

Ndomo A F, Ngamo L T, Tapondjou L A, Tchouanguep F M & Hance T (2008). Insecticidal effects of the powder formulation based on clay and essential oil from the leaves of *Clausena anisata* (Willd.) J. D. Hook ex. Benth. (Rutaceae) against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae*). Journal of Pest Science* 81: 227-234

Papachristos D P & Stamopoulos D C (2002). Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *Journal of Stored Product Research* 38(2): 117-128

Pimentel M A G, Faroni L R D, Silva F H D, Batista M D & Guedes R N C (2010). Spread of phosphine resistance among Brazilian populations of three species of stored product insects. *Neotropical Entomology* 39(1): 101-107

Regnault-Roger C, Vincent C & Arnason J T (2012). Essential oils in insect control: Low-risk products in a high-stakes world. *Annual Review of Entomology* 57: 405-424

Shaaya E & Kostyukovsky M (2011). The use of phytochemicals as fumigants for the control of stored product insect pests. *Journal of Entomological and Acarological Research* 43(2): 245-251

Shaaya E, Ravid U, Paster N, Juven B, Zisman U & Pissarev V (1991). Fumigant toxicity of essential oils against four major stored-product insects. *Journal of Chemical Ecology* 17(3): 499-504

Shaaya E, Ravid U, Paster N, Kostjukovsky M, Menasherov M & Plotkin S (1993). Essential oils and their components as active fumigants against several species of stored product insects and fungi. ISHS Acta Horticulturae 344, *International Symposium on Medicinal and Aromatic Plants*, 22 March 1993, Israel, pp. 131-137

Shanker C & Solanki K R (2000). Botanical insecticides: A historical perspective. *India, Asian Agrihistory* 4(2): 21-30

Taiz L & Zeiger E (2002). Plant Physiology. Sinauer Associates, Sunderland, Massachusetts, pp. 283-308

Tapondjou A L, Adler C, Fontem D A, Bouda H & Reichmuth C (2005). Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* Jacquelin du Val. *Journal of Stored Products Research* 41(1): 91-102

Tapondjou L A, Adler C, Bouda H & Fontem D.A (2002). Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of Stored Products Research* 38: 395-402

Taylor R W D (1994). Methyl bromide- Is there any future for this noteworthy fumigant? *Journal of Stored Products Research* 30: 253-260

Villers P, Navarro S & de Bruin T (2010). New applications of hermetic storage for grain storage and transport; Proceedings of the *10th International Working Conference on Stored Product Protection*; Estoril, Portugal. 27 June-2 July 2010, pp. 446-451

Wang J, Tsai J H, Ding W, Zhao Z & Li J (2001). Toxic Effects of Six Plant Oils Alone and in Combination with Controlled Atmosphere on *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). *Journal of Economic Entomology* 94(5): 1296-1301

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 Relationships Between Propolis Collecting Capability and Morphometric Features of Some Honey Bee Races and Ecotypes in Anatolia

Meral KEKEÇOĞLU[a,d], Nazife EROĞLU[b], Merve KAMBUR[c], Münir UÇAK[c]

[a]*Düzce University, Faculty of Arts and Sciences, Department of Biology, Konuralp, Düzce, TURKEY*

[b]*The Scientific and Research Council of Turkey, Marmara Research Center, Food Institute, Gebze, TURKEY*

[c]*Düzce University, Duzce Vocational School, Beekeeping Program, Düzce, TURKEY*

[d]*Düzce University Beekeeping Reserch Development and Aplication Centre, Düzce, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Meral KEKEÇOĞLU, E-mail: meralkekecoglu@duzce.edu.tr, Tel: +90 (380) 542 11 00/1032
Received: 06 August 2018, Received in Revised Form: 06 November 2018, Accepted: 22 December 2018

AUTHORS ORCID ID
(Meral KEKEÇOĞLU: 0000-0002-2564-8343), (Nazife EROĞLU: 0000-0002-8618-2583), (Merve KAMBUR: 0000-0001-9658-6584), (Münir UÇAK: 0000-0003-1538-6711)

ABSTRACT
Propolis collecting capacity of the honey bee race, *Apis mellifera* L., distributed across Anatolia and Thrace regions of Turkey was investigated and correlated with morphometric characteristics. Thus, the propolis collecting behaviour of honey bee races and ecotypes naturally have been in Turkey, *Apis mellifera caucasica*, *Apis mellifera carnica*, *Apis mellifera syriaca* and Yığılca and Muğla ecotype of *Apis mellifera anatoliaca* were monitored. The mean yield of annual propolis was recorded as the following; Yığılca ecotype (111.6±27.5 g colony) *A. m. caucasica* (104±20.7 g colony), Muğla ecotype (103±34 g colony), *A. m. carnica* (91.16±17.6 g colony), and *A. m. syriaca* (74±6.4 g colony) in descending order. The highest propolis collecting activity was recorded for the Yığılca ecotype of *A. m. anatoliaca* and *A. m. caucasica*. Morphological features of honey bee samples were evaluated by classic morphometric technique to correlate propolis collecting capability and morphological features. Morphometric results of the present study showed that the largest wing and leg lengths belonged to Yığılca ecotype of *A. m. anatoliaca* and *A. m. caucasica*. Furthermore, Pearson correlation showed a significant relationship between some morphometric characteristics including the proboscis and mandibular sections, wing length (WL), wing width (WW), femur length (FL), tibia length (TL), basitarsus length (BL), basitarsus width (BW), and propolis collecting capability ($P<0.05$). Therefore, it seems that the enlargement of certain morphological properties with genetic tendency of the honey bee races and ecotypes, primarily the legs and wings, can lead to better propolis collecting capability.

Keywords: Propolis harvesting; Morphometric characteristics; Correlation*; Apis mellifera* L.; Anatolia

1. Introduction

Morphological differences in honey bee races have led to the most distinctive differences that are associated with pollen and propolis collecting behaviour (Winston 1991). The mouthparts, plumose hairs, and

DOI: 10.15832/ankutbd.447319

broadened hind legs are important morphological characters, especially for propolis collection behaviour (Michener 1974). The hairy coverings of the body, which may consist of branched and unbranched hairy setae, and appendages provide collecting surfaces upon which pollen grains are retained until the bee delivers them to the hive (Goodman 2003). Mouthparts of the bee, including mandibles and the maxillae and tongue (proboscis), are essential features to scrape over any surface, while each hind leg of the worker bee is adapted for holding and transporting of these grains (Goodman 2003). The outer surface of the tibia is characterized by an elongated depression with long hairs covering a receptacle or basket (corbicula) which is a characteristic feature of the Apinae (Winston & Michener 1977; Michener et al 1978). A concave region of the corbicula, formed by exceptional modification of the hind leg, is used as an anchor for pollen and propolis delivering functions (Hodges 1967). The current paradigm is the belief that corbicula initially serve to carry sticky propolis back to the nest as a building material, then the other leg modification of the hind leg occurred for pollen collection (Winston & Michener 1977; Michener et al 1978). Propolis, pollen and nectar collecting behaviours of honey bees have been shown by many studies to correlated with external structures consisting of brushes on the hind legs, corbiculum on hind tibia (Thorp 2000), and the mouthparts and proboscis (Michener et al 1978; Ajao et al 2014).

The amount and quantity of propolis collected by honey bees are related to botanical sources, season, year, propolis collecting techniques and even genetic origin of honey bee races (Mobus 1972; Ghisalberti 1979; Crane 1990). However, there are a few studies indicating that propolis collecting capability is also correlated with certain honey bee races which possess better ability to collect propolis (Ghisalberti 1979; Crane 1990). Some honey bee races such as *Apis dorsata, Apis florea,* and *Apis cerana* do not exhibit propolis collecting behaviour (Wollenweber & Buchmann 1997). Africanized honey bee races have lead to huge success in Brazil (Manrique & Soares 2002), compared to European races of *A. mellifera* for propolis collecting (Garcia et al 2013). Western honey bee races are known as having the more propolis collecting capacity than others (Ruttner 1988b). Grey Caucasian Mountain ecotype of *A. m. caucasica* honey bees was reported to collect much more propolis than *A. m. ligustica* and Far East Dark Forest bees (Thorp 2000). *A. m. carnica* was reported to use propolis rather than bees wax inside the hive (Ghisalberti 1979). The main propolis production of per colony was reported to be 10-300 g for per year (Ochi 1981; Andrich et al 1987). One of the earliest studies reported that annual propolis yield ranged from a minimum of 50 g to a maximum of 600 g for different honey bee races (Ghisalberti 1979).

Because of the excessive use of propolis by *Apis mellifera iberica* and *Apis mellifera intermissa*, these bees can survive winter temperatures at -45 °C and have adapted to climate with temperature extremes (Ruttner 1988a). And also they are known to be susceptible to brood diseases, which could be a further reason for slather use of propolis (Ruttner 1988b).

The objective of the present study was to evaluate the propolis collecting capability of indigenous honey bees, *A. m. caucasica*, *A. m. syriaca*, *A. m. carnica*, Yığılca and Muğla ecotypes of *A. m. anatoliaca*, distributed around Turkey. These races were under controlled conditions in Ankara, Middle Anatolia, Turkey. Additionally, the aim was also to investigate the possible relationship between morphometric features (forewing, hind leg, mandible and proboscis dimensions) and the propolis collecting capability of these honeybee races and ecotypes. Few studies have been conducted thus far, on propolis collecting potential of different honey bees races, and there has been no study reported in the literature focusing on the relationship between phenotypic properties and propolis collecting to date.

2. Material and Methods

2.1. Propolis collecting

Bee colonies with pure queens, the same aged and colony strenght, eight to nine adult frames, were procured from an original and isolated locations for the examination of propolis collecting behaviour in 2015. Colonies lived in their original locations were obtained for experiment. Locations of the races and ecotypes as follows; Muğla ecotype from Muğla province, Yığılca ecotype from Yığılca district of Düzce province, *A. m. carnica* from Kofçaz district of Kırklareli, *A. m. syriaca* from Hatay province, *A. m. caucasica* from Artvin province. Ten colonies were selected for each race and ecotypes. All colonies examined in the

present study were maintained under controlled conditions in the same apiary in Central Anatolia, Turkey, between April-July 2015. Propolis traps were inserted on the top of the hives according to the methods of Şahinler & Yücel (2016) in early spring. Hand collection method was conducted monthly from April to July, 2015. Raw propolis samples were acquired from traps and sorted in order to evaluate propolis harvesting capacity regarding honey bee race. Each trap was initially weighed and subtracted from final gross weight, and then recorded.

2.2. Morphometric analyze of Honey bee

Worker bees were collected from all colonies including three different honey bee races *A. m. caucasica*, *A. m. carnica*, *A. m. syriaca* and two ecotypes of *A. m. anatoliaca* to investigate the relationship between propolis collecting capacity and morphometric measurements of honey bees. Dissection of wings, hind legs, mandible and proboscis were executed (Kekeçoğlu & Soysal 2010).

Preparations of body segments, wing length (WL), wing width (WW), femur length (FL), tibia length (TL), basitarsus length (BL), basitarsus width (BW), whole leg length (WLL), proboscis length (PL) were performed by slight modification according to Kekeçoğlu (2010). All slides were photographed and then measured with a Nikon SMZ745T stereomicroscope equipped with an oculars digital camera system of 1X (except proboscis which required an ocular of 3X), and then digitalized with *de novo* Bs200Pro (BAB Imaging systems, BAB Ltd 1993) (Figure 1a, b, c, d).

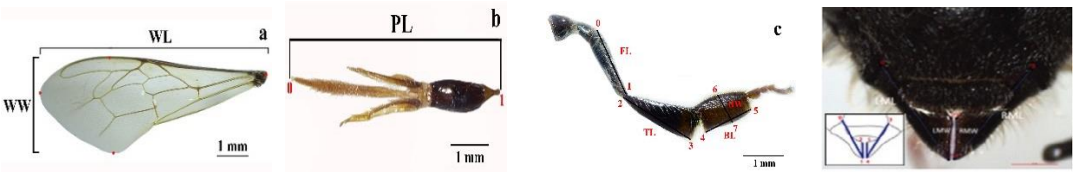


Figure 1- Wing (a), proboscis (b), leg (c) and mandibula (d) morphometric characters of honey bee

2.3. Statistical analysis

Statistical analysis was utilized by using SPSS.15.0 (2005) software. Discriminant function analysis (DFA) was used to classify the colonies and to check the probability that each colony had been correctly classified. ANOVA Posthoc-Tukey Test was performed to explain morphological characteristics which had a particular effect on races and ecotypes. Finally, Pearson's correlation method was applied to investigate any potential relationship between propolis collecting capacity and morphometric measurements of honey bees.

3. Results and Discussion

In this study, propolis collecting capacity of three races (*A. m. caucasica, A. m. syriaca*, and *A. m. carnica*) and two ecotypes (Yığılca, Muğla ecotypes) of *A. m. anatoliaca* in the same apiary were compared during the same season to evaluate propolis collection capability. The amount of propolis collected by each honeybee races were presented in Figure 2. The mean yields were as follows: Yığılca ecotype: 111.6±27.5 g colony; *A. m. caucasica*: 104±20.7 g colony; Muğla ecotype: 103±34 g colony; *A. m. carnica*: 91.16±17.6 g colony; and *A. m. syriaca*: 74±6.4 g colony.

To investigate the relationship between morphometric pattern of honey bees and propolis collecting capacity, eleven morphometric dimension (WL, WW, PL, FL, BL, TL, BW, LML, RML LMW, and RMW) were evaluated. *A. m. caucasica* was found to have the largest size of the WL, TL, LMW, and RMW, while the Yığılca ecotype of *A. m. anatoliaca* had the greatest length of the other 5 characters (WW, PL, FL, BL, BW, LML, RML) (Table 1). According to results of ANOVA, notable significances were found between group variances for WL ($F= 49.624$, $P<0.00$), WW ($F= 26.224$, $P<0.00$), FL ($F= 8.163$, $P<0.00$), TL ($F= 11.055$, $P<0.00$), BU ($F= 24.424$, $P<0.00$), BW ($F= 13.058$, $P<0.00$) and PL ($F= 6.919$, $P<0.00$) charecters.

The PostHoc-Tukey analysis was used to reveal which morphometric characters (WL, WW, FL, TL, BL, BW and PL) differentated honey bee groups. It was monitored that *A. m. caucasica* and Yığılca ecotype of *A. m. anatoliaca* have statistically similar metric features (P>0.05). Therefore, They can be assessed together within the similar group for BL and BW, WL, WW, BL and BW characters. FL and TL variables were significantly different between the groups: *A. m. caucasica-A. m. carnica* (FL: P<0.13, TL: P<0.000, PL), *A. m. caucasica- A. m. syriaca* (FL: P<0.000, TL: P<0.000), Yığılca ecotype-Muğla ecotype (FL: P<0.000, TL: P<0.031).

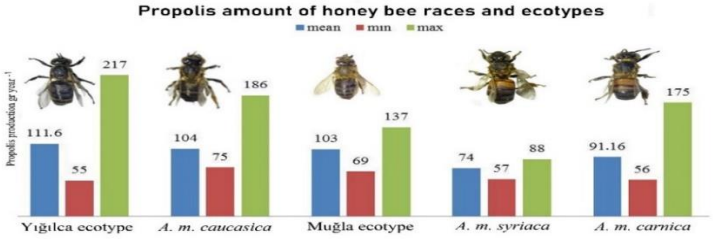


Figure 2- The mean, minimum and maximum propolis amount of three different races and two ecotypes (gr), April-July 2010, Ankara, Turkey

Table 1- The mean and standard deviation of some morphometric characters of honey bee races

Morphometric characters (mm)		*Honey bee race and ecotypes (mean+standard deviation)*				
		Yığılca ecotype (n= 30)	*A. m. caucasica (n= 45)*	*Muğla ecotype (n= 30)*	*A. m. syriaca (n= 60)*	*A. m. carnica (n= 45)*
Wing	WL	9.83±0.15^{bc}	9.87±0.18^{c}	9.39±0.10^{a}	9.39±0.23^{a}	9.68±0.19^{b}
	WW	3.34±0.06^{c}	3.28±0.08^{b}	3.17±0.09^{a}	3.14±0.11^{a}	3.24±0.07^{b}
Proboscis	PL	6.90±0.24^{b}	6.67±0.33^{a}	6.68±0.17^{a}	6.56±0.17^{a}	6.51±0.40^{a}
Leg	FL	3.33±0.07^{c}	3.32±0.13^{b}	3.26±0.10^{ab}	3.22±0.10^{a}	3.25±0.12^{ab}
	TL	3.27±0.10^{bc}	3.31±0.12^{c}	3.18±0.10^{a}	3.18±0.10^{a}	3.20±0.12^{ab}
	BL	2.15±0.09^{c}	2.14±0.10^{c}	2.02±0.08^{ab}	1.98±0.09^{a}	2.06±0.08^{b}
	BW	1.28±0.06^{b}	1.25±0.06^{b}	1.19±0.05^{a}	1.20±0.05^{a}	1.20±0.05^{a}
Mandible	LML	4.68±0.08^{a}	4.56±0.17^{a}	4.45±0.17^{a}	4.45±0.16^{a}	4.41±0.13^{a}
	LMW	1.69±0.04^{a}	1.71±0.07^{a}	1.61±0.09^{a}	1.65±0.07^{a}	1.69±0.08^{a}
	RML	4.60±0.08^{a}	4.47±0.15^{a}	4.36±0.19^{a}	4.43±0.16^{a}	4.31±0.15^{a}
	RMW	1.69±0.05^{a}	1.70±0.06^{a}	1.60±0.07^{a}	1.63±0.09^{a}	1.65±0.18^{a}

FL, femur length; WL, wing length; WW, wing weight; PL, proboscis length; TL, tibia length; BL, basitarsus length; BW, basitarsus width; LL, whole length of leg; LML, left mandibula length; LMW, left mandibula width; RML, right mandibula length; RMW, right mandibula width

Pearson Correlation analysis provided valuable insight to the relationship between morphometric characters and propolis collecting capabilities. The relationship between the length and width of the wings (WL, WW) and propolis collecting capability were found significant statistically (P≤0.008, 0.015). Furthermore, the correlation between length of the leg (FL, TL, BL, BW) and propolis collecting capability were great importance respectively; P≤0.011, 0.002, 0.014 and 0.023. There was a positive correlation between all morphometric features of leg and wing and mean propolis yield, except for proboscis length and mandibula width and length (Table 2).

Table 2- The correlation between some morphometric characters of honey bees and propolis collecting capacity

Correlations	*Morph. features*	*WL*	*WW*	*PL*	*FL*	*TL*	*BL*	*BW*	*LML*	*LMW*	*RML*	*RMW*
Propolis Production (gr)	Pearson Correlation	0.963**	0.945*	0.821	0.965*	0.978**	0.977**	0.927*	0.702	0.764	0.473	0.467
	Sig. (2-tailed)	0.008	0.015	0.089	0.011	0.002	0.014	0.023	0.187	0.167	0.421	0.428

*, correlation is significant at the 0.05 level (2-tailed); **, correlation is significant at the 0.01 level (2- tailed) # FL, femur length; WL, wing length; WW, wing weight; PL, proboscis length; TL, tibia length; BL, basitarsus length; BW, basitarsus width; LML, left mandibula length; LMW, left mandibula width; RML, right mandibula length; RMW, right mandibula width

Morphological biodiversity and foraging behaviours are under the influence of geographic conditions, plant diversity and genetic structure (Michener 2007). As one of the foraging behaviour, propolis collecting performance also closely related with acquired morphologic features (Garcia et al 2013). Mobus (1972) reported that *A. m. caucasica* known to excessive use of propolis has higher propolis collecting ability than other races examined. Silici & Kutluca (2005), Şahinler & Gül (2005) found that Anatolian honey bee (*A. m. anatoliaca*) has better propolis collecting capability than Italian (*A. m. ligustica*), Caucasian (*A. m. caucasica*), and Carniolan (*A. m. carnica*) honey bees in the conditions of Hatay province, where located on the Meditearranean Region. Whereas Kutluca (2003) reported the highest mean propolis yield from Carniolan bee, followed by the Anatolian and Caucasian in Erzurum (Eastern Anatolia). In a controlled apiary in Erzurum, Turkey, Kutluca (2003) measured a mean propolis yield of 11.40±2.19 g colony, 19.20±5.49 g colony and 15.30±5.30 g colony in Caucasian, Carniolan and Anatolian genotypes, respectively. Sahinler & Gül, (2005) studied Italian, Anatolian, Caucasican, and Carniolan honey bees in the Hatay province of Turkey and reported that the highest amount of propolis yield was produced by Anatolian honey bees under the same conditions, namely displaying 39.67 g colony, while Caucasican, Carniolan, and Italian honey bees produced 27.34, 29.63 and 26.12 g colony respectively. According to our findings, the Yığılca ecotype has the most propolis collecting capability (111.6±27.5 g colony) compared to other honeybee races situated in Anatolia under the same environmental conditions in Ankara, Central Anatolia. The other performances are as follows in descending sort; *A. m. caucasica*, *A. m. carnica*, Muğla ecotype and *A. m. syriaca* (Figure 2). The Black Sea region consists of a temperate rainforests characterized by, damp and forested mountains also steeps and hilly grassland while the Anatolian Plateau has a various climate conditions such as hot season, dry summers, long winters, heavy snow fall (Metz 1996; Çakmak 2005). The outcome of this study also showed that "northern honeybee populations (*A. m. carnica*, Yığılca ecotype, *A. m. caucasica*)" have better propolis collecting ability than that of southern honeybee populations (Muğla ecotype, *A. m. syriaca*) (Figure 2) not only in their original locations in the Marmara and Black Sea regions, but also in the Middle Anatolia. Moreover, biochemical compositions of propolis may vary depending on flora, geographic conditions and honeybee races likewise *A. m. caucasica* has more antibacterial propolis compaunds than that of *A. m. carnica* and *A. m. anatoliaca* races (Silici &Kutluca 2005).

Successful adaptations of races to the varied habitats induce for specialization in behavioural and phenotypic characteristics for many taxa (Hepburn & Radloff 1997; Fletcher 1978). Therefore, morphometric characters, namely body size, tongue length, and color and foraging behaviour, propolis and pollen collecting can be vary among to honey bee races (Buttel-Reepen 1906; Alpatov 1929; Skorikov 1929; Maa 1953; Adam 1983). It was indicated that honeybees forage and transport pollen and propolis through externally specialized scrub and corbicula structures (Thorp 1979; Medved et al 2014). Calderone & Page (1988) reported that the differences in the pollen-collecting performances between two artifically selected strains derived from the consequence of phenotypic differences of them. Our findings showed that the segments of the wing, leg and proboscis have statistically significant and high level morphometric variations in northern populations than that of southern populations. It was detected that northern honeybee populations (including Yığılca ecotype of *A. m. anatoliaca* and *A. m. caucasica*) have more propolis collecting capabilities than that of southern populations (Figure 2). It is revealed that the propolis collection capacity can vary between the ecotypes of the same races as far as it is at the subspecific level. Moreover, positive significant correlations were observed between propolis collecting and six morphometric character measurements (WL, WW, FL, TL, BL and BW) (Table 2). Black Sea Region has rainy climate, dense forest and short vegetation period unlike the other regions. Regional differentiations and significant relations indicated that as the body segments developed, honey bees enable to carry more propolis and other forage products. This results emphasized that longer and stronger or shorter body segments may occur depending on adaptation to habitat as an advantage and propolis collection activity is closely related to body segments as well as genetic make up.

4. Conclusions

Results of the present study show that different honey bee races and their ecotypes have the distinctive propolis collecting capability. Therefore, morphometric differences between honey bee races also ecotypes

can be used as an advantage by beekeepers, depending on what traits are in their best interest under the appropriate habitat and environmental conditions.

Acknowledgements

This study was fully supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK), BIDEB-2232 Project No: 113C036, Institution Code: 5134103 under the name of "Investigation of important honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), races and ecotypes in Turkey considering propolis collection capacity and chemical characterization of Turkish propolis".

References

Adam B (1983). In search of the best strains of honey bee. Dadant Sons, Hamilton Illons, N. Bee Books, West Yorkshire

Ajao A M, Oladimeji Y U, Babatundel S K & Obembe A (2014). Differential morphometric patterns of *Apis mellifera* and adaptation to climatic variations Global. *Journal of Bioscience and Biotechnology* 3(1): 34-40

Alpatov W W (1929). Biometrical studies on variation and races of the honey bee (*Apis mellifera* L.). *The Quarterly Review of Biology* 4(1): 1-58

Andrich G, Fiorentini R & Consiglieri A (1987). [Characteristics of some samples of propolis from the Ligurian Coast] Caratteristiche di alcuni tipi di propoli della Riviera Ligure. *Citta delle Api* 28: 30-38

BAB Imaging Systems, BAB Ltd. (1993). Model Bs200Pro. www.bab.com.tr 27.12.2016

Buttel-Reepen H V (1906). Apistica. Beiträge zur Systematik, Biologie, sowie zur geschichtlichen und geographischen Verbreitung der Honigbiene (*Apis mellifera L.*) ihrer Varietaten und der ubrigen *Apis*-Arten. *Mitteilungen Zoologisches Museum Berlin* pp. 117-201

Calderone N W & Page Jr R E (1988). Genotypic variability in age polyethism and task specialization in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology* 22(1): 17-25

Crane E (1990). Bees and beekeeping: science, practice and world resources. Heinemann Newnes pp. 117-142

Çakmak I (2005). The biodiversity of honey bee races in Turkey. *In 3rd European Congress on Social Insects* 22-27 Agust, 2005: 58

Fletcher D J C (1978). The African bee, *Apis mellifera adansonii*, in Africa. *Annual Review of Entomology* 23: 151-171

Garcia R C, Oliveira N T E D, Camargo S C, Pires B G, Oliveira C A L D, Teixeira R D A & Pickler M A (2013). Honey and propolis collecting, hygiene and defense behaviors of two generations of Africanized honey bees. *Scientia Agricola* 70(2): 74-81

Ghisalberti E L (1979). Propolis: a Review. *Bee World* 60(2): 59-84

Goodman L (2003). Form and Function in the Honey Bee, (completed and edited by Richard Cooter and Pamela Munn). International Bee Research Association, Cardiff, U.K. pp. 70-119

Hepburn H R & Radloff S E (1997). Honeybees of Africa. Springer, Berlin, pp. 158

Hodges D (1967). The function of the 'Single hair'on the floor of the honey bees corbicula. *Bee World* 48(2): 58-62

Kekeçoğlu M (2010). Honeybee biodiversity in Western Black Sea and evidence for a new honey bee ecotype in Yığılca Province. *BİBAD* 3(1): 73-78

Kekeçoğlu M & Soysal M I (2010). Genetic diversity of bee ecotypes in Turkey and evidence for geographical differences. *Romanian Biotechnological Letters* 15(5): 5646-5653

Kutluca S (2003). The effects of propolis collecting methods on the colony performance and chemical properties of propolis. Atatürk University Graduate School of Natural and Applied Sciences Department of Animal Sciences, Erzurum, Turkey pp. 70-73

Maa T C (1953). An inquiry into the systematics of the tribus Apidini or honey bees (Hymenoptera). *Treubia* 2(3): 525-640

Manrique A J & Soares A E E (2002). Start of africanized honey bee selection program for increased propolis collecting and its effect on honey production. *Interciencia* 27(6): 312-316

Medved V, Huang Z Y & Popadić A (2014). Ubx promotes corbicular development in *Apis mellifera*. *Biology Letters* 10(1): 20131021

Metz H C (1996). Turkey: a Country Study. Federal Research Division of the Library of Congress, U.S. Government Printing Office, Washington, D.C pp. 175-176

Michener C D (1974). The social behavior of the bees: a comparative study (Vol. 73, No. 87379). Harvard University Press 7-22

Michener C D (2007). The Bees of the World, second edition. Baltimore: Johns Hopkins

Michener C D, Winston M L & Jander R (1978). Pollen manipulation and related activities and structures in bees of the family Apidae. *University of Kansas, Science Bulletin* 51(19): 575-601

Mobus B (1972). The importance of propolis to the honey bee. *British Bee Journal* 19(8): 198-199

Ochi T (1981). A new method to collect propolis. *Honey Bee Science* 2(1): 16

Ruttner F (1988a). Biogeography and taxonomy of honey bees. Springer Verlag, Berlin pp. 165-256

Ruttner F (1988b). Breeding Tecnique and selection for Breeding of Honey bee. G. bread and Sons. Led. Brighton U.K

Silici S & Kutluca S (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *Journal of Ethnopharmacology* 99: 69-73

Skorikov A S (1929). Eine neue Basis für eine Revision der Gattung *Apis* L. *Reports on Applied Entomology* 4: 249-264

SPSS 15.0 (SPSS for Windows, Release 15.0) (2005). Standard Version, SPSS Inc., (www.SPSS.com.tr.)

