KSÜ Tarım ve Doğa Derg
KSU J. Agric Nat
e-ISSN : 2619-9149

T.C.
KAHRAMANMARAŞ
SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

Journal of Agriculture and Nature

Cilt-Volume 27 Sayı-Number 4 Yıl-Year: 2024

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

Dergimiz aşağıdaki indeksler tarafından taranmaktadır (This journal is indexed and abstracted by)

- Emerging Sources Citation Index
- TUBİTAK-TR Dizin
- CAB Abstracts
- CiteFactor
- DRJI (Directory of Research Journal Indexing)
- Google Scholar
- Index Copernicus
- International Directory of Agriculture, Food and The Environment
- Journal Index
- Scientific Indexing Services (SIS)
- The International Plant Names Index

Yazışma Adresi Corresponding Address
Kahramanmaraş Sütçü İmam Üniv.
Tarım ve Doğa Dergisi,
46100 – KahramanmaraşTÜRKİYE
Tel : (+90-344) 300 2108

E-mail: dogabilimleri@ksu.edu.tr
Web: http:dergipark.org.trksudobil
http:dogadergi.ksu.edu.tr

Bu dergi uluslararası hakemli olup yılda 6 kez yayınlanır.
This journal is international peer-reviewed and published 6 issues per year.

Dergimiz, herhangi bir başvuru veya yayımlama ücreti almamaktadır
The Journal doesn't have APC or any submission charges.

Derginin Eski AdıPrevious Name of Journal
KSU Fen ve Mühendislik Dergisi
KSU Journal of Science and Engineering
KSU Doğa Bilimleri Dergisi
KSU Journal of Natural Scienence
Derginin Eski ISSN NumarasıPrevious ISSN Number
1301-2053

Cilt-Volume 27 Sayı-Number 4 Yıl-Year 2024

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
TARIM ve DOĞA DERGİSİ

İÇİNDEKİLER

ARAŞTIRMA MAKALESİ - RESEARCH ARTICLE

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
TARIM ve DOĞA DERGİSİ

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
TARIM ve DOĞA DERGİSİ

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

HAKEMLER (Referees)*

Dr.Öğr. Üyesi	Asli Can	AGCA	Ankara Yıldırım Beyazıt Universitesi, Ankara
Prof. Dr.	Ertuğrul	AĞIRBAŞ	Recep Tayyip Erdoğan Universitesi, Rize
Doç. Dr.	Gülçin Beker	AKBULUT	Malatya Turgut Özal Universitesi, Malatya
Prof. Dr.	Yavuz	AKBAŞ	Ege Üniversitesi, İzmir
Doç. Dr.	Hasan	AKAY	Ondokuz Mayıs Üniversitesi, Samsun
Prof. Dr.	Adem	AKSOY	Atatürk Üniversitesi, Erzurum
Doç. Dr.	Mehmet	AKYÜZ	Kilis 7 Aralık Üniversitesi, Kilis
Ass. Prof.	Solmaz	AZIMI	K.N. Toosi University of Technology, Tahran-İRAN
Prof. Dr.	Mustafa	BAYRAM	Gazıantep Unıversıtesi, Gaziantep
Doç. Dr.	Hakan	BOZDOĞAN	Ahi Evran Üniversitesi, Kırşehir
Doç. Dr.	Bekir	DEMİRTAŞ	Hatay Mustafa Kemal Üniversitesi, Hatay
Doç. Dr	Zeynep	DUMANOĞLU	Bingöl Üniversitesi, Bingöl
Dr.Öğr.Üyesi	Burcu	ERDAL	Bursa Uludağ Üniversitesi, Bursa
Prof. Dr.	Muzaffer	FEYZİOĞLU	Karadeniz Teknik Üniversitesi, Trabzon
Prof. Dr.	Hakan	GEREN	Ege Üniversitesi İzmir
Doç. Dr.	Gamze	GÖGER	Afyonkarahisar Sağlık Bilimleri Üniversitesi Afyon
Doç. Dr.	Murat	GUNEY	Yozgat Bozok Unıversitesi, Yozgat
Dr. Öğr.Üyesi	A. Zeki	HEPÇİMEN	Manisa Celal Bayar Üniversitesi, Manisa
Prof. Dr.	Serra	HEPAKSOY	Ege Üniversitesi İzmir
Doç. Dr.	Sümer	HORUZ	Ercıyes Unıversıtesi, Kayseri
Dr.Öğr.Üyesi	Harun	HURMA	Tekirdağ Namık Kemal Üniversitesi, Twkirdağ
Prof. Dr.	Ali	İSLAM	Ordu Üniversitesi, Ordu
Doç. Dr.	Selin	KALKAN	Giresun Üniversitesi, Giresun
Prof. Dr.	Orkun Barış	KOVANCI	Bursa Uludağ Üniversitesi, Bursa
Prof. Dr.	Müge	KANTAR DAVRAN	Çukurova Üniversitesi, Adana
Dr.Öğr.Üyesi	Mustafa Ali	KAPTAN	Adnan Menderes Unıversitesi, Aydın
Prof. Dr.	Gülay	KAÇAR	Bolu Abant İzzet Baysal Üniversitesi, Bolu
Dr.Öğr.Üyesi	Ece Börtecine	KASAPOĞLU	Çukurova Üniversitesi, Adana
Prof. Dr.	Sevim	KAYA	Gaziantep Üniversitesi, Gaziantep
Doç. Dr.	Ali Rahmi	KAYA	Kahramanmaraş Sütçü İmam Üniv. Kahramanmaraş
Doç. Dr.	Beşir	KOÇ	Bingöl Üniversitesi, Bingöl
Doç. Dr.	Aysen	KOÇ	Yozgat Bozok Üniversitesi, Yozgat

* Soyada göre sıralanmıştır.

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ

TARIM ve DOĞA DERGİSİ

KSÜ Tarım ve Doğa Derg 27 (4), 749-757, 2024
KSU J. Agric Nat 27 (4), 749-757, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1300933

A Diversity Analysis of Fruits of Strawberry Tree (*Arbutus andrachne* L.) Grown in Isparta-Türkiye

Ebru Hatice TIĞLI KAYTANLIOĞLU[1] ✉ Hüseyin FAKİR[2], Uğur ÖZKAN[3], Candan KUŞ ŞAHİN[4]
[1,2]Department of Forest Engineering, Forestry Faculty, Isparta University of Applied Sciences, Isparta, Türkiye, [3]Department of Forest Products Engineering, Forestry Faculty, Isparta University of Applied Sciences, Isparta, Türkiye, [4]Department of Landscape Architecture, Architecture Faculty, Suleyman Demirel University, Isparta, Türkiye
[1]https://orcid.org/0000-0002-9165-6675, [2]https://orcid.org/0000-0002-6606-8011,[3]https://orcid.org/0000-0003-0147-99763
[4]https://orcid.org/0000-0002-0413-2380
✉: ebrukaytanlioglu@isparta.edu.tr

ABSTRACT
The growing locations of plants may affect product quality adversely in several ways. Fruits containing a different percentage of chemicals may cause adverse physicochemical properties. This study, it was evaluated certain physicochemical properties of fruits obtained from Strawberry Tree fruits (*Arbutus andrachne* L.) which were collected from four different geographical locations of stands that are managed Regional Directorate of Forestry. A considerable physicochemical difference was found among the fruit samples. The highest color differences among samples were found with sample IV (ΔE: 15.4), followed by sample I (ΔE: 15.1), sample III (ΔE: 7.6) and sample II (ΔE: 3.6), respectively. However, the size properties (diameter and weight) of samples also show some variations. The highest average diameter (12.42 mm) and weight (1.13 g) with a sugar content of 33.81 °Bx were found to be in sample I. The sugar content difference is realizable result was found to be high at lower diameters but at higher weights. Moreover, the total existence of 43 essential oil compounds is determined for sample I and 35 compounds for sample IV, while 31 of them are similar, which represents 97.07% for sample I and 91.78% for sample IV. These values may be a good indication of the geographical locations of the physicochemical properties of fruits of *A. andrachne.*

Botany

Research Article

Article History
Received : 23.03.2023
Accepted : 24.12.2023

Keywords
Arbutus andrachne's fruit
Color
Morphological properties
Essential oils
Isparta-Türkiye

Isparta-Türkiye'de Yetişen Çilek Ağacı (*Arbutus andrachne* L.) Meyvelerinin Çeşitlilik Analizi

ÖZET
Yetişme ortamları bitkilerden elde edilen ürünlerin kalitesine birçok yönden etkileyebilir. Değişik oranlarda kimyasal madde içeren meyvelerin özelliklerinin farklı olması beklenebilir. Bu çalışmada, Isparta Orman Bölge Müdürlüğü yetki alanındaki dört farklı coğrafi bölgeden toplanan Çilek Ağaçlarından (*Arbutus andrachne* L.) meyvelerin genel özellikleri incelenmiştir. Elde edilen sonuçların istatistiksel analizi sonucu meyve örnekleri arasında, fizikokimyasal özellikler bakımından (renk, ağırlık, uçucu yağ içeriği) önemli farklılıklar bulunmuştur. Numuneler arasında en yüksek renk farkı (ΔE) numune IV (ΔE: 15.4) ile ve daha sonra sırasıyla numune I (ΔE: 15.1), numune III (ΔE: 7.6) ve numune II (ΔE: 3.6) de gözlemlenmiştir. Ayrıca meyvelerin fiziksel boyut özellikleri (çap ve ağırlık) bakımından da önemli farklılıklar gözlemlenmiştir. En yüksek ortalama çap (12.42 mm) ve ağırlık (1.13 g) ile birlikte şeker içeriği (brix sayısı) 33.81 °Bx ile numune I'de hesaplanmıştır. Numuneler arasında şeker oranı, fark edilebilir olarak daha düşük çapa fakat daha yüksek ağırlığa sahip meyvelerde hesaplanmıştır. Ayrıca, numune I için toplam 43, numune IV için ise toplam 35 uçucu yağ bileşiği tespit edilmiş fakat bunlardan 31'inin her iki meyve örneklerinde de ortak olarak bulunduğu anlaşılmıştır. Bu ortak bileşikler, toplam uçucu yağ oranının numune I için %97.07'ini numune IV için ise %91.78'ini oluşturduğu hesaplanmıştır. Bu çalışmada elde edilen verilerden, *A. andrachne* meyvelerinin fizikokimyasal

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 23.05.2023
Kabul Tarihi : 24.12.2023

Anahtar Kelimeler
Arbutus andrachne meyvesi
Renk
Morfolojik özellikler
Uçucu yağlar
Isparta-Türkiye

özelliklerinin, coğrafi yetişme ortamlarının açıklanması bakımından bir gösterge olabileceği olarak değerlendirilmiştir.

Atıf Şekli: Tıglı Kaytanlioglu, E.H.., Fakir , H., Ozkan , U., & Kus Sahin , C. (2024) Isparta-Türkiye'de Yetişen Çilek Ağacı (*Arbutus andrachne* L.) Meyvelerinin Çeşitlilik Analizi. *KSÜ Tarım ve Doğa Derg 27* (4), 749-757. https://doi.org/10.18016/ksutarimdoga.vi. 1300933

To Cite : Tıglı Kaytanlioglu, E.H.., Fakir , H., Özkan , U., & Kuş Sahin , C. (2024). A Diversity Analysis of Fruits of Strawberry Tree (*Arbutus andrachne* L.) Grown in Isparta-Türkiye. *KSU J. Agric Nat 27* (4), 749-757. https://doi.org/10.18016/ksutarimdoga.vi. 1300933

INTRODUCTION

Plant growth is greatly affected by the environment, which needs suitable atmospheric conditions to survive. However, the need for planting must be optimal, otherwise it limits growth or distribution. Some of the important environmental factors that affect plant growth include light, temperature, water, humidity, elevation, and nutrition (Körner et al., 1989; Anonymous, 2024a).

Arbutus andrachne L. is a member of the Ericaceae family which is called the strawberry tree, naturally spread from the East Mediterranean to the Northern Black Sea area in Turkiye (Beyhan, et al., 2020; Santiso Carral, 2015). However, it is an evergreen with orange-red fruits, generally habitat in the form of shrubs or small trees in forest areas where they are tolerant to heat cold, and moisture. It has been reported by researchers that strawberry trees generally bloom between November and March, displaying white to pink panicle-forming bell flowers, and can mature the fruit all year round (Beyhan et al., 2020; Körner et al., 1989; Nemutlu, 2022; Santiso Carral, 2015). It has matured fruits that ripen simultaneously and are prone to getting black spots on its leaves in the fall (Markovski, 2017). It has been proposed that the shape of fruits is heterogeneous in terms of weights, colors, and ripenings (Markovski, 2017; Santiso Carral, 2015). At maturity, the shape of fruits is generally round with a diameter of 20-30 mm and red in different hues (Beyhan et al., 2020; Körner et al., 1989; Nemutlu, 2022; Santiso Carral, 2015). Because some healing properties have been reported (i.e., antiseptic, diuretic, laxative, anti-diabetic, hypertension, and anti-inflammatory), it has begun to increase emphasis on the properties of its fruits (Beyhan et al., 2020; Santiso Carral, 2015). Several phenolic compounds were reported by the authors in *Arbutus* fruits (Miguel et al., 2014). Besides the multiple vitamin and phenolic compounds, the antioxidant activity of strawberry tree fruits was reported to be one of the highest among 28 fruit kinds (Markovski, 2017).

As mentioned above, strawberry trees can be adapted to many climate conditions well and have aesthetic flowers and fruit with a green leaf structure. Hence, distributed in a variety of landscape situations (Nemutlu, 2022). It could be considered to be pivotal in the genesis of architectural plants which can be used in landscaping areas with shrub forms (Anonymous, 2024b). It is recommended to be used as a low-branching specimen plant in residential and commercial courtyards, in raised planters, and around lawns (Nemutlu, 2022; Tatliyer et al., 2019; Anonymous, 2024c). Due to being tolerant of summer drought, the strawberry tree fruits might be attractive to wildlife which may serve as food for birds, with the flowers of the plant pollinated by bees (Markovski, 2017; Nemutlu, 2022; Miguel et al., 2014). Ten different genotypes of *Arbutus andrachne* in Macedonia were evaluated. It was found that one genotype (genotype) had twice as big a fruit mass (1.87 g) as others while it has also dark red coloration (L: 21.02; a: 18.8; b: 12.0) (Markovski, 2017). The *Arbutus* species could be found in a maquis in natural forestlands while different climatic conditions (e.g., light, elevation, direction, or temperature) affect their morphology directly (Santiso Carral, 2015).

The strawberry tree is perhaps one of the best large shrubs or small trees for inland gardens, where it grows slowly, but provides garden value for many years. It has typically red-orange-red strawberry-shaped berries that can impress people, which makes it an ideal eye-catcher in both small and large gardens (Nemutlu, 2022; Anonymous, 2024b,c). However, fruits from *Arbutus andrachne* are found to be used in the food industry as a filler for many products (i.e. dessert, jelly, marmalade, yogurt) (Beyhan et al., 2020; Nemutlu, 2022; Santiso Carral, 2015).

This study aims to give an overview of *Arbutus andrachne* L. and the physicochemical properties of their fruits, together with evaluating their landscape value. It was assumed that native *Arbutus andrachne* L. species present quality and multifunctional species applicable for use in landscaping.

MATERIAL and METHOD

The strawberry trees (*Arbutus andrachne* L.) were found in their natural environment in Eğirdir and Sütçüler Isparta province in Turkey. The fruits of strawberry trees were collected within the vegetation period in 2022, in the natural forest which is managed by the Regional Directorate of Forestry authorities. Due to the difficulties experienced in sampling, fruits were collected from randomly selected healthy trees considering form, age, and general appearance having greenish-reddish colors. The fruits were collected from

four different locations in the same region (I: Northside at elevation 369 m, II: North side at elevation 413 m, III: South side at elevation 364 m, IV: South side at elevation 343 m). Approximately 100 representative samples were collected from all sides of the crown. The collected samples were placed in bags, were labeled after coding, and collection data (collection time, place, and elevation) were marked on the label. Some pomology features (e.g., fruit diameter and weight, color) and essential oil characteristics of collected samples were examined.

The collected fruit samples were manually cleaned from contaminants, and then carefully washed with distilled water. The clean fruits were stored in standard containers at 4 0C until analyzed. Major morphological characteristics affecting fruit properties of size (diameter, mm), weight (g), and color were determined. The fruit diameters and weights were measured by digital calipers (± 0.01 mm) and a digital scale (± 0.01 gr). Degrees Brix (symbol °Bx) is a measure of the dissolved solids in a liquid (e.g. fruit or vegetables) and is commonly used to measure dissolved sugar and/or soluble solid content of a given substrate. Several researchers have already well proposed that there is a close relationship between a product's Brix value and potential sugar content (Kappes et al., 2007; Chauhan et al., 2014). In this case, the Brix (°Bx) level of samples was determined by a hand refractometer (Palm Abbe PA2021, Solon, OH) and reported as sugar content throughout the study. Due to general appearance characteristics, an RGB color model was used to indicate how much of each of the red, green, and blue is included in the samples. The CIE color difference formula was developed to solve the problem of the differences in the evaluation between color meters and the human eye. The ΔE (total color difference) is a difference in color accuracy and standard measurement, created by the Commission Internationale de l'Eclairage (CIE). However, it is based on ΔL*, Δa*, and Δb* color values, all of which provide a complete numerical descriptor of the color in a rectangular coordinate system (Sahin et al., 2011; Sahin et al., 2020). The meanings are as follows:

L* represents lightness, with 0 being a perfect black, with 0% reflectance or transmission, ΔL* represents a lightness difference between measured samples,

- a* represents the redness-greyness of the color. Positive values of a* are red, while negative values are green, Δa* represents the difference in redness or greyness between measured samples,
- b* denotes the yellow-blueness of the color. Positive values of b* are yellow, while negative values are blue, Δb* denotes blueness-yellowness differences between measured samples.
- In the case of the levels mentioned above, the higher the value, the greater the difference in that dimension.

The mathematical expression of the formula, however, is a bit more intimidating:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

The RGB and color coordinate measurements (CIE L*a*b* 1976) were made using a Nix2 handheld colorimeter (Nix sensor LTD, Hamilton, Canada), respectively. A single measurement was recorded for each sample and 10 replicated fruits were measured for each group sampling.

The essential oil of fruits of *A. andrachne* was isolated by water distillation, done using the method described by Sagdic et al. (2013). The ground of the fruits (approximately 100 g) was placed into a flask (1 L) treated with distilled water (1:5 w: v) and hydrodistilled for 1 h with the Clevenger apparatus (Ildam, Turkey). Essential oils were obtained from the cooling tunnel. Following the drying of essential oil with anhydrous sodium sulfate to remove any traces of water and after filtration, it was stored in covered test tubes at -20°C until use. The volatile composition of *A. andrachne* essential oil was determined using a GC-MS system (Shimadzu QP 5050, Japan) equipped with a Quadrupole detector and an FFAP polar capillary column [50 m × 0.32 mm (i.d.), film thickness: 0.25 µm]. The temperature program for the column was set from 120 °C (1 min) to 230°C at a rate of 6 °C/min and then held at 230 °C for 35 min. Helium was used as a carrier gas at a flow of 14 psi. (Split 1:10 mL/min) and the injection volume of each sample was 1 µL. The volatile compounds were identified by using the libraries of Flavor 2, Nist05, and Wiley7n. The peak areas were used directly to give the percentage volatile composition of the essential oil by dividing the area of each peak into the total area under all of the peaks. Figure 1 shows the geological map for collecting samples (A), natural habitats of *Arbutus andrachne* L. (B), and collected fruits (C).

The analysis of the obtained data was made within the scope of the IBM SPSS Statistics 26 program at a 95% confidence level. An analysis of variance (ANOVA) was used to statistically determine color and size variations.

All multiple comparisons were evaluated separately and significant differences between L*, a*, b*, diameter, and weight averages were determined within themselves. If the differences between the averages of the locations were found to be significant as a result of the analysis of variance in terms of the features emphasized, the Duncan test was used to determine the differences between the averages of which locations.

RESULTS and DISCUSSIONS

The fruits of *A. andrachne* were examined at four

different locations in similar regions that coexisted in their natural environment, taking into account that the fruit's colors are differentiated. They appear to be a greenish-reddish color at ambient conditions (Fig. 1C).

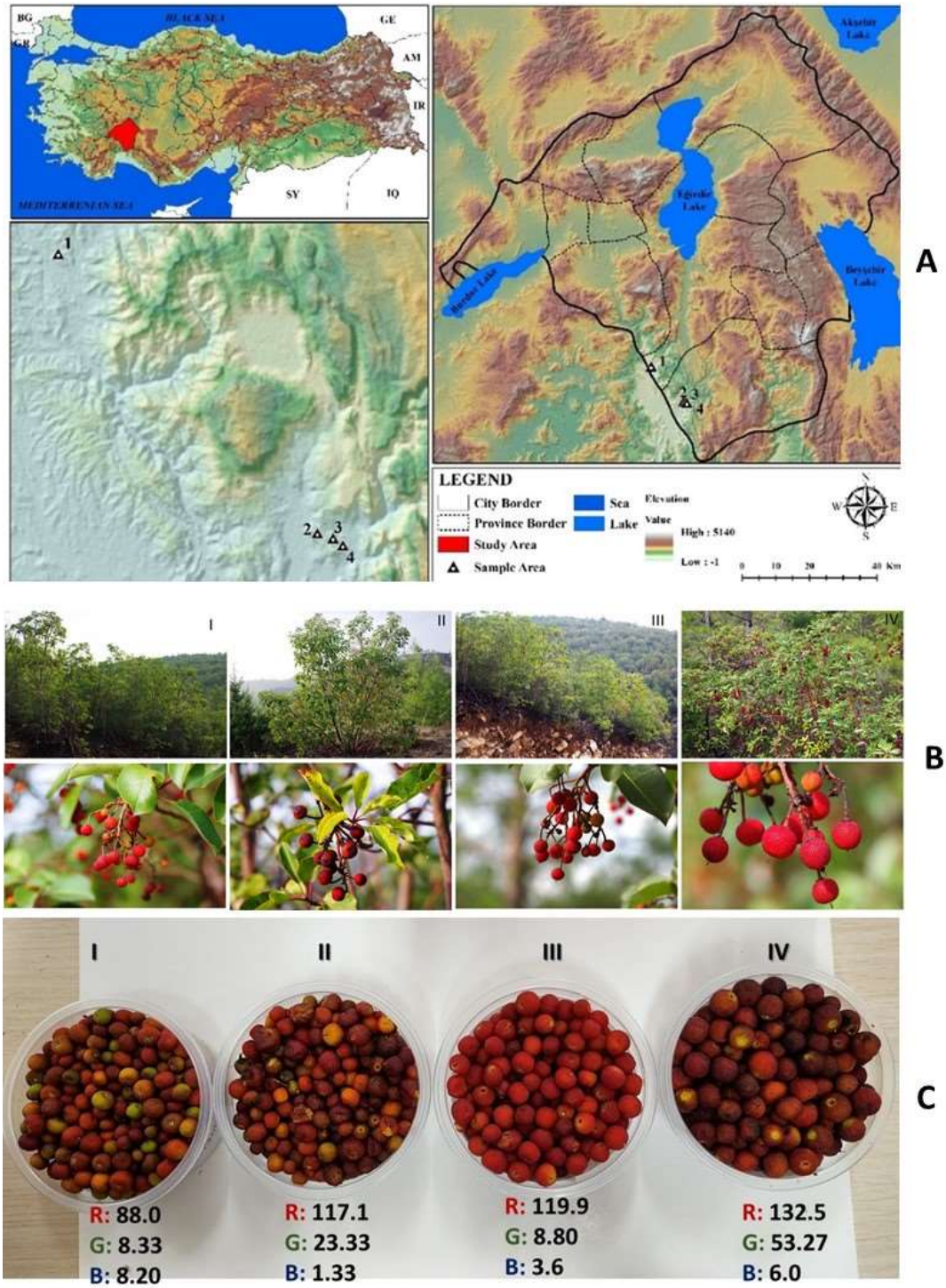


Figure 1. Geological map- (A), natural appearance- (B), and general characteristics (C) of *A. andrachne* fruits.
Şekil 1. A. andrachne meyvelerinin jeolojik haritası- (A), doğal görünümü- (B) ve genel özellikleri (C).

As a result of the analysis of variance in terms of color, diameter, and weight characteristics, the differences between the averages of the locations were found to be statistically significant ($p<0.05$).

However, locational sampling affects variation in the appearance of color, particularly intensity. It was suggested to measure the color intensity of food products using the CIE L*a*b* method (Bible and Singha 1993; Pék et al., 2010). The average color coordinate values of sampling locations are presented

in Table 1. The average color values are found to be L: 25.09 (metric), a: 38.05 (metric), and b: 37.29 (metric), respectively. When considering sampling locations, the highest lightness L: 35.48 (metric) and yellowness b: 46.64 (metric) were found with sample IV but the highest redness value of a: 45.81 (metric) was found with sample III. Although, the visual appearance of a fruit is one of the most important acceptance criteria the collected sample may look aesthetically pleasing. It has been well proposed by several researchers that the higher reddish color could be referred to better ripeness of fruits (Alarcão-E-Silva et al., 2001; Santiso Carral, 2015; Vidrih et al., 2013).

Table 1. The color properties of fruits of *A. andrachne*.
Tablo 1. A. andrachne meyvelerinin renk özellikleri.

Sample	L*	a*	b*
I	16.18±1.66 (D)	37.67±2.70(C)	25.10±2.43 (D)
II	25.82±2.14 (B)	38.97±2.03(B)	40.76±3.21 (B)
III	23.80±0.63 (C)	45.81±2.33 (A)	36.65±1.73 (C)
IV	35.48±1.92 (A)	30.67±4.31 (D)	46.64±2.30 (A)
Average	25.09±1.19	38.05±1.61	37.29±1.58

*Each value represents the mean of at least ten replications. Values sharing the same capital letter (s) within a column are not statistically different at the 0.05 level of confidence.

The quantification of all color values in a simple way is very complicated and includes many phenomenal variations. Therefore, the total color difference (ΔE) which in this study may be useful to determine the color properties, could be used to give an estimation of how the different growing conditions (geographical locations) affect the natural color of *A. andrachne*'s fruits.

Figure 2 shows the different color properties of each group of samples. Among collected fruits, the highest color differences were found with sample IV (ΔE: 15.4), followed by sample I (ΔE: 15.1), sample III (ΔE: 7.6), and sample II (ΔE: 3.6) in that order. In the literature, ΔE values of 2.0 to 3.0 numeric are thought to be observable color differences by observers (Agoston, 2013; Krantz, 1975).

Besides color and general appearances, morphological parameters of fruit (e.g. diameters and weights) have already been reported to be useful methods for determining ripeness characteristics of fruits (Isbilir et al., 2012; Kıvcak et al., 2001; Oliveira et al., 2011). In this regard, the measured average fruit's diameters and weights are presented compared to Table 2. It appears to be a considerable size variation exists among fruit samples ($p<0.05$, $p=0.00$). In four sampling locations, the average diameters are found to be 10.62 mm in diameter and weight 0.77 g, respectively. However, the highest diameter (12.42 mm) and weight (1.13 g) were found to be in sample I. These measured results may be used to distinguish between the geographic sampling locations of *A. andrachne*'s fruits.

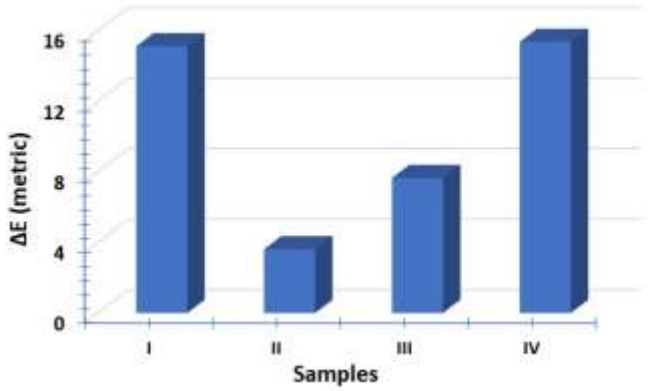


Figure 2. The color properties of fruits of *A. andrachne.*
Şekil 2. A. andrachne meyvelerinin renk özellikleri.

Table 2. The size properties of fruits of *A. andrachne*.
Tablo 2. A. andrachne meyvelerinin boyut özellikleri.

Sample	Diameter (mm)	Weight (g)
I	12.42±0.43 (A)	1.13±0.13 (A)
II	9.78±0.43 (D)	0.65±0.06 (C)
III	10.25±0.52 (B)	0.77±0.07(B)
IV	10.01±0.28 (C)	0.52±0.05 (D)
Average	10.62±0.25	0.77±0.05

*Each value represents the mean of at least ten replications. Values sharing the same capital letter (s) within a column are not statistically different at the 0.05 level of confidence.

The brix value (°Bx) is a numerical index, typically used to determine the ripeness or sugar content of a food sample (Uggla et al., 2005). The measurements exposed that fruit samples did not well correlate with brix (Figure 3), although distinguished from samples by their diameters. The highest value of 33.81 °Bx was found for sample I, followed by 32.97 °Bx (sample II), 32.58 °Bx (sample III), and 27.89 °Bx (sample IV), in that order.

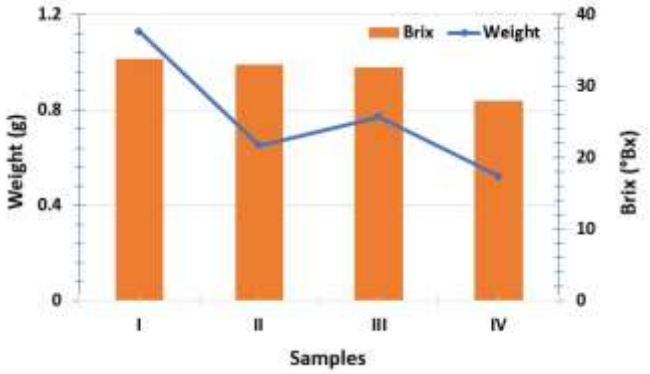


Figure 3. The Brix and weight properties of fruits of *A. andrachne*.

Şekil 3. A. andrachne meyvelerinin briks ve ağırlık özellikleri.

The degree or value of Brix (°Bx) is traditionally used in various food industries (i.e., alcohol, soft drinks, fruit juice honey, and so on.). Because it is generally used to assess the quality of flavor or sweetness, it is an important subject criterion.

It has already been well documented that there is a direct correlation between a food's Brix value and potential sugar content (Kappes et al., 2007; Bolade et al., 2009; Chauhan et al., 2014). In our study, the brix values were measured in the range of 27.89 °Bx (sample IV) to 33.81 °Bx (sample I). But when Figure 4 is carefully analyzed, it is difficult to correlate sampling locations with the diameters of fruit *A. andrachne*.

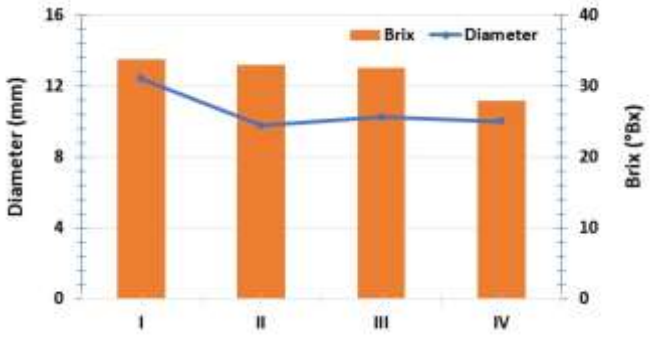


Figure 4. The Brix and diameter properties of fruits of *A. andrachne*.

Şekil 4. A. andrachne meyvelerinin briks ve çap özellikleri.

To find the combined effects of morphological properties (fruit diameters and weights) on fruit's sugar content (°Bx), the obtained values were plotted against them, as shown in Figure 5. The figure suggests that all monitoring properties bring an effect of changing the brix values. However, the sugar content difference is realizable. The sugar content was found to be high at lower diameters but at higher weights, then lowered with increasing diameter and lowering weights. This could be expected considering vast literature information has been reported, on which growth locations impact on maturity and morphological properties of food products (Alarcão-E-Silva 2001; Pék et al., 2010; Vidrih et al., 2013; Ugga et al., 2005; Isbilir et al., 2012; Oliveira et al., 2011; Kivcak et al., 2001).

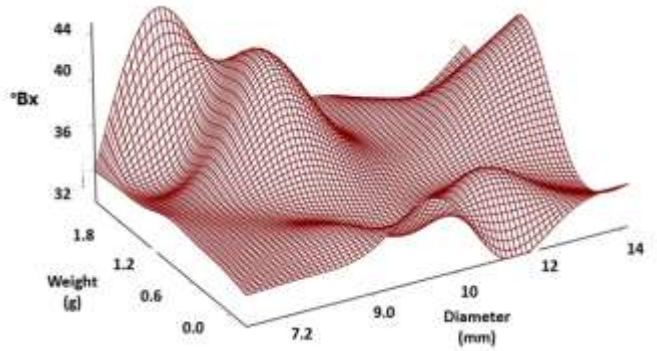


Figure 5. The fruit size effects on brix properties of fruits of *A. andrachne*.

Şekil 5. Meyve boyutunun A. andrachne meyvelerinin briks özelliklerine etkisi.

Some research has already been conducted to determine the chemical composition of physical properties of strawberry tree fruits (Bento and Pereira 2011; Özcan and Hacıseferoğulları 2007). There were reported very useful results considering the medicinal properties of the strawberry tree. In this regard, we have made an effort to determine the essential oil composition of *A. Andrachne*'s fruits. Considering the natural environment, their relative essential oil constituents from two different locations (samples I and IV) are comparatively given in Table 3. However, the total existence of 43 essential oil compounds is determined for sample I and 35 compounds for sample IV, respectively, while 31 of them are similar, which represents 97.07% for sample I and 91.78% for sample IV. The main essential oil components for sample I were found to be Ethyl alcohol (31.89%), (E)-2-Hexenal (16.61%), Hexanal (15.36%), (Z)- 3-Hexen-1-ol (11.02%), Penten-3-one (5.29%), Hexanol (2.86%), Ethyl acetate (2.50%), Cyclopentanol (2.06%), (E)-2-Pentenal (1.43%), 2-Methyl-1-propanol (1.43%), (E)-2-Hexen-1-ol (1.06%). Moreover, the main components for sample 2 were found to be ethyl alcohol (46.66%), (Z)-3-Hexen-1-ol (8.90%), Hexanal (7.29%),2-Methyl-1-

propanol (7.25%), (E)-2-Hexenal (6.17%), Hexanol (5.80%), Ethyl acetate (1.765%), (E)-2-Hexen-1-ol (0.96%), Penten-3-one (0.80%), in that order.

Although the evaluation of all these components is difficult and not intended in this study, major component ratios were considerably different for both groups of samples.

Table 3. The essential oil constituents of fruits of *A. andrachne*.
Çizelge 3. A. andrachne meyvelerinin uçucu yağ bileşenleri.

Compounds	Sample I	Sample IV
Ethyl alcohol	31.89%	46.66%
(E)-2-Hexenal	16.61%	6.17%
Hexanal	15.36%	7.29%
(Z)- 3-Hexen-1-ol	11.02%	8.90%
Penten-3-one	5.29%	0.80%
Hexanol	2.86%	5.80%
Ethyl Acetate	2.50%	1.76%
Cyclopentanol	2.06%	0.47%
(E)-2-Pentenal	1.43%	0.28%
2-Methyl-1-propanol	1.43%	7.25%
(E)-2-Hexen-1-ol	1.06%	0.96%
(E)-2-Heptenal	0.76%	0.06%
2-Butanone, 3-hydroxy- (CAS) Acetoin	0.59%	0.31%
Eucalyptol (1,8-Cineole)	0.57%	0.25%
Linalool	0.50%	0.58%
p-Cymene	0.42%	0.33%
(E,E)-2,4-Hexadienal	0.38%	0.07%
alpha- Pinene	0.32%	0.23%
Nonanal	0.27%	0.34%
Limonene	0.26%	0.15%
Ethyl caproate	0.23%	0.12%
3-Methylbutanal	0.22%	0.91%
Heptanal	0.21%	0.04%
1-Pentanol	0.16%	0.02%
Hexane	0.14%	0.10%
Camphor	0.14%	0.05%
Styrene	0.12%	0.55%
Octanal	0.10%	0.11%
Ethyl carbonate	0.09%	0.05%
2-Methylbutanal	0.06%	0.73%
gamma- Terpinene	0.04%	0.24%
Propyl bromide	0.20%	-
Butyl acetate	0.14%	-
(Z)-2-pentanol	0.87%	-
alpha- Thujene	0.05%	-
Benzaldehyde	0.19%	-
Sabinene	0.07%	-
1-Octen-3-ol	0.04%	-
6-Methyl-5-hepten-2-one	0.10%	-
3-Isobutylcyclohexene	0.12%	-
(E,E)- 2,4-Heptadienal	0.28%	-
Oct-2(E)-enal	0.13%	-
alpha- Terpinolen	0.53%	-
beta- Cyclocitral	0.05%	-
Ionone <(E)-, beta->	0.16%	-
2,3-Butanedione	-	0.08%
3-Methyl-1-butanol	-	3.71%
2-Methyl-1-butanol	-	4.38%
4-Terpineol	-	0.05%
TOTAL	100%	100%

However, it was observed that the components of (E)-2-Hexenal, Hexanal, (Z)- 3-Hexen-1-ol, Penten-3-one, Ethyl Acetate, Cyclopentanol, (E)-2-Pentenal, (E)-2-Hexen-1-ol were found to be higher in sample I whereas Ethyl alcohol, Hexanol, 2-Methyl-1-propanol were higher in sample IV. Moreover, 3-methyl-1-butanol (3.71%) and 2-methyl-1-butanol (4.38%) which have considerably high concentration have

only found in sample IV. It is important to note that sample I contains 14 different components which comprise 2.97% whereas sample IV had 4 different components which comprise 8.72%.

CONCLUSIONS

Species in natural vegetation could be important for plant designs, especially in terms of minimizing maintenance needs. This study was carried out in Isparta province on the *Arbutus andrachne* (Strawberry tree) plant, which is widely distributed in Mediterranean regions. The natural habitats of the *Arbutus andrachne* L. plants in Isparta were examined in situ and fruits were collected and analyzed in terms of selected physicochemical properties. As a result of the study, the characteristics of the fruits were determined and it was then to compare them with different growing locations.

The different parts of plants (i.e., leaves, cones, fruits, flowers) have gained increasing attention and are widely utilized, but there are still some concerns, particularly due to the growing locations' effects on properties. However, special attention should be paid to the use of those plants. The experimental results showed that variations in growing locations had a definite effect on the physicochemical properties of *A. andrachne*. However distinct characteristics were determined by the size, color, sugar content, and essential oil constituents, which were influenced by the geographic locations. However, the characteristics of *A. andrachne*'s fruits did follow some trends, which emphasized that growing conditions affected physical properties. It is important to note that many phenomenological properties were reported for plant substrates and the quantification of all those is very complicated and needs further investigation.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Statement of Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

REFERENCES

Agoston, G. A. (2013). *Color theory and its application in art and design (Vol. 19)*. Springer.

Alarcão-E-Silva, M. L. C. M. M., Leitão, A. E. B., Azinheira, H. G., & Leitão, M. C. A. (2001). The Arbutus Berry: Studies on its color and chemical characteristics at two mature stages. *Journal of Food Composition and Analysis, 14*(1), 27-35.

Anonymous (2024a). Extension.oregonstate.edu. (n.d.). Environmental Factors Affecting Plant Growth. Retrieved from https://extension.oregonstate.edu/gardening/techniques/environmental-factors-affecting-plant-growth

Anonymous (2024b).Architecturalplants.com. (n.d.). Arbutus x Andrachnoides. Retrieved from https://www.architecturalplants.com/product/arbutus-x-andrachnoides/

Anonymous (2024c). Waterwisegardenplanner.org. (n.d.). Arbutus unedo Compacta. Retrieved from https://waterwisegardenplanner.org/plants/arbutus-unedo-compacta/

Bento, I., & Pereira, J. A. (2011). Arbutus unedo L. and its benefits on human health. *J. Food Nutr. Res, 50*, 73-85.

Beyhan, Ö., Demir, T., & Zenginbal, H. (2020). Determination of Some Important Pomological and Biochemical Properties of the Genotypes of Strawberry Tree (*Arbutus unedo* L.) Selected in Bolu Province in Turkey. *Journal of Agricultural Biotechnology, 1*(1), 31-38.

Bible, B. B., & Singha, S. (1993). Canopy position influences CIELAB coordinates of peach color. *HortScience, 28*(10), 992-993.

Bolade, M. K., Oluwalana, I. B., & Ojo, O. (2009). Commercial practice of roselle (*Hibiscus sabdariffa* L.) beverage production: Optimization of hot water extraction and sweetness level. *World Journal of Agricultural Sciences, 5*(1), 126-131.

Chauhan, O. P., Archana, B. S., Singh, A., & Raju, P. S., Bawa, A. S. (2014). A refreshing beverage from mature coconut water blended with lemon juice. *Journal of food science and technology, 51*(11), 3355-3361.

Isbilir, S. S., Orak, H. H., Yagar, H., & Ekinci, N. (2012). Determination of antioxidant activities of strawberry tree (*Arbutus unedo* L.) flowers and fruits at different ripening stages. *Acta Scientiarum Polonorum. Hortorum Cultus, 11*(3), 221-237.

Kappes, S. M., Schmidt, S. J., & Lee, S. Y. (2007). Relationship between physical properties and sensory attributes of carbonated beverages. *Journal of Food Science, 72*(1), S001-S011.

Kivcak, B., Mert, T., Demirci, B., & Baser, K. H. C. (2001). Composition of the essential oil of *Arbutus unedo*. *Chemistry of Natural Compounds, 37*, 445-446.

Körner, C., Neumayer, M., Menendez-Riedl, S. P., & Smeets-Scheel, A. (1989). Functional morphology of mountain plants. *Flora, 182*(5-6), 353-383.

Krantz, D. H. (1975). Color measurement and color theory: II. Opponent-colors theory. *Journal of Mathematical Psychology, 12*(3), 304-327.

Markovski, A. (2017). Morphological characteristics of Greek strawberry tree (*Arbutus andrachne* L.) genotypes. *Acta Agriculturae Serbica, 22*(44), 193-

206.

Miguel, M. G., Faleiro, M. L., Guerreiro, A. C., & Antunes, M. D. (2014). *Arbutus unedo* L.: chemical and biological properties. *Molecules, 19*(10), 15799-15823.

Nemutlu, F. E. (2022). Aesthetıc Plant Suggestıon From Çanakkale Flora To Urban Landscape: *Arbutus unedo* L. *Uluslararası Peyzaj Mimarlığı Araştırmaları Dergisi (IJLAR), 6*(2), 32-41.

Oliveira, I., Baptista, P., Malheiro, R., Casal, S., Bento, A., & Pereira, J. A. (2011). Influence of strawberry tree (*Arbutus unedo* L.) fruit ripening stage on chemical composition and antioxidant activity. *Food Research International, 44*(5), 1401-1407.

Özcan, M. M., & Hacıseferoğulları, H. (2007). The strawberry (*Arbutus unedo* L.) fruits: chemical composition, physical properties and mineral contents. *Journal of Food Engineering, 78*(3), 1022-1028.

Pék, Z., Helyes, L., & Lugasi, A. (2010). Color changes and antioxidant content of vine and postharvest-ripened tomato fruits. *HortScience, 45*(3), 466-468.

Sagdic O., Ozturk I., & Tornuk F. (2013). Inactivation of nontoxigenic and toxigenic *Escherichia coli* O157:H7 inoculated on minimally processed tomatoes and cucumbers: Utilization of hydrosols of Lamiaceae spices as natural food sanitizers. *Food Control, 30*(1), 7-14.

Sahin, C. K., Topay, M., & Var, A. A. (2020). A study on the suitability of some wood species for landscape applications: surface color, hardness and roughness changes at outdoor conditions. *Wood Research, 65*(3), 395-404.

Sahin, H.T., Arslan, M.B., Korkut, S., & Sahin, C. (2011). Color changes of heat-treated woods of red-bud maple, European hophornbeam, and oak. *Color Research & Application, 36*(6), 462-466.

Santiso Carral, X. (2015). Genetic structure and geographic variation in ecophysiological traits of *Arbutus unedo* L. (Ph.D thesis), Unıversıdade De Santıago De Compostela Facultade De Bıoloxía, Santiago de Compostela, Maio 2015 Spain.

Tatliyer A, Kamalak A, Öztürk D. (2019). Sandal Ağacı (*Arbutus andrachne*) Yapraklarının Potansiyel Besleme Değerinin Belirlenmesi. *KSÜ Tarım ve Doğa Derg, 22*(2), 315-321. DOI: 10.18016.ksutarimdoga.vi.478849.

Uggla, M., Gustavsson, K. E., Olsson, M. E., & Nybom, H. (2005). Changes in color and sugar content in rose hips (*Rosa dumalis* L. and *R. rubiginosa* L.) during ripening. *The Journal of Horticultural Science and Biotechnology, 80*(2), 204-208.

Vidrih, R., Hribar, J., Prgomet, Ž., & Poklar Ulrih, N. (2013). The physicochemical properties of strawberry tree (*Arbutus unedo* L.) fruits. *Croatian journal of food science and technology, 5*(1), 29-33.

KSÜ Tarım ve Doğa Derg 27 (4), 758-766, 2024
KSU J. Agric Nat 27 (4), 758-766, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1357294

The Effect of Nettle (*Urtica dioica* L.), Carob (*Ceratonia siliqua* L.), and Chaste (*Vitex agnus-castus* L.) Plants on Fertility in *Caenorhabditis elegans*

Handan SARAC[1]✉ Hülya OZPINAR[2]
[1]Department of Plant and Animal Production, Sivas Technical Sciences Vocational School, Sivas Cumhuriyet University, Sivas, Türkiye,
[2]Department of Pharmaceutical Botany, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Türkiye
[1]https://orcid.org/0000-0001-7481-7978, [2]https://orcid.org/0000-0001-8154-0874
✉: handansarac@cumhuriyet.edu.tr

ABSTRACT
This study was carried out to determine the effects of different concentrations of water extract of Nettle (*Urtica dioica* L.), Carob (*Ceratonia siliqua* L.), and Chaste (*Vitex agnus-castus* L.) plants, which are traditionally used in the treatment of infertility among the people, on fertility in *Caenorhabditis elegans* (*C. elegans*), which is a model organism. Scope of work; 0.1%, 0.05%, 0.02%, and 0.01% concentrations of the water extracts of the plants were applied to the *C. elegans* standard medium and egg counts were made for 3 days according to the Koelle protocol. In addition, one day after each egg count, uncracked eggs were determined, and egg productivity was calculated. The analyses were performed in triplicate, the averages were determined and the differences between the groups were statistically compared with the SPSS program. According to the results obtained from the study, it was determined that Nettle, Carob, and Chaste plants have positive effects on fertility. Among the plants, it was determined that the plant that affected fertility the most was Nettle, followed by Chaste and Carob plants, respectively. In addition, in terms of dosage applications, the best effect in all three plants has been observed at the highest dose of 0.1%.

Botany

Research Article

Article History
Received: 13.09.2023
Accepted: 05.01.2024

Keywords
Urtica dioica L.
Ceratonia siliqua L.
Vitex agnus-castus L.
Caenorhabditis elegans
Fertility

Isırgan (*Urtica dioica* L.), Keçiboynuzu (*Ceratonia siliqua* L.) ve Hayıt (*Vitex agnus-castus* L.) Bitkilerinin *Caenorhabditis elegans*' ta Fertiliteye Etkisi

ÖZET
Bu çalışma, halk arasında geleneksel olarak infertilite tedavisinde kullanıldığı bilinen Isırgan (*Urtica dioica* L.), Keçiboynuzu (*Ceratonia siliqua* L.) ve Hayıt (*Vitex agnus-castus* L.) bitkilerinin su ekstraktının farklı konsantrasyonlarının, bir model organizma olan *Caenorhabditis elegans* (*C. elegans*)' ta fertilite üzerine olan etkilerini belirlemek amacıyla planlanmıştır. Çalışma kapsamında; bitkilerin su ekstraktlarının %0.1, %0.05, %0.02 ve %0.01'lik konsantrasyonları *C. elegans* standart besiyerine uygulanmış ve Koelle protokolüne göre 3 gün boyunca yumurta sayımı yapılmıştır. Ayrıca, her yumurta sayımı yapıldıktan bir gün sonra çatlamayan yumurtalar tespit edilerek, yumurta verimlilikleri de hesaplanmıştır. Analizler üç tekrarlı olarak yapılmıştır ve ortalamalar belirlenerek SPSS programı ile gruplar arasındaki farklar istatistiksel olarak karşılaştırılmıştır. Çalışmadan elde edilen sonuçlara göre, Isırgan, Keçiboynuzu ve Hayıt bitkilerinin fertilite üzerinde olumlu etkilere sahip olduğu belirlenmiştir. Bitkiler arasında fertiliteye en fazla etki eden bitkinin Isıgan olduğu bunu sırasıyla Hayıt ve Keçiboynuzu bitkilerinin takip ettiği belirlenmiştir. Ayrıca, doz uygulamalarında her üç bitkide de benzer şekilde en iyi etki en yüksek doz olan %0.1' lik uygulamada tespit edilmiştir.

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 13.09.2023
Kabul Tarihi : 05.01.2024

Anahtar Kelimeler
Urtica dioica L.
Ceratonia siliqua L.
Vitex agnus-castus L.
Caenorhabditis elegans
Fertilite

To Cite : Sarac, H., & Ozpinar, H., (2024). The Effect of Nettle (*Urtica dioica* L.), Carob (*Ceratonia siliqua* L.), and Chaste (*Vitex agnus-castus* L.) Plants on Fertility in *Caenorhabditis elegans*. *KSU J. Agric Nat 27*(4), 758-766. https://doi.org/10.18016/ksutarimdoga.vi. 1357294

Atıf Şekli: Sarac, H., & Ozpinar, H., (2024). Isırgan (*Urtica dioica* L.), Keçiboynuzu (*Ceratonia siliqua* L.) ve Hayıt (*Vitex agnus-castus* L.) Bitkilerinin *Caenorhabditis elegans*' ta Fertiliteye Etkisi . *KSÜ Tarım ve Doğa Derg 27* (4), 758-766. https://doi.org/10.18016/ksutarimdoga.vi.1357294

INTRODUCTION

Humans' use of plants for medicinal purposes dates back to ancient times. Archaeological findings from the earliest civilizations indicate that people utilized plants to obtain food and address health issues (Özpınar & Yüksek, 2019). Additionally, it is known that many drugs used in modern medicine today are derived from plants (Saraç et al., 2018). Scientific research conducted on the effectiveness and safety of commonly used medicinal and aromatic plants in folk medicine plays a significant role in Turkish traditional medicine and its proper utilization (Fugh-Berman & Kronenberg, 2003). Because, even though herbal remedies have been known for a long time, the specific ways in which each plant affects human physiology are not well understood. Therefore, the scientific community has focused on plant extracts as therapeutic agents for 30-40 years (Bora & Sharma, 2010; Çetin, 2012). Contrary to popular belief, many plants have been discovered to have medicinal effects, and the discovery of such plants continues (Beşen & Beji, 2014). In our country, which has a rich potential in terms of medicinal and aromatic plants, herbal treatment is preferred by the public due to its low cost, easy accessibility, and concerns about the side effects of chemical drugs. It is particularly widely used in the treatment of infertility (Beşen and Beji, 2014; Daştan & Saraç, 2018). Studies conducted in countries where plants affecting fertility are extensively used have found that the medicinal plants used in these studies not only lead to a significant increase in pregnancy rates and ovulation but also result in a decrease in miscarriage rates (Tan et al., 2012; Yılmaz, 2019). In modern times, efforts are being made to document plants that affect fertility. Therefore, their effects are being investigated and extensively discussed through scientific publications and projects (Gaware et al., 2009; Beşen & Beji, 2014). For example, in a study conducted by Telefo et al. (2011) in Cameroon, 46 different plants used in female infertility were documented.

Urtica dioica L. is one of the most common species in the family Urticaceae. In studies with the species, which is a perennial herbaceous plant, the extracts used have been found to have many pharmacological effects such as antioxidant, anti-inflammatory, anti-diabetic, anticancer, antiulcer, etc. (Esposito et al., 2019). *Ceratonia siliqua* L. is a species of the Fabaceae family that is widespread in the Mediterranean region. Due to the many bioactive components contained in the fruit of this species and the products obtained from this fruit; positive effects have been observed on diabetes, inflammation, digestive system disorders, hyperlipidemia, oxidative stress, etc.. (Brassesco et al., 2021). *Vitex agnus-castus* L. species is referred to as the chaste tree that spreads in Central Asia, the Mediterranean Region, and Southern Europe. It is a species of the Verbenaceae family. Studies carried out in recent years show that this type can be effective in the treatment of PMS and menstrual disorders, menopausal problems, and also its antioxidant, antitumoral, antimicrobial, antiepileptic, anti-inflammatory, osteopenic, etc. effects can be observed (Niroumand et al., 2018).

In this study, Nettle *(Urtica dioica* L.), Carob (*Ceratonia siliqua* L.), and Chaste (*Vitex agnus-castus* L.), which have been determined as plant materials, are traditionally used for various human-specific disorders, primarily infertility treatment (Ezer & Avcı, 2004; Ugulu et al., 2009; Edirne et al., 2010; İpekoğlu & Oral, 2019).

Currently, various model organisms are used to demonstrate the effects of many substances, such as plant extracts, on human health. *C. elegans* is a microscopic, non-pathogenic soil nematode (roundworm) that is approximately 1 mm long and 65 µm thick. It freely lives in organic-rich environments such as the soil near tree roots (Hertweck et al., 2003; Ünlü & Erdem, 2010). Due to its fundamental characteristics such as simplicity, transparency, and short lifespan, *C. elegans* has been extensively used as a model organism in basic biological research for many years (Olsen et al., 2006; Porta de la Riva et al., 2012; Savaş et al., 2018; Özpınar, 2020). *C. elegans* can easily sustain its life in laboratory conditions by feeding on bacteria (*Escherichia coli* OP50 strain) on an agar substrate in a petri dish (Ünlü & Erdem, 2010). Additionally, it can be stored indefinitely as a stock at -80°C or in liquid nitrogen (Springer, 2005). Its lifespan under laboratory conditions is approximately 3 weeks, and its life cycle (from embryo to reproductive adult) takes about 3.5 days at 20°C (Olsen et al., 2006) (Figure 1). This study investigated the effects of water extracts obtained from Nettle, Carob, and Chaste plants at concentrations of 0.1%, 0.05%, 0.02%, and 0.01% on *C. elegans*' fertility.