Şahinler N & Gül A (2005). The effects of propolis collecting methods and honey bee genotypes on propolis yield. *Pakistan Journal of Biological Sciences* 8(9): 1212-1214

Şahinler N & Yücel B (2016). Biological activities and using facilities of Turkish propolis. *Works of the Faculty of Agriculture and Food Sciences, University of Sarajevo* 66(1): 82-85

Thorp R W (1979). Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. *Annals of the Missouri Botanical* Garden 66(4): 788-812

Thorp R W (2000). The collection of pollen by bees. *Plant Systematics and Evolution* 222: 211-223

Winston M L (1991). The biology of the honey bee. Harvard University press

Winston M L & Michener C D (1977). Dual origin of highly social behavior among bees. *Proceedings of the National Academy of Sciences* 74(3): 1135-1137

Wollenweber E & Buchmann S L (1997). Feral Honey Bees in the Sonoran Desert: Propolis Sources Other than Poplar (*Populus* spp.). *Zeitschrift für Naturforschung* C 52: 530-535

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

Comparative Biochemical Analysis of High and Low Sucrose Accumulating Sugarcane Varieties at Formative Stage under Heat Stress

Faisal MEHDI[a], Kazim ALI[b], Nesheman HUMA[b], Iqbal HUSSAIN[c], Abid AZHAR[b], Saddia GALANI[b]

[a]*Agriculture and Agribusiness Management (ABM), University of Karachi, Karachi 75270, PAKISTAN*

[b]*The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi 75270, PAKISTAN*

[c]*Department of Botany, Government College University Faisalabad (GCUF), Faisalabad, PAKISTAN*

ARTICLE INFO
Research Article
Corresponding Author: Faisal MEHDI, E-mail: mehdifaisal@yahoo.com, Tel: +90 (345) 607 77 60
Received: 06 September 2018, Received in Revised Form: 03 January 2019, Accepted: 11 February 2019

AUTHORS ORCID ID
(Faisal MEHDI: 0000-0002-8016-8887), (Kazim ALI: 0000-0002-6808-543X), (Nesheman HUMA: 0000-0002-7691-762X), (Iqbal HUSSAIN: 0000-0002-1788-897X), (Abid AZHAR: 0000-0001-9509-1168), (Saddia GALANI: 0000-0002-3195-9447)

ABSTRACT
Sugarcane (*Saccharum officinarum L)* is a valuable cash crop which plays an imperative role in the worldwide economy. However, high temperature has significantly retarded the crop growth and yield by alteration of biochemical pathways. Therefore, the biochemical activities of two sugarcane varieties were explored under heat stress condition. The sugarcane cultivars S2003-US-633 (high sucrose accumulation) and SPF-238 (low sucrose accumulation) were cultivated and subjected to different temperature regimes i.e. control at 30±2 °C, heat stress at 45±2 °C and recovery at 30±2 °C for 24, 48 and 72 hours at formative stage. Detailed profiling of physiochemical attributes, sugar analysis linked with sucrose metabolism enzymes and thermotolerance indicators were investigated. S2003-US-633 exhibited better response in terms of sugar accumulation regulated by sucrose synthase, sucrose phosphate synthase and invertase activities along with more proline accumulation, total soluble protein contents with response to high temperature exposure. While S2003-US-633 is ranked as tolerant variety due to less MDA, H_2O_2 content and electrolytes leakage exhibiting its efficient tolerance mechanism, giving high sugar recovery rate despite harsh environmental conditions. Thus, these findings can be helpful in providing information for engineering sugar improvement along with thermotolerance in sugarcane varieties and providing new avenues towards the economic development of the country.

Keywords: Metabolizing enzymes; Oxidative markers; Sugar recovery; Stress indicators; Thermotolerant; Yield

1. Introduction

Sugarcane is an important crop due to the presence of high sucrose content in its stalk. It plays an important role towards the economic development of the country. In spite of extension in sugarcane production in Pakistan, average sugar recovery is reported 8-9% only which is far beyond from other developed countries (PSMA Report 2007). On the other hand, altering climate conditions along with low sugar recovery potential of sugarcane poses a challenge to sugarcane industry. High glucose, sucrose availability

DOI:10.15832/ankutbd.457918

ıs important for physiologically regulating plant development, sugar signaling and water balance in cell (Liu & Huang 2000; Roitsch & González 2004). Sugar recovery depends on the amount of stored sugar in cane tissue (Joshi et al 2013) involving sucrose metabolizing enzymes such as sucrose synthase, sucrose phosphate synthase, neutral, vacuolar and cell wall invertases. These sucrose metabolizing enzymes in sugarcane genotypes are diversely affected during different growth stages (Tana et al 2014). It has been investigated that accumulation of sucrose in sugarcane tissue is mainly dependent on invertase and SPS activities which are adversely affected by climatic alterations (Ansari et al 2013). However, these climate changes are reported to have a substantial impact on crop production ultimately leading to yield losses with great risk for future global food security (Christensen & Christensen 2007). Rise in current global mean temperature has increased by 0.99 °C (NASA 2017) which is projected to have severe implications on plant growth and development by altering the underlying molecular mechanisms. It has been reported that there are substantial yield reductions observed at temperature more than 45 °C, also affecting the sugar recovery in sugarcane leading to the huge economic losses (Shrivastava et al 2010). This reduction is due to the down regulation of specific genes in carbohydrate metabolism which may lead to the altered activities of carbon metabolism enzymes, compromised starch accumulation and sucrose synthesis due to heat stress (Ruan et al 2010). Like other abiotic stresses, heat stress results in the production of ROS (Potters et al 2007) such as, superoxide anion (O_2^-), H_2O_2 hydroxyl radical $(OH)^1$, which are highly reactive and can alter the metabolism of plant through oxidative damage to membrane, denaturing of protein and nucleic acids leading to cell death. Moreover, H_2O_2 is involved in disruption of various metabolic activities like calvin cycle (Akram et al 2012). Due to the current scenario, development of thermotolerant varieties are the important strategy in adaptation of climate change. In this regard, biochemical characterization is crucial step to recognize varieties with desirable agronomical traits to meet sugar industry requirements. In this study, the effect of heat stress on sugarcane was studied to determine the physiological and biochemical analysis in sugarcane at formative stage to better understand the biochemical response of plants in heat stress condition for improving sugarcane thermotolerance.

2. Material and Methods

In this study, the two cultivars (S2003-US-633 and SPF-238) of sugarcane were cultivated in 20 kg capacity pots having loam soil at net house, The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE) University of Karachi, Karachi, Pakistan in February 2016. Experiment was conducted in Completely Randomized Design (CRD) with three replicates per each treatment group. Half strength Hoagland solution was supplemented weekly for whole experiment. Heat stress was applied by shifting all pots to heat shock room, where white fluorescent tube lights/mercury lamps were used for maintaining photosynthesis active radiation (PAR) ranging from 650-700 µmol m^{-2} s^{-1}. Temperature was set at 45±2 °C and 34±2 °C for day and night respectively while humidity was maintained at 60-70%. For air circulation, fans were adjusted. For recovery experiment, pots were again shifted to growth room at 30±2 °C. Samples were collected for control at 30±2 °C, heat stress treatment at 45±2 °C for 24 h (T24), 48 h (T48) and 72 h (T72) and recovery treatments at 30±2 °C for 24 h (R24), 48 h (R48) and 72 h (R72) and preserved at -80 °C for biochemical analysis.

Extraction and quantification of sugars: For this, 0.5 g fresh sugarcane leaf was homogenized in 2.5 mL ethanol (80%) immediately after sampling. The samples were centrifuged at 1000 rpm for 10 min. Reducing sugar was estimated using dinitrosalicyclic acid DNSA reagent (Miller 1959) and total sugar was quantified by anthrone reagent (Hedge & Hofreiter 1962). Whereas non-reducing sugar was calculated by following formula;

$$\text{Non-reducing sugar (mg mL}^{-1}) = \text{Total sugar - Reducing sugar} \quad (1)$$

Quantification of sugar metabolizing enzymes: For enzyme extraction, 0.5 g leaf tissues were homogenized in 2 mL MOPS-NaOH buffer (pH 7.5) and then centrifuged at 12000 rpm for 30 min. The reaction mixture for Sucrose Synthase (SS) contained; enzymes (10 µL), (0.2 mM) UDPG substrate (25 µL), (4 mM) Fru 6-P, (40 mM) Glu 6-P, (5 mM) MOPS-NaOH (pH -7.5), (5 mM) $MgCl_2$ and (0.1 mM) EDTA. All tubes were placed at 37 °C for 15 min and reaction was stopped by adding 70 µL 30% KOH. The reaction mixture was then placed at 100 °C for 10 min. Then 5 mL 0.14% anthrone in sulphuric acid

was added in reaction. All tubes were again heated at 100 °C for 15 min and OD was taken at 620 nm. For SPS quantification, above-mentioned reaction mixture was used having 40 mM fructose instead of fructose 6 p and Glucose 6 p (Hubbard et al 1989). The activity of cell wall bound invertase was analyzed by using some modification in the procedure of **(**Vorster & Botha 1999). The assay medium consisted of 250 µL K-Phosphate buffer (pH 7.0) for neutral invertase while for cell wall invertase, acetate buffer (pH 3.5) consisting sucrose (4%), 250 µL supernatants and 500 µl deionized water was used and incubated at 37 °C for 60 min. Reaction was terminated by adding 1ml of 1.6% NaOH with DNSA reagent, heated at 100 °C for 10 min. Absorbance was read at 540 nm using known glucose standard curve.

Thermotolerance indicators: For proline estimation, 100 mg leaf tissues were extracted in sulphosalicylic acid (3%) and centrifuged at 10000 rpm for 15 min. In 1ml of supernatant, 1 mL ninhydrin reagent and 1 mL acetic acid were added. The reaction was boiled for 60 min and was placed on ice bath followed by addition of toluene (4 mL). It was vortexed and placed at room temperature for 20 min. Optical density was measured at 520 nm (Bates et al 1973). Total soluble protein was quantified through bradford assay (Bradford 1976). Reaction mixture (3 mL) contained plant extract (50 µL), bradford dye (150 µL) and 0.15 N NaCl (2800 µL), incubated at room temperature for 20 min. Absorbance was measured at 595 nm using known protein standard curve.

Stress induced oxidative markers analysis: Relative membrane permeability (RMP) in terms of percentage was measured by assessing electrolytes leakage using the method by (Yang et al 1996) with the help of electrical conductivity (EC) meter. For H_2O_2 quantification, 100 mg sugarcane leaf tissues were homogenized in 2 mL (0.1%) TCA. After centrifugation, 0.5 mL supernatant was added in (1 mL) phosphate buffer (pH 7.0) and (2 ml) potassium iodide. Then OD was measured at 390 nm. Potassium iodide (2 mL) and potassium buffer (1 mL) were used for blank in the absence of leaf extract. Standard curve of H_2O_2 was constructed by using different concentration of H_2O_2 (Loreto & Velikova 2001).

Determination of lipid peroxidation: Malondialdehyde (MDA) contents were assessed by Heath & Packer (1968). Sugarcane leaf tissues (0.1g) were extracted in 2 mL of tetracholoroacitic acid (5%) and the mixture was centrifuged at 14000 rpm for 10 min. The supernatant (1 mL) was separated and 1 mL thiobarbituric acid (0.5%) was added and the mixture was heated for 20 min. After cooling, the reaction was re-centrifuged at 10,000 rpm for 10 min and then first OD was taken at 532 nm and at 600 nm.

Protein profiling:

SDS-PAGE

Extracted proteins from leaf tissues were resolved on SDS-PAGE gel (Laemmli 1970). Resolving (10%) and stacking gels (4%) were prepared by combination of acrylamide and bis-acrylamide solution, resolving (pH 8.8), stacking buffers (pH 6.8), SDS, fresh APS (5%), TEMED and double distilled water. The gel was run at 100 Volt for 2 hours. The gel was then stained by coomassie dye (G-250) with shaking for overnight at room temperature. Next day the gel was destained by using destaining solution. Then the gel was scanned and documented.

Statistical analysis: Data was statistically analyzed for analysis of variance (ANOVA) using the SPSS package program, version 17.0. Test for normality of data were done using Least Significant Difference (LSD). Statistical significance was determined at $P<0.05$.

3. Results

Sugar analysis: Results revealed that total sugar, reducing and non-reducing sugars had statistically significant differences ($P<0.05$) between cultivars and treatments, while no significant differences ($P>0.05$) between interaction (C x T) were observed. Although, there were varietal differences for all these parameters, but increase in temperature episode caused reduction in total sugar profile (Figure 1a). After 72 hours of thermal stress (T72), pronounced reduction in sucrose was evident in S2003-US-633 (263 µg mL^{-1}) and SPF-238 (228 µg mL^{-1}) as compared to control (712 µg mL^{-1}) and (573 µg mL^{-1}) respectively.

However, recovery treatments greatly triggered regain of sugar loss with passage of recovery time. Same pattern of accumulation upon thermal stress was also observed in reducing and non-reducing sugars (Figure 1b-1c). S2003-US-633 exhibited decline up to 0.12 mg mL^{-1} at T72 from control 0.25 mg mL^{-1} for reducing sugars and 0.45 mg mL^{-1} at T72 from control 0.14 mg mL^{-1} for non-reducing sugars respectively.

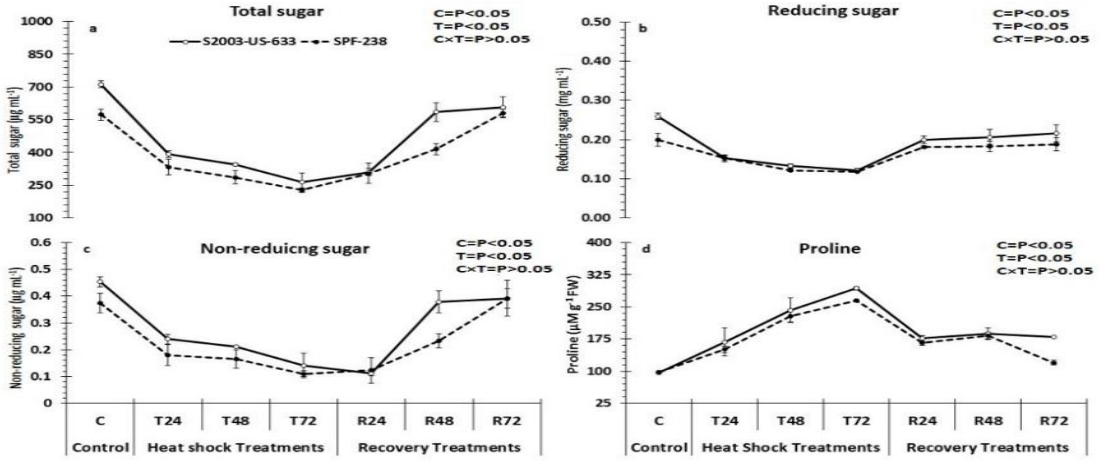


Figure 1- a, Total sugar; b, reducing sugar; c, non-reducing sugar and d, proline of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at 30±2 °C, heat shock treatment at 45±2 °C for 24, 48 and 72hours and recovery treatment at 30±2 °C after 24, 48 and 72 hours

Thermotolerance indicators: Statistical analysis showed significant differences ($P< 0.05$) for proline and total soluble protein contents for cultivars (C) and treatments (T) but there was no statistically significant difference ($P>0.05$) for interaction (C x T) between them. It is clear from the results that free proline accumulation increased by high temperature in both cultivars S2003-US-633 and SPF-238 (Figure 1d). Comparatively, S2003-US-633 had maximum accumulation of total soluble protein in all condition showing better condition of growth. A significant rise in the concentration of proline content observed in both sugarcane varieties under heat shock conditions (T24, T48 and T72), while declined upon recovery. Between these varieties, S2003-US-633 had maximum accumulation of proline (293.5 µmol g^{-1} FW) under heat shock treatment (T72).

Stress induced oxidative markers: There were statistically significant differences for all stress induced damages such as RMP and malondialdehyde contents among cultivars and treatments at the ($P<0.05$) levels respectively. Upon exposure to heat stress, increased EC content was evident at T72 in SPF-238 (28.7%) and S2003-US-633 (24.30%) respectively. During recovery conditions, both cultivars exhibited same pattern for electrolyte leakage (Figure 2b). Similarly, MDA (malondialdehyde) (nmol mL^{-1} FW) was maximum with the progression of heat stress (T24, T48 and T72) in SPF-238. But statistically non-significant differences were found for this attribute only between varieties and treatments (Figure 2c). For hydrogen peroxide contents, there were statistically significant differences ($P<0.05$) between cultivars and treatments while in interaction (C × T) no significant differences were observed. Although heat stress increased production of H_2O_2 in both cultivars but cultivar S2003-US-633 showed minimum accumulation of hydrogen peroxide content as compared to SPF-238. In heat shock conditions H_2O_2 contents of both varieties increased many folds (Figure 2d).

Sugar metabolizing enzymes analysis: The activities of key sucrose metabolizing enzymes (SPS, SS, NIV and CWIN) of two cultivars at vegetative stage under heat stresses are presented in (Figure 3a-3d). Statistical analysis of sugar metabolizing enzymes activities exhibited significant differences ($P<0.05$) for sucrose phosphate synthase and neutral invertase among cultivars (C), treatments (T) but no significant difference ($P>0.05$) was observed for their interaction (C x T) in neutral invertase. The exposure of heat stress declined the enzymes activities in both varieties as compared to the control. Regarding SPS activity, drastic reduction was observed after 72 hours of heat stress in S2003-US-633 (2257 U mL^{-1} min^{-1}) and SPF-238 (2407 U mL^{-1} min^{-1}) and was increased in recovery treatments (R72) (Figure 3a). While, SS activity

exhibited non-significant differences (P>0.05) for cultivars (C) and treatments (T) and significant differences (P<0.05) for interaction (Figure 3b). It is evident from the results that SS activity was sequentially decreased as episodes of heat stress progressed, maximum reduction (69.68 U mL^{-1} min^{-1}) was observed at heat shock treatment (T72) in SPF-238. While both cultivars recovered the maximum SS activity after 72 hours of recovery treatment (R72). For cell wall invertase and sucrose synthase activity, significant differences (P<0.05) were found for treatments (T) while cultivar and their interaction (C x T) were non-significant (P>0.05). Among, heat shock treatments, only 72 hours of heat shock (T72) exhibited decreased activity of SS as compared to other treatments. After 72 hours of heat stress, neutral invertase activity declined in both cultivars S2003-US-633 (0.91 U mL^{-1} min^{-1}) and SPF-238 (0.90 U mL^{-1} min^{-1}), moreover quick recovery was observed in SPF-238 (1.36 U mL^{-1} min^{-1}) as compared to S2003-US-633 (1.11 U mL^{-1} min^{-1}) (Figure 3c). Whereas, cell wall invertase activity declined in heat stress during T24, T48 and T72 hours but there was no significant difference observed in both cultivars (Figure 3d).

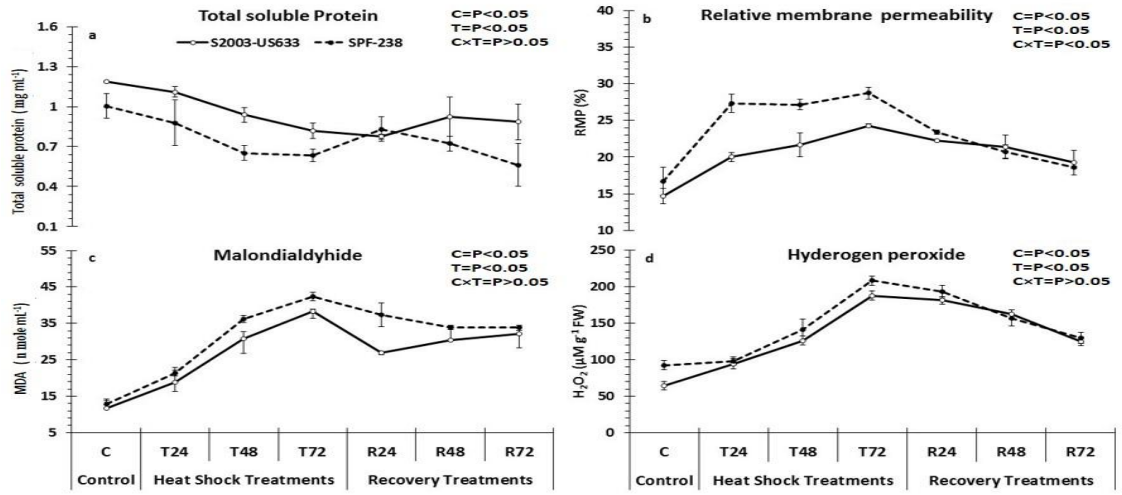


Figure 2- a, Total soluble protein; b, MDA; c, RMP and d, $H_2O_2$of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at 30±2 °C, heat shock treatment at 45±2 °C for T24, T48 and T72 hours and recovery treatment at 30±2 °C after T24, T48 and T72 hours

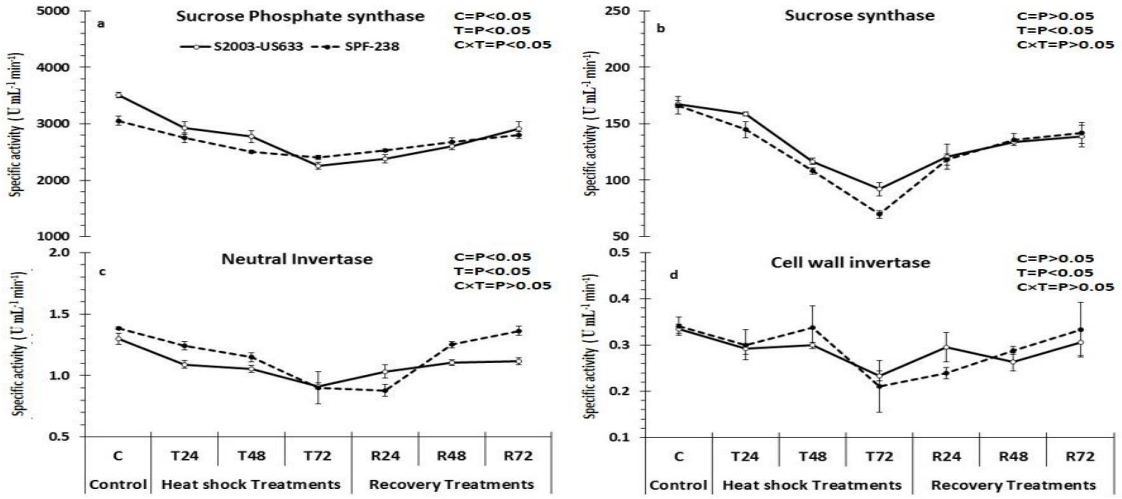


Figure 3- a, SPS; b, SS; c, NIV and d, CWIN of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at 30±2 °C, heat shock treatment at 45±2 °C for T24, T48 and T72 hours and recovery treatment at 30±2 °C after R24, R48 and R72 hours

Protein profiling:

SDS PAGE

At formative stage, protein expression was analyzed through SDS-PAGE for both the varieties (Figure 4). Proteins of different molecular weights were differentially expressed during heat stress. The highest molecular weight protein (≈ 150 kDa) was clearly expressed upon heat stress and recovery treatments. The band intensity of the protein remained stable during all the episodes of heat stress (T24, T48 and T72) but during the recovery phase it was diminished (R24, R48) and then reappeared at R72 hours. Similarly, among high molecular weight proteins 90, 70 and 60 kDa protein bands were consistently expressed during the heat shock treatments but at the initial stages of recovery (R24-R48) these were not visible. Thus, in both varieties same pattern of protein expression was observed in high molecular weight protein but in case of low molecular weight proteins there was a sharp high intensity band observed at approx. 15 kDa (green) in variety S2003-US-633 which was not present in variety SPF-238. During the SDS PAGE analysis 15 kDa protein might be differentially expressed in S2003-US-633 as compared to SPF-238 during the heat shock and recovery phases.