MATERIAL and METHOD

Plant Materials

The Nettle leaves, Carob fruits, and Chaste seeds used as plant material in the study were obtained from an herbalist shop located in Sivas and later diagnosed (Figure 2).

Preparation of Plant Extracts

For extraction, the leaves of Nettle, the fruits of Carob, and the seeds of Chaste were used. The dried plant materials were first ground into a powder using a laboratory-type mill. Then, 100 g of the powdered plant samples were weighed and placed in beakers, to which 500 mL of distilled water (dH_2O) was added as the solvent. The plant samples were macerated at room temperature (25±2°C), 150 rpm, using an electronic

shaker for 24 hours. After maceration, the plant extracts were filtered twice through filter paper (Whatman No. 1). The solvent in the filtrate was removed using a rotary evaporator at 40°C. The resulting dry extracts were stored at +4°C until analysis.

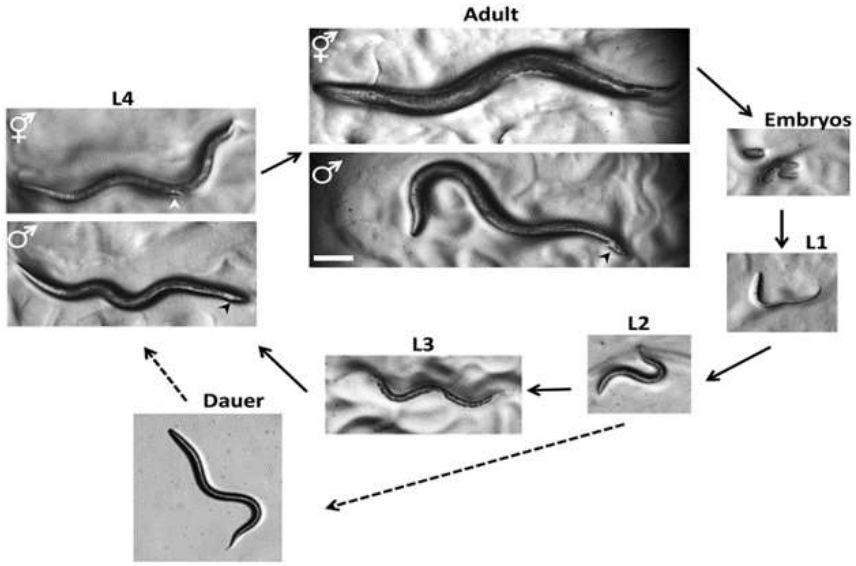


Figure 1. Life cycle of *C. elegans* (Corsi et al., 2015)
Şekil 1. C. elegans'ın yaşam döngüsü (Corsi ve ark., 2015)

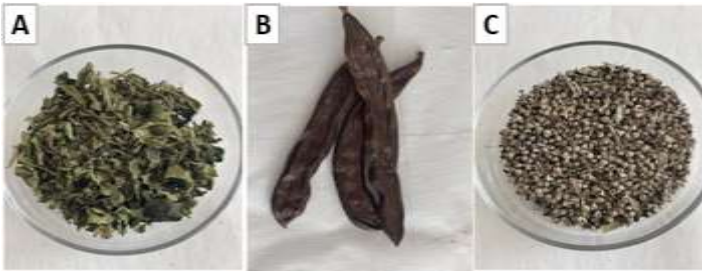


Figure 2. Plant materials used in the study A. Nettle leaf, B. Carob fruit, C. Chaste seed
Şekil 2. Çalışmada kullanılan bitki materyalleri A. Isırgan yaprağı, B. Keçiboynuzu meyvesi, C. Hayıt tohumu

Preparation of TBX Agar Medium for *E. coli* OP50 Strain

To prepare TBX Agar (Tryptone Bile Glucuronide Agar) medium for *E. coli* OP50 strain, 9.125 g of TBX Agar was weighed using a precision balance, and then 250 mL of dH_2O was added to it. The mixture was then placed on a magnetic stirrer to ensure the agar's dissolution, and the medium was autoclaved at 125°C for 15 minutes for sterilization. After autoclaving, the agar medium was allowed to cool down to 55°C.

Purification of *E. coli* OP50 Strain

After preparing the TBX Agar medium for the *E. coli* OP50 strain, approximately 10 mL of the medium was poured into each 60 mm petri dish. The medium was allowed to solidify. Once solidified, the TBX Agar plates were ready for use. Using a sterile loop, *E. coli* OP50 strain was streaked onto the agar surface, near the flame of a Bunsen burner. The plates were then incubated at 37°C for 24 hours. After colony formation was observed, specific blue colonies belonging to the *E. coli* OP50 strain were identified. This step was performed to purify the *E. coli* OP50 strain by identifying the colonies specific to it before transferring the *E. coli* to a liquid medium, to avoid the possibility of contamination.

Preparation of Liquid Culture Medium for *E. coli* OP50 Strain

A liquid culture medium has been prepared for the transfer and propagation of the purified *E. coli* OP50 strain. For this purpose, 9.125 g of Lauryl Sulfate Tryptose Broth (LST Broth) was weighed on a precision balance, and then 250 mL of dH_2O was added. The mixture was autoclaved at 120 °C for 15 minutes to ensure sterilization. Once sterilized in the autoclave, the medium was cooled to 37 °C. Subsequently, a single colony of *E. coli* OP50 strain was inoculated into the LST Broth using a loop taken from a TBX Agar culture. During this process, work was conducted inside a laminar flow (sterile cabinet) and near a Bunsen burner to minimize the risk of contamination. After transferring the liquid culture medium, it was incubated at 37°C in an incubator for 24 hours. Following successful growth, the medium was stored at +4°C for later use (the cloudiness of the liquid indicates the proliferation of *E. coli* OP50 strain).

Preparation of Nematode Growth Media (NGM)

2.5 g of Peptone, 3 g of NaCl, and 20 g of Agar were dissolved in 1 L of dH_2O using a magnetic stirrer until the boiling temperature was reached. The mixture was then autoclaved at 125 °C for 15 minutes and subsequently cooled down to 55 °C. After cooling, the NGM was homogenized by adding the previously prepared and filtered components, including 1 mL of $MgSO_4$ (1M), 1 mL of Cholesterol (5 mg/mL), 1 mL of $CaCl_2$ (1M), and 25 mL of KPO_4 buffer (pH: 7).

Culturing of *C. elegans* on NGM

After homogenization, NGM was poured into 60 mm Petri dishes, approximately 10 mL each, to allow it to solidify into an agar consistency. Once the NGM solidified, about 400 µL of *E. coli* OP50 strain was added to the centre of each dish. The petri dishes with added *E. coli* were then left to dry in a sterile environment for approximately 1-2 days. Care was taken to prevent the NGM from completely drying out to maintain its agar consistency. Once the bacterial solution dried, a small piece was cut from the stock culture and placed upside down onto the newly prepared NGM. This allowed the *C. elegans* to be cultured on the NGM medium, providing an environment suitable for their growth and development.

Preparation of NGM with Plant Extract

The dried plant extracts weighed for 0.01%, 0.02%, 0.05%, and 0.1% concentrations, were individually added to separate NGM. After adding the extracts, the mixture was rapidly stirred and poured into petri dishes to solidify. Control petri dishes were prepared without adding any concentration of plant extract (Figure 3). Petri dishes were prepared in triplicate for all doses of the plant extracts studied. Subsequently, all prepared Petri dishes were wrapped with aluminium foil and stored at +4 °C to be used after the synchronization process of *C. elegans*.

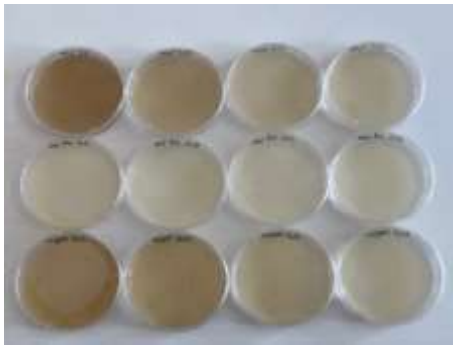


Figure 3. NGMs prepared with water extracts at concentrations of 0.01%, 0.02%, 0.05%, and 0.1%.

Şekil 3. Su ekstraklarının %0.01, %0.02, %0.05 ve %0.1 konsantrasyonları ile hazırlanmış NGM'ler

Synchronization of *C. elegans*

1 g of NaOH was weighed on a precision balance and then 5 mL of dH_2O was added to dissolve it. Subsequently, 1 mL of sodium hypochlorite and 0.5 mL of NaOH solution were transferred to a centrifuge tube. The previously prepared NGM, where *C. elegans* had been cultured, was used to wash the eggs by pipetting with dH_2O. After pipetting, the eggs were also transferred to the centrifuge tube, which was then centrifuged at 3000 rpm for 10 minutes, and the supernatant was discarded. The remaining pellet containing the eggs was transferred to new NGM plates. These eggs constitute synchronized larvae, and when they reach the adult form (L4) by the end of the third day, they are used for fertility analysis (Koelle, 2005).

Fertility Analysis

To determine the effects of plant extracts on fertility, evaluations were conducted on egg counts, the number of individuals hatched from the eggs, and egg productivity. The egg counting for fertility analysis followed the protocol by Koelle (2005). Accordingly, 15 well-fed L4 stage *C. elegans* were transferred to each petri dish prepared with different concentrations of plant extracts (0.01%, 0.02%, 0.05%, 0.1%). After 36 hours, 10 individuals from each dish were transferred to a new petri dish and left at 20°C for 30 minutes. After the specified time, egg counting was performed under a stereo microscope using a 20x objective. Additionally, the number of *C. elegans* individuals hatching from the eggs was counted in the same Petri dishes, and any unhatched eggs were identified.

Statistical Analysis

Fertility analysis was repeated three times for each plant extract and its doses. Statistical analysis of the fertility data was performed using SPSS 22.0 (IBM Corporation, Armonk, New York, United States) software. Differences between the means were determined using the Tukey test with a significance level of $P<0.05$.

RESULTS and DISCUSSION

The effects of water extracts of different concentrations of the Nettle, Carob, and Chaste plants on fertility were determined in the study, and the findings obtained are presented in Tables 1, 2, and 3.

According to the fertility results obtained from a 3-day count of 10 *C. elegans* individuals transferred at the L4 stage, Nettle water extract doses have caused significant increases in both parameters (egg counts and individual counts). This is because significant statistical differences exist between the applied dose groups and the control ($P<0.05$). All applied dose groups have positively influenced fertility. This effect

is proportional to the dose amount. As the dose amount increases, the egg and individual counts increase (Table 1).

All doses of Nettle water extract have increased egg productivity in *C. elegans* compared to the control. Egg productivity was determined as 99.1% at a dose of 0.01%, 99.4% at a dose of 0.02%, 99.2% at a dose of 0.05%, and 99.1% at a dose of 0.1%. The egg productivity of the control is 97.9% (Figure 4).

Table 1. The effect of Nettle water extract on fertility in *C. elegans*
Çizelge 1. Isırgan su ekstraktının C. elegans'ta fertiliteye etkisi

Doses (%)	Egg counts	Individual counts
Control	857.66±4.04^{d}	839.66±4.16^{d}
0.01	958±3^{c}	950.33±4.50^{c}
0.02	964.66±3.21^{bc}	959.66±2.51^{bc}
0.05	969.66±3.78^{b}	962.66±3.21^{b}
0.1	999.33±2.51^{a}	991.33±4.04^{a}

^{a,b,c} Values within a column with different superscripts differ significantly at P<0.05

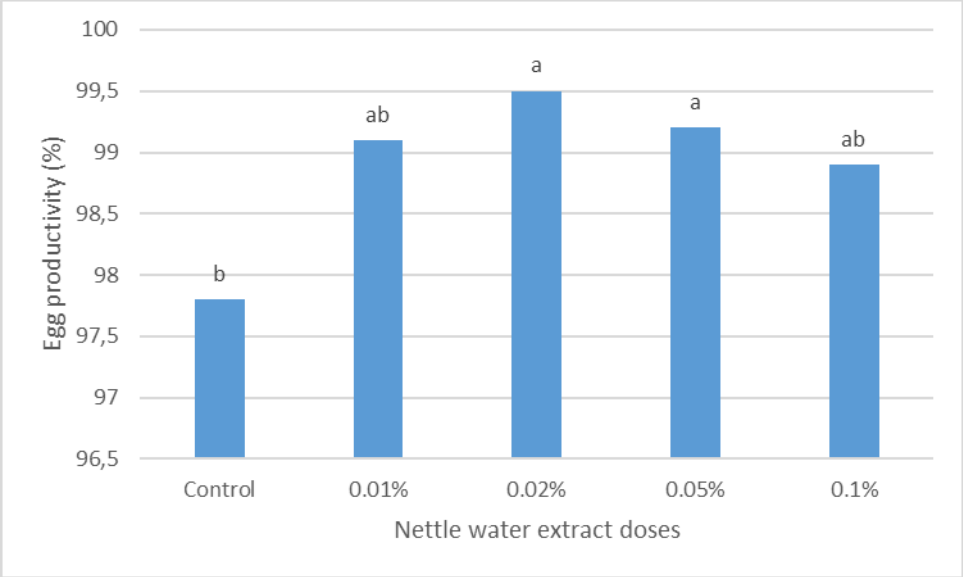


Figure 4. The effect of Nettle plant on egg productivity in *C. elegans*
Şekil 4. Isırgan bitkisinin C. elegans'ta yumurta verimine olan etkisi

Table 2. The effect of Carob water extract on fertility in *C. elegans*
Çizelge 2. Keçiboynuzu su ekstraktının C. elegans'ta fertiliteye etkisi

Doses (%)	Egg counts	Individual counts
Control	866.66±4.16^{b}	846.66±4.72^{c}
0.01	861.66±2.08^{b}	851.66±3.21^{bc}
0.02	864.66±1.52^{b}	856.66±4.16^{ab}
0.05	868.66±3.21^{ab}	860±2^{ab}
0.1	874.66±3.05^{a}	865±3.60^{a}

^{a,b,c} Values within a column with different superscripts differ significantly at P<0.05

When Table 2, which demonstrates the effects of different doses of Carob water extract on fertility, is examined, it is determined that the highest dose of the Carob plant, which is the 0.1% application, and partially the 0.05% application, have a positive effect on egg counts. Compared to the control, no significant difference is observed in other dose applications. When the number of individuals hatched from the eggs is evaluated, a significant increase is determined depending on the dose increase (Table 2). This indicates that the Carob plant has a positive effect on fertility.

The graph showing the egg productivity of different doses of Carob water extract and the control is presented in Figure 5.

When examining Figure 5, it can be observed that all doses of Carob water extract have increased egg productivity compared to the control. The egg productivity, which was 97.6% in the control, reached an average of 99% in Carob extract applied at different concentrations.

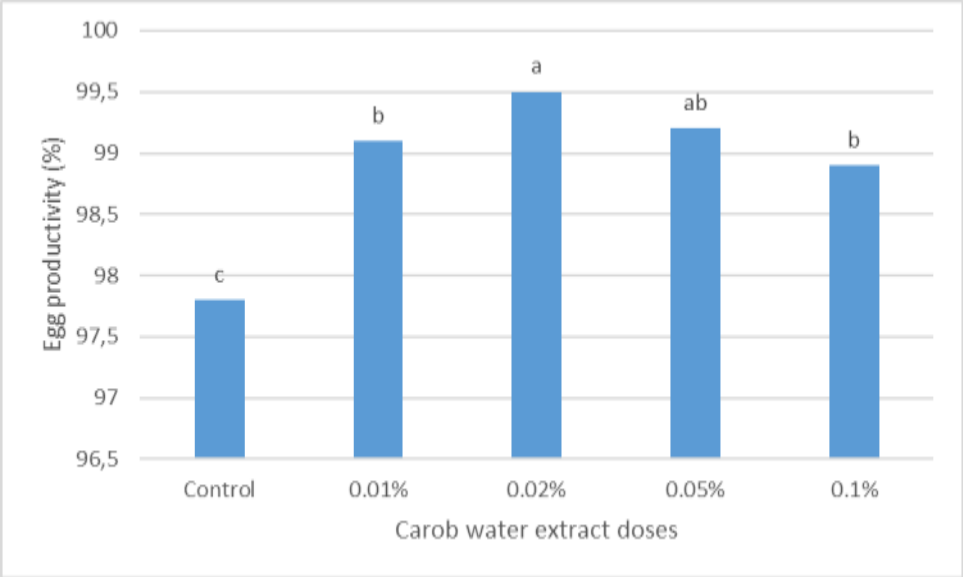


Figure 5. The effect of Carob plant on egg productivity in *C. elegans*
Şekil 5. Keçiboynuzu bitkisinin C. elegans'ta yumurta verimine olan etkisi

All doses of Chaste water extract (0.01%, 0.02%, 0.05%, 0.1%) have shown an increase in egg counts and individual counts in *C. elegans* compared to the control. This difference between the control and dose groups is statistically significant (P<0.05). Chaste water extract has had a positive effect on fertility, increasing egg counts and individual counts with higher doses. The highest numbers of eggs (998.66±3.21) and individuals (988.66±1.52) were observed in the 0.1% dose, while the lowest numbers of eggs (868.66±4.72) and individuals (849.66±4.50) were observed in the control (Table 3).

The graph showing the different doses of water extract of Chaste seeds and the control's egg productivity is given in Figure 6.

Table 3. The effect of Chaste water extract on fertility in *C. elegans*
Çizelge 3. Hayıt su ekstraktının C. elegans'ta fertiliteye etkisi

Doses (%)	Egg counts	Individual counts
Control	868.66±4.72^{e}	849.66±4.50^{e}
0.01	939±4.35^{d}	931.33±3.21^{d}
0.02	951.33±4.04^{c}	946.66±2.51^{c}
0.05	966±4.35^{b}	958.33±2.51^{b}
0.1	998.66±3.21^{a}	988.66±1.52^{a}

^{a,b,c} Values within a column with different superscripts differ significantly at P<0.05

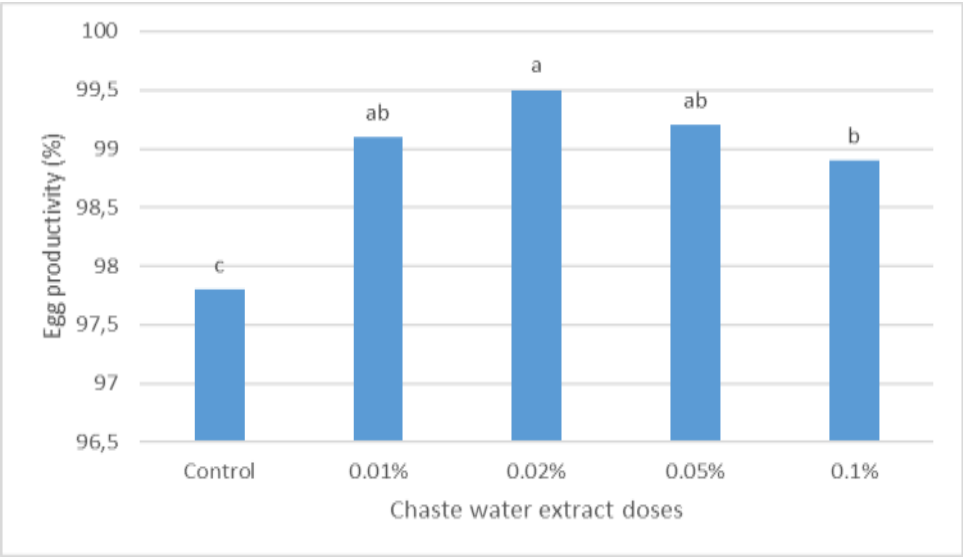


Figure 6. The effect of Chaste plant on egg productivity in *C. elegans*
Şekil 6. Hayıt bitkisinin C. elegans'ta yumurta verimine olan etkisi

The egg productivity of *C. elegans* for the application doses of the Chaste plant at 0.01%, 0.02%, 0.05%, and 0.1% were determined as 99.1%, 99.5%, 99.2%, and 98.9%, respectively. In the control, the percentage of hatched larvae from the eggs is 97.8% (Figure 6).

There are many different studies on the number of eggs and egg productivity of *C. elegans* individuals of different plant extracts and active ingredients (Ozpınar et al., 2017; Özpınar et al., 2017). However, not many studies were found that demonstrate the effects of Nettle, Carob, and Chaste plants on fertility in model organisms found in the conducted literature review.

Yılmaz (2019), in his study investigating the effects of commonly used plants in Turkey, namely Nettle, Rosemary, and Carob, on sperm function parameters under in vitro conditions. He treated sperm samples obtained from 40 patients with extracts of these plants at concentrations of 0.1%, 0.05%, and 0.01% for 30 minutes, 1 hour, and 24 hours, respectively. Sperm motility and vitality parameters were evaluated according to the World Health Organization (WHO) criteria. The study found that progressive sperm motility and vitality were improved with the in vitro application of Nettle, Rosemary, and Carob extracts in normozoospermic samples. Vafaei et al. (2018) reported a study in which they aimed to determine the effects of Carob (*Ceratonia siliqua*) extract on sperm quality, testicular structure, testosterone levels, and oxidative stress in Busulfan-induced infertile mice. In this study, adult male mice were treated with 10 mg/kg of busulfan along with Carob extract at doses of 800, 400, 200, 100, and 50 mg/kg for 35 days. According to the research findings, the administration of 800 mg/kg Carob extract for 35 days improved sperm quality, biochemical parameters, germinal epithelium thickness, and testosterone levels in Busulfan-induced infertile mice. In another study, the effect of orally administered Carob fruit in capsule form and vitamin E on sperm parameters in men with idiopathic infertility was investigated. In the study, it was determined that the daily use of 1500 mg oral Carob fruit capsule for 90 days had a significant effect on sperm motility, but compared to vitamin E, it did not have a significant effect on morphology and count (Sanagoo et al., 2021). Jalili et al. (2014) conducted a study to investigate whether the hydroalcoholic extract of *Urtica dioica* could inhibit the negative effects of nicotine on the viability, count, motility, testicular histology, and testosterone hormone levels of sperm cells. In their research, they determined that the hydroalcoholic extract of *Urtica dioica* could enhance spermatozoa quality and inhibit the negative effects of nicotine on sperm parameters. In a different study, the effects of *Vitex agnus-castus* extract on the reproductive potential of women with premature ovarian ageing (POA) were investigated. The study aimed to determine the impact of *Vitex agnus-castus* extract as a plant that promotes fertility. The results indicated that when used within established dosage guidelines, *Vitex agnus-castus* extract is considered a safe and effective botanical intervention in medical practices for women with POA, encouraging fertility. (Hossein-Rashidi & Nemati, 2017).

CONCLUSION

This study aimed to determine the effects of different concentrations of water extract from Nettle (*Urtica dioica* L.), Carob (*Ceratonia siliqua* L.), and Chaste (*Vitex agnus-castus* L.) plants on fertility in *C. elegans*. It was found that all dosage applications of Chaste and Nettle plants increased the number of eggs and the number of individuals hatched from eggs compared to the control. The Carob plant, on the other hand, showed a positive effect on the number of eggs with its 0.1% and 0.05% applications. The dosage application that yielded the best results in all three plants was 0.1%. Furthermore, according to the study results, it can be said that the water extract of the Nettle plant has a greater impact on fertility overall.

When the data obtained from the study are evaluated as a whole, it is believed that medicinal plants, as one of the traditional methods, can be used to treat infertility, which is widely seen today, instead of using chemically based drugs or lengthy and costly treatment methods. Many plants are being discovered to have more medicinal effects than previously known or can be used in different areas. Scientific research and studies help us better understand the effects of plants on health and lead to discoveries. As more information is obtained about the bioactive compounds found in plants and their effects on the human body, herbal medicines, and treatment methods can evolve. This process is a dynamic field where discoveries are constantly being made in the field of herbal medicine.

ACKNOWLEDGEMENTS

This study was supported by the Sivas Cumhuriyet University Scientific Research Projects Unit (CUBAP) under project number SMYO-028.

Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

1

REFERENCES

Beşen, Ö. G. M. A. & Beji, N. K. (2014). Fertilite ve bitkiler. *Androloji Bülteni 59*, 286-289.

Bora, K. S. & Sharma, A. (2010). Phytochemical and pharmacological potential of *Artemisia absinthium* Linn. and *Artemisia asiatica* Nakai: a review. *J Pharm Res. 3*(2), 325-328.

Brassesco, M. E., Brandao, T. R., Silva, C. L., & Pintado, M. (2021). Carob bean (*Ceratonia siliqua* L.): A new perspective for functional food. *Trends in Food Science & Technology 114*, 310-322.

Corsi, A. K., Wightman, B., & Chalfie, M. (2015). A transparent window into biology: a primer on *Caenorhabditis elegans*. *Genetics 200*(2), 387-407.

Çetin, M. (2012). Hayvan beslemede antibiyotik ve antiparaziterlere alternatif olarak bitkisel ekstraklar ve Pelinotu'nun (*Artemisia Absinthium*) Kullanılması. *KSÜ Doğa Bilimleri Dergisi 15*(4), 58-63.

Daştan, T. & Saraç, H. (2018). Determination of the nutritional element concentrations of the Evelik plant (*Rumex crispus* L.). *Cumhuriyet Science Journal 39*(4),1020-1024.https://doi.org/10.17776/csj.455688

Edirne, T., Arica, S. G., Gucuk, S., Yildizhan, R., Kolusari, A., Adali, E. & Can, M. (2010). Use of complementary and alternative medicines by a sample of Turkish women for infertility enhancement: a descriptive study. *BMC complementary and alternative medicine 10*, 1-7. http://www.biomedcentral.com/1472-6882/10/11.

Esposito, S., Bianco, A., Russo, R., Di Maro, A., Isernia, C., & Pedone, P. V. (2019). Therapeutic perspectives of molecules from *Urtica dioica* extracts for cancer treatment. *Molecules 24*(15), 2753.

Ezer, N. & Avcı, K. (2004). Çerkeş (Çankırı) yöresinde kullanılan halk ilaçları. *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi 24*(2), 67-80.

Fugh-Berman, A. & Kronenberg, F. (2003). Complementary and alternative medicine (CAM) in reproductive-age women: a review of randomized controlled trials. *Reproductive Toxicology 17*(2), 137-152. https://doi.org/10.1016/S0890-6238(02)00128-4.

Gaware, V.M., Parjane, S.K., Merekar Abhijit, N., Pattan, S. R. & Dighe, N. S. (2009). Female infertility and its treatment by alternative medicine: a review. *Journal of Chemical and Pharmaceutical Research 1*(1), 148-162.

Hertweck, M., Hoppe, T. & Baumeister, R. (2003). *C. elegans*, a model for ageing with high-throughput capacity. *Experimental gerontology 38*(3), 345-346. https://doi.org/10.1016/S0531-5565(02)00208-5.

Hossein-Rashidi, B. & Nemati, M. (2017). Effects of *Vitex agnus-castus* extract on the secretory function of pituitary-gonadal axis and pregnancy rate in patients with premature ovarian ageing (POA). *Journal of Herbal Medicine 10*, 24-30. https://doi.org/10.1016/j.hermed.2017.10.003.

İpekoğlu, H. Y. & Oral, H. B. (2019). İnfertilitede kullanilan geleneksel tedavi yöntemleri ve çeşitli uygulamalar: Isparta bölgesi. *Motif Akademi Halkbilimi Dergisi 12*(25), 105-119. https://doi.org/10.12981/mahder.523760.

Jalili, C., Salahshoor, M. R. & Naseri, A. (2014). Protective effect of *Urtica dioica* L against nicotine-induced damage on sperm parameters, testosterone, and testis tissue in mice. *Iranian journal of reproductive medicine 12*(6), 401-408.

Koelle, M. (2005). Quantitation of constitutive egg-laying. Yale University, New Haven, USA.

Niroumand, M. C., Heydarpour, F., & Farzaei, M. H. (2018). Pharmacological and therapeutic effects of *Vitex agnus-castus* L.: A review. *Pharmacognosy Reviews 12* (23).

Olsen, A., Vantipalli, M. C. & Lithgow, G. J. (2006). Lifespan extension of *Caenorhabditis elegans* following repeated mild hormetic heat treatments. *Biogerontology 7*, 221-230. DOI: 10.1007/s10522-006-9018-x.

Özpınar, N. (2020). A model Organism for Antihelminthic Activity: *Caenorhabditis elegans* and *Nigella sativa*. *Türkiye Parazitolojii Dergisi 44*(1), 31. DOI:10.4274/tpd.galenos.2020.6759.

Özpınar, H. & Yüksek, M. (2019). *Stevia rebaudiana* Bertoni bitkisinin yaprak ekstraktının *Caenorhabditis elegans* bireylerinde yumurtadan çıkma ve fiziksel büyüme üzerine etkisi. *International Journal of Academic Medicine and Pharmacy 1*, 1-4.

Ozpinar, H., Ozpinar, N., & Karakus, S. (2017). The effects of Erzincan grape (*Vitis vinifera* spp., Cimin) and benzothiazol on a *Caenorhabditis elegans* organism model. *Pharmacognosy Magazine 13*(Suppl 2), S380.

Özpınar, H.,Özpınar, N., & Karakus, S. (2017). The Physiological and Genotoxic Effects of Water and Ethanol Extracts of Goji Berry (*Lycium barbarum* L.) on Model Organisms. *Journal of Clinical and Experimental Investigations 8*(3), 76-84.

Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A. & Cerón, J. (2012). Basic *Caenorhabditis elegans* methods: synchronization and observation. *JoVE (Journal of Visualized Experiments)* (64), 1-9. DOI: 10.3791/4019.

Sanagoo, S., Farshbaf-Khalili, A., Asgharian, P., Hazhir, S. & Oskouei, B. S. (2021). Comparison of the effect of *Ceratonia siliqua* L. fruit oral capsule and vitamin E on semen parameters in men with idiopathic infertility: a triple-blind randomized controlled clinical trial. *Journal of Complementary and Integrative Medicine 18*(4), 791-796. https://doi.org/10.1515/jcim-2020-0095.

Saraç, H., Daştan, T., Demirbaş, A., Durna Daştan, S., Karaköy, T. & Durukan, H. (2018). Madımak (*Polygonum cognatum* Meissn.) bitki özütlerinin

besin elementleri ve in vitro antikanserojen aktiviteleri yönünden değerlendirilmesi. *Ziraat Fakültesi Dergisi* 340-347.

Savaş, N. 2018. *Avokado (Persea americana) ve Oğulotu (Melissa officinalis) Fonksiyonel Besinlerinin, Caenorhabditis elegans Termotoleransı Üzerine Etkilerinin İncelenmesi*. [Master's thesis, Aydın Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü Beslenme ve Diyetetik Anabilim Dalı]. http://hdl.handle.net/11607/3505.

Springer, W. (2005). *Caenorhabditis elegans as an experimental model organism to study Parkinson's disease-related genes: functional analysis of Parkin and α-synuclein* [PhD Thesis, München, Univ., Diss.].

Tan, L., Tong, Y., Sze, S. C. W., Xu, M., Shi, Y., Song, X. Y. & Zhang, T. T. (2012). Chinese herbal medicine for infertility with anovulation: a systematic review. *The Journal of Alternative and Complementary Medicine 18*(12), 1087-1100. https://doi.org/10.1089/acm.2011.0371.

Telefo, P. B., Lienou, L. L., Yemele, M. D., Lemfack, M. C., Mouokeu, C., Goka, C. S., ... & Moundipa, F. P. (2011). Ethnopharmacological survey of plants used for the treatment of female infertility in Baham, *Cameroon. Journal of Ethnopharmacology 136*(1), 178-187. https://doi.org/10.1016/j.jep.2011.04.036.

Ugulu, I., Baslar, S., Yorek, N. & Dogan, Y. (2009). The investigation and quantitative ethnobotanical evaluation of medicinal plants used around Izmir province, *Turkey. Journal of Medicinal Plants Research 3*(5), 345-367. http://www.academic journals.org/JMPR.

Ünlü, E. & Erdem, C. (2010). Deri yaşlanmasında korunma ve tedavi yöntemleri. *Dermatoz 1*(1), 23-31.

Vafaei, A., Mohammadi, S., Fazel, A., Soukhtanloo, M., Mohammadipour, A. & Beheshti, F. (2018). Effects of Carob (*Ceratonia siliqua*) on sperm quality, testicular structure, testosterone level, and oxidative stress in busulfan-induced infertile mice. *Pharmaceutical Sciences 24*(2), 104-111. doi: 10.15171/PS.2018.16.

Yılmaz, E. (2019). *Türkiye'de Halk Arasında Yaygın Olarak Kullanılan Isırgan, Biberiye ve Keçiboynuzu Bitkilerinin İn vitro Koşullarda Sperm Fonksiyonu Parametrelerine Etkisi*. [Master's thesis, Biruni Üniversitesi, Sağlık Bilimleri Enstitüsü]. https://hdl.handle.net/20.500.12445/1017.

KSÜ Tarım ve Doğa Derg 27 (4), 767-772, 2024
KSU J. Agric Nat 27 (4), 767-772, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1385768

Stem Anatomy of Some *Crepis* L. (Asteraceae) Taxa and Its Taxonomic Significance

Huseyin INCEER[1]✉, Ozge OZGURLUK[2]
Karadeniz Technical University, Faculty of Science, Department of Biology, 61080 Trabzon, Türkiye
[1]https://orcid.org/0000-0003-1344-8747, [2]https://orcid.org/0000-0002-7403-5885
✉: inceer@ktu.edu.tr

ABSTRACT

In this study, the stem anatomy of nine *Crepis* (Asteraceae) taxa was described for the first time from Türkiye. The anatomical data obtained were evaluated in terms of taxonomy with analysis of variance and principal components analysis. The stem mainly consists of a layer epidermis, parenchymatous cortex, collateral vascular bundles, and parenchymatous pith in all taxa examined. Glandular or non-glandular trichomes are found in the epidermal cells of the stem in some species examined. Even though all taxa examined have similar stem anatomy, quantitative traits such as the length or width of the anatomical characters significantly vary among the taxa. In particular, the xylem thickness considerably differs among the taxa studied, according to the results of analysis of variance. Besides, the xylem is thicker in caulescent species than in scapigerous taxa. According to the results of the principal components analysis, the xylem thickness and the epidermis cell length explain most of the total variation with about 82% value among the studied taxa. These results show that xylem thickness and epidermis cell length have a high taxonomic value.

Botany

Research Article

Article History
Received : 03.11.2023
Accepted : 17.01.2024

Keywords
Anatomy
Crepis
Stem
Numerical taxonomy

Bazı *Crepis* L. (Asteraceae) Taksonlarının Gövde Anatomisi ve Taksonomik Önemi

ÖZET

Bu çalışmada, dokuz *Crepis* (Asteraceae) taksonunun gövde anatomisi ilk kez Türkiye'den tanımlandı. Elde edilen anatomik veriler, varyans analizi ve temel bileşenler analizi ile taksonomik yönden değerlendirildi. İncelenen tüm taksonlarda gövde, başlıca tek tabakalı epidermis, parenkimatik korteks, kollateral iletim demetleri ve parenkimatik özden oluşmaktadır. İncelenen bazı türlerde, gövdenin epidermal hüclerinde salgı veya salgısız tüyler bulunur. İncelenen tüm taksonlar benzer gövde anatomisine sahip olmakla birlikte, anatomik karakterlerin uzunluk veya genişlik gibi kantitatif özellikleri, taksonlar arasında önemli ölçüde değişir. Özellikle, ksilem kalınlığı taksonlar arasında oldukça farklıdır. Bununla birlikte, ksilem, gövdeli türlerde, skaplı taksonlara göre daha kalındır. Temel bileşenler analiz sonuçlarına göre, ksilem kalınlığı ve epidermis hücresi uzunluğu yaklaşık %82 değeri ile çalışılan taksonlar arasındaki varyasyonun çoğunu açıklar. Bu sonuçlar, ksilem kalınlığı ve epidermis hücresi uzunluğunun yüksek bir taksonomik değere sahip olduğunu gösterir.

Botanik

Araştırma Makalesi

Article History
Geliş Tarihi : 03.11.2023
Kabul Tarihi : 17.01.2024

Anahtar Kelimeler
Anatomi
Crepis
Gövde
Sayısal taksonomi

To Cite : Inceer, H., & Ozgurluk, O., (2024). Stem Anatomy of Some *Crepis* L. (Asteraceae) Taxa and Its Taxonomic Significance. *KSU J. Agric Nat 27* (4), 767-772. https://doi.org/10.18016/ksutarimdoga. vi.1385768

Atıf Şekli : Inceer, H., & Ozgurluk, O., (2024). Bazı *Crepis* L. (Asteraceae) Taksonlarının Gövde Anatomisi ve Taksonomik Önemi. *KSÜ Tarım ve Doğa Derg 27* (4), 767-772. https://doi.org/10.18016/ksutarimdoga.vi. 1385768

INTRODUCTION

Crepis L. with above 200 species belonging to the tribe Cichorieae is one of the taxonomically difficult genera of the family Asteraceae (Bremer, 1994; Enke, 2009). Its species are widely distributed in the Northern Hemisphere and Africa (Babcock, 1947a, b). Within *Crepis*, delimitation of the species is difficult due to the lack of discriminating characters. High morphological plasticity in the *Crepis* species is also common, which has resulted in a profusion of published names (Iamonico & Iberite, 2023).

According to the classic works of Babcock (Babcock, 1947a, b), *Crepis* is a monophyletic genus. However, recent molecular studies indicate that the genus

Crepis is polyphyletic with split into three statistically well-supported clades (Enke & Gemeinholzer, 2008; Enke, 2009).

The taxonomic significance of stem anatomy has been confirmed in some groups of Asteraceae, such as *Inula* L. and *Tripleurospermum* Sch.Bip. (Karanović et al., 2022; Ozcan & Inceer, 2022). Anatomical characteristics of the stem have also taxonomic value for some members of Cichorieae (Carlquist, 1967; Metcalfe & Chalk, 1979).

The systematic significance of anatomical knowledge for *Crepis* was noted by Inceer et al. (2018) based on achene and leaf characters. However, anatomical data on the stem anatomy of *Crepis* are very scarce. According to current taxonomic literature (Yıldırımlı, 2021), 41 *Crepis* taxa are found in Türkiye, where its endemism rate is about 22%. This study aims to contribute to the anatomical knowledge of *Crepis* with the evaluation of stem anatomical characters of nine *Crepis* taxa from Türkiye using univariate and multivariate analyses.

MATERIALS and METHODS

Plant Materials

The samples of the *Crepis* taxa were collected from natural populations in Türkiye (Table 1), and their stems were fixed in FAA (5 parts formalin: 5 parts acetic acid: 90 parts 70% ethyl alcohol) (Ozcan & Inceer, 2022).

Table 1. Collection data of the *Crepis* taxa examined
Çizelge İncelenen Crepis taksonlarının koleksiyon verileri

Taxon	Locality	Voucher**
C. alpestris (Jacq.) Tausch	Bursa: Uludağ, 2,230 m, 30.7.2013	Inceer 1046
C. amanica Babcock*	Adana: Misis Nur Mountain, 205 m, 18.5. 2013	Inceer 989
C. armena DC.*	Kahramanmaraş: Işık Mountain, 2,550 m, 2.7. 2013	Inceer 1031a
C. aspera L.	Hatay: Near Saint Pierre Church, 160 m, 20.5. 2013	Insert 994
C. aurea (L.) Cass. subsp. *olympica* (K. Koch.) Lamond*	Bursa: Uludağ, 2,035 m, 29.7. 2013	Insert 1043
C. biyhynica Boiss.	Bursa: Uludağ, 2,210 m, 30.7. 2013	Inceer 1045
C. dioritica Schott & Kotschy ex Boiss.*	Niğde: Bolkar Mountains, between Kızıltepe and Karagöl, 2,600 m, 04.7.2013	Insert 1034
C. dioscoridis L.	Muğla: Datça, 80 m, 4.5. 2013	Inceer 984
C. smyrnaea DC. ex Froehlich	Ankara: Mahiye Tepe, 2,043 m, 30.6. 2013	Inceer 1012

*endemic to Türkiye, **vouchers are deposited in the KTUB herbarium

Stem Anatomy

For stem anatomy, transverse sections from the middle parts of the stems fixed in FAA were taken by hand using commercial razor blades (Inceer et al., 2016). The transverse sections were stained with safranin and then mounted in Entellan (Inceer et al., 2016). Five well-permanent slides belonging to five individuals for each taxa were performed (Inceer & Ozcan, 2021). The anatomical structures were examined under the Leica DM 4000B microscope.

Data Analysis

The data obtained from the anatomical characters (epidermis cell length, epidermis cell width, cortex thickness, row number of collenchyma, phloem thickness, xylem thickness, vascular bundle width) were evaluated with analysis of variance (one-way ANOVA, Duncan's multiple-range test), and principal components analysis (PCA). The PCA and ANOVA were performed with using Statistica version 12 and SPSS version 17, respectively.

RESULTS and DISCUSSION

Stem Anatomy

The stem anatomy of nine *Crepis* taxa is presented from Türkiye in detail for the first time. Sytematic aspects for stem anatomy in the studied taxa were provided. The present results show that all taxa studied have similar anatomical structures (Figure 1), but there are significant differences in the dimensions of the anatomical characters among the taxa (Table 2). In particular, the results obtained from ANOVA show that there are considerable differences in the xylem thickness among the taxa studied. Besides, epidermis cell width has a less significant value than other anatomical characters among the taxa.

The stems in the transverse sections were generally more or less rounded in shape. The epidermis covered by a thin cuticle layer is single-layered. The length of the epidermal cells ranges from 12.61±0.93 µm in *C. bithynica* to 16.06±1.26 µm in *C. dioscoridis* (Table 2). The width of epidermal cells varies from 13.83±1.86 µm in *C. alpestris* to 20.54±5.46 µm in *C. aspera*. The cortex is composed of collenchyma, parenchymatous cells as well as endodermis. Its thickness ranges from 83.77±9.86 µm in *C. dioritica* to 147.82±7.76 µm in *C.*

aspera. Within the taxa examined, vascular bundles are collateral, phloem and xylem are clear. Cambium is not visible between the phloem and the xylem. The phloem thickness ranges from 44.94±7.95 µm in *C. dioscoridis* and 121.59±11.50 µm in *C. aspera*. The xylem thickness and vascular bundle wideness vary from 95.16±5.32 µm and 94.55±0.61 in *C. dioritica* to 400.57±4.03 µm and 214.11±1.44 in *C. aspera*, respectively (Table 2). The path is composed of large and round parenchymatic cells (Figure 1). Similar findings are reported from other species of *Crepis* (Metcalfe & Chalk, 1979; Crivellaro & Schweingruber, 2015; Inceer et al., 2016).

Within the studied taxa, *C. alpestris*, *C. aurea* subsp. *olympica* and *C. bithynica* are found in the same ecological environment in the alpine region of Uludağ in Bursa. According to the results of ANOVA, there is no significant difference in all anatomical characters between *C. aurea* subsp. *olympica* and *C. bithynica*. On the other hand, significant differences are found in the cortex thickness, the phloem thickness, and the vascular bundle width between *C. alpestris* and *the* other two taxa.

Some *Crepis* taxa are characterized by having scapiform, that is one-headed stems bearing few or no leaves (Babcock, 1947a, b; Lamond, 1975). The results obtained from stem anatomy indicate there is a significant difference in the xylem thickness between caulescent species and saliferous taxa. As seen in Table 2, except for *C. armena*, the xylem is significantly more thinner in scapigerous taxa, namely *C. alpestris*, and *C. aurea* subsp. *olympica*, *C. bithynica,* and *C. dioritica*, then caulescent species, namely *C. amanica*, *C. aspera*, *C. dioscoridis,* and *C. smyrnaea*.

The present results show that well-lignified sclerenchymatous cells are found between vascular bundles in *C. amanica* and *C. aspera* (Figure 1). A similar finding is reported from the endemic species *Crepis macropus* in Türkiye (Inceer et al., 2016).

Table 2. Differences in anatomical characters (mean value ±SD) of the stem in the *Crepis* taxa studied. Among the taxa in the same column, the mean values with different letters are significant at $p = 0.05$

Çizelge 2 Çalışılan Crepis taksonlarında gövdenin anatomik karakterlerindeki (ortalama değer±standart sapma) farklılıklar.

Taxa	Epidermis cell length (µm)	Epidermis cell width(µm)	Cortex thickness (µm)	Cr	Phloem thickness(µm)	Xylem thickness (µm)	Vascular bundle width(µm)
C. alpestris	12.81±1.06ab	13.83±1.86a	136.64±19.17ef	3-4	76.25±6.34cd	97.60±27.48a	131.76±26.42c
C. amanica	14.03±1.22abc	15.66±2.14a	111.02±14.80cd	3-6	63.64±10.07bc	219.40±6.69c	158.80±8.42d
C. arena	14.64±1.06bcd	16.06±0.93a	105.53±7.64bc	2-3	90.48±3.13e	144.16±10.32b	130.13±4.97c
C. aspera	15.66±0.29cd	20.54±5.46b	147.82±7.76f	2-5	121.59±11.50f	400.57±4.03d	214.11±1.44e
C. aurea subsp. *olympica*	13.62±1.54ab	14.03±1.61a	89.67±9.82ab	2-3	59.58±3.01b	95.57±7.07a	99.43±1.61ab
C. bithynica	12.61±0.93a	14.23±1.76a	89.06±6.01ab	2-3	54.70±7.35ab	103.50±14.49a	98.21±8.21ab
C. dioritica	13.83±1.27abc	14.84±0.93a	83.77±9.86a	1-2	51.04±4.97ab	95.16±5.32a	94.55±0.61a
C. dioscoridis	16.06±1.26d	20.13±2.36b	127.49±18.02de	2-4	44.94±7.95a	130.95±9.61b	117.73±9.60bc
C. smyrnaea	13.83±0.35abc	15.86±0.61a	111.43±14.64cd	2-3	77.88±12.11de	197.44±6.26c	195.40±13.16e
	$F_{8,18} = 3.782$ $p<0.01$	$F_{8,18} = 3.963$ $p<0.01$	$F_{8,18} = 11.843$ $p<0.001$		$F_{8,18} = 27.843$ $p<0.001$	$F_{8,18} = 148.384$ $p<0.001$	$F_{8,18} = 45.935$ $p<0.001$

Aynı sutundaki taksonlar arasındaki farklı harflere sahip ortalama değerler, p = 0.05 düzeyinde önemlidir
Cr: row number of collenchyma

Endemic species *Crepis dioritica* and *C. macropus* were grouped within clade V in the molecular phylogenetic context of the genus *Crepis* (Enke & Gemeinholzer, 2008; Enke, 2009). The present results show that *C. dioritica* has less lignified sclerenchymatous cells than *C. macropus* between vascular bundles. Besides, *C. dioritica* has more thin xylem thickness (95.16±5.32 µm) than *C. macropus* (194.18±15.29 µm, Inceer et al., 2016). On the other hand, the cambium is not visible in *C. dioritica*, while the cambium is visible in *C. macropus* (Inceer et al., 2016). These anatomical traits can be used as additional data to support the morphological separation of *C. dioritica* from its relative *C. macropus*.

Glandular or non-glandular trichomes can be found in epidermal cells of the stem in some members of *Crepis* (Krak & Mraz, 2008). Within the studied taxa, the epidermis contains glandular (uniseriate with unicellular head) and non-glandular trichomes (uniseriate filiform trichomes with elongated apical cells) (Figure 2), except for *C. aurea* subsp. *olympica* and *C. dioscoridis* have stem surfaces without hairs, which is in line with the results of Lamond (1975). Within the studied taxa, the epidermis of *C. armena*, *C. bithynica,* and *C. dioritica* have glandular trichomes. Likewise, glandular trichomes on the stems of *C. dioritica* were previously reported by Lamond (1975).

Within all *Crepis* taxa examined, the collenchyma is under the epidermis. In addition, the cells of this tissue cross vascular bundles. Similar results are reported for the species of *Scorzonera* L. (Makbul et al., 2011). On the other hand, the number of its rows differs among the taxa (Table 2). As seen in Table 2, the highest row number in the collenchyma is found in *C. amanica*, whereas the lowest row number in the collenchyma is present in *C. dioritica*. *Crepis amanica* is an annual species 38-60 cm long and its stem is erect as well as

rather stout (Babcock, 1947b; Lamond, 1975). Hence, the collenchyma as supporting tissue may play an important role in tensile stress in this species.

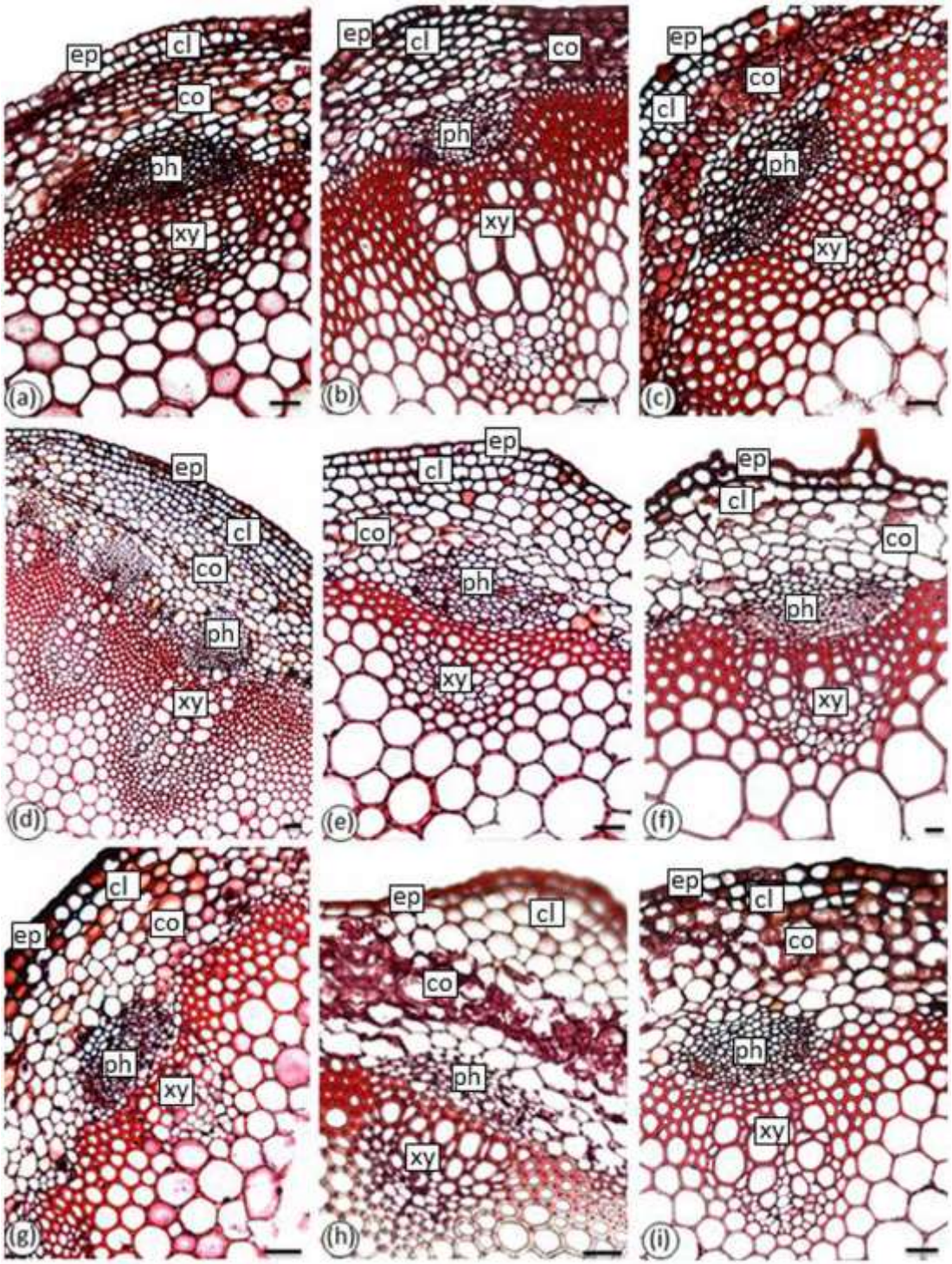


Figure 1. Transverse sections of the stems in the *Crepis* taxa examined; a: *C. alpestris*, b: *C. amanica*, c: *C. armena*, d: *C. aspera*, e: *C. aurea* subsp. *olympica*, f: *C. bithynica*, g: *C. dioritica*, h: *C. dioscoridis*, I: *C. smyrnaea*. ep: epidermis, cl: collenchyma, co: cortex, ph: phloem, xy: xylem. Scale bars: 30 µm (a, b, c, e, g, h, i), 50 µm (d, f)

Şekil 1. İncelenen Crepis taksonlarında gövdelerin enine kesitleri; a:, C. alpestris, b:, C. amanica, c: C. armena, d: C. aspera, e: C. aurea subsp. *olympica, f: C. bithynica, g: C. dioritica, h: C. dioscoridis, I: C. smyrnaea.* ep: epidermis, cl: kollenkima, co: korteks, ph: floem, xy: ksilem. Ölçekler: 30 µm (a, b, c, e, g, h, i), 50 µm (d, f)

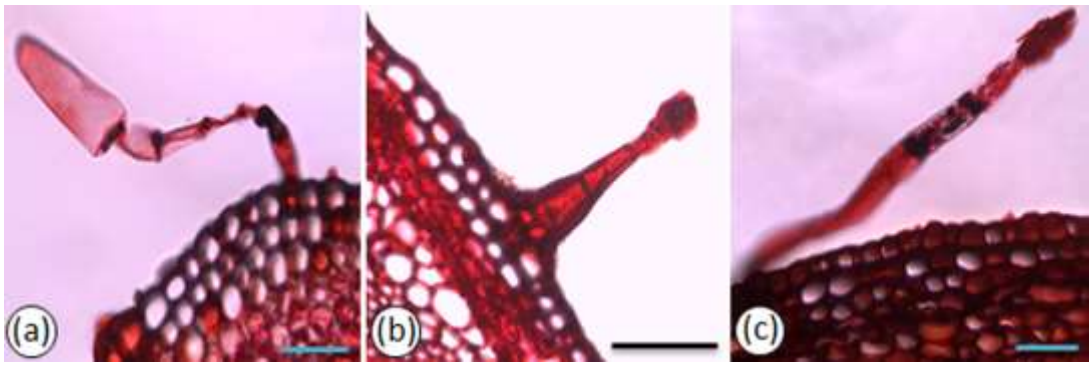


Figure 2. Trichomes in transverse section of the stem in *Crepis*; a: *C. armena*, b: *C. dioritica*, c: *C. smyrnaea*. Scale bars: 50 µm

Şekil 2. Crepis'de gövde enine kesitinde tüyler; a: C. armena, b: C. dioritica, c: C. smyrnaea. Ölçekler: 50 µm

Principal Components Analysis

The results obtained from PCA analysis indicate that PC1 and PC2 are composed of four groups based on anatomical characteristics of the stem (Figure 3). According to PCA data analysis, the first two PC factors accounted for about 82% of the total variance (Figures 3 and 4). As seen in Figure 4, the first factor accounts for about 66% of the total variance, with the mean thickness of the xylem having the highest negative correlation (r = -0.92). The second factor accounts for 16% of the total variance, with epidermis cell length showing a negative correlation (r = -0.62). These findings indicate that xylem thickness and epidermis cell length explain most of the total variation among the studied taxa.