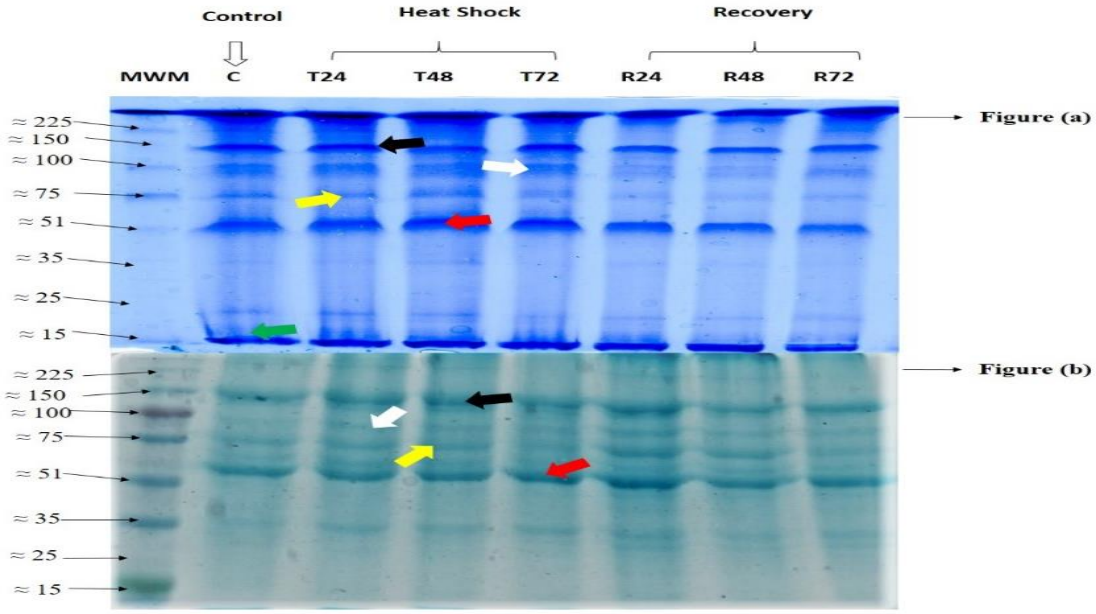


Figure 4- SDS-PAGE protein profiling a, S2003-US-633; b, SPF-238 at formative stage under control at 30±2 °C, heat shock treatment at 45±2 °C for T24, T48 and T72 hours and recovery treatment at 30±2 °C after R24, R48 and R72 hours

4. Discussion

Present study was planned to unravel the sugar metabolism pathways in sugarcane cultivars, for reprograming its resources to cope with unprecedented heat waves without any compromise in its core product (sucrose) accumulation. This differential accumulation of sucrose in sugarcane, found multifarious, not only cultivaral, phenological and temporal (McCormick et al 2008) but also affected by several biotic and abiotic factors (Albacete et al 2011). Regarding sucrose analysis, both sugarcane cultivars showed significant variations depending on duration of stress and recovery treatment. While, reducing and non-reducing sugars analysis revealed same pattern of response for thermal stress by significant decrease. This reduction in sucrose concentration is attributed due to less carbon assimilation and subsequent partitioning of carbon derived energy products including sucrose, from source (leaves) to sink (stem), tissues (Ebrahim et al 1998) or increased respiratory demand. On the other hand, sugar metabolism is regulated by sucrose synthase (SS), sucrose phosphate synthase (SPS) and invertases. Sucrose is catabolized or resynthesized either by sucrose synthase or sucrose phosphate synthase, while invertases are involved in its hydrolyzation,

into smaller sugars. The exposure of heat stresses hampered the activities of SPS, SS and invertases during the exposure of heat stresses in different episodes (T24, T48 and T72) in both sugarcane varieties. But S2003-US-633 exhibited more SPS activity suggesting that SPS may play central role in accumulating of sucrose in sugarcane plants. The high sucrose phosphate synthase activities were associated with high level of sucrose in sugarcane (Botha & Black 2000). A positive correlation of SS, SPS activities with sucrose accumulation was evident in both sugarcane genotypes. In addition, SS, SPS and acid invertase activities were observed in S2003-US-633 (high sucrose accumulation variety) under heat stress. Regarding heat tolerance, S2003-US-633 showed better performance under heat stress conditions by high accumulation of proline as compared to SPF-238. Proline accumulation triggered by biotic and abiotic stresses, act as an electron acceptor, osmolyte and protect the membrane (Abrahám et al 2010) and photosynthetic machinery induced by reactive oxygen species (Hare et al 1998). The increase in lipid peroxidation is also a marker of oxidative stress (Goel & Sheoran 2003) for abiotic and biotic stresses (Apel & Hirt 2004). In this study, marked increase in MDA contents was reported in both sugarcane cultivars under heat shock conditions showing considerable lipid peroxidation of biological membranes leading to the production of ROS along with losing membrane integrity (Boaretto et al 2014). While high temperature also damages cell membrane by losing membrane integrity affecting all other physiological and biochemical processes (Kaur et al 2010). Under heat stress conditions, electrolytes leakage increased many folds as compared to the normal growth condition as well as same recovery pattern were observed of both varieties. This enhanced permeability of membranes severely damaged the mesophyll cells (Zhang et al 2005) and led to the increased electrolytes leakage at high temperature in leaves sugarcane (Savchenko et al 2002). More proline accumulation, total protein contents, total sugars, reducing sugars, non-reducing sugars content while less MDA content, H_2O_2 content and less electrolytes leakage (EC) with response to high temperature exposure in S2003-US-633, ranked as tolerant variety. SDS-PAGE analysis revealed that high and low molecular weight proteins bands raging from 15kDa to 150 kDa protein in S2003-US-633. It is assumed that HSPs (60, 70 and 90 kDa) might be play role in development of stress tolerance without inhibiting the activities of sugar metabolizing enzymes. On the other hand, accumulation of proline content that act as an osmolyte and the oxidative makers, responsible to stabilize the protein in stressed condition. It can be concluded that S2003-US-633 proved more thermotolerant variety with great sugar accumulation under different regimes Thus, these biochemical attributes can index the degree of tolerance of sugarcane crop to exhibit adaptability under stressful conditions providing the insights to molecular breeders to identify the thermotolerant sugarcane varieties with improved recovery of sugarcane.

Acknowledgements

The authors are indebted to Higher Education Commission (HEC) for providing funds for this research work

References

Abrahám E, Hourton-Cabassa C, Erdei L & Szabados L (2010). Methods for determination of proline in plants. In Plant Stress Tolerance pp. 317-331

Akram N A, Ashraf M & Al-Qurainy F (2012). Aminolevulinic acid-induced changes in some key physiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus L.)* plants under saline regimes. *Scientia Horticulturae* 142: 143-148

Albacete A, Grosskinsky D K & Roitsch T (2011). Trick and treat: a review on the function and regulation of plant invertases in the abiotic stress response. *Phyton* 50(2): 181-204

Ansari M I, Yadav A & Lal R (2013). An-overview on invertase in sugarcane. *Bioinformation* 9(9): 464

Apel K & Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* 55: 373-399

Bates L S, Waldren R P & Teare I D (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* 39(1): 205-207

Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1-2): 248-254

Boaretto L F, Carvalho G, Borgo L, Creste S, Landell M G, Mazzafera P & Azevedo R A (2014). Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. *Plant Physiology and Biochemistry* 74: 165-175

Botha F C & Black K G (2000). Sucrose phosphate synthase and sucrose synthase activity during maturation of internodal tissue in sugarcane. *Functional Plant Biology* 27(1): 81-85

Christensen J H & Christensen O B (2007). A summary of the prudence model projections of changes in European climate by the end of this century. *Climatic Change* 81(1): 7-30

Ebrahim M K, Zingsheim O, El-Shourbagy M N, Moore P H & Komor E (1998). Growth and sugar storage in sugarcane grown at temperatures below and above optimum. *Journal of Plant Physiology* 153(5-6): 593-602

Goel A & Sheoran I S (2003). Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biologia Plantarum* 46(3): 429-434

Hare P D, Cress W A & Van Staden J (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell & Environment* 21(6): 535-553

Heath R L & Packer L (1968). Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125(1): 189-198

Hedge J E & Hofreiter B T (1962). Estimation of starch by anthrone reagent. *Methods in Carbohydrate Chemistry* pp. 9-10

Hubbard N L, Huber S C & Pharr D M (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (Cucumis melo L.) fruits. *Plant Physiology* 91(4): 1527-1534

Joshi B J, Krishnaveni S, Vijayalakshmi D, Sudhagar R & Raveendran M (2013). Activities of enzymes involved in synthesis and degradation of sucrose in popular sugarcane varieties. *Asian Journal of Experimental Biology* 4(2): 237-244

Kaur S, Singh H P, Mittal S, Batish D R & Kohli R K (2010). Phytotoxic effects of volatile oil from Artemisia scoparia against weeds and its possible use as a bioherbicide. *Industrial Crops and Products* 32(1): 54-61

Laemmli U K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259): 680

Liu X, Huang B (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science* 40: 503-513

Loreto F & Velikova V (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* 127(4): 1781-1787

McCormick A J, Watt D A & Cramer M D (2008). Supply and demand: sink regulation of sugar accumulation in sugarcane. *Journal of Experimental Botany* 60(2): 357-364

Miller G L (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3): 426-428

NASA (2017). NASA' s Goddard Institute for Space studies (GISS). https://climate.nasa.gov/vital-signs/global-temperature/ (July 2017)

PSMA (2007) (Pakistan sugar mill association). *Annual Report* pp. 4

Potters G, Pasternak T P, Guisez Y, Palme K J & Jansen M A (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends in Plant Science* 12(3): 98-105

Roitsch T & González M C (2004). Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* 9(12): 606-613

Ruan Y L, Jin Y, Yang Y J, Li G J & Boyer J S (2010). Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Molecular Plant* 3(6): 942-955

Savchenko G E, Klyuchareva E A, Abramchik L M & Serdyuchenko E V (2002). Effect of periodic heat shock on the inner membrane system of etioplasts. *Russian Journal of Plant Physiology* 49(3): 349-359

Shrivastava P K, Kaleriya K A, Parmar V & Patil R G (2010). Time Series Analysis of Temperature Variations and its Impact on Sugarcane Crop. *In National Symposium on Climate Change and Rainfed Agriculture, Organized by Central Research Institute for Dryland Agriculture at Hyderabad February 18-20*

Tana B, Chanprame S, Tienseree N & Tadakittisarn S (2014). Relationship between invertase enzyme activities and sucrose accumulation in sugarcane *(Saccharum spp.). Kasetsart Journal: Natural Science* 48: 869-879

Vorster D J & Botha F C (1999). Sugarcane internodal invertases and tissue maturity. *Journal of Plant Physiology* 155: 470-477

Yang G, Rhodes D & Joly R J (1996). Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. *Functional Plant Biology* 23(4): 437-443

Zhang J H, Huang W D, Liu Y P & Pan Q H (2005). Effects of temperature acclimation pretreatment on the ultrastructure of mesophyll cells in young grape plants *(Vitis vinifera L. cv. Jingxiu)* under cross-temperature stresses. *Journal of Integrative Plant Biology* 47(8): 959-970

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 Seedling Resistance of Spring Wheat Lines to *Fusarium culmorum*

Elfinesh Shikur GEBREMARIAM[a], Aziz KARAKAYA[b], Gul ERGINBAS-ORAKCI[c], Abdelfattah A. DABABAT[c], Timothy C. PAULITZ[d]

[a]*Hawassa University, College of Agriculture, School of Plant and Horticultural Sciences, P. O. Box 5, Hawassa, ETHIOPIA*

[b]*Ankara University, Department of Plant Protection, Faculty of Agriculture, Dışkapı, 06110 Ankara, TURKEY*

[c]*CIMMYT (International Maize and Wheat Improvement Center), Yenimahalle, Ankara, TURKEY*

[d]*United States Department of Agriculture, Agricultural Research Service, Wheat Health, Genetics and Quality Research Unit, Pullman, WA, USA*

ARTICLE INFO
Research Article
Corresponding Author: Aziz KARAKAYA, E-mail: karakaya@agri.ankara.edu.tr, Tel: +90 (312) 596 12 58
Received: 02 October 2018, Received in Revised Form: 15 January 2019, Accepted: 13 February 2019

AUTHORS ORCID ID
(Elfinesh Shikur GEBREMARIAM: 0000-0001-7802-4502), (Aziz KARAKAYA: 0000-0003-3019-9009),
(Gul ERGINBAS-ORAKCI: 0000-0003-2734-4316), (Abdelfattah A. DABABAT: 0000-0002-3172-0452),
(Timothy C. PAULITZ: 0000-0002-8885-3803)

ABSTRACT
Wheat diseases are one of the constraints limiting wheat yields wherever the crop is grown. Fusarium crown rot, incited by *Fusarium culmorum*, is one of the most important diseases limiting wheat yields especially in dryland areas. Although there are no wheat varieties which are fully resistant to crown rot, the use of varieties showing some degree of resistance is the most reliable and cost effective method to control this disease. In this study, seedling reactions of 165 spring wheat breeding lines (*Triticum aestivum* L.) obtained from CIMMYT, Mexico were determined under growth room conditions using an aggressive isolate of *Fusarium culmorum.* Crown rot severity was assessed using a 1-5 scale. The mean disease severity scores for the lines tested ranged from 1.4 to 4.4. Two out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and had scores of 1.4. Twenty lines showed moderately resistant (MR) reaction and had scores ranging from 1.6 to 2.4. The scores of both the R and MR lines were not significantly different from scores of MR control cultivars. Sixty-three percent of the lines were moderately susceptible (MS). Out of the 165 lines tested, 39 were susceptible (S) in their reaction. The promising wheat lines that showed some degree of resistance to *Fusarium culmorum* in the present study can serve as useful sources of genetic resistance in breeding for Fusarium crown rot.

Keywords: Wheat; *Triticum aestivum*; *Fusarium culmorum*; Disease resistance

1. Introduction

Wheat (*Triticum* spp.) plays a tremendous role in human nutrition. It serves as a staple food for 40% of the world's population (Bockus et al 2010). Its high yield and nutrition, ease of grain storage and processing it into different food forms made wheat the major diet component (Curtis 2002; Shewry 2009). It provides essential amino acids, minerals, vitamins and dietary fiber (Shewry 2009; Bockus et al 2010). Wheat serves

DOI: 10.15832/ankutbd.466442

as a source of more calories and protein to the world's diet than any other food crop. In Turkey, wheat contributes to more than half of the calories and protein in the diet (Hanson et al 1982).

Turkey is an important wheat producer in the world with annual production of around 22.1 million tons from a total wheat production area of 7.77 million ha in 2013 (Anonymous 2014). The average yield of wheat in Turkey is 2.8 tons ha^{-1} (Anonymous 2014), however, the yield varies from 1 ton ha^{-1} in the Eastern region to 3 tons ha^{-1} in the European part of Marmara region (Braun et al 2001). In Turkey wheat accounts for 3.9% of total world wheat production, more than 32% of total cultivated land and 60% of cereal production (Geçit et al 2009).

Cereal diseases exist wherever the crops are grown. Soilborne diseases including crown rot are important diseases of cereals in the world, particularly in areas where cereal based rotations and marginal growing conditions are common. Despite their economic importance, some soilborne diseases are given less attention because of the difficulty in working with them (Wallwork 2000; Singleton 2002). Soilborne pathogens of cereals invade crown and root tissues and interfere with nutrient and water uptake which lead to economic yield losses (Singleton 2002). Damage caused by *Fusarium* species on small grain cereals include rotting of seeds, seedlings, crowns, roots, basal stems or heads (Paulitz et al 2002). Fusarium crown rot also causes damping-off, reduction in grain and straw yields and grain quality (Smiley et al 2005). Crown rot pathogens cause yield losses due to damaged seedlings, improper grain filling and lodging (Schilling et al 1996). The disease is of economic importance in dryland wheat producing regions including Turkey, Europe, North and South Africa, Australia, North and South America and West Asia (Smiley et al 2005; Chakraborty et al 2006; Bockus et al 2010; Gebremariam et al 2018a). World-wide losses exceeding 30% have been documented (Cook 1968, 1992; Mishra 1973; Klein et al 1991; Burgess et al 2001; Hekimhan et al 2004). Yield losses ranging from 24% to 43% caused by crown rot diseases have been recorded on common bread wheat cultivars in Turkey (Nicol et al 2001; Hekimhan et al 2004).

Management of crown rot has relied on cultural practices that only provide partial control and are not reliable for limiting damage caused by the disease (Cook 1981; Smiley & Patterson 1996; Paulitz et al 2002). Although there are no fully resistant wheat cultivars to crown rot disease (Pereyra et al 2004; Wisniewska & Kowalczyk 2005), use of genotypes that show some degree of resistance/tolerance is the most reliable and efficient approach to reduce yield losses due to Fusarium crown rot disease (Cook 2001).

The disease crown rot is also known by different common names including Fusarium crown rot, dryland foot rot, dryland root rot, Fusarium root rot and common root rot (Paulitz et al 2002). Crown rot is caused by a complex of fungal pathogens which include *F. culmorum* (W. G. Smith) Sacc. (Bockus et al 2010). These pathogens may occur singly, but they often exist together in the same fields and even within individual plants, and there may be difference in dominance of different pathogens at a specific location from year to year (Smiley & Patterson 1996). In Turkey and other parts of the world *F. culmorum* and *F. pseudograminearum* are the two most commonly reported damaging *Fusarium* species causing the disease (Cook 1992; Aktaş et al 1999; Burgess et al 2001; Tunali et al 2006). In Turkey, *F. pseudograminearum* is relatively common in the Marmara region while *F. culmorum* is more prevalent in the Central Anatolian Plateau (Burgess et al 2010).

In this study, reactions of 165 spring wheat lines obtained from CIMMYT were determined using an aggressive isolate of *Fusarium culmorum*. An abstract of this study has been published previously (Gebremariam et al 2018b).

2. Material and Methods

Fusarium culmorum isolate Fc2 determined as the most aggressive isolate in Gebremariam (2015) study was used in assessing reactions of 165 lines of spring wheat (*Triticum aestivum* L.) obtained from CIMMYT, Mexico. Monosporic cultures of *F. culmorum* isolate were grown on synthetic nutrient-poor agar (SNA) for 10-14 days at a temperature of 25 ºC day/20 ºC night, with 12 h photoperiod under cool white and black fluorescent light to initiate spore formation. Autoclavable plastic bags (25 cm x 38 cm) were filled with wheat bran (150 g) and moistened with distilled water (50 mL). It was then sterilized at a

temperature of 121 ºC for 15 min. The sterilization was repeated two times at an interval of 24 h. The cultures were cut into pieces and put into plastic bags containing sterile wheat bran and incubated for 10-14 days under the same incubation conditions mentioned above. After 3 days, flasks and bags were shaken daily to provide uniform colonization of grains. The wheat bran colonized by spores of *F. culmorum* isolate was air dried under aseptic conditions before use. Spore suspensions were made by putting enough amount of wheat bran colonized by spores of the isolate in sterile distilled water, mixed well to let the spores become suspensed in water, filtered using several layers of cheesecloth and the concentration adjusted to $1x10^6$ spores/ml after counting spore number using a haemocytometer. The amount of suspension was adjusted according to the number of seedlings inoculated. One percent NaOCl solution was used for 3 min for surface disinfestation of wheat seeds. Surface disinfested seeds were rinsed twice in sterile distilled water. Seeds were then placed in Petri dishes with a stack of filter paper saturated with sterile distilled water and kept in an incubator at a temperature of 23 ºC for 3-4 days for germination.

Single pre-germinated seed was placed in each plastic tube (2.5 cm in diam. x 16 cm in length) containing 55 g of sterile potting mixture of sand: soil: organic matter (50:40:10, v/v/v), covered with thin layers of same soil mixture and moistened. Plants were then kept in a growth chamber at a condition of 16 h photoperiod under artificial light, 25/15($\pm$5) ºC day and night temperatures and relative humidity of 60/80 ($\pm$10)% (Mitter et al 2006). Plants were supplied with water whenever necessary. One week after planting, plants were inoculated with 1 mL of spore suspension ($1x10^6$ spores mL^{-1}) amended with 0.1% v/v Tween 20 on stem bases (~ 0.5 cm above the soil) (Mitter et al 2006) using an aseptic pipette. Nine wheat cultivars (Table 1) were used as controls. The control cultivars were inoculated with the same amount and concentration of spore suspension. Each treatment (each wheat germplasm) was replicated 5 times. Treatments were arranged in Randomized Complete Block Design (RCBD) and plants were covered with plastic for 48 h to maintain high humidity and darkness required for fungal incubation (Mitter et al 2006). Plants were then placed at the same light, temperature and humidity conditions mentioned above. Plants were provided with an appropriate amount of water every day for the duration of the experiment. The experiment was repeated.

Nine weeks after inoculation, soil was washed off plants and leaf sheaths were removed. Scoring for the typical symptoms of browning on the crown and the main stem base was carried out using a 1-5 scale (1: 1-9%, 2: 10-29%, 3: 30-69%, 4:70-89%, 5: 90-99%) modified from Wildermuth & McNamara (1994) according to Nicol et al (2001). Scale values were square root transformed and data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure of SPSS (IBM SPSS Statistics 21) and means were compared using Tukey's HSD test (P= 0.05).

Table 1- Wheat genotypes used as controls in the screening experiment

Wheat genotype	*Accession no*	*CID*[1]	*Reaction*[2]	*Wheat type*[3]	*Sources*[4]
Adana-99			MS	SW	TK
Altay-2000	010627		MR/MS	WW	TK
Carisma			MR	WW	IT
Suntop		200000963	MR	SW	AUS
Emu Rock		200000805	MS	SW	AUS
Janz	960370	4982215	MS	WW	AUS
Seri-82	951027		S	SW	MX
Kutluk-94	950660		S	WW	TK
Süzen-97	950283		S	WW	TK

[1]CID, Cross Identification; [2]MS, moderately susceptible; MR, moderately resistant; MS, moderately susceptible; S, susceptible
[3]SW, spring wheat; WW, winter wheat; [4]TK, Turkey; IT, Italy; AUS, Australia; MX, Mexico

3. Results and Discussion

Based on the result of aggressiveness tests, the most aggressive *F. culmorum* isolate Fc2 was used to screen wheat germplasm for their reaction (Gebremariam 2015).

The mean disease severity scores for the lines tested ranged from 1.4 to 4.4 with an average of 3.1 (Table 2). Two lines out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and had scores of 1.4. Twenty lines (lines 5, 100, 143, 163, 32, 138, 86, 89, 104, 123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) showed a moderately resistant (MR) reaction and had scores ranging from 1.6 to 2.4. The scores of both the R and MR lines were not significantly different from scores of MR control cultivars Suntop (1.6), Carisma (1.8) and Altay-2000 (2.4). Sixty-three percent of the lines were moderately susceptible (MS). The scores of MS lines ranged from 2.6 to 3.4 which were not significantly different from the MS control cultivars Adana-99 (2.6), Janz (2.6) and Emu Rock (2.6). Out of the 165 lines tested, 39 were susceptible (S) in their reaction. These S lines had scores ranging from 3.6 to 4.4 which were not significantly different from the score of the S control cultivars Süzen-97 (3.6) and Kutluk-94 (4.0).

Table 2- Mean disease severity scores and reactions of wheat lines and control cultivars tested against *Fusarium culmorum* isolate Fc2

Germplasm[a]	*Score*[1]	*Reaction*[2,3]
158, 147	1.4[a*]	R
5, 100, 143	1.6 [ab]	MR
Suntop	1.6 [ab]	MR
163, 32, 138	1.8 [abc]	MR
Carisma	1.8 [abc]	MR
86, 89, 104, 123, 153, 161	2.0 [abcd]	MR
8, 34, 142	2.2 [abcde]	MR
9, 15, 47, 116, 146	2.4 [abcdef]	MR
Altay-2000	2.4 [abcdef]	MR
33, 54, 57, 76, 78, 87, 115, 120, 135, 137, 156, 157, 169	2.6 [abcdefg]	MS
Adana-99	2.6 [abcdefg]	MS
Janz	2.6 [abcdefg]	MS
Emu Rock	2.6 [abcdefg]	MS
67	2.8 [bcdefgh]	MS
22, 28, 30, 43, 75, 82, 85, 93, 95, 102, 103, 109, 114, 117, 118, 124, 127, 130, 148, 152, 155, 159, 160, 165, 168	3.0 [cdefghi]	MS
Seri-82	3.0 [cdefghi]	MS
12, 13, 16, 21, 40, 41, 44, 61, 65, 66, 74, 79, 80, 88, 96, 105, 111, 112, 119, 121, 122, 129, 132, 134, 139, 145, 154,	3.2 [defghij]	MS
2, 4, 10, 14, 17, 18, 23, 24, 26, 27, 29, 31, 35, 45, 46, 48, 55, 56, 63 ,64, 77, 91, 94, 97, 98, 101, 107, 108, 113, 125, 126, 133, 141, 144, 149, 151, 162, 166,	3.4 [efghij]	MS
3, 68, 71, 73, 128, 136, 19, 25, 36, 42, 51, 52, 58, 62, 84, 92, 99, 106, 110, 164, 37, 39, 49, 60, 70, 72, 81, 131	3.6 [fghij]	S
Süzen-97	3.6 [fghij]	S
49, 60, 70, 72, 81, 131	3.8 [ghij]	S
6, 20, 38, 69, 83, 150, 167	4.0 [hij]	S
Kutluk-94	4.0 [hij]	S
7,11,53	4.2 [ij]	S
59	4.4 [j]	S

*, values that share a letter are not significantly different at 0.05 level, according to Tukey's HSD test; [1], score of each germplasm is the mean of five replicates; [2], R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; [3], score ranges for corresponding reaction R=1-1.4, MR= 1.5-2.4, MS= 2.5-3.4, S= 3.5-4.4 and HS= 4.5-5, [a] , Adana-99. Altay-2000, Seri-82, Kutluk-94, Süzen 97, Carisma, Janz, Emu Rock and Suntop are control cultivars

Screening for resistance to Fusarium crown rot of wheat was started in Australia in 1960s (McKnight & Hart 1966; Purss 1966). Although most wheat varieties are susceptible, partial resistance to crown rot occurs. Screening for resistance and susceptibility can be carried out using seedling and adult plant tests and positive correlations between crown rot ratings in greenhouse and field trials have been documented (Klein et al 1985; Wildermuth & McNamara 1994; Mitter et al 2006; Li et al 2008). Therefore, the seedling

bioassay which is time saving and avoids effects of other seasonal or environmental factors can be used to screen large quantities of germplasms rapidly and promising materials can be taken to field testing. Wheat varieties vary in their reaction to crown rot, ranging from very susceptible to moderately resistant (Wallwork 2000). However, there are no fully resistant wheat cultivars to this disease (Pereyra et al 2004; Wisniewska & Kowalczyk 2005). The genotypes tested in our study showed differences in reaction ranging from resistant (R) to susceptible (S) to *F. culmorum* isolate Fc2. Two lines (147, 158) were resistant in their reaction and had scores of 1.4. Thirteen percent of the lines tested showed consistently resistant/ moderately resistant (R/MR) reaction to *F. culmorum* isolate Fc2. Differences in reaction ranging from moderately resistant (MR) to susceptible (S) in wheat genotypes against *F. culmorum* have also been reported from Turkey (Demirci 2003). The search for resistance initially should focus upon only one species and expand later to include other species (Paulitz et al 2002; Miedaner et al 2012). For crown rot, a high correlation between the resistance to *F. graminearum* and *F. culmorum* in wheat and rye has been documented (Miedaner 1997). Therefore, the wheat lines that showed some degree of resistance to *F. culmorum* in our research can serve as useful sources of genetic resistance in breeding for *F. culmorum* in particular or can be expanded and used to search for resistance to other *Fusarium* species. These lines can also be used to reduce yield losses due to Fusarium crown rot and carryover of inoculum to the subsequent years.

The lines that showed consistent resistant/moderatly resistant (R/MR) reactions can serve as useful sources of genetic resistance in breeding for Fusarium crown rot. Plant breeders attempting to incorporate resistance to crown rot into cereal crops in Turkey should focus on screening with *F. culmorum* isolates.

In areas where the damaging *F. culmorum* is prevalent, integrated management options should include crop rotation with at least 2 years break from wheat, use of varieties showing some degree of resistance to the disease, selecting proper nitrogen fertilization rates and irrigation management to maintain continuous moisture throughout the growing season.

4. Conclusions

Fusarium crown rot incited by *Fusarium culmorum* is one of the most important diseases limiting wheat yields. In this study, seedling reactions of 165 spring wheat breeding lines (*Triticum aestivum* L.) obtained from CIMMYT, Mexico were determined under growth room conditions using an aggressive isolate of *Fusarium culmorum.* Two out of the 165 lines tested (lines 147 and 158) were resistant (R) in their reaction and twenty lines (lines 5, 100, 143, 163, 32, 138, 86, 89, 104, 123, 153, 161, 8, 34, 142, 9, 15, 47, 116, 146) showed moderately resistant (MR) reaction. These promising wheat lines that showed some degree of resistance to *Fusarium culmorum* can serve as useful sources of genetic resistance in breeding for Fusarium crown rot.

References

Aktaş H, Kınacı E, Yıldırım, A F, Sayın L & Kural A (1999). Konya yöresinde hububatta sorun olan kök ve kök boğazı çürüklüğü etmenlerinin hububatta verim komponentlerine etkileri ve mücadelesi üzerinde araştırmalar. In: H Ekiz (Ed.), *Orta Anadolu'da Hububat Tarımının Sorunları ve Çözüm Yolları Sempozyumu*. 8-11 Haziran 1999, Konya, Turkey, pp. 392-403

Anonymous (2014). Website: F.A.O. http://faostat.fao.org, Accessed online on October, 12, 2014

Bockus W W, Bowden R L, Hunger R M, Morrill W L, Murray T D & Smiley R W (Eds.). (2010). Compendium of wheat diseases and pests. 3rd edition. The American Phytopthological Society Press, St. Paul, Minnesota

Braun H J, Zencirci N, Altay F, Atli A, Avci M, Eser V, Kambertay M & Payne T S (2001). Turkish wheat pool. In: A. P. Bonjean & W. P. Angus, (Eds.), *World Wheat Book - A History of Wheat Breeding*. Lavoisier Publishing, Paris, pp. 851-879

Burgess L W, Backhouse D, Summerell B A & Swan L J (2001). Crown rot of wheat. In: B. A. Summerell, J. F. Leslie, D. Backhouse, W. L. Bryden & L. W. Burgess, (Eds.), *Fusarium - Paul E. Nelson Memorial Symposium* (November 1997: Pennsylvania State University). American Phytopathological Society Press, St. Paul, Minnesota, pp. 271-295

Burgess L W, Bentley A R & Wallwork H (2010). Crown rot of wheat. In: J M Nicol, A R Bentley, P J Ferrar. (Eds.), *Soilborne pathogens of wheat: Their biology, economic importance and integrated control.* 4th International master class in soilborne pathogens of wheat. Anadolu Agricultural Research Institute, Eskişehir, Turkey, pp. 45-50

Chakraborty S, Liu C J, Mitter V, Scott J B, Akinsanmi O A, Ali S, Dill-Macky R, Nicol J, Backhouse D & Simpfendorfer, S (2006). Pathogen population structure and epidemiology are keys to wheat crown rot and Fusarium head blight management. *Australian Plant Pathology* 35: 643-655

Cook R J (1968). Fusarium root and foot rot of cereals in the Pacific Northwest. *Phytopathology* 58: 127-131

Cook R J (1981). Fusarium diseases of wheat and other small grain in North America. In: P. E. Nelson, T. A. Toussoun, & R. J. Cook (Eds.), *Fusarium*: Diseases, Biology and Taxonomy. Pennsylvania State University, Pennsylvania, pp. 39-52

Cook R J (1992). Wheat root health management and environmental concerns. *Canadian Journal of Plant Pathology* 14: 76-85

Cook R J (2001). Management of wheat and barley root diseases in modern farming systems. *Australasian Plant Pathology* 30(2): 119-126

Curtis B C (2002). Wheat in the World. In: B C Curtis., S Rajaram & H Gomez Macpherson (Eds.),. *Bread wheat improvement and production*. F.A.O., Rome, pp. 1-17

Demirci F (2003). Bazı buğday çeşitlerinin önemli kök ve kök boğazı hastalık etmenleri (*Fusarium* spp., *Bipolaris sorokiniana*)'ne karşı reaksiyonlarının belirlenmesi. *Tarım Bilimleri Dergisi* 9(4): 460-466

Gebremariam E S (2015). Determination of *Fusarium* species associated with crown rot of wheat in Turkey and assessment of resistance status of some wheat genotypes to *Fusarium culmorum*. Ph. D. thesis. Ankara University, Graduate School of Natural and Applied Sciences. Ankara, Turkey

Gebremariam E S, Sharma-Poudyal D, Paulitz T C, Erginbas-Orakci G, Karakaya A & Dababat A A (2018a). Identity and pathogenicity of *Fusarium* species associated with crown rot on wheat (*Triticum* spp.) in Turkey. *European Journal of Plant Pathology* 150(2): 387-399

Gebremariam E S, Karakaya A, Erginbas-Orakci G & Dababat A A (2018b). Assessment of the seedling resistance of some spring wheat lines to *Fusarium culmorum*. *ICAPS 2018 International Conference on Advances in Plant Sciences*. April 25-27, 2018. Sarajevo, Bosnia and Herzegovina

Geçit H H, Çiftçi C Y, Emeklier Y, Ikincikarakaya S, Adak M S, Kolsarıcı Ö, Ekiz H, Altınok S, Sancak C, Sevimay C S & Kendir H (2009). Tarla Bitkileri. Ankara Üniversitesi Ziraat Fakültesi Yayınları. Yayın No: 1569. Ders Kitabı: 521

Hanson H, Borlaug N E & Anderson R G (1982). Wheat in the third world. Wetview Press, Colorado

Hekimhan H, Bağcı A, Nicol J M, Arısoy Z, Taner S & Şahin S (2004). Dryland root rot: A major threat to winter cereal production under sub-optimal growing conditions. *4th Internation Crop Science Congress*, September 26 - October 1 2004. Brisbane, Australia, pp. 283

Klein T A, Liddell C M, Burgess L W & Ellison F W (1985). Glasshouse testing for tolerance of wheat to crown rot caused by *Fusarium graminearum* group 1. In: C A Parker, A D Rovira, K J Moore, & P T W Wong (Eds.), *Ecology and Management of Soilborne Plant Pathogens*. American Phytopathological Society Press, St. Paul, Minnesota, pp. 167-168

Klein T A, Burgess L W & Ellison F W (1991). The influence and spatial patterns of wheat plants infected by *Fusarium graminearum* group 1 and the effect of crown rot on yield. *Australian Journal of Agricultural Research* 42: 399-407

Li Y, Liu C, Chakraborty S, Manners J & Kazan K (2008). A simple method for the assessment of crown rot disease severity in wheat seedlings inoculated with *Fusarium pseudograminearum*. *Journal of Phytopathology* 156: 751-754

McKnight T & Hart J (1966). Some field observations on crown rot disease of wheat caused by *Fusarium graminearum*. *Queensland Journal of Agricultural and Animal Sciences* 23: 373-378

Miedaner T (1997). Breeding wheat and rye for resistance to Fusarium diseases. *Plant Breeding* 116(3): 201-220

Miedaner T, Risser P, Paillard S, Schnurbusch T, Keller B, Hartl L, Holzapfel J, Korzun V, Ebmeyer E & Utz H F (2012). Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations. *Molecular Breeding* 29: 731-742

Mishra C B P (1973). Untersuchungen über Fusarium-Arten an Weizenkaryopsen und Nachweis ihrer Pathogenität als Fußkrankheitserreger. *Archives of Phytopathology and Plant Protection* 9(2): 123-132

Mitter V, Zhang M C, Liu C J, Ghosh R, Ghosh M & Chakraborty S (2006). A high-throughput greenhouse bioassay to detect crown rot resistance in wheat germplasm. *Plant Pathology* 55: 433-441

Nicol J M, Rivoal R, Trethowan R M, van Ginkel M, Mergoum M & Singh R P (2001). CIMMYT's approach to identify and use resistance to nematodes and soilborne fungi, in developing superior wheat germplasm. In: Z Bedo & L Lang (Eds.), Wheat in a Global Environment. *Proceedings of the 6th International Wheat Conference*, 5-9th June 2000, Budapest, Hungary, pp. 381-389

Paulitz T C, Smiley R W & Cook R J (2002). Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Canadian Journal of Plant Pathology* 24: 416-428

Pereyra S A, Dill-Macky R & Sims A L (2004). Survival and inoculum production of *Gibberella zeae* in wheat residue. *Plant Disease* 88: 724-730

Purss G S (1966). Studies of varietal resistance to crown rot of wheat caused by *Fusarium graminearum* Schw. *Queensland Journal of Agricultural and Animal Sciences* 23: 475-498

Schilling A G, Moller E M & Geiger H H (1996). Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum, F. graminearum,* and *F. avenaceum*. *Phytopathology* 86: 515-522

Shewry P R (2009). Wheat. *Journal of Experimental Botany* 60(6): 1537-1553

Singleton L L (2002). Diseases of roots and rowns. In: B C Curtis, S Rajaram & H Gomez Macpherson, H. (Eds.), *Bread wheat İmprovement and Production*. F.A.O., Rome

Smiley R W & Patterson L M (1996). Pathogenic fungi associated with Fusarium foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Disease* 80: 944-949

Smiley R W, Gourlie J A, Easley S A, Patterson L M & Whittaker R G (2005). Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease* 89: 595-604

Tunali B, Nicol J M, Erol F Y & Altiparmak G (2006). Pathogenicity of Turkish crown and head scab isolates on stem bases on winter wheat under greenhouse conditions. *Journal of Plant Pathology* 5: 143-149

Wallwork H (2000). Cereal root and crown diseases. Grains Research and Development Corporation, Adelaide, Australia.

Wildermuth G B & McNamara R B (1994). Testing wheat seedlings for resistance to crown rot caused by *Fusarium graminearum* Group 1. *Plant Disease* 78: 949-953

Wisniewska H & Kowalczyk K (2005). Resistance of cultivars and breeding lines of spring wheat to *Fusarium culmorum* and powdery mildew. *Journal of Applied Genetics* 46: 35-40

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

Meta-analysis of the Effects of Salinity Stress on Cotton (*Gossypium* spp.) Growth and Yield in Iran

Azadeh RAZAJI[a], Farzad PAKNEJAD[b], Maryam MOAREFI[c], Abdolmajid MAHDAVI DAMGHANI[d], Mohammad NABI ILKAEE[e]

[a]*Student of Phd, Faculty of Agriculture, Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, IRAN*

[b]*Professor, Faculty of Agriculture, Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, IRAN*

[c]*Assistant Professor, Faculty of Agriculture, Department of Plant breeding, Karaj Branch, Islamic Azad University, Karaj, IRAN*

[d]*Associate Professor of Agro-ecology, Environmental Sciences Research Institute, Department of Agr-oecology, Tehran, IRAN*

[e]*Associate Professor, Faculty of Agriculture, Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, IRAN*

ARTICLE INFO
Research Article
Corresponding Author: Farzad PAKNEJAD, E-mail: farzadpaknejad@yahoo.com, Tel: +98 (0263) 441 81 43
Received: 28 November 2018, Received in Revised Form: 05 January 2019, Accepted: 31 January 2019

AUTHORS ORCID ID
(Azadeh RAZAJI: 0000-0001-9128-0631), (Farzad PAKNEJAD: 0000-0003-0951-2072), (Maryam MOAREFI: 0000-0003-2726-3508), (Abdolmajid MAHDAVI DAMGHANI: 0000-0003-4280-7200), (Mohammad NABI ILKAEE: 0000-0002-1374-9462)

ABSTRACT
Research on the impact of inputs has a long history in the country; however, because of the dispersion and diversity in the results of various experiments, it is difficult to achieve a general conclusion. In this study, a meta-analysis approach has been used to overcome this problem in order to achieve a single result by integrating and re-analyzing the findings of independent experiments. For this purpose, scientific articles published by researchers of the country regarding the effect of salinity stress on cotton yield were investigated. Articles were selected so that, in their meta-analysis of data required to perform there. The data necessary for the implementation of the meta-analysis is present in them. The results of this study showed that from 1996 to 2017; 15 papers and thesis were published on the effects of salinity stress on yield, early maturing and number of bolls in upland cotton (*Gossypiumhirsutum* L.). With increasing salinity stress, yield and number of bolls decreased. The standardized values for salinity stress effect on cotton yield were significant in all five treatments (control via salinity, control via 2-3 dS m^{-1}, control via 4-5 dS m^{-1}, control via 6-7 dS m^{-1}, control via 8-9 dS m^{-1}) compared to control ($P<0.001$). The standardized values of the effect of salt stress on cotton aging were significant in all five treatments compared to control. The standardized values of the effect of salt stress on cotton aging were significant in all five treatments compared to control. Results showed increasing salinity stress can cause early maturing in cotton. The standardized values for the effect of salinity stress on number of bolls per cotton plant, in the comparison of the total treatments of salinity stress, 2-3 dS m^{-1}, 6-7 dS m^{-1} and 8-9 dS m^{-1} were significant compared to control ($P<0.001$). In general, the results of this study showed that salinity stress can reduce yield of cotton by reducing the number of bolls and also can cause early maturing on cotton.

Keywords: Upland cotton (*Gossypiumhirsutum* L.); Yield, early maturing; Salinity; Meta-analysis

1. Introduction

Upland cotton (*Gossypiumhirsutum* L.) plays an important role in supplying food (oil and protein), human

DOI: 10.15832/ankutbd.489187

fiber and livestock feed, and the need for cotton fabrics is constantly increasing in the world. It is the most important natural fiber of the world as a product of agriculture, industry and commerce, and now it accounts for 55% of the total natural fiber production of the world. Cotton seed oil is one of the best-quality vegetable oils. Cotton seeds are the second largest oilseed in the world after soybeans (Ahmadi & Aghaalikhani 2012).

Cotton seed meal has 33 to 43 percent protein and is used as a protein supplement in the ration of the animal. In the cottony regions of Iran, Golestan province has a long history of cultivating this plant and due to its climatic conditions, the province has long been specialized in the production of cotton. For this reason, once known as the cotton capital and white golden land. Cotton produced in this province is considered one of the best-quality cotton with medium-long fibers in the world due to high effective fiber length, microny or Suitable elegance, high strength, elasticity and uniformity (Ahmadi & Aghaalikhani 2012).

Numerous studies in the past have been carried out on various fields of agricultural sciences. For example, a lot of articles and research can be found on the effects of planting dates, density, environmental stresses, various fertilizers and other items. Although each research has a special value separately, but it is necessary to examine the results of this research in conjunction with each other to arrive at a conclusion about the factors affecting the yield of crops. For example, the effect of seed size on germination percentage has different results, some suggesting that smaller size seeds have a higher germination or emergence percentage (Stamp 1990). Some studies have found that the larger size seeds have a higher percentage (Baloch et al 2001; Cordazzo 2002). Cordazzo also, in some reports, it has been shown that seed size does not affect the percentage of germination (Bretagnolle et al 1995; Guillemin & Chauvel 2011). If a researcher wants to review all these studies, it will be difficult to come up with a conclusion that larger seeds are better or smaller. Because the contradictory results are obtained, and each has sufficient scientific credibility. On the other hand, it is rarely possible for two identical studies to reach the same results. If the result of this compilation is the result of a statistical analysis of a large number of different papers and researches, statistically, it can be said that what the results was obtained in this series of studies. In this way, statistical analysis is called meta-analysis. The term meta-analysis was first used by Glass (1976) at the American Educational Research Association. The meta-analysis objective is to obtain more information from existing information that is achieved by overlapping the results of smaller studies and with one or more statistical analyzes. Thus, results that may not be discovered in smaller studies can be obtained by using meta-analysis of dozens of small studies. The need to summarize various researches has already been taken into consideration. To this end, some researchers are reviewing articles in which specific case studies have been carried out and are trying to summarize the effect. However, in most of them, there is no statistical method for reviewing and summarizing the results of research (for example, Matthews et al 2012; Taylor & Salanenka 2012). Recently, a handful of researchers in agronomic science have used a meta-analysis method to compare the results of various researches (Wang et al 2013). However, searching on various internal sites such as sid, Magiran and Google showed that no research papers in agricultural fields have been used by this statistical method in Iran, and this method has been used only in the sciences and social sciences. The purpose of this study was to introduce a meta-analysis method for reviewing various agronomic studies and increasing appetite and knowledge in agronomic sciences.

Due to the lack of water resources in the country, identifying appropriate management and agronomic approaches is essential for the use of lower quality water (salty and brackish). Many areas of the country that are dedicated to cotton cultivation face the problem of soil water and soil salinity in these areas, salt-resistant products such as cotton are cultivated. Hanson et al (2006) examined the drip irrigation system with salt water on cotton. With the application of 314 to 473 mm of water during the growth period, seed yield was changed from 3.51 to 3.63 t ha^{-1} and its yield was 1.11 to 1.19 t ha^{-1}. Simsek et al (2004) Due to the problems caused by the use of salt water and consequently land saltiness, it is necessary to use drip irrigation for cotton cultivation in the near future. Yazar et al (2002) reported that using traditional irrigation systems for cotton cultivation caused irrigation water losses, reduced water use efficiency, and increased salinity and drainage problems. He also believes that salt and water stress during the growing season will reduce the production of the boll and the fall of the bolls, which reduces yields. Yazar et al (2002) results showed that cotton is more sensitive to salinity in early stages of growth and flowering compared to the rest of the growth stages. The height of the plant was affected by salinity and the dry weight of the seeds was

reduced by application of saline water, so that the dry weight of the seeds in soil salinities 7.7 and 12.5 dS m^{-1}, compared with 2 dS m^{-1} decreased by 52% and 84%, respectively. Therefore, the purpose of this research is meta-analysis of the effects of salinity stress on growth and yield of cotton in Iran.

2. Material and Methods

In this study, the effects of salinity stress on early maturing, yield, and number of bolls and height of cotton in Iran were evaluated and articles related to the subject of the research were collected. After collecting data, it is typically determined in the scale of the effect size (Gurevitch & Hedges 1999), and for the purpose of comparison, the confidence intervals are determined around the averages or slopes. The full description of the method of statistical computations of the meta-analysis has been presented by Gurevitch & Hedges (1999). Further steps are briefly described. The first step in the implementation of the meta-analysis is calculated of the standard difference between the mean of the control treatment and the experimental treatments, which is called the effect of 1 (d). Thus, for each of the 46 independent experiments investigated in this meta-analysis, a value of d is computed (Equation 1). It should be noted that the effect size was calculated separately for both fertilizer levels and for each fertilizer level.

$$d = \frac{\overline{X}_t - \overline{X}_c}{S_P} \times J \tag{1}$$

In which, respectively, the mean of control and fertilizer treatments, the S_p is standard deviation of the combined mean and J correctional points for the bias of the standard deviations of the mean values. The values of J and S_p are calculated from equations 2 and 3 respectively:

$$J = 1 - \left[\frac{3}{4(df_c + df_t) - 1} \right] \tag{2}$$

$$S_p = \sqrt{\frac{df_c(S_c^{\,2}) + df_t(S_t^{\,2})}{df_c + df_t}} \tag{3}$$

In which S_c and S_t respectively, the standard deviation of the mean of control and fertilizer treatment, df_c and df_t respectively, are the degree of freedom of control and fertilizer treatment. If the values of the standard deviations of the meanings are not mentioned in the article, we can estimate the value of Sp based on the error of the test (MSE) variance in the articles presented in Equation 4:

$$S_p = \sqrt{\left(\frac{n_c + n_t - 2}{n_c + n_t} \right) MSE} \tag{4}$$

Where; n_c and n_t are the number of replicas of control and treatment respectively.

Undoubtedly, all the tests under investigation do not have the same precision. Therefore, it is necessary to calculate for each experiment proportional to its accuracy, and then the amount of the effect of each experiment can be adjusted to it. To do this, first, the variance of the effect size for each experiment (V_d) is calculated (Equation 5):

$$V_d = \left[\frac{n_c + n_t}{n_c \times n_t} \right] + \left[\frac{d^2}{2n(n_c + n_t)} \right] \tag{5}$$

The contrary of this variance is weight of that test, so any test with a smaller variance will have more weight: Finally, a total or aggregate effect (d^*) is calculated, which in fact is the standardized difference between control and fertilizer treatments for all under consideration experiments (Equation 6):

$$d^* = \frac{\sum w_i d_i}{\sum w_i} \tag{6}$$

And its standard deviation (S_{d*}) will also be obtained from Equation 7:

$$S_{d*} = \sqrt{\frac{1}{\sum w_i}} \tag{7}$$

The last step of the meta-analysis is the significance test d^*, with the definiteness of S_{d*}, we can calculate the confidence interval d^*. If this confidence interval is overlapping with zero, the size of the cumulative cohesive effect (d^*) is meaningless and the control Treatment is different from other treatments, otherwise the difference in treatment from control is significantly greater than zero. All calculations and graphs were done in Excel.

3. Results and Discussion

3.1. Summary of the results of the experiments under investigation on salinity stress

The results of this study showed that from 1996 to 2017, 15 papers and theses were published on the effects of salinity stress on yield, earliness and number of bolls in cotton. Of these, 15 papers have been the subject of treatment. 13 papers with 2-3 dS m^{-1} treatment, 12 papers with 5 dS m^{-1} treatment, 11 papers with a treatment of 6-7 dS m^{-1}, and 11 papers with a treatment of 8-9 dS m^{-1} (Figure 1). With increasing Intensity of salinity stress, the yield (Figure 2) and number of bolls (Figure 4) decreased. Seed yield was 3008 kg ha^{-1} in control treatment, which was statistically significant with salinity stress of 8-9 dS m^{-1}. Early maturing in control treatment was obtained 70.8 days after planting. This difference was statistically significant with salinity stress treatment of 8-9 dS m^{-1} (Figure 3). The number of bolls in the control treatment was 21.41, which was statistically significant with salinity stress of 8-9 dS m^{-1}. The results showed that in all cases, the effect of treatments with stress was less than control. The lowest yield was obtained in treatment of 8-9 dS m^{-1}. The shortest processing time was obtained in treatment of 8-9 dS m^{-1}. The results showed that in all cases, number of bolls per plant was lower in stress plants than control. The lowest number of bolls per plant was obtained at 8-9 dS m^{-1} treatment.

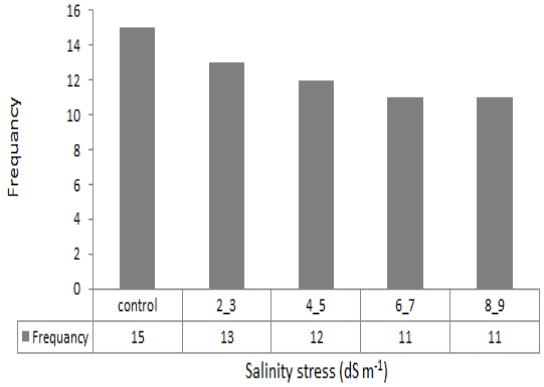


Figure 1- Frequently distribution of treatments in terms of salinity stress

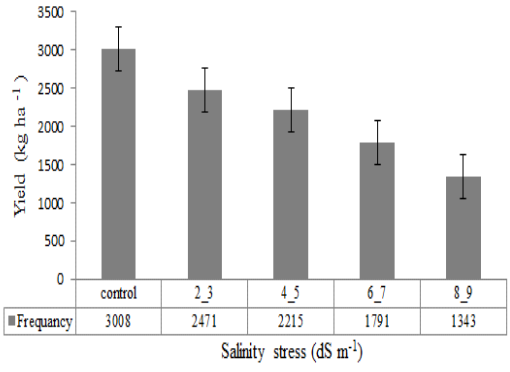


Figure 2- Seed yields of treatments in terms of salinity stress

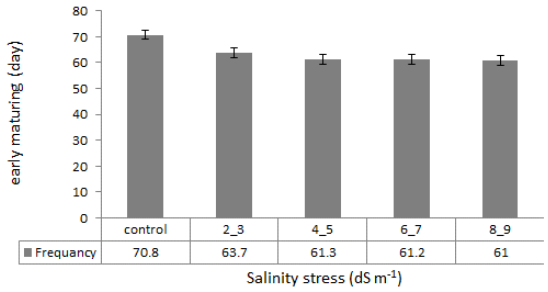


Figure 3- Early maturing in terms of salinity stress stress

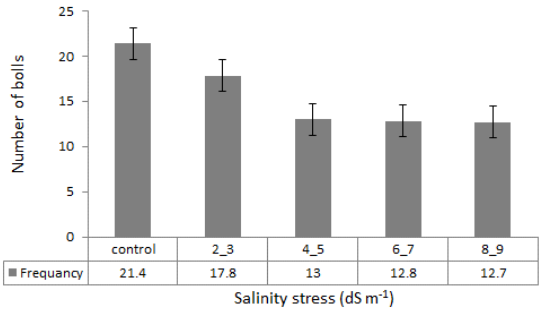


Figure 4- Number of bolls in terms of salinity

3.2. Statistical comparison between salinity stress levels

Effect size values for each of the four traits under study were normal distribution, and the normal distribution of this distribution is a prerequisite for continued meta-analysis. Gurevitch & Hedges (1999) stated that if the distribution of the magnitude of the effect size is not normal, then the logarithm of the difference between the mean of the control and the experimental treatments for the implementation of meta-analysis should be used. The size of the effect can indicate the extent of the dispersion and the difference between the data in different experiments. The magnitude of the effect shown in the plot of the effect size and accumulation graph is measured by the t test and, if it is meaningful, indicates that the difference between the different experiments was significant for a specific trait such as yield under a particular treatment, such as drought stress. On the other hand, if the standard deviation, which is shown in the plot of the effect size and accumulation graph and funnel graph, be higher, the probability of a significant trait such as yield under a treatment, such as drought stress, is reduced. In this study, in the plot of the effect size and accumulation, the size of the effect of the point and the standard deviation are shown respectively by the vertical and horizontal lines connected to the point respectively (half the standard deviation above the point and half the sub-point). Thevertical axis numbers in the accumulation graph represent the magnitude of the effect, as well as the difference between the vertical axis numbers representing the standard deviation. The standardized values for salinity stress effect on cotton yield were significant in all five treatments compared to control (P<0.001). It should be noted that the effect size for each trait is the difference between mean salinity of the mean of control treatment (without stress), so the positive values indicate that the average treatment with salinity stress is higher than the control treatment (Figure 5).