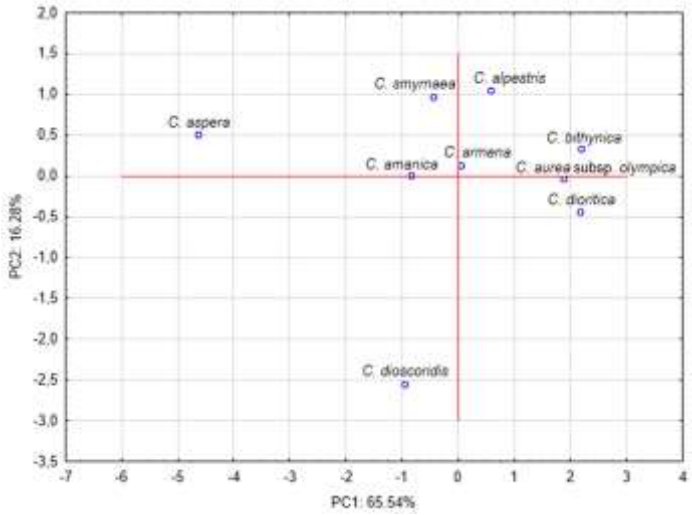


Figure 3. Results from the PCA of the *Crepis* taxa based on stem anatomy

Şekil 3. Crepis taksonlarının gövde anatomisine dayalı temel bileşenler analiz sonuçları

CONCLUSION

This is the preliminary study on the taxonomic evaluation of anatomical characters of the stem using analysis of variance and principal components analysis. According to the results of these analyses, the xylem thickness and epidermis cell length have a high taxonomic value at an interspecific level to infer anatomical variations among the studied taxa. In particular, the xylem is thicker in caulescent species (*C. amanica*, *C. aspera*, *C. dioscoridis,* and *C. smyrnaea*) than scapigerous taxa (*C. alpestris*, *C. aurea* subsp. *olympica*, *C. bithynica* and *C. dioritica*). The caulescent species *C. amanica* and *C. aspera* have also well-lignified sclerenchymatous cells between vascular bundles in their stems. On the other hand, the collenchyma may play an important role in tensile stress in *C. amanica*.

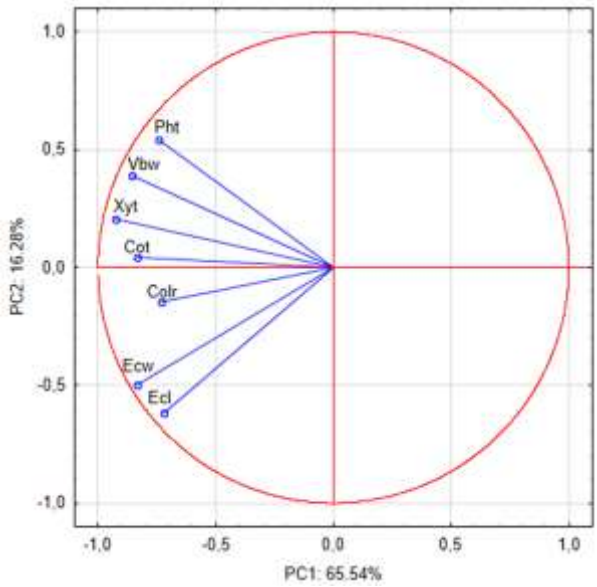


Figure 4. Results from the PCA of stem anatomy in *Crepis*; ct: cortex thickness, cr: row number of collenchyma, ecl: epidermis cell length, ecw: epidermis cell width, pht: phloem thickness, xyt: xylem thickness, vb: vascular bundle width

Şekil 4. Crepis'de gövde anatomisinin temel bileşenler analiz sonuçları; ct: korteks kalınlığı, cr: kollenkima sıra sayısı, ecl: epidermis hücre uzunluğu, ecw: epidermis hücre genişliği, pht: floem kalınlığı, xyt: ksilem kalınlığı , vbw: iletim demet genişliği

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Nursen Aksu Kalmuk for field excursions, Research Assistant Rabia Sagan for taking trichome images, and the Scientific and Technological Research Council of Turkey (TUBITAK, Project No. 112T132) for its financial support. This study is a part of the master thesis of Ozge Ozgurluk.

Researchers Contribution Rate Declaration Summary

Huseyin Inceer: Conceptualization, Data curation, Visualization, Formal analysis, Investigation, Methodology, Software, Funding acquisition, Writing-original draft, Writing-review & editing. Ozge Ozgurluk: Methodology, Data curation.

Conflicts of Interest Statement

The article's authors declare that they do not have any conflict of interest.

REFERENCES

Babcock, E. B. (1947a). The genus *Crepis* I: The taxonomy, phylogeny, distribution and evolution of *Crepis*. *University of California Press, Berkeley, and Los Angeles.*

Babcock, E. B. (1947b). The genus *Crepis*. II: Systematic treatment. *University of California Press, Berkeley, and Los Angeles.*

Bremer, K. (1994). Asteraceae, Cladistics and Classification. *Timber Press, Portland.*

Carlquist, S. (1967). Anatomy and systematics of *Dendroseris* (sensu lato). *Brittonia, 19*, 99-121.

Crivellaro, A., & Schweingruber, F. H. (2015). Stem anatomical features of dicotyledons: Xylem phloem, cortex, and perderm characteristics for ecological and taxonomical analysis. *Kessel Publishing.* DOI: 10.5282/ubm/data.56

Enke, N., & Gemeinholzer, B. (2008). Babcock revisited: new insights into generic delimitation and character evolution in *Crepis* L. (Compositae: Cichorieae) from ITS and matK sequence data. *Taxon, 57*, 756-68. DOI: 10.2307/25065905

Enke, N. (2009). Contributions towards a revised infrageneric classification of *Crepis* (Cichorieae, Compositae). *Willdenowia, 39*, 229-245.

Lamond, J. M. (1975). *Crepis* L. In P. H. Davis (Ed.), Flora of Turkey and the East Aegean Islands, vol. 5. *Edinburgh University Press, Edinburgh,* 814-841.

Iamonico, D., & Iberite, M. (2023). On the typification of the Linnaean name *Crepis foetida* (Asteraceae). *Taxon, 72*(5), 1109-1111. DOI: 10.1002/tax.12345

Inceer, H., Aksu Kalmuk, N., Imamoglu, K. V., Duman, O., Hayirlioglu-Ayaz, S., & Arslan, G. (2016). Micromorphological, anatomical, and cytogenetical studies in endemic *Crepis macropus* Boiss. & Heldr. (Asteraceae) from Turkey. *Acta Botanica Croatica, 75*, 173-178.

Inceer, H., Aksu Kalmuk, N., Imamoglu, K. V., Arslan, G., Duman, O., Hayırlıoglu-Ayaz, S., & Guner, G. (2018). Achene anatomy and stomatal characteristics of eighteen *Crepis* L. (Asteraceae) taxa from Turkey with notes on their systematic significance. *Nordic Journal of Botany, 36*(8), e01829. DOI: 10.1111/njb.01829

Inceer, H., & Ozcan, M. (2021). Taxonomic evaluations on the anatomical characters of leaf and achene in Turkish *Tripleurospermum* with its relative *Matricaria* (Asteraceae). *Flora, 275,* 151759. DOI: 10.1016/j.flora.2020.151759

Karanović, D., Zorić, L., Zlatković, B., & Luković, J. (2022). Leaf and stem anatomy and micromorphology of four Inuleae (Compositae) genera, with notes on their taxonomic significance. *Nordic Journal of Botany, 2022*(7), e03566. DOI: 10.1111/njb.03566

Krak, K., & Mraz, P. (2008). Trichomes in the tribe Lactuceae (Asteraceae) – taxonomic implications. *Biologia, 63*(5), 616-630.

Makbul, S., Coskuncelebi, K., & Beyazoglu, O. (2011). Notes on the stem anatomy of *Scorzonera* (Asteraceae) taxa from Northeast Turkey. *Phytologia Balcanica, 17*(1), 113-121.

Metcalfe, C. R., & Chalk, L. (1979). Anatomy of dicotyledons. *Clarendon, Oxford.*

Ozcan, M., & Inceer, H. (2022). Stem anatomical survey of the genera *Matricaria* and *Tripleurospermum* (Asteraceae) from Turkey with its taxonomical and ecological implications. *Botanica Serbica, 46*(1), 143-151. DOI: 10.2478/botser-2022-0009

Yıldırımlı, Ş. (2021). A new species, *Crepis vuralii* Yıld. (Asteraceae) from Turkey. *OT Sistematik Botanik Dergisi, 28*(1-2), 149-156.

KSÜ Tarım ve Doğa Derg 27 (4), 773-781, 2024
KSU J. Agric Nat 27 (4), 773-781, 2024
DOI:10.18016/ksutarimdoga.vi.1329172

The Evaluation of psbA-trnH IGS Sequences in The Genus *Potentilla* L. as Barcoding Region

Aykut YILMAZ[1]
[1]Uşak University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Uşak, Türkiye
aykut.yilmaz@usak.edu.tr

ABSTRACT

With more than 400 species, the genus *Potentilla* has a wide distribution causing morphological variations. Besides the wide distribution, many conditions such as hybridization, introgression, autopolyploidy, and allopolyploidy are observed in the genus. These can lead to misidentifications and taxonomic problems. DNA barcoding studies are important for species identification and solving taxonomic problems. However, preferred DNA sequences may have different effects among plant groups. Therefore, which DNA regions should be preferred is important to obtain more comprehensive results. In this research, psbA-trnH IGS sequences belonging to the *Potentilla* taxa were examined based on their compatibility and analyzed variable sites, passim-info sites, and nucleotide frequencies (%) for the region relevant by using Molecular Evolutionary Genetics Analysis (MEGA 11). Finally, a Maximum Parsimony (MP) dendrogram was performed to evaluate the *Potentilla* taxa taxonomically and phylogenetically. As a result of this study, it can be stated that the taxa belonging to the genus *Potentilla* were well grouped in the dendrogram and the use of psbA-trnH IGS sequences is strongly recommended for further studies.

Molecular Biology

Research Article

Article History
Received : 18.07.2023
Accepted : 03.12.2023

Keywords
Potentilla
DNA barcoding
psbA-trnH IGS
MEGA 11
Dendrogram

Barkodlama Bölgesi Olarak *Potentilla* L. Cinsinde psbA-trnH IGS Sekanslarının Değerlendirmesi

ÖZET

Potentilla cinsi 400'den fazla türle, morfolojik değişimlere neden olan geniş bir yayılışa sahiptir. Geniş yayılışın yanı sıra, cinsteki hibridizasyon, introgression, otopoliploidi ve allopoliploidi gibi birçok durum gözlemlenir. Bunlar, yanlış tanımlamalara ve taksonomik problemlere yol açabilir. DNA barkodlama çalışmaları, tür tanımlama ve taksonomik problemlerin çözümünde önemlidir. Ancak, tercih edilen DNA sekansları, bitki grupları arasında farklı etkilere sahip olabilir. Bu nedenle, hangi DNA bölgelerinin tercih edilmesi gerektiği daha kapsamlı sonuçlar elde etmek için önemlidir. Bu çalışmada, *Potentilla* taksonlarına ait psbA-trnH IGS sekansları uyumlulukları açısından incelendi ve variable bölgeler, parsim-info bölgeler ve ilgili bölge için nükleotit frekansları (%) Molecular Evolutionary Genetics Analysis (MEGA 11) kullanılarak analiz edildi. Son olarak, Maximum Parsimony (MP) dendrogram taksonomik ve filogenetik olarak *Potentilla* taksonlarını değerlendirmek için oluşturuldu. Çalışmanın sonucu olarak *Potentilla* cinsine ait taksonların dendrogramda iyi gruplandığı ve psbA-trnH IGS sekanslarının daha sonraki çalışmalarda kullanımının fayda sağlayacağı söylenebilir.

Moleküler Biyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 18.07.2023
Kabul Tarihi : 03.12.2023

Anahtar Kelimeler
Potentilla
DNA barkodlama
psbA-trnH IGS
MEGA 11
Dendrogram

Atıf İçin : Yılmaz, A., (2024). Barkodlama Bölgesi Olarak *Potentilla* L. Cinsinde psbA-trnH IGS Sekanslarının Değerlendirmesi. *KSÜ Tarım ve Doğa Derg 27* (4), 773-781. DOI: 10.18016/ksutarimdoga.vi.1329172.

To Cite: Yılmaz, A., (2024). The Evaluation of psbA-trnH IGS Sequences in The Genus Potentilla L. as Barcoding Region. *KSU J. Agric Nat 27* (4), 773-781. DOI: 10.18016/ksutarimdoga.vi.1329172.

INTRODUCTION

The genus *Potentilla* L. (Rosaceae) comprises more than 400 species with a wide distribution area in the world (Sojak, 2008; Sojak, 2009; Persson et al., 2020a; Yılmaz, 2023a,b). Especially temperate zones of the northern hemisphere and its boreal parts are the regions where the genus *Potentilla* has the highest species number and diversity (Bean, 2015; Yılmaz, 2023a).

There are still many problems in the evaluation of the genus *Potentilla* taxonomically and aspect of its species identification concept. In other words, species

names, and their positions as taxonomically and phylogenetic relationships based on variations determined are controversial for the genus and continue the discussions based on the many genera circumstances within the family Rosaceae by the taxonomists (Lundberg et al., 2009; Dobes & Paule, 2010; Eriksson et al., 2022). Besides the changes in species names, many taxa have been transferred to other genera or described within different genera every day. Especially, the relationships between the genus *Potentilla* and *Sibbaldia* L. based on the identification of some species, in addition to their taxonomic positions of taxa evaluated within *Potentilla* or *Sibbaldia* are highly variable and complex (Lundberg et al., 2009; Eriksson et al., 2015). Similar relationships are observed between the genus *Potentilla* and *Argentina* Hill. in the studies by Sojak (2010) and Feng et al. (2014).

Essentially, the mistakes in the definition and taxonomic status of species are the most important reasons for making problematic and hard to understand the genus. Although morphological characters are very useful and important in the species description and even determination of variations, it is inadequate in plant groups such as *Potentilla* that have species showing high variations. Polyploidy is a common observed and well-known phenomenon for *Potentilla* species. Furthermore, this process is very significant to understanding the speciation and later in the evolution of the *Potentilla* taxa. It is stated that many species evolved by hybridization and polyploidization (Töpel et al., 2011; Persson et al., 2020b). In other words, it can be stated that both hybridization and polyploidization are important processes in the evolution of *Potentilla* taxa (Potter et al., 2007; Paule et al., 2011, 2012; Persson et al., 2020a). As a result, many *Potentilla* taxa have variable chromosome numbers from diploid to hexadecaploid (16x) (Kalkman, 2004). Besides the *Potentilla* taxa with variable chromosome numbers, it is observed that Potentilla species are exhibiting different chromosome numbers in different locations (IPCN; Kechaykin et al., 2016; Schanzer et al., 2020).

Another case increasing the complexity of *Potentilla* taxa in the aspect of taxonomics and phylogenetics is species diversity and their distributions. The genus *Potentilla* is one of the biggest plant groups evaluated within the family Rosaceae according to the species number and distribution. In other words, the genus is represented by the habitats showing distribution in many parts of the world. All of these increase hybridization, polyploidy, and the presence of taxa that exhibit intermediate morphological characters between parent plants in the genus, as a result of introgression and genetic drift.

The inadequacy of morphological characters caused by all these stated cases makes it necessary to determine the new characters. Determination of molecular characters and their use for the description of problematic taxa in the aspect of specified features especially gives very important results to overcome such difficulties besides a more detailed evaluation of the genus as phylogenetically. Especially, molecular studies based on DNA sequence information of nuclear or chloroplast genomes have been frequently used in different plant groups. However, species identification and separation ability of the barcoding region preferred may exhibit variability in different plant groups. For this reason, the using separately of different DNA sequences containing gene and spacer regions with as many taxa as possible provides important information about which DNA sequences must be preferred and which region combinations are more useful for analysis. One of these which is been the most important and frequently preferred for efficient identification is a psbA-trnH region.

In this study, psbA-trnH intergenic spacer (IGS) sequence information acquired from the National Center for Biotechnology Information (NCBI) was examined for 77 *Potentilla* taxa

i. to understand the species identification and discrimination abilities
ii. to evaluate phylogenetic relationships of *Potentilla* taxa based on the region preferred
iii. to make a comparison with previous studies
iv. finally to make recommendations about the DNA barcoding region preferred for further studies.

MATERIALS and METHODS

The sequence information of the psbA-trnH region for all *Potentilla* taxa was first acquired from the NCBI database. After all, data belonging to the psbA-trnH sequence which are shared by different researchers were collected, and it was observed the regions for sequences related such as psbA gene/psbA-trnH IGS/trnH gene, psbA-trnH IGS and psbA gene/psbA-trnH IGS. Finally, the psbA-trnH IGS sequences were extracted from these regions to make the most accurate analysis using as many taxa as possible and thus to reveal the discrimination ability of the region examined more clearly. Although the sequence lengths of many *Potentilla* taxa for the region examined are compatible, it was observed the presence of incompatibility in some taxa uploaded to NCBI by different researchers. Essentially, it is commonly observed that situations do not match the sequences in the aspect of their lengths in many plant groups (Yılmaz & Yeltekin, 2022). Therefore, the sequences for each *Potentilla* taxon were analyzed based on their compatibility, and compatible sequences were mostly preferred for analysis (Appendix). Moreover, some *Potentilla* taxa whose sequences were uploaded by different researchers were represented by a few

samples in the phylogenetic tree to show the accuracy of the data in NCBI. In total, 77 *Potentilla* taxa belonging to 91 samples for the psbA-trnH IGS region were investigated to evaluate the phylogenetic relationships and to understand the species identification ability of the region preferred.

The analysis for the determination of variable sites, passim-info sites, base substitutions, and nucleotide frequencies, besides the phylogenetic tree, was performed by using Molecular Evolutionary Genetics Analysis (MEGA 11) (Tamura et al., 2021). Firstly, all sequences for the *Potentilla* taxa were aligned and then variable sites and parsim-info sites were computed. Base substitution probabilities were determined and shown in Table 1. Furthermore, transitional and transversional base substitutions (%), in addition to transition/transversion ratios for purines and pyrimidines were determined and shown in Table 2. Nucleotide frequencies % as A+T/U and G+C of psbA-trnH IGS sequences belonging to *Potentilla* taxa were also computed.

Finally, the Maximum Parsimony (MP) dendrogram that bootstrap values are reported on branches with the option of hide values lower than 50% was provided to evaluate the genus *Potentilla* as taxonomically and phylogenetically, besides species identification and separation abilities of the region examined for *Potentilla* taxa. The positions with gaps and missing data appearing as a result of multiple sequence alignments were eliminated with the complete deletion option of the program for more effective analyses, so it is aimed to provide more meaningful and comprehensive results.

RESULTS and DISCUSSION

psbA-trnH IGS sequences belonging to the *Potentilla* taxa were extracted from psbA/trnH gene regions provided by the NCBI database. In this study, a total of 91 samples representing 77 *Potentilla* taxa were analyzed in the aspect of species identification and separation ability of psbA-trnH IGS sequences. Some taxa were represented by a few samples and these taxa were preferred from the data set loaded by different times and researchers to evaluate the sequence compatibilities and discrepancies in the NCBI database. For this aim, firstly all psbA-trnH IGS sequences of *Potentilla* taxa were aligned and then variable and parsimony informative nucleotides were determined. Variable sites and parsimony informative sites were observed in 344 and 115 nucleotides, respectively. In addition to the determination of variable and parsimony informative nucleotides of *Potentilla* taxa for the psbA-trnH IGS region, the probabilities of base substitution were computed. The highest base substitution was observed at the rate of 25.4% from C to T. After that, the second highest base substitution was observed at the rate of 14.67% from G to A (Table 1). Moreover, the rates (%) of transitional and transversional base substitutions were computed as 53.31% and 46.69%, respectively, by using the substitution rate from one base to another base from Table 1.

Table 1. The probability of substitution (r) from one base (row) to another base (column) for psbA-trnH IGS sequences (Transitional substitutions are shown in bold)

Çizelge 1. psbA-trnH IGS sekansları için bir bazdan diğerine değişim olasılıkları (Transisyonel baz değişimleri koyu renkli gösterilir)

	A	T	C	G
A	-	9.01	1.97	**7.69**
T	8.11	-	**5.55**	4.25
C	8.11	**25.4**	-	4.25
G	**14.67**	9.01	1.97	-

The transition/transversion rate was determined as 1.80 for purines (k_1), and 2.81 for pyrimidines (k_2), besides the overall transition/transversion rate (R=0.82). Finally, nucleotide frequencies for psbA-trnH IGS sequences belonging to the *Potentilla* taxa were analyzed as 73.34% (A+T/U) and 26.66% (G+C). As a result, it can be stated that psbA-trnH IGS sequences for *Potentilla* taxa consist of A and T/U bases at high levels.

All information such as alignment length, variable site, passim-info site, transitional substitutions, transversional substitutions, transition/transversion rates, and nucleotide frequencies were shown in Table 2.

Table 2. The information of taxa examined based on psbA-trnH IGS sequences

Çizelge 2. psbA-trnH IGS sekansları temelinde incelenen taksonların bilgisi

Taxon	Alignment length (bp)	Variable site	Parsim-info site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion rate Purines (k_1)	Pyrimidines (k_2)	Overall (R)	Nucleotide freq. (%) A+T/U	G+C
77	802	344	115	53.31	46.69	1.80	2.81	0.82	73.34	26.66

MP dendrogram was drawn to show the phylogenetic relationships of the taxa examined and to determine the species identification ability for the *Potentilla* taxa of sequences (Figure 1).

Many researchers in their studies based on phylogenetic relationships of the *Potentilla* taxa state

the presence of six major clades (Anserina, Argentea, Alba, Fragarioides, Ivesioid, and Reptans) in the genus (Dobes & Paule, 2010; Töpel et al., 2011; Feng et al. 2017). The dendrogram separated the taxa into seven groups based on psbA-trnH IGS sequences. The taxa resolved in Group I (*P. caulescens*, *P. nitida*, *P. Biflora*and *P. alba*) are evaluated in the Alba clade. In other words, taxa belonging to the Alba clade were grouped and formed a distinct group in the phylogenetic tree. Similarly, it was observed that the taxa evaluated in the Reptans clade (*P. indica*, *P. erecta,* and *P. reptans*) were clustered together in Group II.

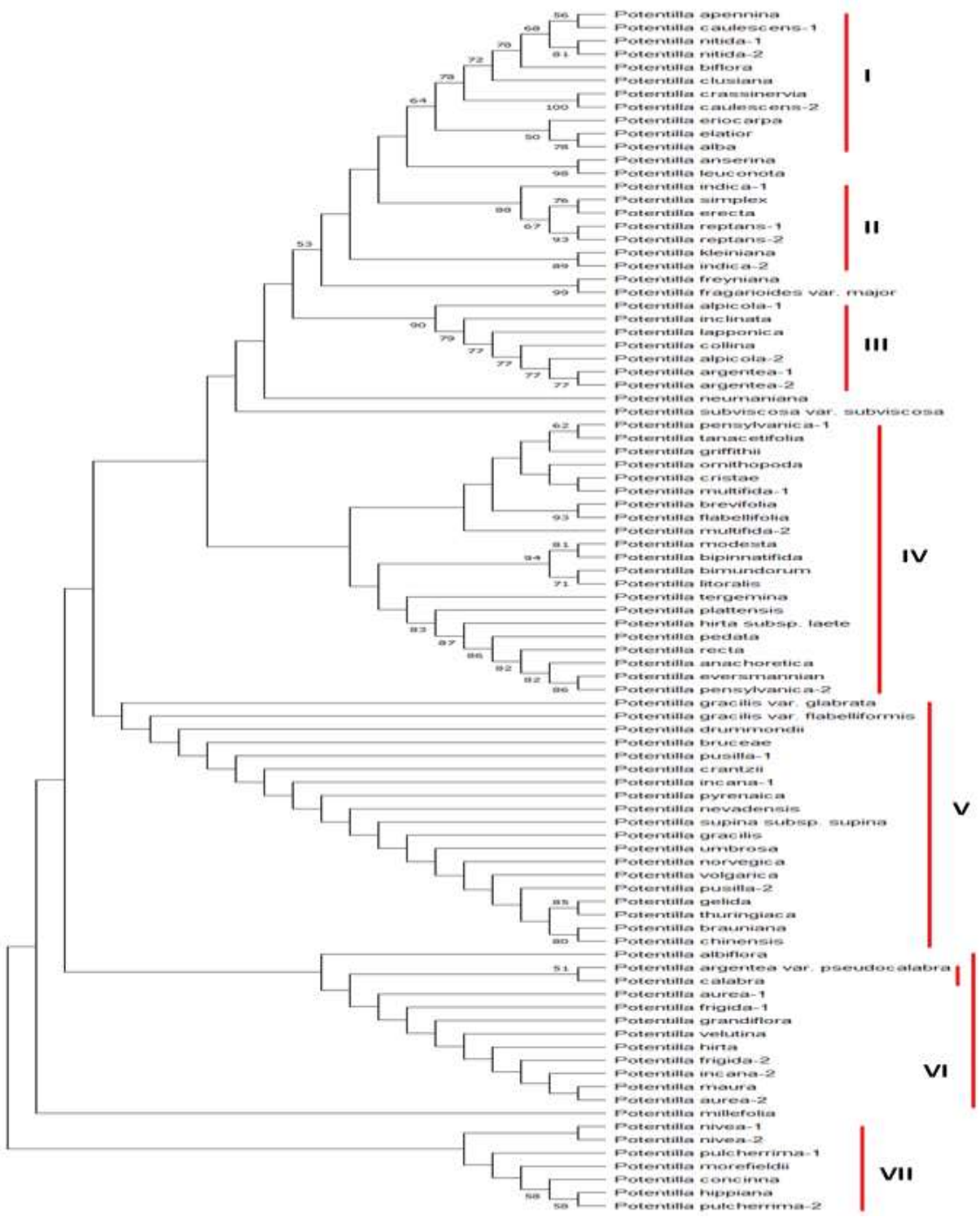


Figure1. MP tree provided from psbA-trnH IGS sequences of *Potentilla* taxa
Şekil 1. Potentilla taksonlarının psbA-trnH IGS sekanslarından elde edilen MP ağacı

The taxa belonging to the Argentea clade were represented by the highest species number in the MP dendrogram and clustered in other groups. Furthermore, the taxa evaluated in the Argentea clade formed the outmost groups in the dendrogram. Paule et al. (2012) state in their study based on implications of hybridization and cytotypic differentiation in the speciation of *P. alpicola* that "Populations of *P. collina* have been regarded rather as recent derivatives of the hexaploid *P. argentea*. The observation of clones within both *P. alpicola* and *P. collina* suggested a possible apomictic mode of reproduction". Moreover, the case study of *P. alpicola* made by Paule et al. (2012) shows that processes such as apomixis play a significant role in the creation of polymorphism in the genus *Potentilla*. In this study, when the relationships among *P. alpicola*, *P. collina,* and *P. argentea* were investigated, it was observed that the five samples of these taxa were clustered together in Group III and separated from other taxa belonging to the Argentea clade. In other words, it can be stated that these three taxa are closely related to each other phylogenetically.