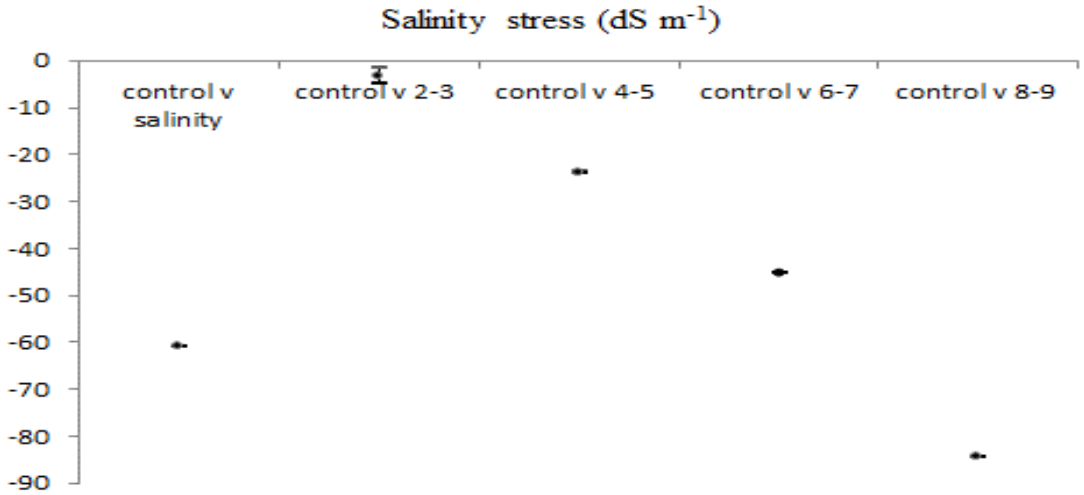


Figure 5- Comparison of the effects of different levels of salinity stress on performance. Vertical lines the confidence interval is the size of the combined effect of the stress test on the test. The first comparison is the control against the average of treatments with salinity stress

The standardized values for the effect of salt stress on early maturing of cotton were significant in all five treatments compared to control. The results showed that in all cases, early maturing tension treatments were less than control (Figure 6).

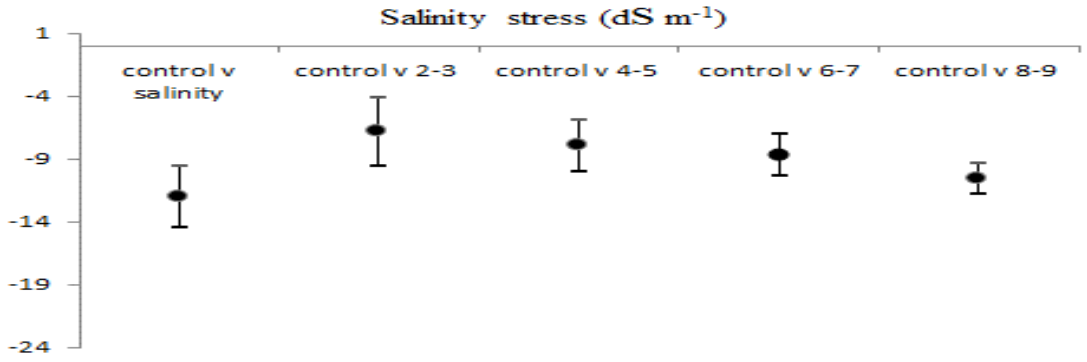


Figure 6- Comparison of the effects of different levels of salinity stress on prematurely. Vertical lines of the confidence interval of the size of the tuned combined effect among the trials is under investigation. The first comparison is the control against the average of treatments with salinity stress

The standardized values for the effect of salinity stress on number of bolls per cotton plant were significant in comparison to total salinity treatments, 2-3 dS m^{-1}, 6-7 dS m^{-1} and 8-9 dS m^{-1} with control ($P<0.001$) (Figure 7).

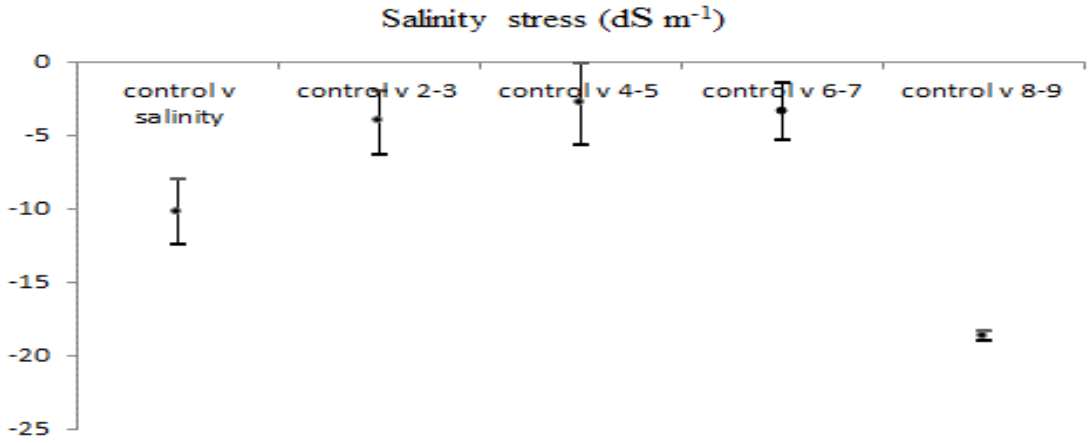


Figure 7- Comparison of the effects of different levels of salinity stress on number of bolls per plant. Vertical lines of the confidence interval of the size of the tuned combined effect among the trials is under investigation. The first comparison is the control against the average of treatments with salinity stress

3.3. Accumulation graph and funnel graph

Studies data are located on the vertical axis of the accumulation graph. In some cases, studies are sorted according to the year of publication, and in some cases, based on sample size. In any case, a better sorting criterion is the criterion for most uniformity. Studies are divided into two groups of meaningful and non-significant, which, if the treatments cut off the zero line, are not significant. If the treatments do not interrupt the zero line, this study is significant. Also, if meaningful research is done, the right side of the axis is zero, that is, drought stress will increase the desired trait if left side of it indicates that the trait was reduced to drought stress.

From the collected and reviewed research collections, Figure 8, has been obtained and its accumulation graph is related to the impact of cotton yield on the salinity stress as outlined below. Regarding the diagrams, among studies in which salinity stress reduced yield, one can refer to the study by Nikzadfar et al (2012) and Anagholi et al (2016). Among these papers, Nikzadfar et al (2012) have the highest weight and the lowest confidence intervals. As it is seen, the research used in this trait shows the effect of treatments very close to zero and with a low confidence interval. One of the high-weight researches is the paper by Nikzadfar et al (2012), Afrasiab et al (2015).

In the funnel graph, Figure 9 shows that the articles are in the 95% range and have a lower and more inferior bias, which is considered to be a positive result of the research. As shown in Figure 9, only 1 study with negative effect on the effect of salinity stress on the performance was not significant and was located outside the funnel.

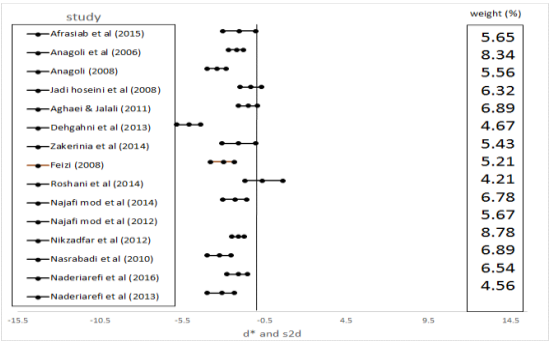


Figure 8- Accumulation graph, effect of yield on salinity stress

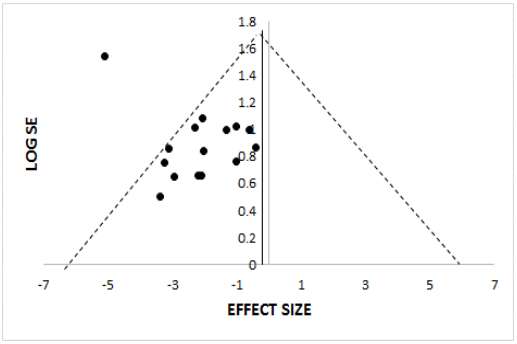


Figure 9- Funnel graph, effect of yield on salinity stress

From the collected and reviewed research collections, Figure 10, has been obtained and its accumulation graph relates to the study of the effect of cotton aging on the salinity stress described below. Regarding the charts, among which studies have shown that salinity stress aging can be reduced, one can refer to the study by Nikzadfar et al (2012) and Anagohli et al (2016). Between these articles, the article of Naderiarefi et al (2016), has the highest weight and the lowest confidence interval. As you can see, the research used in this trait shows the effect of treatments near zero. Among the high-weight researches, Najafi Mod & Colleagues (2014) are mentioned. On the funnel graph, graph 11, shows that the articles are in the 95% range, with a lower and more inefficient amount of paper, which is considered to be a positive result of the research. As it is estimated from Figure11, only 2 positive-for-effect paper have no significant effect on the effect of salinity stress on outbreak.

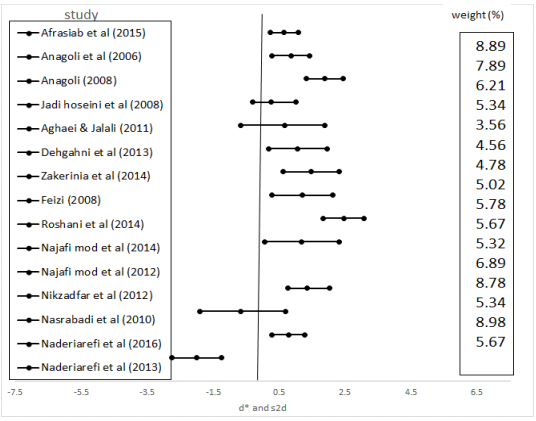


Figure 10- Accumulation graph, early maturing impact on salinity stress

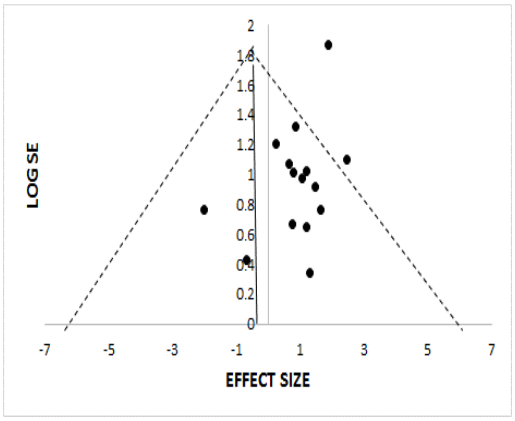


Figure 11- Funnel graph, early maturing impact on salinity stress

From the collected and reviewed research collections, Figure 12, has been obtained and its accumulation chart is related to the study of the impact of the number of cotton balls against the salinity stress that is plotted below. According to the diagrams, from studies in which salinity stress reduces the number of bolls, can be noted the study of Anagholi et al (2016). Among these articles, Afrasiab et al (2015) have the highest weight and minimum confidence intervals. As can be seen, the research used in this trait shows the effect of treatments near zero and with a low confidence interval. One of the high-weight researches is the Feizi's research (2008).

In the case of the funnel, Figure13 shows that the papers are in the range of 95%, the bias is less and its unbiased is more, which is considered to be a positive result of the research. As shown in Figure13, one negative and one positive study on the effect of salinity stress have not significant effect on number of bolls and have been out of the funnel.

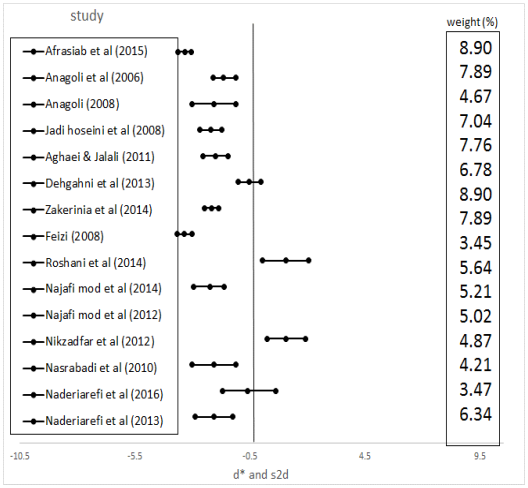


Figure 12- Accumulation graph, number of bolls affected by salinity stress

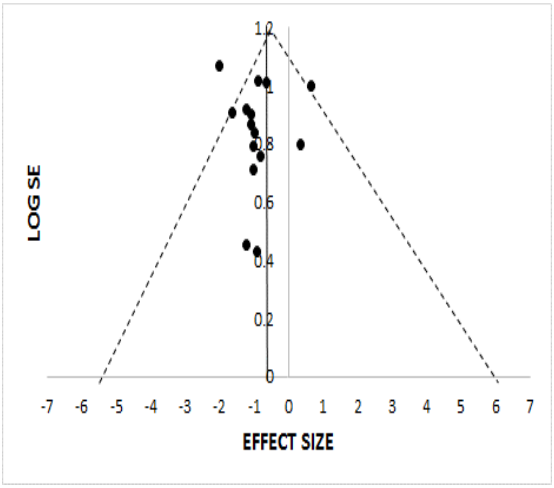


Figure 13- Funnel graph, number of bolls affected by salinity stress

Since plant growth has a close relationship with soil salinity of the root development zone, so, under irrigation water salinity conditions, the plant generates more energy to absorb water and food. In this condition, the plant components are affected by soil water salinity and due to salt tolerance, they have a severe decrease compared to conventional conditions. The set of these factors will reduce the height, reduce the number of bolls per plant, and ultimately reduce plant yield. On the other hand, salt stress can lead to premature production and, consequently, reduced yield (Afrasiab et al 2015). In case of increasing salinity of water relative to optimal conditions, reduction of yield components such as number of bolls per plant is significant, which, in turn, affects performance.

Product performance is affected by the amount of water salinity. So that whatever the salinity decreases, the yield loss is also reduced. The results from other studies show that By increasing salinity of irrigation water and causing osmotic pressure, plant root strength decreases to absorb the water in the root environment, therefore, the accumulation of solutes in the root zone causes less water and nutrient absorption by the plant (as compared to the proper irrigation water for salinity) In this situation, the yield of the plant is reduced Yang et al (2011).

4. Conclusions

In general, the results of this study showed that salinity stress can reduce yield of cotton by reducing the number of bolls and also can cause early maturing on cotton. The lowest and highest yield and number of bolls in cotton were observed respectively in 8-9 dS m^{-1} treatment and control. Also the shortest processing time was obtained in treatment of 8-9 dS m^{-1}. The result of meta-analysis indicated that salinity effects were

significant in reducing yield number of bolls and increasing early maturing in cotton. Regarding the diagrams, among studies in which salinity stress reduced yield, one can refer study by Nikzadfar et al (2012). Among these papers, Nikzadfar et al (2012) have the highest weight and the lowest confidence intervals. As it is seen, the research used in trait shows the effect of treatment very close to zero and with a low confidence interval. One the high-weight researches are the paper by Nikzadfar et al (2012), Afrasiab et al (2015). Regarding the charts, among which studies have shown that salinity stress can be reduced aging, one can refer to the Nikzadfar et al (2012) and Naderifar et al (2016), has the highest weight and lowest confidence interval. As you can see, the research used in this trait shows the effect of treatments near zero. Among the high-weight researches, Najafi & Colleagues (2014) are mentioned. According to the diagrams, from studies in which salinity stress reduces the number of bolls, can be noted the study of Anagholi et al (2016). Among these articles, Afrasiab et al (2015) have the highest weight and minimum confidence intervals. As can be seen, the research used in this trait shows the effect of treatments near zero and with a low confidence interval. One of the high-weight researches is the Feizi's research (2008). At end it is necessary to apply special programs to reduce the effect of salinity stress on cotton plant. On the other hand, given the growing trend of soil salinity in Iran, the sensitivity of this is more and more understandable.

References

Afrasiab P, Delbari M, Asadi R & Mohammadi E (2015). Effect of soil section and water salinity on yield and components yield of cotton. *Journal of Plant Production Research* 22(3): 295-311

Ahmadi M & Aghaalikhani M (2012). An analysis of energy consumption of cotton cultivation in Golestan province to offering strategy for increasing resources productivity. *Journal of Agro Ecology* 4(2): 151-158

Anagholi A, Rousta M J & Azari A (2016). Selection of salt tolerance varitieis of cotton by using of tolerant indices. *Arid Bio Scientific and Research Journal* 2: 1-9

Baloch H A, DiTommaso A & Watson A K (2001). Intrapopulation variation in Abutilon theophrasti seed mass and its relationship to seed germinability. *Seed Science Research* 11: 335-343

Bretagnolle F, Thompson J D & Lumaret R (1995). The influence of seed size variation on seed germination and seedling vigor in diploid and tetraploid DactylisglomerataL. *Annals of Botany* 76: 605-615

Cordazzo C V (2002). Effect of seed mass on germination and growth in three dominant species in southern Brazilian coastal dunes. Brazil. *Journal of Biology* 62: 427-435

Feizi S (2008). Watering irrigation management on soil salinity in a period of rotation. *Water Management and Irrigation Journal* 5(1): 11-25

Glass G V (1976). Primary, secondary and meta_analysis. Educational Research, 5. pp. 23-34

Guillemin J P & Chauvel B (2011). Effects of the seed weight and burial depth on the seed behavior of common ragweed (Ambrosia artemisiifolia). Weed Biology and Management 11: 217-223

Gurevitch J & Hedges L V (1999). Statistical issues in ecological meta-analysis. *Ecology* 80: 1142-1149

Hanson B R, Hutmacher R B & May D (2006). Drip irrigation of tomato and cotton under shallow saline ground water conditions. *Irrigation and Drainage Systems* 20: 155-177

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: S69-S73

Naderiarefi A, Ahmadi A, Tavakoli A R, Vafaietabar M A R & Sabikdast M (2016). The effect of issue stress on physiological characteristics of leaf and drought resistance of different cotton genotypes. *To Agricultural Crop Journal* 18(4): 987-999

Najafi Mod M H, Alizadeh A, Davari K, Kafi M & Shahidi A (2014). Study on the yield assesse indexes and determinate optimum depth irrigation for two cultivars of cotton on different level of water salinity. *Journal of Water and Soil* 26(4): 990-998

Nikzadfar M, Fathi D & Sohrabi B (2011). The effect of various level of water and nitrogen fertilizers on quality and quality of cotton. *First National Conference on Meterology and Water Management*, *Tehran University,* Fall; 20-21 Desember

Simsek M, Kakira M & Tonkaz T (2004). The effects of different drip irrigation regimes on water melonyield and yield components under semi-arid climatic conditions. *Journal of Agriculture Research* 55: 1149-1157

Stamp N E (1990). Production and effect of seed size in a grassland annual (Erodiumbrachycarpum, Geraniaceae). *American Journal of Botany* 77: 874-882

Taylor A G & Salanenka Y A (2012). Seed treatments: phytotoxicity amelioration and tracer uptake. *Seed Science Research* 22: S86-S90

Wang M, Wang Q & Zhang B (2013). Response of miRNA and their targets tosalt and drought stresses in cotton (Gossypiumhirsutum L.). *National Library of Medicine* 530(1): 26-32

Yang G, Tang H, Nie Y & Zhang H (2011). Response of cotton growth, yield and biomass to nitrogen split application. *European Journal of Agronomy*. pp. 34-45

Yazar A, Sezen S N & Severen S (2002). LEPA and trickle irrigation of cotton in the Southeastern Anatolian project (GAP) area in Turkey. *Agriculture Water Management* 54: 89-203

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

Influence of Toposequence on Physical and Mineralogical Properties of Soils Developed on Basaltic Parent Material under Sub-humid Terrestrial Ecosystem

Tülay TUNÇAY[a], Orhan DENGİZ[b], Ali İMAMOĞLU[c]

[a]*Soil Fertilizer and Water Resources Center Research Institute, Ankara, TURKEY*

[b]*Ondokuz Mayis University Agricultural Faculty Department of Soil Science and Plant Nutrition, Samsun, TURKEY*

[c]*Nevşehir Hacıbektaş Veli University, Faculty Art and Science, Geography Department, Nevşehir, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Orhan DENGİZ, E-mail: odengiz@omu.edu.tr, Tel: +90 (505) 438 33 53
Received: 19 December 2018, Received in Revised Form: 16 January 2019, Accepted: 11 February 2019

AUTHORS ORCID ID
(Tülay TUNÇAY: 0000-0002-5719-8391), (Orhan DENGİZ: 0000-0002-0458-6016), (Ali İMAMOĞLU: 0000-0001-9197-1029)

ABSTRACT
Understanding of soil forming factors is crucial to define physical, mineralogical and morphological properties of soils. In addition to that soil formations were highly associated with slope positions which have influence on pedogenetic and weathering process of the soils. Because, topography or relief affects how water and other material are added to and removed from soils. The aim of this study is to enhance our understanding of the role toposequence in controlling chemical weathering, influence on physical and mineralogical properties in soils developed on basaltic parent material under sub-humid terrestrial ecosystem in Black Sea region of Turkey using geochemical and mineralogical data obtained from X-ray diffraction and Scanning Electron Microscope analysis. For this purpose, four representative profiles formed on different topographic positions of transect were investigated and designated according to Soil Survey Staff (2014)/IUSS Working Group WRB (2015) classification systems. The results clearly showed that topography strongly affects soil physical, mineralogical and morphological characteristics either directly or indirectly in the local region even soils formed on the same parent material with the same climatic condition. This case was also explained with chemical weathering indices (Chemical Index of Alteration and Chemical Index of Weathering) in this study.

Keywords**:** Toposequence; Weathering indices; Soil formation; Clay minerals

1. Introduction

Understanding of soil forming factors (e.g. parent material, climate, topography, vegetation and time) is crucial to define physical, mineralogical and morphological properties of soils. It is well known that the chemical and mineralogical composition and the physical structure of the parent material set the initial conditions of the incoming soil (Jenny 1941; Voortman 2011). Rolling topography is also another factor influence soil formation due to erosional losses, and affecting the distribution of vegetation (Florinsky & Kuryakova 1996; Sebastiá 2004), provides climatic conditions (Grzyl et al 2014; Ridolfi et al 2008). In

DOI: 10.15832/ankutbd.499353

addition, topography or relief is the most important factor for soil formation affects how water and energy were added and/or lost from soil (Dengiz & Başkan 2010). Arnold (2006) indicated that a reference relief unit was a catchment or watershed area and the analysis of lateral transfers on, in and through the soils had to be considered to understand the functioning of the landscape units. The systems could be open or close relative to the flow of water and energy. Therefore, Moore et al (1992) reported that correlations between quantified terrain attributes and measured soil attributes toposequence can be assessed.

Moreover, mineral weathering, fundamental part of pedological evaluation, also provides an essential role by transforming bedrock to weathered rock and consequently to soil that supply nutrients to ecosystems (Berner et al 1983; White & Brantley 1995; Dixon et al 2009; Lybrand & Rasmussen 2018; Tunçay et al 2019). In this case, during weathering, the elements are leached and accumulated in different ways due to several pedogenic processes that influence different elements and produce different results. These processes include dissolution of primary minerals, formation of secondary minerals, redox reactions, transportation of material and ion exchange (Middleburg et al 1988; Dengiz 2010). Therefore, it is important to understand how a soil is formed from bedrock and to examine how chemical or physical weathering influences the geochemical evolution of soils. As basalts locate a large part of the earth surface weathering, basalt formed by the solidification of molten materials that originated within the earth is important soil material global weathering and carbon cycling which covered a large part of the earth surface (Price et al 2005; Braun et al 2009; Heckman & Rasmussen 2011). When basalts weather in subhumid condition, a rapid loss of cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and relative accumulation of Si^{4+}, Al^{3+} and Fe^{3+} that yield clay minerals develops (Eggleton et al 1987; Chorover et al 2004). Furthermore, many researchers indicated that the weathering of volcanic minerals is recognized to make a significant contribution to the global silicate cycle (Louvat & All`egre 1998; Dessert et al 2001; Kısakürek et al 2004), thus influencing carbon dioxide drawdown and climate control, since carbon dioxide is consumed particularly in Ca and Mg silicates weathering. For instance, on Deccan Traps in India, with an estimated area of 106 km^2, thought to account for 5% of the global silicate weathering flux (Gaillardet et al 1999). In total, basalt rocks may account for over 30% of the global carbon dioxide drawdown in silicate weathering (Dessert et al 2003).

There are many variables including geologic, climatic and topographic state that potentially affect chemical weathering rates in earth crust (Stallard & Edmond 1983; Grantham & Velbel 1988). Chemical weathering indices such as Chemical Index of Alteration (used for chemical weathering in sediment area) and Chemical Index of Weathering are used to define weathering profiles (Price & Verbal 2003). Changes in the weathering index with depth commonly are gradual or continuous, steady and systematic for homogeneous parent rocks (Sutton & Maynard 1992) reflecting continuous leaching of elements as weathering progresses on an initially homogenous parent material. Numerous studies have been conducted on determination the extent of weathering process, soil formation (Munroe et al 2006; Anderson et al 2007; Zhang et al 2007; Brantley 2008).

The purpose of this study is to further our understanding on the role toposequence on chemical weathering, along with its influence on physical and mineralogical properties in soils developed on basaltic parent material under sub humid terrestrial ecosystem in Black Sea region of Turkey using geochemical and mineralogical data obtained from X-ray diffraction (XRD) and Scanning Electron Microscope (SEM) analysis.

2. Material and Methods

2.1. Field description

The study was conducted throughout a transverse section between Bafra Plain and Canik Mountain located around 20 km west of Samsun Province in the central Black Sea region of Turkey (Figure 1), where is situated at coordinates 4597065 N – 253437 E and 4595005 N - 251693 E (UTM-36N/WGS84, m).

The study area extended from 10 m to 300 m a.m.s.l (above mean sea level) and contains four distinct landscape features (foot slope, back slope, lowland plateau, and shoulder) that represent the changes in geomorphology, topographical gradient, parent material and soil characteristics. The underlying bedrock is

primarily made up of Quaternary basaltic colluvial deposits on the foot slope and lowland plateau, and Mesozoic basalt and marl-limestone and on the back slope and highland plateau, respectively. The region is under semi-humid climate conditions in which summers are warmer than winters (mean monthly temperature in July is 22.2 ºC, and in January 6.9 ºC). The mean annual temperature is 13.6 ºC, 764.3 mm precipitation, and evapotranspiration is 726.7 mm yr^{-1} (TSMS 2018). The soil temperature regime is classified as mesic, and the moisture regime is ustic (USDA Soil Survey Staff (1999). Pasture and forests are the dominant land covers. A small part of the study area is made up of a slightly-sloped (0.0 to 2.0%) low plateau, whereas mountainous areas, and areas sloping at angles that range from moderate to severe (3% to 20%), are prevalent in other regions. Only a limited region of the foot slope and lowland plateau is suitable for agricultural management.