P. argentea var. *pseudocalabra* was evaluated as an intermediate between hexaploid *P. argentea* and *P. calabra* by Paule et al. (2011). Here, *P. calabra* and *P. argentea* var. *pseudocalabra* were clustered very closely in Group VI and showed similarity phylogenetically based on psbA-trnH IGS sequences.

Especially, molecular studies based on DNA sequence information of nuclear or chloroplast genomes have been frequently used in different plant groups. Furthermore, combined analysis containing two or more genes and spacer regions has been used by many researchers to provide better phylogenetic resolution and identification in plant groups. However, the species identification and discrimination capabilities of DNA sequences preferred in the relevant plant group should be known to obtain maximum benefit from the DNA region used and then to use the right region combinations, because the DNA regions preferred are not equally effective in different plant groups.

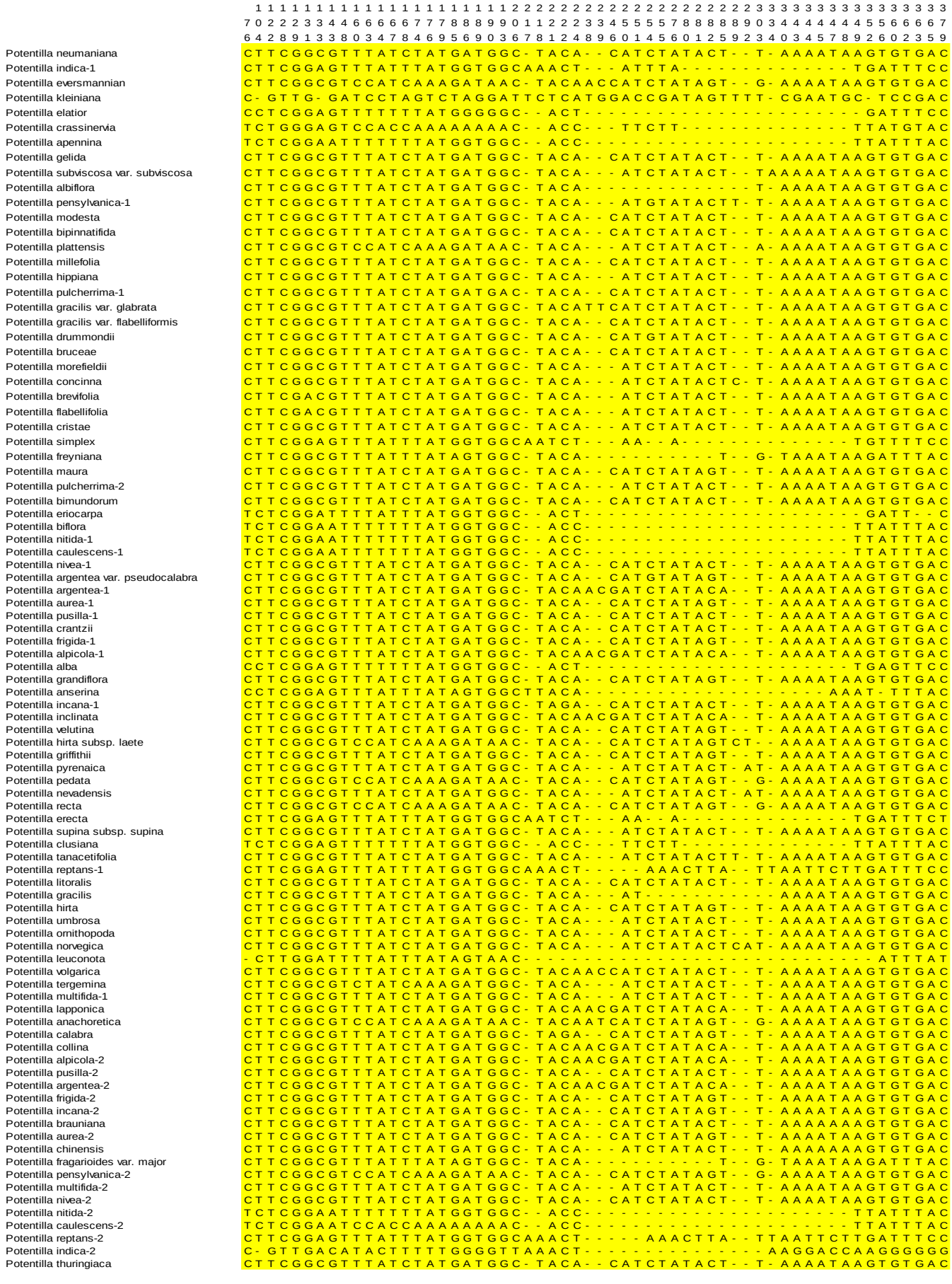
Santos and Pereira (2018) state in their study that cpDNA sequences are very important in species identification and phylogenetic analysis in plants, in addition to the importance of the region and region combinations. Furthermore, they used the SPInDel (Species Identification by Insertions/Deletions) approach to achieve better identification of plant species by using the combination of variable length sequences in cpDNA. As a result, when considered alone, the regions having low discrimination ability increased the separation ability with right region combinations. Similarly, Gontcharov et al. (2004) state that combined analysis is better than single-gene analysis in the aspect of phylogenetic resolution and is supported by morphological information.

In this concept, the studies based on species identification abilities of different DNA sequences have high importance to get better results in the future with the right region combinations. It is stated in the study on the importance of trnL/trnF IGS region in the taxonomy of the genus *Potentilla* L. by Yılmaz (2023a) that the region preferred has high variable sites and grouping ability, in addition to strongly recommended for further studies. Similarly, both ITS1 and ITS2 sequences between rDNA genes in the study made by Yılmaz (2023b) are strongly recommended in the phylogenetic evaluation of *Potentilla* taxa, besides their determination of contribution levels. However, rbcL sequences were insufficient in the identification and grouping of some *Potentilla* taxa according to the phylogenetic tree (Yılmaz, 2023c). In other words, it can be stated that rbcL sequences in the comparison of other DNA regions provide less information in the solving of taxonomic problems and analysis of phylogenetic relationships.

Here, psbA-trnH IGS sequences were examined in the aspect of species identification and separation abilities for *Potentilla* taxa, besides phylogenetic evaluation.

Loera-Sanchez et al. (2020) state in their study based on the identification of forage legumes and grasses using trnH-psbA sequences that the barcoding region examined is a promising candidate for efficient identification and plant species richness assessments. However, Yılmaz (2021) states in the study based on comparisons of nuclear and chloroplast DNA regions in the aspect of their species identification abilities for *Crocus* L. taxa that the DNA region belonging to partial psbA gene-psbA/trnH IGS-partial trnH gene have not enough sequence variations (30 nucleotides) for the identification of species, although it phylogenetically separated some *Crocus* taxa.

As a result of this study, it can be stated that the psbA-trnH IGS region separated the many *Potentilla* taxa from each other and grouped them based on the clades according to the phylogenetic tree. However, it was insufficient in the complete solution of still existing problems and the discrimination of all taxa phylogenetically. The determinations of variable and parsimony informative nucleotides give very important information based on the phylogenetic and taxonomic relationships for plant groups analyzed, besides the species identification ability of the region examined. Furthermore, the inconsistency between both of them may show the sequence incompatibility for the taxa analyzed. In this study, 344 variable sequences and 115 parsimony informative sites were determined for psbA-trnH IGS sequences of *Potentilla* taxa. When the DNA sequences of each taxa were examined, it was observed the variability in the nucleotide sequences and sequence lengths of some taxa. This is the main reason for the presence of a high


Potentilla neumaniana
Potentilla indica-1
Potentilla eversmannian
Potentilla kleiniana
Potentilla elatior
Potentilla crassinervia
Potentilla apennina
Potentilla gelida
Potentilla subviscosa var. subviscosa
Potentilla albiflora
Potentilla pensylvanica-1
Potentilla modesta
Potentilla bipinnatifida
Potentilla plattensis
Potentilla millefolia
Potentilla hippiana
Potentilla pulcherrima-1
Potentilla gracilis var. glabrata
Potentilla gracilis var. flabelliformis
Potentilla drummondii
Potentilla bruceae
Potentilla morefieldii
Potentilla concinna
Potentilla brevifolia
Potentilla flabellifolia
Potentilla cristae
Potentilla simplex
Potentilla freyniana
Potentilla maura
Potentilla pulcherrima-2
Potentilla bimundorum
Potentilla eriocarpa
Potentilla biflora
Potentilla nitida-1
Potentilla caulescens-1
Potentilla nivea-1
Potentilla argentea var. pseudocalabra
Potentilla argentea-1
Potentilla aurea-1
Potentilla pusilla-1
Potentilla crantzii
Potentilla frigida-1
Potentilla alpicola-1
Potentilla alba
Potentilla grandiflora
Potentilla anserina
Potentilla incana-1
Potentilla inclinata
Potentilla velutina
Potentilla hirta subsp. laete
Potentilla griffithii
Potentilla pyrenaica
Potentilla pedata
Potentilla nevadensis
Potentilla recta
Potentilla erecta
Potentilla supina subsp. supina
Potentilla clusiana
Potentilla tanacetifolia
Potentilla reptans-1
Potentilla litoralis
Potentilla gracilis
Potentilla hirta
Potentilla umbrosa
Potentilla ornithopoda
Potentilla norvegica
Potentilla leuconota
Potentilla volgarica
Potentilla tergemina
Potentilla multifida-1
Potentilla lapponica
Potentilla anachoretica
Potentilla calabra
Potentilla collina
Potentilla alpicola-2
Potentilla pusilla-2
Potentilla argentea-2
Potentilla frigida-2
Potentilla incana-2
Potentilla brauniana
Potentilla aurea-2
Potentilla chinensis
Potentilla fragarioides var. major
Potentilla pensylvanica-2
Potentilla multifida-2
Potentilla nivea-2
Potentilla nitida-2
Potentilla caulescens-2
Potentilla reptans-2
Potentilla indica-2
Potentilla thuringiaca

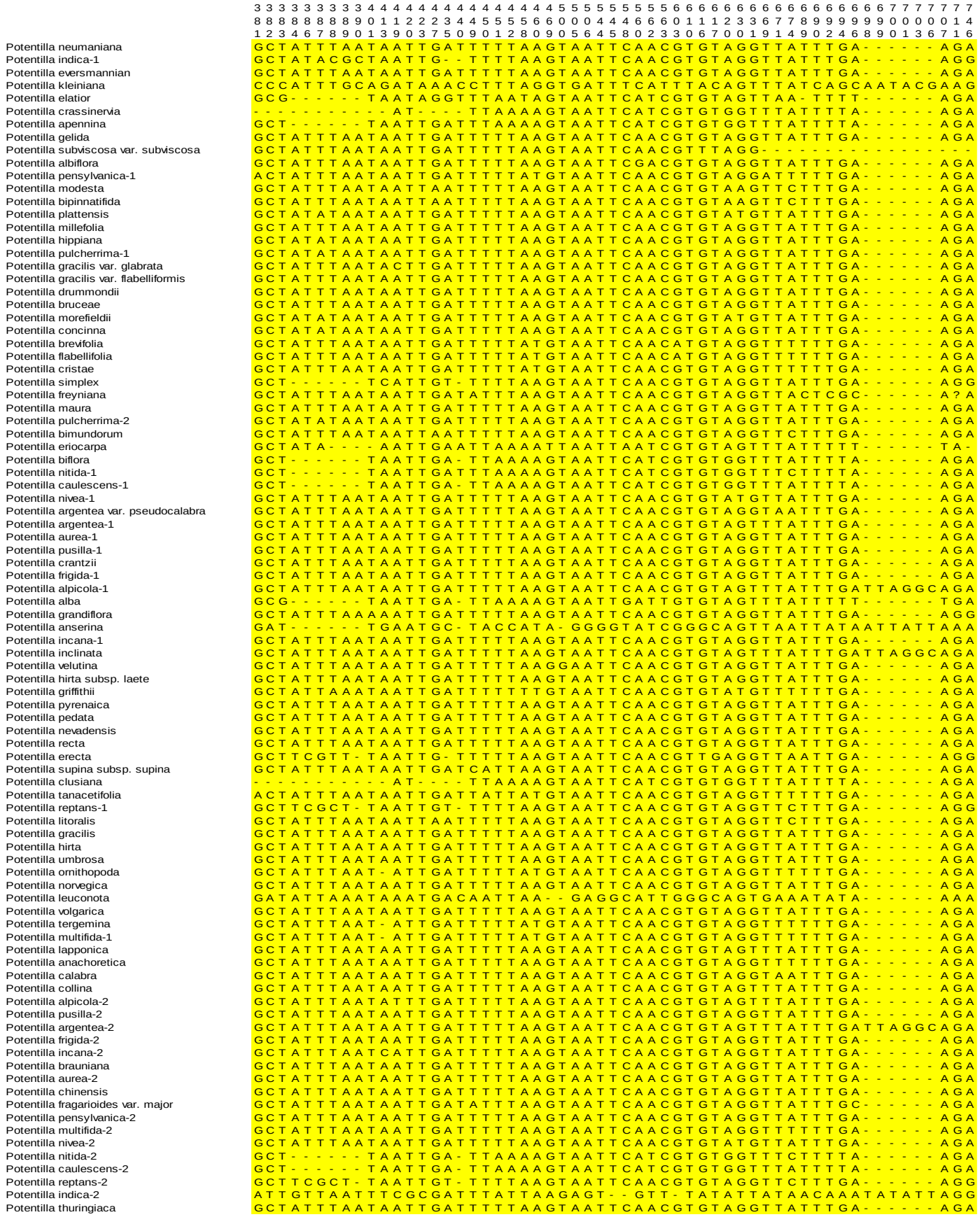


Figure2. *Potentilla* taxa and Parsimony informative sites belonging to psbA-trnH IGS sequences (The numbers show variable nucleotides)

Şekil 2. Potentilla taksonları ve psbA-trnH IGS sekanslarına ait Parsimony informative bölgeler (Sayılar değişken nükleotitleri gösterir)

level of the variable nucleotide number. In other words, most of the sequence variations between *Potentilla* taxa examined are caused by the change of only a nucleotide. Parsimony informative sites observed in 115 nucleotides show the accuracy of this (Figure 2).

CONCLUSION

Although morphological characters are very important tools in the identification and classification of species, many times they can be also reasons for misclassifications in some plant groups because of some situations such as wide geographical distribution, geomorphological structure and climatic changes, hybridization, introgression, autopolyploidy, and allopolyploidy. The genus *Potentilla* with species over 400 exhibits all situations that may cause changes in morphological characters and thus misidentification. In other words, the discrepancies observed in the MP dendrogram can be caused by situations such as misidentifications of the taxa, missing data caused by sequencing, or labeling errors in accessions. Although combined analysis containing different DNA sequences is very important in the solving of problems stated, it is necessary to have knowledge about which DNA regions are more useful and then, which region combinations will provide more advantage.

Finally, it can be stated that the taxa belonging to the genus *Potentilla* were well grouped in the dendrogram and the using of psbA-trnH IGS sequences is strongly recommended for further studies with the combination of the regions with the ability to reveal the phylogenetic relationships.

ACKNOWLEDGMENTS

The author would like to thank NCBI for the sequence information used in this study and for the authors for sharing the sequence information in NCBI.

Author's Contributions [Century12 bold]

The contribution of the author is 100 %.

Statement of Conflict of Interest

The author has declared no conflict of interest.

REFERENCES

Bean, A. R. (2015). Notes on Potentilla (Rosaceae) and related genera in Australia. *Muelleria, 33*, 75-83.

Dobes, C., & Paule, J. (2010). A comprehensive chloroplast DNA-based phylogeny of the genus Potentilla (Rosaceae): Implications for its geographic origin, phylogeography and generic circumscription. *Molecular Phylogenetics and Evolution, 56*(1), 156-175. DOI: 10.1016/j.ympev. 2010.03.031

Eriksson, T., Lundberg, M., Töpel, M., Östensson, P., & Smedmark, J. E. E. (2015). Sibbaldia: a molecular phylogenetic study of a remarkably polyphyletic genus in Rosaceae. *Plant Systematics and Evolution, 301*(1), 171-184. DOI: 10.1007/s00606-014-1063-7

Eriksson, T., Persson, N. L., & Smedmark, J. E. E. (2022). What is Potentilla? A phylogeny-based taxonomy for Potentillinae (Rosaceae). *Taxon, 71*(3), 493-505. DOI: 10.1002/tax.12822

Feng, T., Moore, M. J., Sun, Y., Meng, A., Chu, H., Li, J., & Wang, H. (2014). A new species of Argentina (Rosaceae, Potentilleae) from Southeast Tibet, concerning the taxonomic status of the genus. *Plant Syst Evol, 301*(3), 911-921. DOI: 10.1007/s00606-014-1063-7

Feng, T., Moore, M. J., Yan, M. H., Sun, Y. X., Zhang, H. J., Meng, A. P., Li, X. D., Jian, S. G., Li, J. Q., & Wang, H. C. (2017). Phylogenetic study of the tribe Potentilla (Rosaceae), with further insight into the disintegration of Sibbaldia. *Journal of Systematics and Evolution, 55*(3), 177-191. DOI: 10.1111/jse.12233

Gontcharov, A. A., Marin, B., & Melkonian, M. (2004). Are Combined Analyses Better Than Single Gene Phylogenies? A Case Study Using SSU rDNA and rbcL Sequence Comparisons in the Zygnematophyceae (Streptophyta). *Molecular Biology and Evolution, 21*(3), 612-624. DOI: 10.1093/molbev/msh049

IPCN. Index to Plant Chromosome Numbers. (1979). In IPCN Database: Goldblatt, P., Johnson, D. E., eds. St. Louis, MO: Missouri Botanical Garden: USA, Available online: www.tropicos.org/project/ipcn (accessed on 21 June 2023).

Kalkman, C. (2004). Potentilla. In: Kubitzki K, ed. Flowering plants-Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales. *Berlin: Springer*, 366.

Kechaykin, A. A., Skaptsov, M. V., Smirnov, S. V., Kutsev, M. G., & Shmakov, A. I. (2016). Study of genome size representatives of the genus Potentilla L. (Rosaceae Juss.). *Biol. Bull. Bogdan Chmelnitskiy Melitopol State Pedagog. Univ., 6*, 229-233.

Loera-Sanchez, M., Studer, B., & Kölliker, R. (2020). DNA barcode trnH psbA is a promising candidate for the efficient identification of forage legumes and grasses. *BMC Research Notes, 13*, 35. DOI: 10.1186/s13104-020-4891-y

Lundberg, M., Töpel, M., Eriksen, B., Nylander, J. A., & Eriksson, T. (2009). Allopolyploidy in Fragariinae (Rosaceae): Comparing four DNA sequence regions, with comments on classification. *Molecular Phylogenetics and Evolution, 51*(2), 269-280. DOI: 10.1016/j.ympev.2008.12.003

NCBI, National Centre of Biotechnology Information,

https://www.ncbi.nlm.nih.gov/genbank.

Paule, J., Sharbel, T. F., & Dobeš, C. (2011). Apomictic and sexual lineages of the Potentilla argentea L. group (Rosaceae): cytotype and molecular genetic differentiation. *Taxon, 60*, 721-732.

Paule, J., Scherbantin, A., & Dobeš, C. (2012). Implications of hybridization and cytotypic differentiation in speciation assessed by AFLP and plastid haplotypes–a case study of Potentilla alpicola La Soie. *BMC Evolutionary Biology, 12*, 132. DOI: 10.1186/1471-2148-12-132

Persson, N. L., Toresen, I., Andersen, H. L., Smedmark, J. E. E., & Eriksson, T. (2020a). Detecting destabilizing species in the phylogenetic backbone of Potentilla (Rosaceae) using low-copy nuclear markers. *Annals of Botany Plants, 12*(3), plaa017. DOI: 10.1093/aobpla/plaa017

Persson, N. L., Eriksson, T., & Smedmark, J. E. E. (2020b). Complex patterns of reticulate evolution in opportunistic weeds (Potentilla L., Rosaceae), as revealed by low-copy nuclear markers. *BMC Evolutionary Biology, 20*, 38. DOI: 10.1186/s12862-020-01614-w

Potter, D., Eriksson, T., Evans, R. C., Oh, S., Smedmark, J. E. E., Morgan, D. R., Kerr, M., Robertson, K. R., Arsenault, M., Dickinson, T. A., & Campbell, C. S. (2007). Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution, 266*(1-2), 5-43. DOI: 10.1007/s00606-006-0510-2

Santos, C., & Pereira, F. (2018). Identification of plant species using variable length chloroplast DNA sequences. *Forensic Science International: Genetics, 36*, 1-12. DOI: 10.1016/j.fsigen.2018.04.013

Schanzer, I. A., Fedorova, A. V., Shelepova, O. V., & Suleymanova, G. F. (2020). Molecular Phylogeny and Phylogeography of Potentilla multifida L. agg. (Rosaceae) in Northern Eurasia with a Special Focus on Two Rare and Critically Endangered Endemic Species, P. volgarica and P. eversmanniana. *Plants, 9*, 1798. DOI: 10.3390/plants9121798

Soják, J. (2008). Notes on Potentilla XXI. A new division of the tribe Potentilla (Rosaceae) and notes on generic delimitations. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie, 127*(3), 349-358. DOI: 10.1127/0006-8152/2008/0127-0349

Sojak, J. (2009). Potentilla (Rosaceae) in the former USSR; second part: comments. Notes on Potentilla XXIV. *Feddes Repertorium, 120*, 185-217.

Sojak, J. (2010). Argentina Hill., a genus distinct from Potentilla (Rosaceae). *Thaiszia-J. Bot., 20*, 91-97.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027. DOI: 10.1093/molbev/msab121

Töpel, M., Lundberg, M., Eriksson, T., & Eriksen, B. (2011). Molecular data and ploidal levels indicate several putative allopolyploidization events in the genus Potentilla (Rosaceae). *PLoS Currents, 3*, RRN1237. DOI: 10.1371/currents.RRN1237

Yılmaz, A., & Yeltekin, Y. (2022). The Evaluations Of Taxonomic Classifications In The Genus Trifolium L. Based On ITS Sequences. *Sakarya University Journal of Science, 26*(3), 545-553. DOI: 10.16984/saufenbilder.971792

Yılmaz, A. (2021). The Evaluations and Comparisons of Nuclear and Chloroplast DNA Regions Based on Species Identification and Phylogenetic Relationships of Crocus L. Taxa. *Journal of the Institute of Science and Technology, 11*(2), 1504-1518.

Yılmaz, A. (2023a). The importance of trnL/trnF IGS region in the taxonomy of the genus Potentilla L. *Trakya Univ J Nat Sci, 24*(1), 71-76. DOI: 10.33483/trkjnat.101080

Yılmaz, A. (2023b). The importance of ITS1-ITS2 sequences in the phylogenetic evaluation of Potentilla L. taxa and comparison of these sequences as noncoding regions. *International Studies in Health Sciences.* DOI: 10.1016/j.hj.2022.03.005

Yılmaz, A. (2023c). Phylogenetic relationships of Potentilla L. taxa based on rbcL sequences. *Scope and Importance of Agricultural Studies.* DOI: 10.1016/j.hj.2022.03.005.

APPENDIX

GQ384973, LC632299, MN871351, JF708223, GQ385036, GQ385035, GQ385033, GQ385029, GQ385027, GQ385025, GQ385023, GQ385022, GQ385021, GQ385020, GQ385017, GQ385016, GQ385014, GQ385013, GQ385012, GQ385010, GQ385009, GQ385008, GQ385007, GQ385006, GQ385005, GQ385004, GQ384999, GQ384998, GQ384997, GQ384995, GQ384993, GQ384992, GQ384991, GQ384990, GQ384989, GQ384988, GQ384987, GQ384986, GQ384984, GQ384983, GQ384982, GQ384981, GQ384980, GQ384976, GQ384975, GQ384974, GQ384972, GQ384970, GQ384969, GQ384968, GQ384964, GQ384963, GQ384962, GQ384961, GQ384960, GQ384959, GQ384958, GQ384957, GQ384956, GQ384955, GQ384954, GQ384952, GQ384951, GQ384950, GQ384948, GQ384947, GQ384946, MN871395, MN871365, MN871363, MN871352, MN871335, HM776571, JX276877, JX276876, JX276873, JX276869, JX276827, JX276825, JX276784, JX276782, OQ161262, LC703124, MF543685, MF543674, DQ778819, HG800560, HG800559, HE966756, HE966755, HQ433307

KSÜ Tarım ve Doğa Derg 27 (4), 782-792, 2024
KSU J. Agric Nat 27 (4), 782-792, 2024
DOI:10.18016/ksutarimdoga.vi.1339345

Exploring the Potential of *Psephellus huber-Marathi* (Wagenitz) Wagenitz: A Comprehensive UHPLC-MS/MS Analysis of Phytochemical Composition and Evaluation of Antioxidant, Antimicrobial, and Antiproliferative Activities

Tugrul Cagri AKMAN[1]✍, Samed SİMSEK[2], Zeynep AKSİT[3], Ali AYDİN[4], Mustafa Abdullah YİLMAZ[5]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yildirim University, 25240, Erzincan, Türkiye, [2]Department of Medical Services and Techniques, Çayırlı Vocational School, Erzincan Binali Yıldırım University, 24500, Erzincan, Türkiye, [3]Department of Food Engineering, Erzincan Binali Yildirim University, 25240, Erzincan, Türkiye, [4]Department of Basic Medical Science, Faculty of Medicine Bozok University, 66900, Yozgat, Türkiye, [5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakır, Türkiye

[1]https://orcid.org/0000-0002-5439-950X, [2]https://orcid.org/0000-0001-8451-3425, [3]https://orcid.org/0000-0002-0349-0223
[4]https://orcid.org/0000-0002-9550-9111, [5]https://orcid.org/0000-0002-4090-7227
✉: eczcagri87@gmail.com

ABSTRACT

Psephellus huber-morathii (PH) is an endemic species in the Eastern Anatolia Region. In this study, besides the biological activities of the 70% methanol extract of PH, its comprehensive phytochemical composition was investigated. The phenolic composition of the PH extract was analyzed using the UHPLC-MS/MS method. To evaluate its antimicrobial characteristics, the microdilution technique was employed. The antioxidant capabilities and total phenolic and flavonoid contents of the extract were determined using the spectrophotometer. Additionally, the effects of the extracts on cell proliferation and NCI-60 survival parameter values were assessed using the MTT assay. Quinic acid and chlorogenic acid were major compounds. The extract showed high antioxidant activity in DPPH (IC_{50}:13.9±0.4 µg mL^{-1}) and FRAP (61.3±2.3 mg TE g $extract^{-1}$) methods. The total phenolic and flavonoid contents of the extract were found as 52.2±1.9 mg GAE g $extract^{-1}$ and 28.6±0.9 mg QE g $extract^{-1}$, respectively. Discussion: The extract showed low antiproliferative activity against normal lung and retinal cell lines, promising anticancer effects on gynecological and colon cancer cells, and moderate antimicrobial activity against *Bacillus cereus* and *Enterococcus faecium*. The study demonstrated the medicinal potential and value of PH extract against infectious diseases and cancer.

Biochemistry

Research Article

Article History
Received : 08.08.2023
Accepted : 19.12.2023

Keywords
Psephellus huber-Marathi
Antimicrobial
Antioxidant
Antiproliferative
LC-MS/MS

Psephellus huber-Marathi (Wagenitz) Wagenitz'in Potansiyelini Keşfetme: Fitokimyasal Bileşiminin Kapsamlı Bir UHPLC-MS/MS Analizi ve Antioksidan, Antimikrobiyal ve Antiproliferatif Aktivitelerinin Değerlendirmesi

ÖZET

Psephellus huber-morathii (PH), Doğu Anadolu Bölgesi'nde endemik bir türdür. Bu çalışmada PH'nin %70 metanol ekstraktının biyolojik aktivitelerinin yanı sıra kapsamlı fitokimyasal bileşimi de incelenmiştir. PH ekstraktının fenolik bileşimi, UHPLC-MS/MS yöntemi kullanılarak analiz edildi. Antimikrobiyal özelliklerini değerlendirmek için mikrodilüsyon tekniği kullanıldı. Ekstrenin antioksidan özellikleri, toplam fenolik ve flavonoit içerikleri spektrofotometre kullanılarak belirlendi. Ek olarak, ekstraktların hücre proliferasyonu ve NCI-60 sağkalım parametre değerleri üzerindeki etkileri, MTT deneyi kullanılarak değerlendirildi. Kinik asit ve klorojenik asit majör bileşiklerdi. Ekstrakt, DPPH (IC_{50}:13.9±0.4 µg mL^{-1}) ve FRAP (61.3±2.3 mg TE g $ekstrakt^{-1}$) yöntemlerinde yüksek antioksidan aktivite göstermiştir. Ekstraktın toplam fenolik ve flavonoid içerikleri sırasıyla 52.2±1.9 mg GAE g $ekstrakt^{-1}$ ve 28.6±0.9 mg QE g $ekstrakt^{-1}$ olarak bulundu. Ekstrakt, normal akciğer ve retina hücre hatlarına karşı düşük antiproliferatif aktivite, jinokolojik ve kolon kanser hücreleri üzerinde umut verici bir antikanser etki ve

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 08.08.2023
Kabul Tarihi : 19.12.2023

Anahtar Kelimeler
Psephellus huber-morathii
Antimikrobiyal
Antioksidan
Antiproliferatif
LC-MS/MS

Bacillus cereus ve *Enterococcus faecium*'a karşı orta derecede antimikrobiyal aktivite gösterdi. Çalışma, PH ekstraktının bulaşıcı hastalıklara ve kansere karşı tıbbi potansiyelini ve değerini göstermiştir.

Atıf İçin : Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Exploring the potential of Psephellus huber-morathii (Wagenitz) Wagenitz: A comprehensive UHPLC-MS/MS analysis of phytochemical composition and evaluation of antioxidant, antimicrobial, and antiproliferative activities. *KSÜ Tarım ve Doğa Derg 27* (4), 782-792. https://doi.org/10.18016/ksutarimdoga.vi.1339345.

To Cite: Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Exploring the potential of Psephellus huber-morathii (Wagenitz) Wagenitz: A comprehensive UHPLC-MS/MS analysis of phytochemical composition and evaluation of antioxidant, antimicrobial, and antiproliferative activities. *KSU J. Agric Nat 27* (4), 782-792. https://doi.org/10.18016/ksutarimdoga.vi.1339345

INTRODUCTION

Medicinal plants have been used for a long time by many communities as they are rich in natural phytochemicals (phenolic acids, flavonoids, tannins, phenolic diterpenes, terpenoids, vitamins, essential oils, and other biological metabolites). It is crucial to discover the phytochemicals of medicinal plants, to research and assess their therapeutic and bioeconomic potentials, the possible applications in the medical industries, and biological functions. Particularly phenolic derivatives of bioactive chemicals have biological effects that are anti-inflammatory, antibacterial, antimicrobial, and antioxidant. They are also used successfully as a natural medicine and various treatment methods due to their antiproliferative properties that suppress various tumors or prevent a poor prognosis (Ríos & Recio, 2005; Chanda & Nagani, 2013; Tarhan et al., 2022). Türkiye has a unique and diverse botanical heritage with over 11,700 plant species. Numerous species are both endemic and have medicinal uses. Plants show many medicinal properties and biological activities with many important compounds such as alkaloids, sesquiterpene lactones, and flavonoids in their compositions (Güçlü et al., 2022). For this reason, elucidating the phytochemical compositions and biological activities of plants will guide their medicinal uses. One of the significant plant families in Türkiye is Asteraceae, which comprises 190 species, 112 of which are endemic. *Psephellus huber-morsathii* (PH) (*synonym Centaurea huber-morathii*) is a member of the Asteraceae family and an endemic species in Erzincan, Türkiye (Aydin et al., 2013). There is no study in the literature about the traditional use of PH in Türkiye. However, many plants from the Psephellus genus are utilized in traditional medicine in Türkiye. It was reported that plants from the Psephellus genus have antipyretic, hemorrhage, and wound-healing effects (Altundağ et al., 2013; Özcan, 2013) and they are used as antidiabetic, antibacterial, and for stomach disorders (Orallo et al., 1998; Arif et al., 2004; Güven et al., 2005). Also worldwide, Psephellus species are traditionally used for treating diarrhea, dandruff, rheumatism, inflammation, digestive issues, hypertension, fever, infections, and menstrual problems alone or in combination with other herbs (Farrag et al., 1993; Barrero et al., 1997; Orallo et al., 1998; Khammar & Djeddi, 2012).

The pharmacological and medicinal properties of PH are not investigated in detail. There are limited studies in the literature that investigate the biological activities and phytochemical profile of PH. In previous studies, the phytochemical profile of the methanol extract of PH seeds by HPLC (Shoeb et al., 2007), its antibacterial, and antioxidant activity (Sarker, 2005), and its antiproliferative activity against colon cancer (Sarker et al., 2007) were reported. In addition, the essential oil profiles of the seeds were determined (Başer et al., 2006). However, there is only one study examining the total phenolic content (TPC), antioxidant, antimicrobial, antityrosinase, and anticholinesterase activities of the methanol extract of PH aerial parts (flowers, leaves, stem, and roots) (Korkmaz et al., 2019). Several studies reported the phytochemical compositions, and antioxidant, cytotoxic, and anti-inflammatory activities of other *Psephellus* species (Shoeb et al., 2007; Zengin et al., 2010; Polatoglu et al., 2015; Demiroz, Nalbantsoy, Kose, & Baykan, 2020). The phytochemical composition of PH was determined by the high-sensitivity liquid chromatography-mass spectrometry (UHPLC-MS/MS) technique in any study. Also, the total flavonoid content (TFC), the cytotoxic activity, and its efficacy against other types of cancer (brain and gynecological) of PH were not studied. In this study, the phytochemical composition of the hydroalcoholic extract of PH was determined by the UHPLC-MS/MS method. Apart from the antioxidant and antibacterial properties of PH extract, total phenolic, and flavonoid contents, antiproliferative and cytotoxic activities of the plant were evaluated for the first time.

MATERIALS and METHODS

Plant materials

PH was collected during the flowering period from subalpine grasslands on Pöske Mountain in Erzincan Province, Türkiye, on 26 June 2022 (2069 meters above sea level). The plant was dried in dark suitable drying areas at room temperature and the taxonomic descriptions of its materials were provided by

taxonomist Prof. Dr. Ali Kandemir, Erzincan Binali Yildirim University, Faculty of Arts and Sciences, Department of Biology. Authentic samples (ID number: 10862) were stored in the Biology Department Herbarium, Erzincan Binali Yıldırım University.

Preparation of extracts

Air-dried aerial parts of the plant were finely ground using a laboratory mill (5-20 mm). 10 g plant materials were macerated overnight with 500 mL of 70% methanol (the ratio of methanol: water, 70:30, v/v) and filtered. The extraction process was repeated three times. The methanol was evaporated, and the remaining water was lyophilized to give an amorphous solid. The yield of the extraction procedure was 19%. The obtained powder (1.9 g) was stored at +4 °C for LC-MS/MS characterization and antioxidant, antimicrobial, and antiproliferative activity studies.

Mass and chromatographic conditions

A Shimadzu-8040 model ultra-high performance liquid chromatography device (UHPLC) equipped with an autosampler (SIL-30AC), a column oven (CTO-10ASvp), dual pumps (LC-30AD), a degasser (DGU-20A3R) and MS/MS detector (8040) was used for the phytochemical characterization of PH. The phenolic compounds were quantified using a validated UHPLC method. Table 1 displays the chromatographic conditions of the method (Yilmaz, 2020).

Table 1 Chromatographic conditions.
Çizelge 1 Kromatografik şartlar

Parameters	*Conditions*
Column	Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm)
Temperature	40°C
Mobil phase	Mobile phase A: (Water: 5 mM ammonium formate: 0.1% formic acid) Mobile phase B: (Methanol: 5 mM ammonium formate: 0.1% formic acid)
Flow rate	0.5 mL min^{-1}
Injection volume	5 µL
Gradient Elution	20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min)

A Shimadzu LCMS-8040 model mass spectrometer with negative electrospray ionization mode (ESI) in the UHPLC system was used for detection. Mass conditions and collision energies (CE) were optimized for the qualitative and quantitative determination of phytochemical compounds. Mass conditions are given in Table 2. Data from the mass detector was recorded and processed by LabSolutions software (Shimadzu). The 56 compounds were identified and quantified using the multiple reaction monitoring (MRM) mode.

Table 2 Mass conditions
Çizelge 2 Kütle şartları

Parameters	*Conditions*
Mass spectrometer	Shimadzu LCMS-8040
Ionization Mode	Electrospray ionization (ESI)
Drying gas (N2) flow	15 L min^{-1}
Nebulizing gas (N2) flow	3 L min^{-1}
DL temperature	250°C
Heat block temperature	400°C
Interface temperature	350°C

Antioxidant activity assays

In-vitro free radical scavenging (DPPH·), the ferric ion reducing antioxidant power (FRAP), TPC, and TFC of extract of PH were assessed as antioxidant parameters by using spectrophotometric techniques.

DPPH· free radical scavenging activity

Shimada's method was used for the free radical scavenging activity of the extract with a small modification (Sipahi et al., 2022). Stock solutions of extract and standard (1 mg mL^{-1}) were prepared. A 0.026 M DPPH· (2,2-diphenyl-1-picryl hydrazine) solution was prepared using methanol. 5, 10, 25, 50, 75, 100, 200, and 250 µg mL^{-1} solutions were prepared by diluting the stock solution to 3 mL with methanol. 1 mL of DPPH· solution was added to each solution. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbances of all solutions at 517 nm against a blank were recorded using a spectrophotometer. The absorbance values obtained were converted to % activity, and the IC_{50} (µg mL^{-1}) for PH extract was calculated. Trolox was used as a positive control. All tests were repeated three times, and the test data were given as mean ± standard deviation.

$$\%\ activity_{DPPH} = 100 \times [Acontrol-(Asample-Ablank)] / Acontrol$$

Ferric-ion reducing antioxidant power (FRAP) assay

Oyaizu's method was applied with minor modifications for the ferric-ion-reducing power of the extract (Aksit et al., 2022). Trolox was used as the reference standard. Stock solutions of the extract and Trolox were prepared at a concentration of 1 mg mL^{-1}, separately. 0.25 mL of the extract was made up to 1.25 mL with 0.2 M phosphate buffer (pH 6.6) and then 1.25 mL $K_3Fe(CN)_6$ (1%) was added. The mixture was incubated at 50 °C for 20 minutes and cooled at room conditions. Following incubation, 1.25 mL trichloroacetic acid (10%) and 0.25 mL iron (III) chloride (0.1%) were added and the final mixture was vortexed. The absorbance of the mixture at 700 nm was measured. A calibration curve was created from different concentrations (5-400 µg mL^{-1}) of Trolox and, the result was converted to mg Trolox equivalent (TE)

activity g extract^{-1} using that calibration curve. The tests were done six times, and the results were given as mean values with standard deviations.

Total phenolic content

The TPC of the extract was determined by spectrophotometer using the Folin-Ciocalteu's reagent (Aksit et al., 2022). Gallic acid was used as a standard in the study. The stock solutions of both gallic acid and extract were prepared (1 mg mL^{-1}). 0.1 mL of the stock solution of the extract was diluted with 4.5 mL of distilled water. It was made up of 5 mL with 0.3 mL of Na_2CO_3 (2%) and 0.1 mL of Folin-Ciocalteu reagent. After 10 minutes at room temperature, it was vortexed and kept in the dark for 120 minutes for incubation. The absorbance at 760 nm of the solution was recorded. The calibration curve (y=0.117x-0.011) of gallic acid consisting of different concentrations (1, 5, 10, 25, 50, 100, 250, 400, and 800 µg mL^{-1}) was used for calculations. TPC was expressed as mg gallic acid equivalent phenolic substance per g of extract.

Total flavonoid content

Aluminum chloride was used to determine the TFC in the extract with the spectrophotometric method (Aksit et al., 2022). Quercetin was used as a standard. 1 mg mL^{-1} stock solution of the extract and quercetin was prepared in methanol. 4.7 mL of methanol, 0.1 mL of $AlCl_3$ (10%), and 0.1 mL of 1 M ammonium acetate solution were added to 0.1 mL of the stock solution of the extract, and vortexed. The mixture was incubated for 45 minutes. After the incubation, the absorbance at 415 nm of the mixture was recorded. The calibration curve of quercetin was generated using different concentrations (1, 5, 10, 25, 50, 100, 250, 400, and 800 µg mL^{-1}). Finally, after the results were converted to mg quercetin equivalent (QE) g extract^{-1} using the calibration curve, the TFC of the extract was reported as the mean and standard deviation.

Determination of the minimum inhibitory concentration (MICs)

The microdilution technique was utilized in the antimicrobial test to determine the antimicrobial characteristics of PH extract against four Gram-positive: (Elshikh et al., 2016) *S. aureus* (ATCC 6538), *L. monocytogenes* (ATCC 51774), *B. cereus* (ATCC 10876), *E. faecium* (ATCC 8459), and as well as four Gram-negative; *P. fluorescens* (ATCC 13525), *P. aeruginosa* (ATCC 15442), *E. coli* (ATCC 25922), *S. enteritidis* (ATCC 13076). To obtain serial dilution of test material (from 0.1 to 1.95 µg mL^{-1}), 100 µL of test solution prepared in dimethyl sulfoxide (DMSO) (10%, w/v) was added to the first row of the 96-well plate. The other wells were filled with 50 µL of sterile Muller Hinton Broth (MHB), then 50 µL volume of the first well was transferred to the latter wells. 10 µL of bacterial suspension (1x10^8 CFU mL^{-1}) was added to the corresponding well and incubated at 37 ℃ for 18 hours. After, the addition of 30 µL of resazurin solution (0.02%) to each well the plates were incubated for 6 hours above-mentioned condition. For the setting of the sterile control well, 50 µL of DMSO solution (10% w/v), 10 µL of MHB, 30 µl of indicator solution, and 10 µL of bacterial solutions were added for each bacterium. Negative control was prepared by the addition of 30 µL of indicator solution and 60 µL of MHB. The color change from purple to pink indicated a non-inhibited bacterial action. The concentration level at which color change was observed was noted as MIC values in µg mL^{-1} for each bacterial strain assayed. Tetracycline was used as the positive control.

Antiproliferative activity

Cancer cell lines and cell culture

In this work, the following cell types were used: HeLa (ATCC, CCL-2) and A2780 (RRID, CVCL-0134) gynecological cancer cell lines, SW620 (ATCC, CCL227) and HT29 (ATCC, HTB-38) colon cancer cell lines, A172 (ATCC, CRL-1620) and C6 (ATCC, CCL-107) brain cancer cell lines, and Beas2B (ATCC, CRL-9609) normal lung cell lines, RPE (ATCC, CRL-4000) normal retinal cell lines, and HSF (ATCC, CRL-7449) normal skin cell lines. In a laminar cabinet, all cell preparation processes were completed in a sterile setting. The cell lines were employed once they had reached confluence in a supplemented DMEM medium with 10% FBS and 2% PenStrep solution at 37°C, 5% CO_2 conditions. 10,000 cells per well were sown on measuring plates. Test extracts were introduced after 16 hours of pre-incubation, and after 24 hours of incubation, measurements were made.

Antiproliferative activity assay

The effects of the extracts on cell proliferation and NCI-60 survival parameter values were assessed using the MTT assay. Following a 24-hour incubation period with test extracts and cancer cell lines, this test technique was used. The optical density of the cells treated with the DMSO was taken to be 100% and the findings were expressed as % cell inhibition. So, using the formula [1-(A test substance/A solvent control) x 100, the % inhibition was determined. The IC_{50} concentrations of test extracts were determined using the MTT technique on cells with increasing concentrations of each test extract (1.96, 3.91, 7.81, 15.63, 31.25, 62.5, and 125.0 µg mL^{-1}) across a predetermined range. It was examined by applying a logarithmic function to the absorbance-derived logarithmic curve after the NCI-60 survival parameters were measured using the following formulas (GI_{50}, TGI, and LC_{50}):

Cell proliferation:

[(Ti-Tz)/(C-Tz)]x100 if Ti>/=Tz (cytolytic effect) or

[(Ti-Tz)/Tz]x100 if Ti<Tz (cytocidal or cytotoxic effect)

(Tz; zero point, C; control growth, Ti; inhibition by test substance).

GI_{50}: Concentration value that reduces growth by 50%

([(Ti-Tz)/ (C-Tz)]x100=50),
TGI: Concentration value that reduces growth by 100% (Ti=Tz),
LC_{50}: concentration value that by 50% kills cells in the medium ([(Ti-Tz)/Tz]x100=-50).

Cytotoxicity test

The LDH technique was used to assess if the test extracts were cytotoxic or cytostatic to cells. Depending on the extracts examined, a rise in LDH in the culture supernatant will occur if more cells perish throughout the incubation time. The cytoplasmic enzyme lactate dehydrogenase (LDH), which is stable, is present in the majority of cells. The manufacturer's instructions for using the LDH cell cytotoxicity kit were followed for this purpose. Briefly, NAD^+ is reduced to $NADH/H^+$ by the LDH resulting in lactate-to-pyruvate conversion. Then, a catalyst (diaphorase) transfers H/H^+ from $NADH/H^+$ to the tetrazolium salt which is reduced to formazan. All measurements were made in triplicate. IC_{50} concentrations of test substances were used. In a nutshell, the formula below was used to calculate and assess the change in the quantity of formazan produced as a result of LDH enzyme activity:
% Cytotoxicity = [(Substance Absorbance - Low Control / High Control - Low Control) x 100].

Statistical analysis

The results of LC-MS/MS analyses, and antioxidant and antiproliferative activity assays were statistically analyzed using the SPSS Statistics (IBM v.20, Chicago, IL, USA). The results were expressed as mean and standard deviation.

RESULTS and DISCUSSION

In the study, the dried aerial parts of endemic *Psephellus huber-morathii* were used and these parts of the plant were extracted with 70% methanol. The phytochemical profile of the extract was determined and the total phenolic content, total flavonoid content, and antioxidant, antimicrobial, and antiproliferative activities of the hydroalcoholic extract were investigated.

LC-MS/MS characterization

There is no tandem mass spectrometry and electrospray ionization to liquid chromatography application in the literature for the evaluation of the phenolic composition of PH. In the study, the plant extract was screened by the UHPLC-MS/MS method, which can recognize 56 phytochemicals. In the method, negative ionization, which shows higher sensitivity for phenolic compounds and flavonoids, was preferred. These phytochemicals were determined by examining the molecular ions and MS/MS fragments and the associated collision energies for these fragments, and their amounts were expressed as mg analyte g extract^{-1}. (Figure 1)
The phenolic composition of the extract is listed in Table 3. In the extract of PH, the flavonoid phytochemicals such as protocatechuic aldehyde, vanillin, coumarin, luteolin-*7-O*-glucoside, quercetin-*3*-glucuronide, rutin, isoquercitrin, hesperidin, apigenin-*7*-glucoside, quercitrin, kaempferol-*3-O*-glucoside, nicotiflorin, quercetin, naringenin, luteolin, kaempferol, apigenin, chrysin, acacetin were detected and quantified.

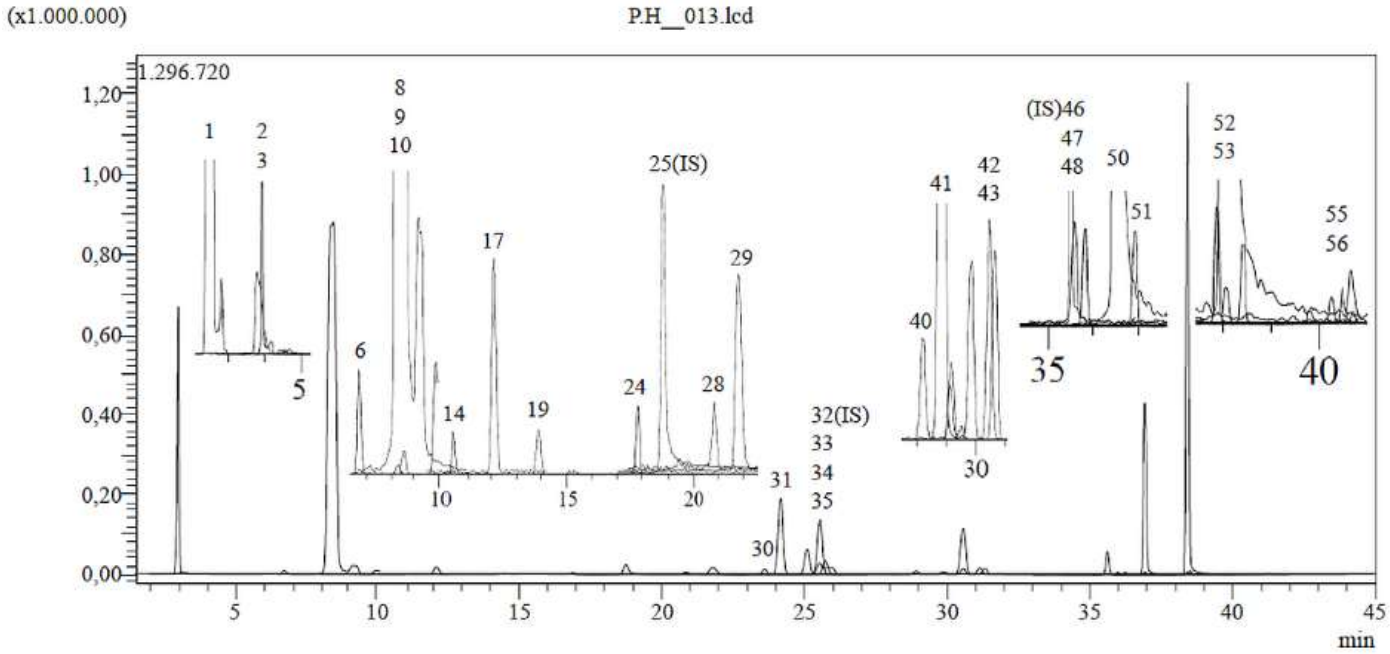


Figure 1. MRM chromatogram of the hydroalcoholic extract of PH obtained by UHPLC-MS/MS.
Şekil 1. UHPLC-MS/MS ile elde edilen PH hidroalkolik ekstraktının MRM kromatogramı

Table 3 Phytochemical compounds of PH extract analyzed by UHPLC-MS/MS (mg g extract^{-1})
Çizelge 3 UHPLC-MS/MS ile analiz edilen PH ekstraktının fitokimyasal bileşikleri (mg g extract^{-1})

No	*Analyte*	*mg g extract^{-1}±SD*	*No*	*Analyte*	*mg g extract^{-1}±SD*
1	Quinic acid	44.95±3.17	29	Salicylic acid	0.10±0.00
2	Fumaric acid	0.22±0.00	30	Luteolin-*7-O*-glucoside	0.40±0.01
3	Aconitic acid	0.24±0.01	31	Quercetin-*3*-glucuronide	9.04±1.34
4	Gallic acid	<LOD	32	Rutin-D3-IS	n.a.
5	Epigallocatechin	n.d.	33	Rutin	3.22±0.89
6	Protocatechuic acid	0.30±0.04	34	Isoquercitrin	6.62±1.47
7	Catechin	<LOD	35	Hesperidin	1.26±0.07
8	Gentisic acid	0.02±0.00	36	*O*-Coumaric acid	<LOD
9	Chlorogenic acid	29.00±4.63	37	Genistin	<LOD
10	Protocatechuic aldehyde	0.04±0.00	38	Rosmarinic acid	n.d.
11	Tannic acid	n.d.	39	Ellagic acid	n.d.
12	Epigallocatechin gallate	<LOD	40	Apigenin-*7-O*-glucoside	0.24±0.01
13	1,5-dicaffeoylquinic acid	n.d.	41	Quercitrin	6.82±1.09
14	4-*OH* Benzoic acid	0.15±0.04	42	Kaempferol-*3-O*-glucoside	0.78±0.09
15	Epicatechin	<LOD	43	Nicotiflorin	0.74±0.08
16	Vanillic acid	n.d.	44	Fisetin	<LOD
17	Caffeic acid	0.13±0.03	45	Daidzein	<LOD
18	Syringic acid	<LOD	46	Quercetin-D3-IS	n.a.
19	Vanillin	0.05±0.01	47	Quercetin	0.15±0.03
20	Syringic aldehyde	<LOD	48	Naringenin	0.02±0.00
21	Daidzin	<LOD	49	Hesperetin	<LOD
22	Epicatechin gallate	<LOD	50	Luteolin	1.30±0.06
23	Piceid	<LOD	51	Genistein	0.01±0.00
24	*p*-Coumaric acid	0.03±0.00	52	Kaempferol	0.04±0.01
25	Ferulic acid-D3-IS	n.a.	53	Apigenin	2.43±0.53
26	Ferulic acid	<LOD	54	Amentoflavone	n.d.
27	Sinapic acid	<LOD	55	Chrysin	0.01±0.00
28	Coumarin	0.02±0.00	56	Acacetin	0.01±0.00

*n.d.: Not detected, n.a.: Not Applicable, SD: Standard deviation, LOD: Limit of detection

Many organic acids and phenolic acids in the plant extract were identified and quantified: quinic acid, fumaric acid, aconitic acid, protocatechuic acid, gentisic acid, chlorogenic acid, 4-*OH* benzoic acid, caffeic acid, *p*-coumaric acid, salicylic acid. According to these results, quinic acid (44.95 mg analyte g extract^{-1}) and chlorogenic acid (30.00 mg analyte g extract^{-1}) are major compounds of PH. Besides the major compounds, PH contains high amounts of quercetin-*3*-glucuronide (9.04 mg analyte g extract^{-1}), isoquercitrin (6.62 mg analyte g extract^{-1}), quercitrin (6.82 mg analyte g extract^{-1}) and apigenin (2.43 mg analyte g extract^{-1}).