Figure 1- Location map of the study area

2.2. Methods

In view of the hypothesis that topography, parent material, and climate–vegetation cover may be the fundamental factors that control the mass balance in soil development, soils were investigated throughout a transverse section (diagonally in the direction from south-western to north-western) using four representative profiles (Figure 2).

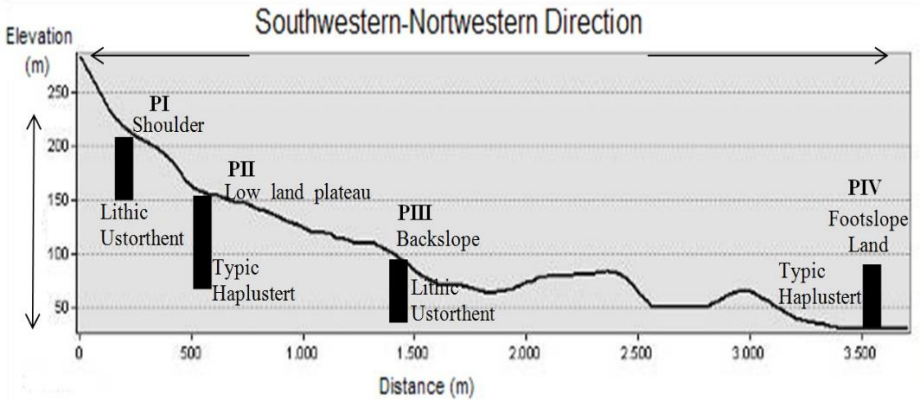


Figure 2- Transect of the four different soil profiles on basalt parent material on different topographic positions

The morphological features of the four profiles in the field were determined, followed by sample collection using genetic horizons and classification in compliance with the Soil Survey Staff (2014). To investigate the physical, chemical, and mineralogical characteristics of the soil, twelve disturbed and twelve undisturbed soil samples were collected. Then, for laboratory analysis, the samples were first air-dried and passed through a 2-mm sieve. pH and electrical conductivity (EC) in 1:2.5 (w/v) in soil/water suspension by pH-meter and EC-meter, respectively; $CaCO_3$ content by the volumetric method (Soil Survey Staff 1992). The particle-size distribution was determined by employing the hydrometer method (Bouyoucos 1951). By following the method proposed by Blacke & Hartge (1986), the bulk density of the samples was determined using the undisturbed soil core.

The saturated hydraulic conductivity of the samples was determined using the undisturbed soil cores by adopting the procedure proposed by Klute & Dirksen (1986). The void ratio of the samples was calculated from the ratio of the volume of the void to that of the solids (Munsuz 1982). The porosity of the samples was calculated from the ratio of the volume of voids to the total soil volume (Munsuz 1985), and the aggregate stability was determined using the wet-sieving method proposed for undisturbed soil cores (Kemper 1965). The moisture contents of the undisturbed soil cores at pressures of 0, 1, 2, 5, 10, 33, 50, 100, 500, and 1500 kPa were determined (Klute 1986).

Following the degradation of organic matter with dilute and Na–acetate-buffered H_2O_2 (pH 5), the clay fraction (< 2 μm) was determined using soil dispersion with a sodium metaphosphate (calgon) and sedimentation in water. Cu Kα radiation at an angle of 2θ ranging from 2° to 30°, with steps of 0.02° 2θ and a counting time of 2 seconds per step, specimens oriented on glass slides were analysed with X-ray diffraction (XRD). Then, Mg and K saturation, along with ethylene glycol solvation (EG) methods were applied, respectively, followed by heating at 550 °C for 2 hours. The minerals and their relative abundances were determined using the diagnostic XRD spacing of the minerals, and then evaluated using their XRD relative peak intensities obtained from the XRD graphs (Whittig & Allardice 1986). Selected soil samples were also studied with a scanning electron microscope (SEM). In this analysis, microprobe process was not done. The samples were mounted onto aluminium stubs and coated first with carbon and then with gold. This double coating proved superior to a coating of carbon or gold alone. Each specimen was studied at magnifications ranging from 250 to 20.000.

Various indices have been proposed to describe the weathering in soils (Harnois 1988; Nesbit & Young 1989). The common principles upon which all indices are based on is determined by the varying ratios of some basic cations (Ca, Mg, K, and Na) to acidic cations of Al and Si. In this study, the following indices were used to quantify the weathering rates of the profiles:

a. Chemical Index of Alteration (CIA) (Nesbitt &Young 1982):

$$CIA = (100)\ [Al_2O_3/(Al_2O_3 + CaO + Na_2O + K_2O)]$$

b. Chemical Index of Weathering (CIW) (Harnois 1988):

$$CIW = (100)\ [Al_2O_3/(Al_2O_3 + CaO + Na_2O)]$$

3. Results and Discussion

3.1. The clay mineralogy of the soils

The type and amount of clay minerals have an important and active role both in the physical and chemical processes and even in the biological processes in the soils. Therefore, determining the type and amount of clay minerals is of importance to illustrate soil genesis. Clay types differ from each other in their shrinkage or expansion due to their crystal structure, the different chemical compounds they contain and the alterations caused by temperature fluctuations.

Figure 3 shows genetic horizons of the studied soil profiles, the XRD diagrams of the parent materials,

and the SEM images (Figure 4) of the primary minerals and clay minerals. In the XRD, since 2θ values beyond 24.5 are within the range of alkali feldspar rather than clay minerals such as smectite or illite. Quartz mineral, which is among the primary minerals, is found in all the profile samples of both lines; however, the amount of quartz increased from the surface horizons to the parent material.

Moreover, Table 1 shows the prevalent clay minerals and primary minerals, and their proportion to each other, both in the genetic horizons and in the parent material. As seen in Table 1, the prevalent clay minerals suite were smectite, illite, kaolinite and vermiculite, while quartz, zeolite, with rather low Ca-feldspar and K-feldspar were the primary minerals. These findings are in agreement with the SEM images (Figure 4). However, all clay minerals were not present in all profiles, and their relative proportions in each profile and horizon differed, especially in terms of the geochemical alterations during the pedological processes.

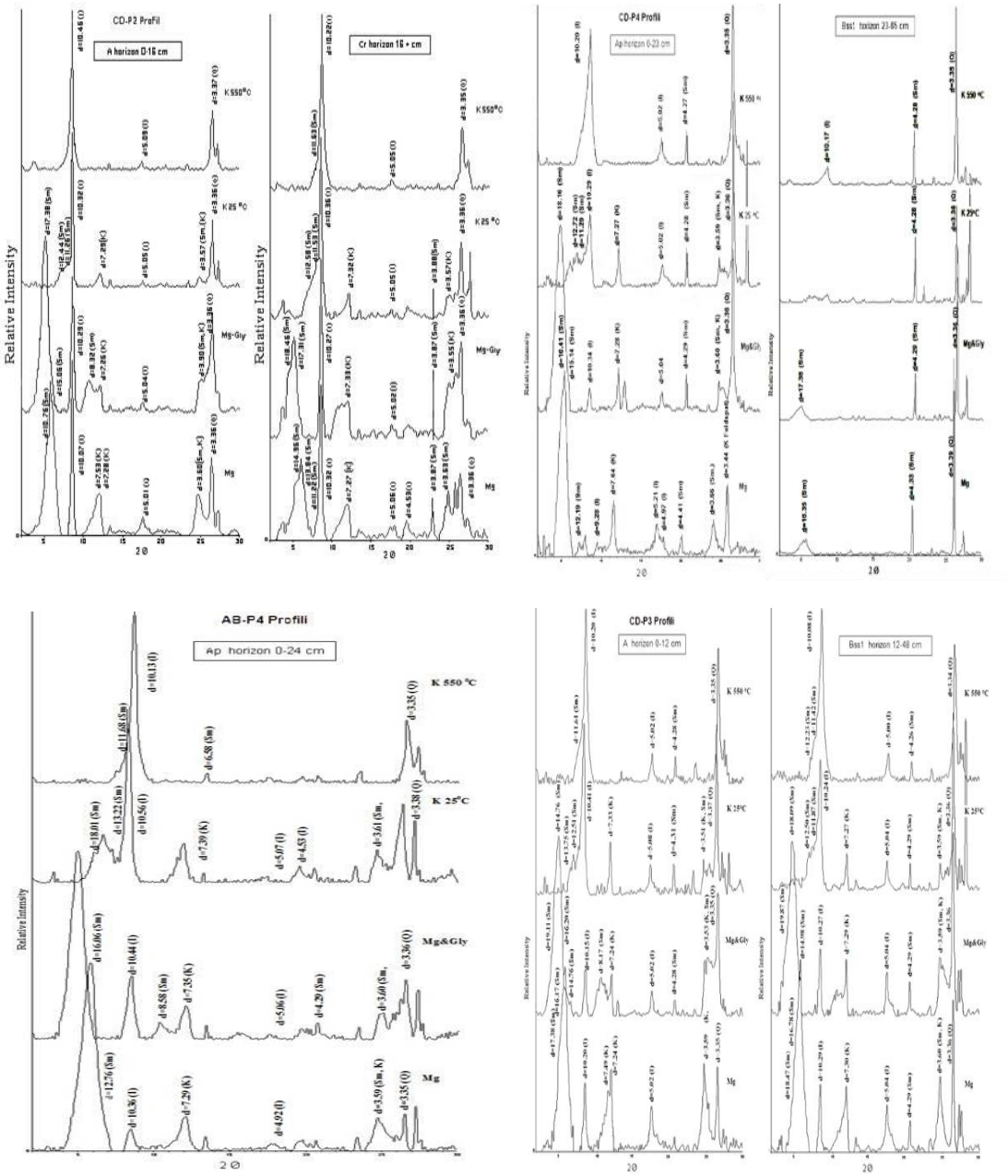


Figure 3- X-ray diffractograms of surface and subsurface horizons for each profile

Table 1- The relative ratios of the clay fractions and clay minerals both in the horizons of the profiles

Horizon	*Depth (cm)*	*Clay minerals*			*Primary minerals*				
		Vermiculite	*Illite*	*Kaolinite*	*Smectite*	*Quartz*	*Zeolite*	*Ca Feldspar*	*K Feldspar*
PI/Shoulder									
A	0-16	-	++++	+++	++	+++	-	-	-
Cr	16+	-	+++	++	+++	+++	-	-	-
PII/Low land plateau									
A	0-12	-	+++	++	++++	+	-	-	-
Bss1	12-48	-	+++	+++	++++	++	-	-	-
Bss2	48-89	-	+++	+++	++++	++	-	-	-
C	89+	-	+++	++	++++	++	-	-	-
PIII/Backslope									
Ap	0-24	-	++	+++	+++	+++	-	-	-
Cr	24+	-	-	-	-	-	-	-	-
PIV/Footslope									
Ap	0-23	-	+++	++	++++	+	-	-	+
Bss1	23-65	-	++	-	+++	++	-	-	-
Bss2	65-106	-	++	+	+++	+	-	-	-
C	106 +	++	+++	++	++++	++	-	-	-

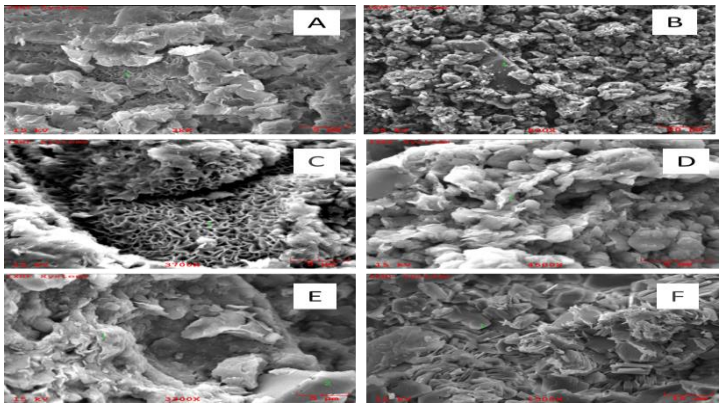


Figure 4- SEM images of clay minerals and primary mineral of soil profiles samples (A: 1: Smectite; B: 1: Quartz; C: 1: Smectite; D: 1: Illite; E: 1: Illite, 2: Feldspar and F: Kaolinite)

The profiles on the south western-north western direction reveal that, especially the soils formed on foot slope and bottom lands (Profiles PII and PIV), smectite was dominant, followed by illite, kaolinite and then vermiculite. Moreover, all profiles on this section contained the quartz at amounts ranging from low to moderate, and the tendency of its ratio to increase in the profiles from the surface to the parent material was noteworthy. In addition, albeit at a low amount, K-feldspar was observed in the surface layers of the soils in the bottom land. In their study on the effects of aspect on the weathering rates and clay mineralogy of the soils formed on andesite/trachyandesite parent materials under semi-arid conditions, Şenol et al (2014) attributed the higher amounts of K-feldspar and quartz on the northern slope soils, compared to those in the southern slope profiles, to the greater weathering over short distances due to aspect. The researchers also reported that smectite-illite-interlayered clay minerals were present in the southern slope and, in order of their abundance, illite, kaolinite and smectite were present in the northern section of the slope.

The investigation of the mineral contents of the PIII and PI profiles, classified as Lithic Ustorthent in the slope lands revealed that, among the clay minerals and primary minerals, the soils of the bottom-slope lands (PIII) contained moderate abundances of kaolinite, smectite and quartz minerals, respectively, and a low illite, whereas the upper-slope land soils, Lithic Ustorthent, mainly contained illite, followed by kaolinite and a low amount of smectite clays. Except for the surface soil of the bottom lands that contain low amounts of K-feldspar, the primary minerals that include zeolite and Ca- and K-feldspars were not

found in the soils on the section from the bottom lands. In another study focusing on the genesis and clay mineralogy of the soils formed on different vectors, physiographic units, and geological units, Dengiz et al (2006) investigated five different soil profiles that were formed on the low and high terraces in Gölbaşı province of Ankara, on the DII, DIII and DIV plateau plains, and on different geological formations. The researchers found that the dominant clay minerals in the PI and PIII profiles, which were opened on the DII plateau plain and low terrace and classified as Entisols (Lithic Xerorthent) - contained low amounts of illite in addition to the prevalent quartz, calcite, and chlorite minerals. The PII and PV profiles that were on the DIII and DIV plateau plains, and classified as Inceptisols, had important differences in their formations and clay minerals because of their different vectors. The PIII profile that was on the high terrace plains and classified as Mollisols had a more advanced soil formation than that of the other profiles and contained illite, smectite and palygorskite clay minerals.

Illite is the dominant clay mineral in Profile I, whereas in Profile II and IV smectite was main clay mineral. XRD findings indicated distribution of clay mineral type in surface horizons vary, as follows: PI: illite > kaolinite > smectite; PII: smectite > illite > kaolinite; PIII: kaolinite > smectite > illite and PIV: smectite > illite > kaolinite (Table 1 and Figure 4).

3.2. Weathering indices

Table 2 shows weathering rates derived from the geochemical properties of the profiles in the southwest–northeast direction. The CIA value is based on chemical weathering and the removal of basic cations of Ca, Na, and K from minerals, and it represents the ratios of primary and secondary minerals in soils. CIA was calculated using the formula proposed by Nesbitt &Young (1982). It represents the degree of hydrolytic weathering and alteration of feldspars to clays, and helps to determine the relative clay amount. The CIA value of soils or sediments that contain high amounts of intensely weathered residual clays, such as kaolinite or gibbsite, is 100, while the CIA value of unweathered, upper-crustal rock is 50 (Fedo et al 1995). The average CIA value of rocks such as shale ranges from 70 to 75. CIA values are divided into five groups: very slightly weathered (50 to 60), slightly weathered (60 to 70), moderately weathered (70 to 80), highly weathered (80 to 90) and extremely weathered (90 to 100).

Table 2- The weathering indices for the profiles classified according to Soil Survey Staff/IUSS Working Group WRB on the section

Horizon	*Depth (cm)*	*CIA*	*CIW*
PI- Shoulder (Lithic Ustorthent/Eutric Regosol)			
A	0-16	55.7	62.0
Cr	16+	53.6	59.2
PII- Low land plateau (Typic Haplustert/Haplic Vertisol)			
A	0-12	65.4	74.2
Bss1	12-48	60.4	65.8
Bss2	48-89	60.9	79.7
Cr	89+	50.8	75.1
PIII- Backslope (Lithic Ustorthent/Eutric Regosol)			
Ap	0-24	58.5	63.9
Cr	24+	59.8	62.6
PIV- Footslope (Typic Haplustert/Haplic Vertisol)			
Ap	0-23	61.9	67.7
Bss1	23-65	73.8	80.4
Bss2	65-106	42.3	44.5
C	106+	41.3	43.4

CIA, chemical index of alteration; CIW, chemical index of weathering

There were important differences among the CIA values in the profiles on transect due to the different topographic positions. Moreover, there were significant differences among the horizons of the PIV (Typic Haplustert) where the lowest and highest CIA values were determined. Although the CIA values of the profiles on the high-slope lands were in the very slightly-weathered class, the CIA values of other profiles were either in the slightly weathered or moderately weathered class. The CIA rates of the surface horizons

of all profiles were higher than that of the lower horizons, especially if the parent material was considered. As a result of the obstruction of the soil formation because of the continuous transport of the slope soils and upper soil to the bottom lands, the CIA values of the PIII and PI, Lithic Ustorthent in the study area, which indicate the weathering rate of the profiles, were lower than that of the other soils, which explains the weak weathering in the profiles. On the other hand, the most intense weathering occurred in PIV profile, which is located in the bottom lands, and in which the CIA values differ more dramatically depending on the parent material.

The CIW value of unweathered rock is 50 and reaches 100 in intensely weathered environments, and the CIW rate increases with increasing weathering. As with the case for CIA values, the inter-profile CIW values differed significantly and ranged from 43.4 to 80.4. The highest CIW values of the soils were determined in the PIV (Typic Haplustert), and the distribution pattern of the CIW in the profile was similar to that of the CIA. Again, as in the case of CIA as revealed by applying the same classification used for CIA to CIW - the CIW values of the PIII and PI coded profiles were in the same class due to the obstruction of the soil formation by erosion. This serves as additional evidence for the similarity between the weathering processes that occur in the soils despite the slight differences in morphology.

3.3. The physical properties of the soils on the south western-north eastern section

Soil physical properties showed variability as a result of dynamic interactions among natural environmental factors of climate, parent material, erosion and topography (Dengiz et al 2006; Dengiz & Başkan 2010; Kibar et al 2012; Dengiz et al 2013). Table 3 shows the results of the physical soil analyses of the disturbed and undisturbed soil samples of PI, PII, PIII and PIV designated on the southwest-northeast section. Among the physical soil properties, the differences between the bulk density, void ratio, porosity, and water-resistant aggregate weight of the soil horizons were determined statistically significant ($P<0.01$).

Table 3- OM and some physico-chemical properties of the soils located in different topographical positions in the south western-north eastern direction

Horizon	*$pH_{(H2O)}$ 1/2.5*	*EC (dS m^{-1})*	*$CaCO_3$ (%)*	*OM (%)*	*Clay (%)*	*Silty (%)*	*Sand (%)*	*Texture class*	*BD (g cm^{-3})*	*VR*	*P (%)*	*WRAS (%)*
PI- Shoulder (Lithic Ustorthent/Eutric Regosol												
A	7.03	0.19	0.50	2.25	34.4	25.5	40.1*	CL	1.53b	0.7d	42.4bc	52.0a*
PII- Low land plateau (Typic Haplustert/Haplic Vertisol)												
Ap	7.05	0.16	0.79	1.71	41.5*	24.2	34.3	C	1.48c	0.8c	44.1ab	11.5d
Bss1	7.72	0.19	0.29	1.69	68.5*	18.3	13.2*	C	1.48c	0.7d	42.4bc	7.9e*
Bss2	7.79	0.34	1.37	0.59	49.8*	26.4	23.8*	C	1.53b	0.8b	45.2ab	7.2e*
PIII- Backslope (Lithic Ustorthent/Eutric Regosol)												
Ap	7.87	0.55	0.49	2.35	32.1*	27.9*	40.0*	CL	1.41e*	0.9a*	46.7a*	49.1b
PIV- Footslope (Typic Haplustert/Haplic Vertisol)												
Ap	7.50	0.17	0.20	1.65	56.2	23.1	20.7	C	1.45d	0.8b	45.2ab	7.2e*
Bss1	7.30	0.44	0.98	1.26	62.6	12.8*	24.6	C	1.57a*	0.7e*	40.9c*	7.7e*
Bss2	8.25	0.17	1.10	1.09	68.4	15.8	15.8	C	1.45d	0.8b	45.1ab	18.0c

OM, organic matter; C, Clay; CL, Clay Loamy; BD, bulk density; VR, void ratio; P, porosity; WRAS, water resistance aggregate btability; *****, the values showed dark colour indicates the highest and lowest values. The difference between the averages showed the same letter is not significant according to the Duncan test at the 0.05 level

Although the texture classes of the soil horizon samples were clayey, the bulk densities of the soil horizon samples were generally high. Obtaining high bulk density values (1.57 g cm^{-3}) - despite expecting low values - by considering the clay structure and moderately high clay content of the soils was regarded as a normal consequence of the compaction that naturally occurs in the soils depending on the climate conditions during sampling, and which increases for greater soil depths. Moreover, observing the highest bulk density in the bottom land and tilled land (PIV Typic Haplustert), and the location of the horizon (Bss1) with the highest bulk density just below the plough-till depth (23 to 65 cm), indicate that the high values may also be due to agricultural activities. Again, the void ratio and porosity values, which are known to be significantly affected by compression in the soil, revealed that the void ratio and porosity values are

decreased in soil horizons with increased compression and bulk density (PIV). It is known that there is a negative relationship between the bulk density in soil, and the porosity and void ratio. Indeed, the results given in Table 3 agree with this negative relationship. The highest void ratio and porosity values were observed in the horizon with the lowest bulk density (PIII/Ap), while the results obtained in the soil horizon with the highest bulk density (PIV) were the opposite. Furthermore, the fact that the soil horizon with the highest void ratio and porosity (PIII/Ap) was within dry agricultural land derived from forestlands having a high organic matter content (2.35%) are also among the important indicators of the effects of organic matter on the results. Table 3 also reveals the positive effects of organic matter on water-resistant aggregates. Among the horizons with the highest water-resistant aggregate, the A horizon in the PI profile and the Ap horizon in the P4 profile had organic matter contents of 2.25% and 2.35%, respectively, which were the highest organic matter values obtained in the toposequence. Again, when the results given in the Table are co-evaluated with the organic matter contents, it can be argued that the colloidal organic matter has a greater effect on the aggregate stability than the colloidal clay content. In agreement with this argument, the two soil horizons with the highest aggregate stability (PI/A and PIII/Ap) had the lowest clay content (34.4% and 32.1%) and the highest sand content (40.1% and 40.1%) among all the soil horizons identified in the section. This result is important evidence for the more effective role of colloidal organic matter in aggregate formation than that of the colloidal clay content.

Table 4 shows water retention of horizons (PI, PII, PIII and PIV) that were designated on the southwest-northeast section. The changes in the moisture content among the soil horizons at different matric potentials (0, 1, 2, 5, 10, 33, 100, 500 and 1500 kPa) were found as statistically significant ($P<0.01$) (Table 4).

Table 4- The water retention of the soil horizons of the profiles located in different topographic positions in the south western-north eastern direction

Horizon	*0 (kPa)*	*1 (kPa)*	*2 (kPa)*	*5 (kPa)*	*10 (kPa)*	*33 (kPa)*	*100 (kPa)*	*500 (kPa)*	*1500 (kPa)*
PI- Shoulder (Lithic Ustorthent/Eutric Regosol									
A	37.8d*	34.2d*	33.7c*	32.5d*	31.8d*	31.0d*	24.6d*	21.7f*	20.1f*
PII- Low land plateau (Typic Haplustert/Haplic Vertisol)									
A	41.3c	38.1bc	37.61b	37.1c	36.7c	36.01c	30.9c	26.7e	23.3e
Bss1	47.2a*	45.4a*	44.9a*	44.4a*	43.7a*	42.81a*	39.2a*	36.7a*	34.4a*
Bss2	45.7ab*	43.2a*	42.4a*	41.4b	40.0b	39.19b	36.9a*	33.5b	30.1b
PIII- Backslope (Lithic Ustorthent/Eutric Regosol)									
Ap	42.6c	39.7b	38.4b	36.6c	35.5c	34.58c	31.1bc	30.2c	29.0bc
PIV- Footslope (Typic Haplustert/Haplic Vertisol)									
Ap	41.2c	39.1b	37.9b	36.6c	35.7c	34.53c	29.4c	27.3de	25.6de
Bss1	40.9c	38.2bc	37.5b	36.6c	35.9c	35.60c	33.9b	30.0cd	28.0bcd
Bss2	43.1bc	35.5cd	32.3c*	31.8d*	30.7d*	28.80d*	28.4c	28.1cde	26.9cd

*, the values showed dark colour indicates the highest and lowest values. The difference between the averages showed the same letter is not significant according to the Duncan test at the 0.05 level

When the changes in moisture at different matric potentials (0, 1, 2, 5, 10, 33, 100, 500 and 1500 kPa) are evaluated together, the Bss1 horizon in the PII profile had the highest moisture content, and its moisture exchange with the other soil horizons was statistically significant ($P<0.01$). However, at certain matric potentials (0, 1, 2 and 100 kPa), the Bss1 and Bss2 horizons in the PII had the highest moisture content, and their moisture contents at these matric potentials were statistically the same for each other. Among all matric potentials, the lowest moisture content was determined in the A horizon of PI.

Although the results may lead us to regard the clay content of the soils as the soil property that primarily determines the water retention capacity of the soil horizons at different matric potentials, the co-evaluation of the moisture content results given in Table 4, with the other physical soil properties given in Table 3, reveal that the rather than clay content, the clay type in the soil horizons from the transect had a greater effect on the water retention capacity of the soil than the clay content of the soil horizon. This argument is further supported by the results that showed that the Bss2 horizon of the PIV profile had the lowest moisture content at the matric potentials of 2, 5, 10 and 33 kPa, although it had the highest clay content (68.4%)

among all the soil horizons of the section. Moreover, the low water-retention capacity of the Bss2 horizon of the PIV can also be associated with the lowest degree of weathering observed in the horizon when compared to the other horizons in the section, which was also indicated by the CIA and CIW values (Table 2). Also, conforming to the above-mentioned evaluations for the clay type, the Ap, Bss1 and Bss2 horizons of the PIV profile with higher clay and organic matter contents had lower water retention than the Bss2 horizon of the PII profile - which had relatively lower clay and organic matter contents at almost all of the investigated matric potentials. Furthermore, it can be argued that the PII profile, which had higher water retention at all matric potentials, was more greatly affected by soil formation processes, due to the generally higher weathering indices (CIW) of its Bss1 and Bss2 horizons, compared to those of the other soil horizons; in addition, its high water-retention capacity can be attributed to the presence in these horizons of smectite-type clays able to swell to a high degree.

4. Conclusions

In this study, features of pedogenic evolution were tested that influence toposequence on physical, mineralogical properties and weathering rates derived from the geochemical in soils developed on basaltic parent material under sub-humid climate condition. For that reason, four representative profiles located from the south western to the north western were investigated and classified according to Soil Survey Staff (2014)/IUSS Working Group WRB (2015) classification systems. According to these systems, PI and PIII developed on shoulder and back slope were classified as Lithic Ustorthent/Eutric Regosol while, PII and PIV formed on low land plateau and footslope positions were classified Typic Haplustert and Haplic Vertisol. In this present research, it was determined that the main limitation soil forming factor on profiles development in hillslope positions (shoulder and back slope) where covered by weak vegetation is soil erosion in the study area. Therefore, soils can be described as young soils. On the other hand, soils in lower slope position (low land plateau-foot slope) have development sub surface horizon due to high clay content (generally smectite) and no interruption events. Main subsurface diagnostic horizon of these soils is slickensides (Bss horizon). This case also explained with chemical weathering indices such as CIA, CIW and investigated matric potentials which related with clay minerals. Among all the investigated matric potentials of profiles, Lithic Ustorthent soils, PI and PIII, have the lowest moisture content. Moreover, it can be said that higher water retention at all matric potentials, was more greatly affected by soil formation processes due to the generally higher weathering indices (CIA and CIW) of the slickensides horizon compared to those of the other soil horizons.

Consequently, the results clearly showed that topographic condition strongly effects on soil physical, mineralogical and morphological characteristics either directly or indirectly in the local region even soils formed on the same parent material and under the same climatic condition. This case was also explained with chemical weathering indices in this study. Therefore, the solution to stimulate soil forming and preventing soil erosion lies in directly addressing and removing the causes, e.g., stopping deforestation and the grazing on slope land and compensating the local people who are negatively affected, making contour farming and reduced tillage practices compulsory on certain slopes and soil types, and zoning high quality agricultural land to protect it from development. In addition, more scientific researches should be developed related with this topic to create site management plans in decision making and implementation in future.

Acknowledgements

The authors gratefully acknowledge the scientific research grant (TÜBİTAK-213O073) from the Scientific and Technological Research Council of Turkey.

References

Anderson S P, von Blanckenburg F & White A F (2007). Physical and chemical controls on the Critical Zone. *Elements* 3: 315-319

Arnold R W (2006). Soils: Basic Concepts and Future Challenges, ed. Giacono Certini and Riccardo Sclenghe. Published by Cambridge University Press, pp. 1-10

Blacke G R & Hartge K H (1986). Bulk density. In Klute, A. (ed). Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd ed. *Agronomy* 9: 363-382

Bouyoucos G J (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. *Agronomy Journal* 43: 435-438

Brantley S L (2008). Understanding soil time. *Science* 321: 1454-1455

Braun J J, Descloitres M, Riotte J, Fleury S, Barbiero L, Boeglin J L, Violette A, Lacarce E, Ruiz L, Sekhar M, Kumar M S M, Subramanian S & Dupre B (2009). Regolith mass balance inferred from combined mineralogical, geochemical and geophysical studies: Mule Hole gneissic watershed, South India. *Geochimica et Cosmochimica Acta* 73: 935-961

Berner R A, Lasaga A C & Garrels R M (1983). The carbonate-silicate geochemical cycle and its effect on atmospheric carbon dioxide over the past 100 million years. *American Journal of Soil Science* 283: 641-683

Chorover J, Amistadi M K & Chadwick O A (2004). Surface charge evolution of mineral–organic complexes during pedogenesis in Hawaiian basalt. *Geochimica et Cosmochimica Acta* 68: 4859-4876

Dengiz O (2010). Morphology, Physico-Chemical Properties and Classification of Soils on Terraces of the Tigris River in the South-East Anatolia Region of Turkey. *Journal of Agricultural Sciences*, 16: 205-212

Dengiz O & Başkan O (2010). Characterization of soil profile development on different landscape in semiarid Region of Turkey a case study; Ankara-Soğulca catchment. *Anadolu Tarım Bilimleri Dergisi* 25: 106-112

Dengiz O, Göl C, Karaca S & Yüksel M (2006). Effects of different landscape position and parent material on soil variability and land use in both sides of Acıçay River-Çankırı. 5th International Soil Meeting (ISM) on Soil Sustaining Life on Earth, Managing Soil and Technology: May 22-26, 2006 Şanlıurfa, Turkey 745-752

Dengiz O, Sağlam M Özaytekin HH & Başkan O (2013). Weathering rates and some physico-chemical characteristics of soils developed on a calcic toposequences. *Carpathian Journal of Earth and Environmental Sciences* 8: 13-24

Dessert C, Dupre B & Francois L M (2001). Erosion of Deccan Traps determined by river geochemistry: impact on the global climate and the 87Sr/86Sr ratio of seawater. *Earth Planet Science Lett*er 188: 459-474

Dessert C, Dupr´e B, Gaillardet J, Franc¸ois L M & All`egre C J (2003). Basalt weathering laws and the impact of basalt weathering on the global carbon cycle. *Chemical Geology* 202: 257-273

Dixon J L, Heimsath A M & Amundson R (2009). The critical role of climate and saprolite weathering in landscape evolution. *Earth Surface Processes Landforms* 34: 1507-1521

Eggleton R A, Foudoulis C & Varkevisser D (1987). Weathering of basalt. Changes in rock chemistry and mineralogy. *Clays & Clay Minerals* 35: 161-169

Fedo C M, Nesbitt H W & Young G M (1995). Unraveling the effects of potassium metasomatism in sedimentary rocks and paleosols with implications for paleoweathering conditions and provenance. *Geology* 23: 921-924

Florinsky I V & Kuryakova G A (1996). Influence of topography on some vegetation cover properties. *Catena* 27: 123-141

Gaillardet J, Dupre P, Louvat C J & All`egre C J (1999). Global silicate weathering and CO_2 consumption rates deduced from the chemistry of the large rivers. *Chemical Geology* 159: 3-30

Grantham J H & Velbel M A (1988). Influence of climate and topography on rock fragment abundance in modern fluvial sands of the southern Blue Ridge Mountains, North Carolina. *Journal of Sedimentary Petrology* 58: 219-227

Grzyl A, Kiedrzyński M, Zielińska K M & Rewicz A (2014). The relationship between climatic conditions and generative reproduction of a lowland population of Pulsatilla vernalis: The last breath of a relict plant or a fluctuating cycle of regeneration. *Plant Ecology* 215: 457-466

Harnois L (1988). The CIW Index: A new chemical index of weathering. *Sedimentary Geology* 55: 319-322

Heckman K & Rasmussen C (2011). Lithologic controls on regolith weathering and mass flux in forested ecosystems of the south western USA. *Geoderma* 164: 99-111

IUSS Working Group WRB (2015). World References Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps Update 2015, World Soil Rep., 106. FAO, Rome, 203 pp

Jenny H (1941). Factors of Soil Formation: A System of Quantitative Pedology. McGraw-Hill, New York

Kibar M, Deniz K & Sarıoğlu F E (2012). The morphology, mineralogy, geochemistry and physical implications of foid bearing syenite and syenite-carbonate rocks contact zone soils: Kırşehir-Akpınar-Buzlukdağ, Turkey. *Eurasian Journal of Soil Science* 2: 69-74

Kısakürek B, Widdowson M & James R H (2004). Behaviour of Li isotopes during continental weathering: the Bidar laterite profile, India. *Chemical Geology* 212: 27-44

Klute A (1986). Water retention. Laboratory methods. In Klute, A. (ed). Methods of soil analysis. Part 1. Phsical and mineralogical methods. 2nd ed. *Agronomy* 9: 635-662

Klute A & Dirksen C (1986). Hydraulic conductivity and diffusivity: Laboratory Methods. Methods of Soil Analysis– Part I. Editör: Klute, A. Madison, Wisconsin: American Society of Agronomy 687-732

Louvat P & All`egre C J (1998). Riverine erosion rates on Sao Migual volcanic island, Azores archipelago. *Chemical Geology* 148: 177-200

Lybrand R A & Rasmussen C (2018). Climate, topography, and dust influences on the mineral and geochemical evaluation of granitic soils in southern Arizona, *Geoderma* 314: 245-261

Middleburg J J, van der Weijden C H & Woittiez J R W (1988). Chemical processes affecting the mobility of major, minor and trace elements during weathering of granitic rocks. *Chemical Geology* 68: 253-273

Moore I D, Gessler P E, Nielsen G A & Peterson G A (1992). Soil attribute prediction using terrain analysis. *American Journal of Soil Science Society* 2: 443-452

Munroe J S, Farrugia G & Ryan P C (2006). Parent material and chemical weathering in alpine soils on Mt. Mansfield, Vermont, USA. *Catena* 70: 39-48

Munsuz N (1982). Toprak Su ilişkileri. Ankara Üniversitesi Ziraat Faktültesi Yayınları: 798

Munsuz N (1985). Toprak Mekaniği ve Teknolojisi. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 922, Ders Kitabı: 260, Ankara

Nesbitt H W & Young G M (1982). Early proterozoic climates and plate motions inferred from major element chemistry of Lutites. *Nature* 299(5885): 715-717

Price J R & Velbel M A (2003). Chemical weathering indices applied to weathering profiles developed on heterogeneous felsic metamorphic parent rocks. *Chemical Geology* 202: 397-416

Price J R, Velbel M A & Patino L C (2005). Rates and timescales of clay-mineral formation by weathering in saprolitic regoliths of the southern appalachians from geochemical mass balance. GSA Bull. 117: 783-794

Ridolfi L, Laio F & D'Odorico P (2008). Fertility island formation and evolution in dryland ecosystems. *Ecology and Society* 13: 439-461

Sebastiá M T (2004). Role of topography and soils in grassland structuring at the landscape and community scales. *Basic and Applied Ecology* 5: 331-346

Soil Survey Staff (1992). Procedures for Collecting Soil Samples and Methods of Analysis For Soil Survey. Soil Survey Investigations Report No. 42, Washington DC, USA

Soil Survey Staff (1999). Soil Taxonomy. A Basic of Soil Classification for Making and Interpreting Soil Survey. USDA Handbook No: 436, Washington D.C. USA

Soil Survey Staff (2014). Keys to Soil Taxonomy. United States Department of Agriculture Natural Resources Conservation Service Washington D.C. USA

Stallard R F & Edmond J M (1983). Geochemistry of the Amazon 2: the influence of geology and weathering environment on the dissolved load. *Journal of Geophysical Research-Oceans* 88: 9671-9688

Sutton S J & Maynard J B (1992). Multiple alteration events in the history of a sub-Huronian regolith at Lauzon Bay, Ontario. *Canadian Journal of Earth Science* 29: 432-445

Şenol H, Özaytekin H H, Akgül M & Alaboz P (2014). Yarı kurak şartlarda Trakit/Trakitandezit ana materyal üzerinde oluşan toprakların ayrışma oranları ve kil minerolojine bakının etkisi. *Tarım Bilimleri Dergisi* 20: 288-301

Tunçay T, Dengiz O, Bayramin İ, Kılıç Ş & Başkan O (2019). Chemical weathering indices applied to soils developed on old lake sediments in a semi-arid region of Turkey. *Eurasian Journal of Soil Science* 8(1): 60-72

TSMS (2018). Turkish State Meteorological Services, https://mgm.gov.tr (last access: 20 November 2018)

Voortman R (2011). Explorations into African land resource ecology on the chemistry between soils, plants and fertilizers (PhD dissertation). Faculty of Economics and Business, Vrije Universiteit, pp. 264

White A & Brantley S (1995). Chemical weathering rates of silicate minerals; an overview. *Rev. Mineral. Geochem* 31: 1-22

Whittig L D & Allardice W R (1986). X-ray diffraction techniques. In: Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. (A. Klute, ed., 2nd eds.), ASA publication 9, Madison, Wisconsin, pp. 55-86

Zhang G, Pan J, Huang C & Gong Z (2007). Geochemical features of a soil chronosequence developed on basalt in Hainan Island, China. *Revista Mexicana de Ciencias Geológicas* 24: 261-269

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 Impact of Livestock Supports on Production and Income of the Beef Cattle Farms: A Case of Samsun Province, Turkey

Nevra ALHAS EROGLU[a], Mehmet BOZOGLU[b], Abdulbaki BILGIC[c]

[a]*Regional Directorate of Turkish Statistical Institute, Samsun, TURKEY*

[b]*Department of Agricultural Economics, College of Agriculture, Ondokuz Mayis University, Samsun, TURKEY*

[c]*Department of Agricultural Economics, College of Agriculture, Ataturk University, Erzurum, TURKEY*

ARTICLE INFO
Research Article
Corresponding Author: Nevra ALHAS EROGLU, E-mail: nevraalhas@tuik.gov.tr, Tel: +90 (505) 778 92 79
Received: 26 November 2018, Received in Revised Form: 28 January 2019, Accepted: 12 February 2019

AUTHORS ORCID ID
(Nevra ALHAS EROGLU: 0000-0002-1188-8274), (Mehmet BOZOGLU: 0000-0001-8333-1865), (Abdulbaki BILGIC: 0000-0002-1003-7072)

ABSTRACT
Although the beef cattle sector has been considerably supported during the last two decades, Turkey could not get its self-sufficiency yet. The objective of this case study was to examine the impacts of livestock supports on production and income of beef cattle farms. The survey data was collected from randomly selected 171 cattle farms in Samsun province of Turkey. The Treatment Effect Model was used to measure the impacts of livestock supports on beef meat production and gross profit of the farms. The results indicate that the farmers, who have larger land and herd, higher education level, keeping farm records, are mechanized and specialized in beef cattle breeding were more likely to benefit from livestock supports than their counterparts. The Treatment Effect Model highlights that livestock support has a statistically significant effect on the amount of beef meat produced whereas it has no statistically significant effect on the gross profits of the farms. The research recommended that the livestock supports are necessary for the sustainability of beef cattle farms. The farms should be encouraged to get records via Farm Accountancy Data Network and the record keeping farms should be supported by higher amounts.

Keywords: Beef cattle breeding; Livestock supports; Impact assessment; Treatment effect; Turkey

1. Introduction

The livestock sector is of an important place in the agricultural sector of the world. Thus, 38.6% of the gross production value of agricultural production in the world was provided by the livestock sector in 2016. In the same year, in Turkey, the livestock sector provided 34.5% of total agricultural production value (FAO 2018). In the last decade, the livestock sector has been substantially supported both in Turkey and in the world due to its essential role by meeting food demand and supplying input to the agro-food industry. Therefore, the evaluation of the impacts of supports on production and income of the beef cattle farms is essential to develop more efficient support policies.

The development of the livestock sector is important to meet the food need of countries. Therefore, it is essential to maintain the sustainability of the sector, to improve it by means of supports and to assess

DOI: 10.15832/ankutbd.487493

the impacts of the supports. In the period of 2000-2016, in Turkey, the budget of total agricultural supports had nominally changed from $4.5 to $3.97 billion whereas the budget of livestock supports had increased from $0.02 to $1.02 billion. The share of the livestock supports in the total agricultural supports had increased from 0.5% to 25.7% in the same period (Anonymous 2015).

The main output of the sector is definitely beef meat. In the last two decades, the number of slaughtered beef had increased by 214% and the production of beef meat had increased by 351% in Turkey (TurkStat 2018a). While the producer prices had increased nominally, there had not been a significant increase in reel prices except for the last few years. Because of inadequacies in domestic beef meat production, continuously rises in consumer prices, the country had to import breeding material, live animal or beef meat. Therefore, there had been an important rise in import especially after the year 2010.

The costs of the beef cattle breeding activity is another disputable field. The major cost of the beef meat production is feed. This cost constituted about 40.95% (Alhas Eroğlu 2017), 33.1% (Çelik & Sarıözkan 2017) and 48.3% (Özkan & Erkuş 2003) of the total cost of production. The increase in this main cost element pushes up beef meat prices. The parity between beef meat and forage increased by 25.7%, whereas the parity between beef meat and milk increased by 28.1% during the period of 1994-2017. This data could be interpreted in favor of the cattle beef producers but the negative developments for dairy sector have affected dairy cattle sector and resulted in difficulties in obtaining breeding material and increasing in beef meat production costs and prices. The government has supported forage and roughage crops to increase production. These supports had increased both in sown areas and production of forage and roughage crops. Nevertheless, rising especially in price of concentrate feeds have negatively affected the production cost of beef meet.

The livestock supports have been provided with farms in the last twenty years in order to get sustainability in the sector. Of all livestock supports, both breeding male cattle and forage crop support are of great importance and directly affect the beef cattle farms. Breeding male cattle supports have been granted for the farms that slaughtered one year old and at least 200 kg carcass weighted male cattle and record it to the official system. It was granted since 2011 but the unit price of the support has been decreasing over the time. The forage crop support has been granted for farms in order to decrease the feed cost. The farmers who have grown clover, corn, sainfoin, etc. at least 1 ha officially recorded land could provide with support. Contrary to breeding male support, the unit price of forage crop support has risen over the years. Although the sector has been seriously supported for 15 years, there have been some considerable problems such as supplying adequate breeding materials, rising feed cost and beef meat prices and increasing import. Therefore, the impacts of the cattle support on production and prices make these policies controversial. Though the sector received 30% of total agricultural supports, the cattle breeding sector has some important obstacles such as depending on external inputs like feed and breeding material, the prevalence of small-scale farm sizes. Therefore, the efficiency of the support policies has been disputable (Anonymous 2018). The assessment of the impacts of direct supports is highly essential for the beef cattle sector.

Literature review shows that the number of the studies concerning the impacts of agricultural support policies had considerably increased in recent year. The majority of this literature addressed the impacts of decoupled payments. Although the impacts of agricultural supports had been explored in a broad context including farmer, farm structure, input use, production, income and environment, the emphasis of the literate has been mainly on production and income of farms. The literature about the impacts of agricultural supports on production and income has mainly concentrated on the three different scenarios such as supports have (i) no effect, (ii) positive effect and (iii) negative effect. A number of studies identified decreasing impacts of agricultural supports on production and income (Chau & de Gorter 2000; Moss et al 2002; Breen et al 2005; Goodwin & Mishra 2005; Goodwin & Mishra 2006; Shrestha et al 2007; Gorton et al 2008; Acs et al 2010; Morgan-Davies et al 2012; Kazukauskas et al 2014). Some of them highlighted that the changes of supports decreased production due to the reduction of cattle number (Moss et al 2002; Shrestha et al 2007; Acs et al 2010; Morgan-Davies et al 2012), while Breen et al (2005) stated that some of the producers terminated production activity. Chau & de Gorter (2000) and Gorton et al (2008) stressed that thank to the supports, the farms continued to produce regardless of profit.

Numerous research results indicated that the agricultural supports had an increasing impact on production and income of the farms (Hennessy 1998; Sckokai & Moro 2006; Revell & Oglethorpe 2003; O'Donoghue & Whitaker 2010; Majewski et al 2011; Viaggi 2011; El Benni et al 2012; Bartolini & Viaggi 2013; Severini & Tantari 2013). In addition to income-boosting effect, the agricultural supports decrease volatility and inequality in income (El Benni et al 2012; Severini & Tantari 2013). However, Hennessy (1998) and Sckokai & Moro (2006) stated that agricultural supports have decreased the degree of risk by reducing income variability faced by farmers. Some researcher stressed that without agricultural supports the further number of producers would be likely to end up their production (Majewski et al 2011; Viaggi 2011; Bartolini & Viaggi 2013).

Some literature highlighted that agricultural supports had neither an increasing nor decreasing impact on production and income (Douarin et al 2007; Genius et al 2008; Lobley & Butler 2010; Weber & Key 2012; Giannoccaro & Berbel 2013; Latruffe et al 2013). Thus, 66% of the producers in the study of Latruffe et al (2013) and 62% of the producers in the study of Lobley & Butler (2010) did not change the amount of production in case of no support scenario.

Contrary to the international literature, in Turkey, there have been limited studies concerning the impact of supports on the livestock sector. Of all, the impacts of supports were examined at the levels of provincial, regional or countrywide using secondary data or primary survey data (Topçu 2008; Yılmaz et al 2008; Topçu et al 2008; Demir 2009; Demir & Yavuz 2010; Keskin et al 2010; Aksoy et al 2012; Özüdoğru & Tatlıdil 2012). Aksoy et al (2012) indicated that the livestock supports during the period of 2002-2009 had not an effect on milk production and suggested that it is essential to design support policies at the regional level. This suggestion was also emphasized by other scholars in order to increase efficiency (Demir 2009; Keskin et al 2010; Demir & Yavuz 2010). While Yılmaz et al (2008) stated that the supports increase the inequality of income distribution; Özüdoğru & Tatlıdil (2012) indicated that unionized producers could reduce their cost by benefiting from supports. These empirical studies mostly examined the effects of supports on production and income of the dairy farms. Despite the increase in beef prices in Turkey and government supports, the beef meat production could not be increased to the expected levels of the country. Therefore, it is essential to evaluate the impacts of cattle support policies at the farm level. The objective of this study was to explore the impacts of cattle breeding supports on the production and gross profit of beef cattle farms in Samsun province of Turkey by using the treatment effect model (TEM). The average treatment effect (ATE) and the average treatment effect on the treated farms (ATET) have been put forward in terms of direction, size, and statistics in the current study.

This paper is structured as follows: after the introduction, main developments of the beef cattle sector in Turkey and the essence of the study was described in the next section. The data and methodology were specified in the third section. In the fourth section, descriptive statistics and the model results were introduced. In the last section, conclusions with policy recommendations were presented.

2. Material and Methods

2.1. Research area

The research area, Samsun province, has located in the Black Sea Region. It has 9352 km² acreages and it consists of approximately 1% of total area of Turkey. In 2017, 1.61% of the total agricultural land in Turkey has located in Samsun province (TurkStat 2018b). In the same year, the value shares of crop production, livestock and animal products of Samsun province in the country value were 2.40%, 1.80%, and 0.72%, respectively. The share of the total production value of Samsun province in Turkey was 1.82% (TurkStat 2018c).

The support of breeding male cattle has been given to the farms since 2011 and Samsun province had received about 1.3% of the total support (Anonymous 2016). Whereas, the share of Samsun province in total cattle and beef cattle number of Turkey were 2.42% and 2.69%, respectively (TurkStat 2018c). This figure indicates that Samsun could not sufficiently benefit from the supports.

2.2. Material

The research population consisted of 799 beef cattle farms which were members of two Beef and Lamb Producers Associations in Samsun province. In the study, 137 farms were selected randomly using strata sampling method with the farms that have less than 130 cattle and 34 cattle farms having than 130 cattle were determined using census method[1]. The total number of surveyed beef cattle farms was 171. The sampling method is determined with 99% a confidence interval and 1% of error. The sampling procedure was presented in Equation 1 and Table 1 (Yamane 2001).

$$n = \frac{N\sum(N_h S_h^2)}{N^2 D^2 + \sum(N_h S_h^2)} \quad (1)$$

Where; n, sample size for the strata of I and II (137); N, population size (761); N_h, number of units in the strata of h; S_h, the standard deviation in the strata of h; $D^2 = d^2/Z^2$; d, level of precision (acceptable sampling error); z, the value from z score table.

Table 1- The population and sample of the research

Strata	*Strata range (per cattle)*	*N*	*n*	*Method*
I	1-59	628	110	Sample
II	60-129	133	27	Sample
III	130+	34	34	Census
Total	---	799	171	-

2.3. Method

In this research, Treatment Effect Model was used to analyze the impacts of supports on production and gross profit of beef cattle farms. TEM is used to estimate the impacts of a treatment and evaluate the probable outcome of it. In the model, there is a treatment that is farms which receive a support from the government. This model is used to determine the difference between the state where the farmer does not receive support and the state he/she receives. If the difference is positive and statistically significant, there is an incentive for the sustainability of support; otherwise, it means that other plans should be considered. However, the information of the farmer in the absence of support is sometimes not fully achieved. For this purpose, it integrates the farmers who do not receive support but they are totally similar in socio-demographic and economic characteristics with farmers who receive the support in order to put the difference between the two states. The aim of the empirical model is to determine whether this treatment has an impact on response variable and if has, the direction of this impact (Hsieh 2009).

Let outcome y_j, treatment t_j, error term ε_j and the vector of all exogeneous covariates $z_j = (w_j x_j)$, the equation can be denoted;

$$E(y_j | x_j, t_j, \varepsilon_j) = \exp(x_j\beta + \delta t_j + \varepsilon_j) \quad (2)$$

Where; y_{0j} is potential outcome without treatment ($t_j = 0$), y_{1j} is potential outcome with treatment ($t_j = 1$), β_0 and β_1 are coefficients for the control and treatment regimens and let the potential outcome model be;

$$E(y_{0j} | x_j, \varepsilon_j) = \exp(x_j\beta_0 + \varepsilon_{0j})$$

$$E(y_{1j} | x_j, \varepsilon_j) = \exp(x_j\beta_1 + \varepsilon_{1j})$$

[1] Although there were 38 cattle farms in the third strata, 4 of them were unanswered because of repetition and merging of farms.

$$t_j = \begin{Bmatrix} 1, w_j\gamma + u_j > 0 \\ 0, otherwise \end{Bmatrix} \quad (3)$$

In the binary model, y_{0j} and y_{1j} have never been observed together and it can be denoted;

$$y_j = t_j y_{1j} + (1 - t_j)\, y_{0j} \quad (4)$$

Average treatment effect and average treatment effect on the treated are the major parameters of the TEM model. ATE refers to the average treatment effect and it is the average difference of treatment and control potential outcomes and estimated by;

$$ATE = E\left[\{\exp(x_j\beta_1) - \exp(x_j\beta_0)\}\exp\left(\frac{\sigma^2}{2}\right)\right]$$

$$= E\{E(y_{1j} - y_{0j}|z_j)\} = E(y_{1j} - y_{0j}) \quad (5)$$

On the other hand, ATET refers to the average treatment effect on the treated and it is the average effect of treatment on outcome compared with no treatment for a random draw from the subpopulation selecting (or assigned) no treatment (Rubin 1974; Heckman & Robb 1985; Terza 1998; Angrist 2001).

$$ATET = \left[\{\exp(x_j\beta_1) - \exp(x_j\beta_0)\}\exp\left(\frac{\sigma^2}{2}\right)\frac{\Phi(\rho\sigma + w_j\gamma)}{\Phi w_j\gamma}|t_j = 1\right]$$

$$= E\{E(y_{1j} - y_{0j}|z_j, t_j = 1)|t_j = 1\} = E(y_{1j} - y_{0j}|t_j = 1) \quad (6)$$

In this study, livestock supports are taken account as treatment and the impacts of this treatment on beef meat production and gross profit were estimated. The model is based on the three assumptions such as the livestock supports have (i) no effect, (ii) increase effect or (iii) decrease effect on the amount of beef meat produced and gross profit of the farms.

3. Results and Discussion

3.1. Descriptive analysis

The descriptive statistics are presented in Table 2. The results of the study indicate that 50.9% of beef cattle farms were specialized in the beef cattle breeding and 57.3% of them were keeping physical or financial records. About 25% of cattle farms employed permanent labor for beef cattle breeding, whereas 59.1% of them employed temporary labor. About 64% of total farms were small-scale owning less than 60 cattle. The average gross profit of farms was $ 60435.23[2]. About 95% of beef cattle farms benefited from $ 4969.73 of total agricultural support, whereas 80.1% of farms benefited from $ 2425.19 of fattening male cattle and forage crop supports. The share of fattening male cattle and forage crop supports into the total agricultural supports was 48.8%. The farms spent 90% of the support revenues for agricultural activities.

The cattle farms were averagely 8.25 km far from the district center and 40.4% of them have located in Bafra and Çarşamba districts. The average agricultural land was 10.7 ha and 32.2% of farms had 10 ha or more agricultural land. However, average agricultural land was found 15.8 ha for the unionized and 7.1 ha non-unionized farmers in the study of Özüdoğru & Tatlıdil (2012). The average agricultural land of Goodwin & Mishra (2005; 2006), Latruffe et al (2013) and Giannoccaro & Berbel (2013) is above the result of this research whereas it is below in the research of Majewski et al (2011). About 82% of the farms grew forage crops.

[2] In the field research period, the average exchange rate of TL/$ is 2.33

The average membership duration in Turkish Beef and Lamb Producers Associations was 4.29 years and 3.5% of farm managers participated in the governance of the association. Besides, 61.4% of beef cattle farms were a member of other farmer organizations. Majewski et al (2011) stated the proportion of unionization as 54% whereas it was estimated 55% and 79.7% in Giannoccaro & Berbel (2013) and Gorton et al (2008), respectively. About 76% of the farms had non-agricultural income. About 91% of the farms own one or more agricultural machines and 53.8% of the farms had tractor and trailer.

The farms had averagely 6.84 household members. About 73% of householders' main profession was a farmer, whereas 14% of them perform the beef cattle breeding activity with other profession. Therefore, 87.7% of the farms directly conduct cattle breeding activity. While 68.18% of households were within the economic active age group (15-64 age), main profession of 83.6% of them is farmer. The average age of managers was 49.16 years and average experience in beef cattle farming was 20.19 years. About 64.91% of managers graduated from primary school, whereas 17.5% of them graduated from high school or above. The share of high school graduates was found as 65% in the study of Majewski et al (2011) and 13.5 years in the study of Gorton et al (2008).

Table 2- Descriptive statistics of beef cattle farms

Variables	*Mean*	*Std. dev.*
Cattle farming		
Specialized in beef cattle breeding (Yes= 1, No= 0)	0.509	0.501
Keeping record (Yes= 1, No= 0)	0.573	0.496
Temporary labor employment (Yes= 1, No= 0)	0.591	0.493
Permanent labor employment (Yes= 1, No= 0)	0.251	0.435
Cattle farm size is between 1 and 59 beefs (Yes= 1, No= 0)	0.643	0.480
Gross profit ($)	60435.23	109034.38
Benefit from cattle breeding supports (Yes = 1, No= 0)	0.801	0.400
Farm structure		
Distance of the farm to the district center (km)	8.257	6.855
The farm is situated in Bafra ve Çarşamba districts (Yes= 1, No= 0)	0.404	0.492
Household size (unit)	6.842	3.767
Farm size is over 10 ha (Yes= 1, No= 0)	0.322	0.468
Growing fodder crop (Yes= 1, No= 0)	0.819	0.386
Membership duration into the Beef and Lamb Producers Association (year)	4.292	1.903
Participation in the management of the Management of Beef and Lamb Producers Association (Yes= 1, No= 0)	0.035	0.185
Membership of other farmer organizations (Yes= 1, No= 0)	0.614	0.488
Have non-agricultural income (Yes= 1, No= 0)	0.760	0.428
Tractor and trailer ownership (Yes= 1, No= 0)	0.538	0.500
Other agricultural machines ownership (Yes= 1, No= 0)	0.906	0.292
Manager		
The main profession as farmer (Yes= 1, No= 0)	0.877	0.329
The cattle farming experience (year)	20.199	11.022
High school or higher education (Yes= 1, No= 0)	0.175	0.381
Agricultural supports ($)	4969.73	8238.30
Cattle breeding supports ($)	2425.19	3614.39

About 71% of cattle farms were satisfied with the cattle breeding activity. Nevertheless, the major reason of dissatisfaction was stated as high cost of production and inadequacy of supports. About 49% of the farms intended to increase the number of cattle in the near future. The fundamental problems of the farms were stated as increase in forage prices, an inadequacy of support policies and negative effects of cattle import on production and prices. Aydın et al (2010) found that 31.7% of the farmers have not considered the increase in supports as a solution for a rise in beef meat prices. However, Goodwin & Mishra (2005) highlighted that 54% of the farmers consider the costs as the main element of production decision.

The amount of supports was not seen sufficient by 66.7% of the farmers and 40.4% of the farmers stated that support payments were not paid on time. Almost half of the farmers stated that the announcement of supports was not enough and the application procedure of the supports takes much time due to the red tape (Table 3).

Table 3- The problems facing with utilization of supports

Problem area	*1. Strongly disagree*	*2. Disagree*	*3. Neutral*	*4. Agree*	*5. Strongly agree*	*Total score*	*Rank*
The amount of support is not sufficient	1.2	7.6	1.2	23.3	66.7	764	1
Supports are not paid on time	11.7	40.4	1.1	21.1	25.7	528	2
The request of support takes much time due to red tape	15.8	35.1	1.7	18.7	28.7	529	3
The announcement of supports isn't adequate	25.1	29.2	0.6	32.2	12.9	476	4
The supports could be confiscated due to debt, sponsorship etc.	29.9	33.9	2.3	14.0	19.9	445	5

The main expectations of the farms on cattle breeding were stated respectively as getting stability in cattle and meat prices, reducing forage prices, enhancing and revising supports in respect to quality, hygiene and amount of meat, paying supports on time and enhancing extension opportunities (Table 4).

Table 4- The expectations of the farms on cattle breeding

Type of expectation	*1. Strongly disagree*	*2. Disagree*	*3. Neutral*	*4. Agree*	*5.Strongly agree*	*Total score*	*Rank*
The price of cattle and meat should be stable	0.6	0	1.2	18.7	79.5	815	1
The price of forage should be decreased	0.6	1.2	2.3	16.4	79.5	809	2
The amount of supports should be increased	0	4.7	1.2	12.3	81.9	806	3
The supports should be paid on time	0	0	4.7	41.2	54.1	764	4
The producers should be provided with more extension opportunities about breeding	1.8	2.3	1.8	47.4	46.8	744	5
The supports should be focused on quality, hygiene and amount of production	8.2	2.9	3.5	31.0	54.4	719	6
Membership fee of the unions should be lessened.	1.2	14.0	10.5	28.7	45.6	690	7
The supports should be seasonally organized	2.9	26.3	10.5	21.6	38.6	627	8

3.2. Treatment effect model results

TEM model was analyzed for both production (beef meat) and gross profit. The log-likelihood value and sigma (σ) value was measured -241.445 and 0.776 for beef meat model and -340.112 and 1.495 for gross profit model. Rho (ρ) parameter shows that one standard deviation in the probability of benefit from supports resulted in 0.39 standard deviation in beef meat production and 0.02 standard deviation in gross profit. But these effects were not found statistically significant.

Even though there is no relationship between the probability of cattle breeding supporting system and the amount of meat production and gross profit when taking non-controlled factors into account (e.g., correlation coefficients), the results of the model highlight that cattle breeding supports had positive statistically significant effect on production of beef meat. Therefore, these supports are essential to boost beef meat production and to ensure economic sustainability of beef cattle sector. On the other hand, cattle breeding supports had statistically insignificant effect on gross profit. By the way, this effect indicates that cattle farms could not be financially well-managed. Besides, the statistically insignificant effect can be explained by the high costs of production in the farms that was not get benefit from the livestock supports.

According to the beef meat production and gross profit TEM results, cattle farms which are situated in Çarşamba and Bafra districts and keeping farm records were more likely to benefit from livestock supports than their counterparts. On the other hand, the gross profit model resulted that the farms that have land larger than 10 ha were also more likely to benefit from livestock supports. The effect of location can be explained by some reasons. First of all, beef cattle farms in Bafra and Çarşamba the districts were relatively large-scaled, closer to district center and had easier access to information sources of supports. Besides, one of the Beef and Lamb Producers Association has located in Bafra district. The higher probability of benefiting from subsidy for the farms keeping records can be explained by their advantages such as management, planning, and technology. Lastly, the farms that have 10 ha or larger land grow forage crops and provide the roughage requirement of their farms. Therefore, it enables the farms to decrease their production costs and increase their profitability.

The results of beef meat production and gross profit models were presented in Table 5. The results indicate that supports, specialization, keeping a record, employing permanent labor and having higher mechanization level had statistically significant positive effects on beef meat production. On the other hand, the location of the farm and non-agricultural income had a statistically significant negative effect on beef meat production. Benefiting from higher supports by the farms in Çarşamba and Bafra districts decrease production risk and negatively affect the expansion of the scale of farms. The farms that have non-agricultural income produce less beef meat than the other farms. This shows that that non-agricultural income prevents farms from specialization and expansion of the scale.

The results of the gross profit model indicate that the participation into the management of Beef and Lamb Producers Association, higher mechanization and education level of a manager (high school or over) had statistically significant positive effects on beef meat production, whereas ownership of tractor and trailer had statistically significant negative effects. The farms whose manager participated in the management of Beef and Lamb Producers Association had higher gross profit than their counterparts. Because they had more chance to get technical assistance, cheaper input and market their products with better conditions by the association. Although the gross profit of the farms which had the modern machines for cattle farming was higher than the others, the gross profit of the farms which had tractor and trailer was lower than the other farms. The farms who own tractor and trailer concentrate on crop production and their beef meat production and gross profit was lower than their counterparts. Lastly, the farms whose education level of a householder with high school or higher education had higher gross profit than their counterparts. This parameter shows that education had a positive effect on the profitability of the farm because education is essential on adoption and application of new technologies.

ATE and ATET of beef meat production and gross profit models are presented in Table 6. Cattle breeding supports increase the meat production by 11760 kg and the gross profit by $ 8025.75 on average. Among the farms that are supported (treated), the beef meat production of a farm increase by 12620 kg when it is supported compared with the case that it is not supported and the coefficient of production is statistically significant. On the other hand, though the gross profit also increases by $ 7811.15 in this comparison, the results of the research highlight that the coefficient of gross profit is not statistically significant. Therefore, we can say that cattle breeding supports significantly increase average meat production in the region. At the same time, this increase is more important among the beneficiaries (e.g., the treated farms). However, although the increase in gross profit is not statistically significant, the estimated values can be attributed to increased production costs. The optimal use of inputs used in meat production can make gross profit more advantageous and make the use of supports more sustainable.

Table 5- TEM results for beef meat production and gross profit of cattle farms

Variables	*Probability of being supported*		*Production value (log)*		*Probability of being supported*		*Gross profit*	
	Coefficient	*t-value*	*Coefficient*	*t-value*	*Coefficient*	*t-value*	*Coefficient*	*t-value*
Constant	1.6702	0.9825	-1.7181***	-2.8464	1.2876	0.8007	-1.0174	-0.6983
Cattle farming								
Specialized in beef cattle breeding	0.7328	1.5828	0.6573***	3.0660	0.6588	1.4490	0.3557	0.8852
Keeping record	0.8850*	1.7513	0.4359**	2.1190	0.8832*	1.8484	0.3594	0.6986
Temporary labor employment	-0.1737	-0.4387	0.1807	1.0282	-0.0944	-0.2588	0.1928	0.5114
Permanent labor employment	-0.2803	-0.3016	0.5103**	2.3811	-0.0004	-0.0005	0.6437	1.5389
Cattle farm size is between 1 and 59 beefs	-1.4818	-1.3863	-	-	-1.2239	-1.4250	-	-
Farm Structure								
Distance of the farm to the district center (km)	-0.0053	-0.1239	0.0077	0.5301	-0.0074	-0.1718	0.0342	1.2735
The farm is situated in Bafra and Çarşamba districts	1.1204**	2.2191	-0.4423*	-1.8916	1.0572**	2.2412	-0.4358	-1.0114
Household size (unit)	0.0189	0.2570	0.0211	0.8065	0.0174	0.2484	-0.0273	-0.6112
Farm size is over 10 ha	0.7427	1.1769	-0.1640	-0.7802	0.8162*	1.7266	-0.0107	-0.0210
Growing fodder crop	-	-	-0.3469	-1.5379	-	-	-0.4494	-1.0910
Membership duration into the Beef and Lamb Producers Association (year)	-0.1576	-1.4325	0.0155	0.3450	-0.1451	-1.1662	0.1262	1.2560
Participation in the Management of Beef and Lamb Producers Association	-1.0688	-0.7190	0.5110	0.7160	-0.9306	-0.6811	1.5275**	2.5064
Membership of other farmer organizations	-0.6708	-1.1975	-0.0249	-0.1278	-0.7035	-1.3009	-0.6753	-1.6539
Have non-agricultural income	0.2437	0.4600	-0.3456*	-1.6560	0.2112	0.4240	0.0589	0.1398
Tractor and trailer ownership	-0.2379	-0.2751	0.6042*	1.7977	-0.2302	-0.3154	1.4370*	1.9332
Other agricultural machines ownership	0.1434	0.2615	-0.2719	-1.4830	0.0882	0.2074	-0.6655*	-1.7311
Manager								
The main/second profession as farmer	0.0045	0.0071	-0.1769	-0.7496	0.1953	0.3301	0.2530	0.3623
The cattle farming experience (year)	0.0076	0.3603	-0.0008	-0.1040	0.0095	0.4628	-0.0016	-0.1092
Education level is equal or over high school	-0.1937	-0.3101	0.2292	1.0478	-0.2353	-0.3742	0.9100**	2.2800
Benefit from cattle breeding supports	-	-	1.4778***	3.1693	-	-	0.2760	0.1619
σ	0.7761***	12.2346			1.4953***	15.308		
ρ	-0.3969	-1.0522			-0.0277	-0.0322		
Log-likelihood	-241.444				-340.112			

***, significant at 1%; **, significant at 5%; *, significant at 10%

Table 6- ATE and ATET of production and gross profit

Variables	*Production*		*Gross profit*	
	Coefficient	*t-value*	*Coefficient*	*t-value*
ATE	1.176***	3.135	0.080	0.110
ATT	1.262***	3.020	0.078	0.100

***, significant at 1%; **, significant at 5%; *, significant at 10%

4. Conclusions

Although the number of cattle and beef meat production has been substantially raised by means of supports in Turkey in recent years, the domestic production could not fulfill the demand of beef meat yet and therefore the demand has been met by a great amount of import. In this sense, the evaluation of the impacts of livestock supports on beef cattle farms is essential to analyze the efficiency of resource utilization, self-sufficiency, and sustainability of beef cattle sector. This research seeks to identify the impact of cattle breeding supports on production and income of beef cattle farms via the case of Samsun province.

The results of this study highlight that the farms have larger land and herd, specialized in beef cattle breeding, using modern devices and machines, and keeping records were more likely to benefit from livestock supports than their counterparts. The essential result of the study indicates that the supports could increase the production and income of the farms. Nevertheless, the cattle breeding supports had only a statistically significant effect on the beef meat production. As the supports had a significant contribution to the self-sufficiency of beef meat production, the farms should be continuously supported in order to increase the production in spite of no effect on gross profit. The statistical insignificance of gross profit model can be explained with the inability of farms to transform the physical product to fiscal return. The reason why the farms could not able to achieve sufficient gross profit can be explained by about half of the farms have lack of record and could be hardly managed. Therefore, cattle farms should be encouraged to keep financial records via mandatory of Farm Accountancy Data Network and supports should be revised in the form that the more detail financial record the farms have, the more support they could be granted. Although the specialization of about half of the farms in beef cattle breeding is essential, the dependency on external input for breeding cattle has increased the farm costs. This dependency raises the requirement of capital and reduces the economic profitability and sustainability in the long run. The supports would be effective in this sense and they should be revised in order to encourage the farms to produce their own breeding cattle and reduce the costs.

Cross-sectional and province-based data were used in this study. Therefore, it is proposed that the data should be expanded to regional and countrywide studies using either panel or single cross-sectional data in order to consider the wide perspective of the sector. Future researches should also examine all supply chain of beef meat sector. In this study, only support based economic sustainability was examined. It is also recommended that social, environmental and politic sustainability of the cattle farming should be analyzed in order to get a complete view of the sector.

Acknowledgements

This study is quoted from a part of the PhD thesis of Nevra ALHAS EROĞLU. It was supported by The Scientific and Technological Research Council of Turkey and Ondokuz Mayis University.

References

Acs S, Hanley N, Dallimer M, Gaston K J, Robertson P, Wilson P & Armsworth P R (2010). The effect of decoupling on marginal agricultural systems: Implications for farm incomes, land use and upland ecology. *Land Use Policy* 27(2): 550-563

Aksoy A, Terin M & Keskin A (2012). Türkiye süt sığırcılığında ıslah ve destekleme politikalarının bölgesel etkileri üzerine bir araştırma. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi* 43(1): 59-64

Alhas Eroğlu N (2017). Samsun ilinde hayvancılık desteklerinin besi işletmelerinin üretim ve gelirlerine etkileri. PhD Thesis, Ondokuz Mayis University (Published), Samsun, Turkey

Angrist J D (2001). Estimation of limited dependent variable models with dummy endogenous regressors: Simple strategies for empirical practice. *Journal of Business and Economic Statistics* 19(1): 2-28

Anonymous (2015). Kırmızı Et Stratejisi. Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Livestock, Ankara, Turkey

Anonymous (2016). http://www.tuketbir.org.tr/basin_detay.asp?gidenID=MzI=

Anonymous (2018). Kalkınma Bakanlığı, 11. Kalkınma Planı, Tarım ve Gıdada Rekabetçi Üretim, Özel İhtisas Komisyonu Raporu, Ankara, Turkey

Aydın E, Can M F, Aral Y, Cevger Y & Sakarya E (2010). Türkiye'de canlı hayvan ve kırmızı et ithalatı kararlarının sığır besicileri üzerine etkileri. *Veteriner Hekimler Derneği Dergisi* 81(2): 51-57

Bartolini F & Viaggi D (2013). The common agricultural policy and the determinants of changes in EU farm size. *Land Use Policy* 31: 126-135

Breen J P, Hennessy T C & Thorne FS (2005).The effect of decupling on the decision to produce: An Irish case study. *Food Policy* 30(2): 129-144

Chau N H & De Gorter H (2000). Disentangling the production and export consequences of direct farm income payments. In: *American Agricultural Economics Association Meetings*, July 30-August 2, No: 21854, Tampa, Florida

Çelik C & Sarıözkan S (2017). Kırşehir ili merkez ilçede sığır besiciliği yapan işletmelerin ekonomik analizi. *Harran Üniversitesi Veteriner Fakültesi Dergisi* 6(1): 38-45

Demir N (2009). Destekleme politikalarının hayvancılık sektörü üzerine etkilerinin bölgesel karşılaştırmalı analizi. PhD Thesis, Atatürk University (Published), Erzurum, Turkey

Demir N & Yavuz F (2010). Hayvancılık destekleme politikalarına çiftçilerin yaklaşımlarının bölgelerarası karşılaştırmalı analizi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi* 41(2): 113-121

Douarin E, Bailey A, Davidova S, Gorton M & Latruffe L (2007). Structural, location and human capital determinants of farmers' response to decoupled payments. *EU FP6 Project IDEMA (Impact of Decoupling and Modulation in the Enlarged EU: a sectorial and farm level assessment)*, Deliverable 14

El Benni N, Finger R & Mann S (2012). Effects of agricultural policy reforms and farm characteristics on income risk in Swiss agriculture. *Agricultural Finance Review* 72(3): 301-324

FAO (2018). Databases. Retrieved in September 10, 2018 from http://www.fao.org/faostat/en/#data/QV

Genius M, Karagiannis G & Tzouvelakas V (2008). Assessing European farmers' intentions in the light of the 2003 CAP reform. In: Paper Presented at the 109th European Association of Agricultural Economics (EAAE) Seminar "The CAP after the Fischer Reform: National Implementations, Impact Assessment and the Agenda for Future Reforms', 20-21 November, Viterbo, Italy

Giannoccaro G & Berbel J (2013). Farmers' stated preference analysis towards resources use under alternative policy scenarios. *Land Use Policy* 31: 145-155

Goodwin B K & Mishra A K (2005). Another look at decoupling: Additional evidence on the production effects of direct payments. *American Journal of Agricultural Economics* 87(5): 1200-1210

Goodwin B K & Mishra A K (2006). Are "decoupled" farm payments really decoupled? An empirical evaluation. *American Journal of Agricultural Economics* 88(1): 73-89

Gorton M, Douarin E, Davidova S & Latruffe L (2008). Attitudes to agricultural policy and farming futures in the context of the 2003 CAP reform: A comparison of farmers in selected established and new Member States. *Journal of Rural Studies* 24: 322-336

Heckman J J & Robb R (1985). Alternative methods for evaluating the impact of interventions. *Journal of Econometrics* 30: 239-267

Hennessy D (1998). The production effects of agricultural income support policies under uncertainty. *American Journal of Agricultural Economics* 80(1): 46-57

Hsieh Y (2009). Lecture Note on Treatment Effects Analysis. New York University, pp. 1

Kazukauskas A, Newman C & Sauer J (2014). The impact of decoupled subsidies on productivity in agriculture: across-country analysis using micro data. *Agricultural Economics* 45: 327-336

Keskin A, Dağdemir V & Yavuz F (2010). Türkiye et sığırcılığında ıslah ve destekleme politikalarının bölgesel etkileri üzerine bir çalışma. In: *Türkiye 9. Ulusal Tarım Ekonomisi Kongresi*, 22-24 Eylül, Şanlıurfa, Türkiye

Latruffe L, Dupuy A & Desjeux Y (2013). What would farmers' strategies be in a no-CAP situation? An illustration from two regions in France. *Journal of Rural Studies* 32: (10-25). pp. 14

Lobley M & Butler A (2010). The impact of CAP reform on farmers' plans for the future: some evidence from South West England. *Food Policy* 35(44): 341-348

Majewski E, Sulewski P, Raggi M & Viaggi D (2011). Differences in possible reactions of EU farmers from selected European regions to CAP Change. *Acta Scientiarum Polonorum Oeconomia* 10(1): 45-56

Morgan-Davies C, Waterhouse T & Wilson R (2012). Characterization of farmers' responses to policy reforms in Scottish hill farming areas. *Small Ruminant Research* 102(2-3): 96-107

Moss J, McErlean S, Kostov P & Patton M (2002). Analysis of impact of decoupling on agriculture in the UK. Food and Agricultural Policy Research Institute, University of Missouri

O'Donoghue E J & Whitaker J B (2010). Do direct payments distort producers' decisions? An examination of the Farm Security and Rural Investment Act of 2002. *Applied Economic Perspectives and Policy* 32(1): 170-193

Özkan U & Erkuş A (2003). Bayburt ilinde sığır besiciliğine yer veren tarım işletmelerinin ekonomik analizi. *Tarım Bilimleri Dergisi* 9(4): 467-472

Özüdoğru T & Tatlıdil F F (2012). Amasya Damızlık Sığır Yetiştiricileri Birliğine üye olan ve olmayan işletmelerin ekonomik analizi ve süt sığırcılığına yönelik desteklerin gelire etkisi. *Selçuk Tarım ve Gıda Bilimleri Dergisi* 26(3): 42-29

Revell B & Oglethorpe D (2003). Decoupling and UK agriculture: a whole farm approach. Study Commissioned by DEFRA, London

Rubin D B (1974). Estimating casual effects of treatments in randomized and nonrandomized studies. *Journal of Educational Psychology* 66(5): 688-701

Sckokai P & Moro D (2006). Modeling the reforms of the common agricultural policy for arable crops under uncertainty. *American Journal of Agricultural Economics* 88(1): 43-56

Severini S & Tantari A (2013). The effect of the EU farm payments policy and its recent reform on farm income inequality. *Journal of Policy Modeling* 35: 212-227

Shrestha S, Hennessy T & Hynes S (2007). The effect of decoupling on farming in Ireland: A regional analysis. *Irish Journal of Agricultural and Food Research* 46: 1-13

Terza J V (1998). Estimating count data models with endogenous switching: sample selection and endogenous treatment effects. *Journal of Econometrics* 84(1): 129-154

Topçu Y (2008). Çiftçilerin tarımsal destekleme politikalarından faydalanma istekliliğinde etkili faktörlerin analizi: Erzurum ili örneği. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi* 21(2): 205-212

Topçu Y, Uzundumlu A S & Kızıloğlu S (2008). Sığır besiciliği işletmelerinin başarısızlığında etkili faktörlerin analizi: Erzurum ili örneği. *Alınteri* 15(B): 19-25

TURKSTAT (2018a). Retrieved in agricultural prices and economic accounts and livestock statistics. Retrieved in September 10, 2018 from http://www.tuik.gov.tr/PreTablo.do?alt_id=1004

TURKSTAT (2018b). Retrieved in crop production statistics. Retrieved in September 10, 2018 from https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul

TURKSTAT (2018c). Retrieved in statistical indicators, regional statistics. Retrieved in September 10, 2018 from https://biruni.tuik.gov.tr/bolgeselistatistik/tabloOlustur.do#

Viaggi D (2011). Approaches to research in support to agricultural policy: The experience of the CAP-IRE Project. *Acta Scientiarum Polonorum Oeconomia* 10(2): 83-94

Weber J G & Key N (2012). How much do decoupled payments affect production? An instrumental variable approach with panel data. *American Journal of Agricultural Economics* 94(1): 52-66

Yamane T (2001). Elementary Sampling Theory. Prentice Hall Inc., Englewoods, New Jersey

Yılmaz H, Demircan V & Dernek Z (2008). Türkiye tarımında doğrudan gelir desteği uygulamaları (Isparta ili üreticileri açısından bir değerlendirme). *Doğuş Üniversitesi Dergisi* 9(2): 248-265

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. In the discipline encompassing the science of food, our journal only publishes novel and high quality original research papers only about basic and applied research topics in food science, food chemistry, and food microbiology and biotechnology. Articles regarding the topics of food engineering and nutrition are out of scope.

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. Authors will normally receive reviewers' comments within 8 weeks of submission.

Manuscript Submissions: Manuscript should be submitted to journal's online submission system by the corresponding author.

All submissions should in-clude following documents:

1. Title page with author names, titles, addresses and contact information (in Word format).
2. Manuscript (in Word format). All author names, titles and affiliations should be excluded.
3. 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.
4. Submission Check List (in PDF format).
5. Document showing the result of iThenticate (max. 24% match accepted).
6. 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),
- Abstract (maximum 250 word),
- Introduction,
- Material and Methods,
- Results and Discussion,
- 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 canbe suitable for reproduction. Label each 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/s2). 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).