Two HPLC studies were reported for the phenolic compounds of PH (Korkmaz et al., 2019; Shoeb et al., 2007). Korkmaz et al. identified 7 compounds in the methanol extract of the plant and quantified two of them. In parallel with this study, they reported that the plant contains *p*-coumaric acid (2.21 mg g extract^{-1}). They also suggested that the plant contains benzoic acid (11.55 mg g extract^{-1}) as the main compound. Shoeb et al. reported that they isolated kaempferol and afzalin from plant seeds by HPLC. Only the qualitative analysis of phytochemical compounds was performed in this study, and their amounts were able to not determined. Octanol, hexadecanoic acid, *p*-cymene, and caryophyllene oxide were found in the essential oil analysis of plant seeds (Başer et al., 2006). This study is the first comprehensive report on the organic acid and flavonoid compounds of PH hydroalcoholic extract.

Antioxidant activities, TPC and TFC

The results of the TPC, TFC, FRAP, and DPPH· activities of the extract are shown in Table 4.

Both TPC and TFC as well as DPPH· and FRAP activities of the plant extract were determined quickly by UV-visible spectrophotometer. In this investigation, the TPC and TFC of PH extract were calculated as 52.19±1.97 mg GAE (gallic acid equivalent) and 28.57±0.94 mg QE per gram extract. Additionally, the extract concentration required to achieve 50% inhibition of the DPPH· radical (IC_{50}) was calculated as 13.18±0.91 µg mL^{-1}, compared to 8.25±1.16 µg mL^{-1} for the positive control. Similar results were observed for the FRAP test, which measures the ability to reduce Fe^{3+} to Fe^{2+}, where one gram of extract had a reducing power equal to 61.27±2.33 mg Trolox.

Table 4 The results of the TPC, TFC, FRAP, and DPPH · activities of the extract
Çizelge 4 Ekstraktın TPC, TFC, FRAP ve DPPH · aktivitelerinin sonuçları

Example	*DPPH· scavenging IC_{50} (µg mL^{-1}) ±SD*	*Total phenolics mg GAE g extract^{-1} ±SD*	*Total flavonoids mg QE g extract^{-1} ±SD*	*Ferric Ion Reducing power mg TE g extract^{-1}±SD*
The extract	13.90±0.5	52.19±2.0	28.57±1.0	61.27±2.3
Trolox	8.25±1.2	-	-	-

SD: Standard deviation, *IC_{50}:* Half maximal inhibitory concentration, GAE: gallic acid equivalent, QE: quercetin equivalent, TE: Trolox equivalent

In the literature, TPC values of water and methanol extracts of PH were reported as 13.9±0.460 mg GAE g extract^{-1} and 10±0.268 mg GAE g extract^{-1}, respectively. The DPPH· activity of these extracts was expressed as IC_{50} value of 0.3379±0.00 mg mL^{-1} and 0.2073±0.00 mg mL^{-1}. It was discovered that FRAP activities were 841±4.70 µM TE and 666±3.21 µM TE. (Korkmaz et al., 2019b) In another study, the methanol extract of plant seeds showed 10 times higher antioxidant activity than Trolox in the DPPH test (Sarker et al., 2005).

In this study, the antioxidant activity, TPC, and TFC of PH hydroalcoholic extract were investigated for the first time. The extract showed high antioxidant activity in DPPH and FRAP tests. It also has rich TPC and TFC. These findings can be explained by the high yield (19%) of the 70% methanol extraction procedure. The rich phytochemical profile of the hydroalcoholic extract better reflects the antioxidant ability, TPC, and TFC of the plant. The antioxidant activity and TPC findings in this study support previous findings about PH (Sarker et al., 2005; Korkmaz et al., 2019).

Estimation of antimicrobial activities of PH

The minimum inhibitory concentration was defined if an extract has strong activity when the MIC is less than 100 µg mL^{-1}, moderate activity when it is between 100 and 500 µg mL^{-1}, and weak activity when it is between 500 and 1000 µg mL^{-1}. The extract is inactive if the MIC is more than 1000 µg mL^{-1} (Holetz et al., 2002; Khan et al., 2009). Antimicrobial activities of the extract are given with MIC values in Table 5.

Table 5 MIC values of PH extract (µg mL^{-1})
Çizelge 5 PH ekstraktının MIC değerleri (µg mL^{-1})

Microorganisms	*PH extract*	*Tetracycline*
Pseudomonas fluorescence	250	3.91
Pseudomonas aeruginosa	500	7.81
Escherichia coli	250	7.81
Salmonella enteritidis	250	3.91
Listeria monocytogenes	500	3.91
Staphylococcus aureus	250	1.95
Bacillus cereus	125	7.81
Enterococcus faecium	125	1.95

Accordingly, the extract showed moderate antimicrobial activity in almost all of *S. aureus, L. monocytogenes, B. cereus, E. faecium*, *P. fluorescens*, *P. aeruginosa*, *E. coli*, and *S. enteritidis.* The extract had a lower MIC value (125 µg mL^{-1}) against gram-positive bacteria *B. cereus* and *E. faecium*. On the contrary, the extract showed a low antimicrobial activity against *L. monocytogenes* and *P. aeruginosa* with a MIC value of 500 µg mL^{-1} (Table 5). This assay demonstrated that the hydroalcoholic extract of PH was more effective against *B. cereus* and *E. faecium* than other bacteria tested.

In the literature, the antimicrobial activity of PH was investigated by Korkmaz et al. (2019), the methanol extract of PH showed moderate antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli*. (MIC values 100-500 µg mL^{-1}) However, it was reported that the methanol extract has weaker activity against *L. monocytogenes* (Korkmaz et al., 2019). In the study, it was determined that the hydroalcoholic extract showed moderate activity against *L. monocytogenes*. On the other hand, Sarker et al. (2005) reported that the seed extract did not show any antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli.* It was reported that plant seeds show low and moderate antimicrobial activity only against *Citrobacter freundii* and *Enterococcus faecalis* (MIC values 100-1000 µg mL^{-1}) (Sarker et al., 2005).

Antiproliferative activity

Evaluation of PH extract according to NCI-60 screening methodology

Numerous antiproliferative medicines used in contemporary chemotherapy still do not exhibit the intended therapeutic qualities because of the tumors' dynamic mutational loads. Additionally, their therapeutic efficacy decreases over time due to chemotherapeutic agent resistance mechanisms and side effects. Because of this, a lot of research is being done to obtain new antiproliferative drugs. In this aim, the MTT test was used to assess the effects of PH extract on cell proliferation (NCI-60 screening approach) and IC_{50} values. It can be seen that the tested extract has a higher lethal concentration (LC_{50}) value than the control antiproliferative drug, 5-Fluorouracil (5FU) when LC_{50} values of the extract on Beas2B, RPE, and HSF control cells are examined. This suggests that the extract does not have unfavorable toxicity. High LC_{50} values suggest that the cytotoxic effects of the extract are lower, which is

desired, as indicated in the NCI-60 screening protocol. PH extract showed high LC_{50} (>1000 µg mL^{-1}) values on Beas2B, RPE, and HSF control cells. This result was interpreted as a desirable good situation when compared with 5FU (417.72 µg mL^{-1}) (Table 6).

Table 6 GI_{50}, TGI, LC_{50}, and IC_{50} values for PH extract (µg mL^{-1})
Çizelge 6 PH ekstraktının GI_{50}, TGI, LC_{50} ve IC_{50} değerleri(µg mL^{-1})

Cell Lines	*PH extract*				*5FU*			
	GI_{50}	*TGI*	*LC_{50}*	*IC_{50}*	*GI_{50}*	*TGI*	*LC_{50}*	*IC_{50}*
Beas2B**	1.04	309.23±9.9	>1000	97.66±4.1	1.43	33.08±1.8	417.72±10.8	34.62±1.7
RPE**	1.71	>1000	>1000	94.58±2.9	1.54	55.37±3.6	423.82±8.3	65.30±2.1
HSF**	1.97	42.91±2.2	>1000	61.68±2.4	1.41	30.64±2.2	357.86±7.8	32.43±2.0
A172**	1.54	>1000	>1000	106.40±3.2	1.38	45.67±2.0	336.86±9.4	45.88±2.1
C6**	1.52	>1000	>1000	126.78±3.8	1.39	39.87±1.5	348.65±9.1	46.11±2.4
HeLa**	2.35	209.83±3.5	>1000	82.28±2.9	1.27	37.18±1.4	393.06±9.5	32.74±1.7
A2780**	2.55	>1000	>1000	131.03±3.2	1.29	48.77±2.0	386.42±8.5	49.94±1.9
SW620**	2.54	>1000	>1000	142.72±3.2	1.59	47.12±1.9	391.24±10.1	53.41±2.7
HT29**	1.28	>1000	>1000	96.25±2.7	1.29	35.36±1.9	411.54±9.2	43.18±2.1

GI_{50}: Growth Inhibition, TGI: Total Growth Inhibition, LC_{50}: Lethal concentration, IC_{50}: Half maximal inhibitory concentration, 5FU: 5-Fluorouracil
*Percent inhibition noted is mean values ± SDs of three independent measures. (SD: Standard deviation)
** If percent inhibition is smaller than 10, the SD value is <0.5.

Considering the Total Growth Inhibition (TGI) and Inhibitory Concentration (IC_{50}) values of the extracts for control cells, the extract was not toxic against normal cells. It showed higher TGI and IC_{50} values on Beas2B, RPE, and HSF normal cells than 5FU (control drug), respectively. As can be observed, the extract's toxic values fell within acceptable ranges because its TGI and IC_{50} values for HSF normal cells were greater than those of 5FU. Examination of the Growth Inhibition (GI_{50}) values of the PH extract showed that the GI_{50} value of the extract on Beas2B control cells (1.04 µg mL^{-1}) was lower than the GI_{50} value of 5FU (1.43 µg mL^{-1}). In RPE and HSF control cells, the extract's GI_{50} values were higher than 5FU. A low GI_{50} value indicates greater cytostatic effects of the extract on Beas2B cells, which is desirable. This feature allows the extract to be used in a wide and safe dose range for chemotherapeutic treatment (Table 6).

The antiproliferative effect of the extract on glioblastoma (A172 and C6), gynecological (HeLa and A2780), and colon (SW620 and HT29) cancer cell lines were also evaluated in the study (Table 6).

The extract showed similar efficacy to 5FU on colon and glioblastoma cell lines in terms of GI_{50} growth inhibition. However, on HeLa and A2780, the extract had a higher GI_{50} value (2.35 and 2.55 µg mL^{-1}). TGI and IC_{50} values of the extract were also found to be higher than 5FU for all these cancer cell lines (Table 6). The high inhibition values measured indicate that the extract is more effective at killing cancer cells than the control antiproliferative drug. The high LC_{50} (389.16->1000 µg mL^{-1}) values caused by the extract in glioma, gynecological, and colon cancer cells compared to 5FU indicate that the safe dose range of the extract in cancer treatment is wide. As a result, concentration modifications will be carried out more easily in a wide range (Table 6).

The different inhibition effects of the extract in each cell line are due to the differences in the cellular biological mechanisms that take an active role against the extract. In conclusion, when we look at all NCI-60 survival parameters, the fact that the extract has low GI_{50} and high LC_{50} values against cancer cells shows that it is a potential candidate for antiproliferative drug development. On the other hand, the GI_{50}, TGI, LC_{50}, and IC_{50} values of the extract on normal Beas2B, RPE, and HSF cell lines are within the desired limits is proof of its reliability (Table 6). All these findings prove that the extract of PH is specifically effective against cancer.

Tumor Specificity Index (TSI) was obtained by dividing the sum of the IC_{50} values from normal cells (Beas2B, RPE, and HSF) by the sum of the IC_{50} values of each cancer cell (A172, C6, HeLa, A2780, SW620, and HT29). According to this data, the tumor-specificities of PH extract to cancer cells are ideal because of the same selectivity as the positive control drug. In addition, the TSI value of the extract was the best for HeLa cells (TSI, 1.04) (Table 7).

Cytotoxicity test

For this, a kit is used to assess the activity of cytoplasmic lactate dehydrogenase (LDH), which leaks from the damaged plasma membrane into the environment. The level of leakage that might result in indirect membrane damage is indicated by LDH activity. Utilizing IC_{50} concentrations, the percent cytotoxicity caused by the extract was evaluated in this investigation. Accordingly, PH extract showed 10.0±0.8, 12.0±1.0, and 9.5±0.6 percent cytotoxicity values against Beas2B, RPE, and HSF at IC_{50}

concentration, respectively. On the other hand, the percent cytotoxic effect of the extract on cancer cell lines was determined as 11.4±1.0% for A172, 10.5±0.9% for C6, 9.9±0.6% for HeLa, 9.7±0.8% for A2780, 10.0±0.9% for SW620 and 11.9±1.0% for HT29 (Table 8). In the literature, the cytotoxicity activity of PH was investigated in a single study. In this study, it was reported that methanol extracts of plant seeds have remarkable cytotoxic effects against colon cancer using the MTT test. (IC_{50}: 33.0 g mL^{-1}) As can be seen, the plant extract showed higher cytotoxic activity against colon cancer cells compared to the seed extract.

Table 7 Tumor Specificity Index (TSI) for PH extract*
*Çizelge 7 PH ekstraktı için Tümör Özgüllük İndeksi (TSI)**

Cell Lines	*PH extract (µg mL^{-1})*		*5FU (µg mL^{-1})*	
	IC_{50}	*TSI*	*IC_{50}*	*TSI*
Beas2B, RPE, and HSF	84.64	1	44.11	1
A172	106.40	0.81	45.88	0.96
C6	126.78	0.68	46.11	0.96
HeLa	82.28	1.04	32.74	1.35
A2780	131.03	0.66	49.94	0.88
SW620	142.72	0.60	53.41	0.82
HT29	96.25	0.90	43.18	1.02

IC_{50}: Half maximal inhibitory concentration, TSI: Tumor Specificity Index, 5FU: 5-Fluorouracil
*TSI is calculated by dividing the average normal cell line IC_{50} by the IC_{50} of each cancer cell line.

Table 8 % Cytotoxicity values for PH extract at IC_{50} concentrations against the cells*
*Çizelge 8 Hücrelere karşı IC_{50} konsantrasyonlarında PH ekstraktı için % sitotoksisite değerleri**

	Beas2B	*RPE*	*HSF*	*A172*	*C6*	*HeLa*	*A2780*	*SW620*	*HT29*
The extract	10.0±0.8	12.0±1.0	9.5±0.6	11.4±1.0	10.5±0.9	9.9±0.6	9.7±0.8	10.0±0.9	11.9±1.0
5FU	10.1±1.0	10.7±1.0	10.9±1.1	10.8±1.0	11.5±1.1	11.9±1.1	10.1±1.0	11.0±1.3	10.9±1.2

*Percent cytotoxicity was noted as mean values ± SDs of three independent measures. (SD: Standard deviation)

CONCLUSION

The study is the first to report comprehensive phytochemical compounds and antiproliferative activity of the *Psephellus huber-morathii* which is endemic in Türkiye. When the MTT proliferation test and LDH cytotoxicity test findings were interpreted together, the extract showed an acceptable antiproliferative effect against cancer cells. It has minimal cytotoxicity against normal cells and a wide range of concentrations for dose adjustment. For this reason, we suggest further investigation of *Psephellus huber-Marathi* extract through preclinical and clinical studies. In this study, the extract showed moderate antimicrobial activity (100-500 µg mL^{-1}) against various Gram-positive (*S. aureus, L. monocytogenes, B. cereus,* and *E. faecium*) and Gram-negative bacteria (*P. fluorescens, P. aeruginosa, E. coli,* and *S. enteritidis*) that cause many infectious diseases. Moreover, the hydroalcoholic extract both exhibited high antioxidant activity and contained significant TPC and TFC. The phenolic compounds determined in the extract play an important role in these biological activities. Additionally, the extract exhibited promising antiproliferative effects against gynecological (IC_{50}=82.28±2.9) and colon (IC_{50}=96.25±2.7) cancer lines. These findings contribute to the understanding of *Psephellus huber-Marathi* as a valuable source of bioactive compounds with potential therapeutic applications. Further investigations are warranted to explore the underlying mechanisms and validate their efficacy in preclinical and clinical studies. The medical potential of *Psephellus huber-marathon* against cancer and infectious diseases should be supported by *in vivo* studies and used in alternative treatments.

ACKNOWLEDGMENT

The authors thank Dr. Hüseyin AKŞİT and Prof. Dr. Ali Kandemir for the collection and taxonomic description of the plant samples.

Author's contribution

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Aksit, H., Aksit, Z., Simsek, S., Aydin, A., Yilmaz, M. A., Kandemir, A., & Koksal, E. (2022). LC-MS/MS profiling phytochemical content of *Echinophora chrysantha* (Apiaceae) and antiproliferative, antioxidant activity. *Pharmacy & Pharmacology International Journal, 10*(5), 190–194. https://doi.org/10.15406/PPIJ.2022.10.00384

Altundağ, E., Altundağ, B. G. E., & Gürdal, B. (2013). Anatomical characteristics of *Centaurea glastifolia* L. (*Asteraceae*) used as folk medicine in East Anatolia. *Journal of Faculty of Pharmacy of Istanbul University, 40*, 57-64. Retrieved from https://dergipark.org.tr/en/pub/iujfp/issue/578/5676

Arif, R., Küpeli, E., & Ergun, F. (2004). The biological activity of *Centaurea* l. species (Review). *Fen Bilimleri Dergisi, 17*(4), 149–164.

Aydin, Ö., Çoşkunçelebi, K., Gültepe, M., & Güzel, M. E. (2013). A contribution to the taxonomy of *Centaurea* including *Psephellus* (*Asteraceae*) based on anatomical and molecular data. *Turkish Journal of Botany, 37*(3), 419–427. https://doi.org/10.3906/bot-1204-25

Barrero, A. F., Herrador, M. M., Arteaga, P., Cabrera, E., Rodriguez-Garcia, I., Garcia-Moreno, M., & Gravalos, D. G. (1997). Cytotoxic activity of flavonoids from *Carthamus arborescens*, *Ononis natrix* ssp. *ramosissima* and *Centaurea malacitana*. *Fitoterapia (Milano), 68*(3), 281–283.

Başer, K. H. C., Özek, G., Özek, T., & Duran, A. (2006). Composition of the essential oil of *Centaurea huber-Marathi Wagenitz* isolated from seeds by micro distillation. *Flavor and Fragrance Journal, 21*(3), 568–570. https://doi.org/10.1002/FFJ.1620

Chanda, S., & Nagani, K. (2013). In vitro and in vivo methods for anticancer activity evaluation and some Indian medicinal plants possessing anticancer properties: An Overview. *Journal of Pharmacognosy and Phytochemistry, 2*(2). Retrieved from www.phytojournal.com www.phytojournal.com

Demiroz, T., Nalbantsoy, A., Kose, F. A., & Baykan, S. (2020). Phytochemical composition and antioxidant, cytotoxic, and anti-inflammatory properties of *Psephellus goeksunensis* (Aytaç & H. Duman) Greuter & Raab-Straube. *South African Journal of Botany, 130*, 1–7. https://doi.org/10.1016/ J.SAJB.2019.11.021

Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of the minimum inhibitory concentration of biosurfactants. *Biotechnology Letters, 38*(6), 1015–1019. https://doi.org/10.1007/S10529-016-2079-2/ TABLES/3

Farrag, N., Abdel-Aziz, E., El-Domiaty, M., & El Shafea, A. (1993). Phytochemical investigation of *Centaurea araneosa* growing in Egypt. *Zagazig Journal of Pharmaceutical Sciences, 2*(1), 29–45. https://doi.org/10.21608/ZJPS.1993.188986

Güçlü, G., Ergül, M., Uçar, E., Eruygur, N., Ataş, M., & Aşkın AKPULAT, H. (2022). Anticancer, Antioxidant, Antimicrobial and Enzyme Inhibitory Activities of *Inula aucheriana*. *2022 KSU J. Agric Nat, 25*(5), 946–954. https://doi.org/10.18016/ksutarimdoga.vi.985837

Güven, K., Çelik, S., & Uysal, I. (2005). Antimicrobial activity of *Centaurea* species. *Pharmaceutical Biology, 43*(1), 67–71. https://doi.org/10.1080/13880200590903390

Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V., & Dias Filho, B. P. (2002). Screening of some plants used in Brazilian folk medicine for the treatment of infectious diseases. *Memórias Do Instituto Oswaldo Cruz, 97*(7), 1027–1031. https://doi.org/10.1590/S0074-02762002000700017

Khammar, A., & Djeddi, S. (2012). Pharmacological and Biological Properties of some *Centaurea* Species. *European Journal of Scientific Research, 84*(3), 398–416. Retrieved from http://www.EuropeanJournalofscientificresearch.com

Khan, N. A., Quereshi, S., Pandey, A., & Srivastava, A. (2009). Antibacterial Activity of Seed Extracts of Commercial and Wild *Lathyrus* Species. *Turkish Journal of Biology, 33*(2), 165–169. https://doi.org/10.3906/biy-0806-25

Korkmaz, N., Sener, S. O., Balturk, N., Kanbolat, S., Badem, M., Aliyazicioglu, R., Ozgen, U., Kandemir, A., Karaoglu, S. A. (2019). Determination of Phenolic Contents by HPLC, Antioxidant, Antimicrobial, Antityrosinase, and Anticholinesterase Activities of *Psephellus huber-marathon*. *Journal Of Pharmaceutical Research International, 26*(1), 1–10. https://doi.org/10.9734/JPRI/2019/V26I130125

Orallo, F., Lamela, M., Camiña, M., Uriarte, E., & Calleja, J. M. (1998). Preliminary study of the potential vasodilator effects on rat aorta of centaurein and centaureidin, two flavonoids from *Centaurea corcubionensis*. *Planta Medica, 64*(2), 116–119. https://doi.org/10.1055/S-2006-957386/BIB

Özcan, M. (2013). Morphological and anatomical aspects of *Psephellus pulcherrimus* (syn: *Centaurea pulcherrima* var. *freynii*) (*Cardueae, Asteraceae*) growing in Turkey. *Journal of Forestry Faculty, 14*(1), 104-112.

Polatoglu, K., Sen, A., Bulut, G., Bitis, L., & Gören, N. (2015). The essential oil composition of *Centaurea stenolepis* Kerner. from Turkey. *Http://Dx.Doi.Org/10.1080/0972060X.2014.935090, 17*(6), 1268–1278. https://doi.org/10.1080/ 0972060X.2014.935090

Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology, 100*(1–2), 80–84. https://doi.org/10.1016/J.JEP.2005.04.025

Sarker, S. D., Shoeb, M., Celik, S., Jaspars, M., Nahar, L., Kong-Thoo-Lin, P., & MacManus, S. M. (2007). Extracts of *Centaurea bornmuelleri* and *Centaurea huber-morathii* inhibit the growth of colon cancer cells in vitro. *Advances in Traditional Medicine, 7*(4), 336–340. https://doi.org/10.3742/OPEM.2007.7.4.336

Sarker, S. D., Kumarasamy, Y., Shoeb, M., Celik, S., Eucel, E., Middleton, M., & Nahar, L. (2005). Antibacterial and antioxidant activities of three

Turkish species of the genus *Centaurea*. *Advances in Traditional Medicine, 5*(3), 246–250. https://doi.org/10.3742/OPEM.2005.5.3.246

Shoeb, A., MacManus, S., Jaspars, M., Nahar, L., Kong-Thoo-Lin, P., Celik, S., & Sarker, S. (2007). Lignans and Flavonoids from the Seeds of *Centaurea bornmuelleri* Hausskn. Ex. Bornm. and *Centaurea huber-morathii* Wagenitz. *Polish Journal of Chemistry, 81*(1), 39–44. https://doi.org/10.2/JQUERY.MIN.JS

Sipahi, H., Orak, D., Reis, R., Yalman, K., Şenol, O., Palabiyik-Yücelik, S. S., … Yesilada, E. (2022). A comprehensive study to evaluate the wound healing potential of okra (*Abelmoschus esculentus*) fruit. *Journal of Ethnopharmacology, 287*, 114843. https://doi.org/10.1016/J.JEP.2021.114843

Tarhan, L., Ozturk Urek, R., Oner, A., & Nakiboglu, M. (2022). Evaluation of phenolic profiles, antioxidant activities, and cytotoxic and apoptotic potentials of *Phlomis angustissima* and *Phlomis fruticosa*, medicinal plants from Turkey. *European Journal of Integrative Medicine, 55*, 102188. https://doi.org/10.1016/J.EUJIM.2022.102188

Yilmaz, M. A. (2020). Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC-MS/MS method validation. *Industrial Crops and Products, 149*, 112347. https://doi.org/10.1016/J.INDCROP.2020.112347

Zengin, G., Cakmak, Y. S., Guler, G. O., & Aktumsek, A. (2010). In vitro antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from the Central Anatolia region of Turkey. *Food and Chemical Toxicology, 48*(10), 2638–2641. https://doi.org/10.1016/J.FCT.2010.06.033

KSÜ Tarım ve Doğa Derg 27 (4), 793-801, 2024
KSU J. Agric Nat 27 (4), 793-801, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1285786

Investigation of New Potential Uses of Menengiç (*Pistacia terebinthus*) for Various Areas

Meltem ASAN OZUSAGLAM[1]✍
[1]Department of Molecular Biology and Genetic, Faculty of Science and Letters, Aksaray University, Aksaray, Türkiye
[1]https://orcid.org/0000-0002-3638-1306
✉: meltemozusaglam@gmail.com

ABSTRACT

Pistacia terebinthus has been used extensively in alternative medicine. The inhibitory activity of ethanol fruit extract from *P. terebinthus* fruit on food-borne and clinical test microorganisms was obtained to determine the potential use of the extract for food and pharmaceutical industries as a natural antimicrobial source. The antibacterial activity of the ethanol extract on fish-originated pathogen bacteria was also determined for its potential use in the feed industry. In addition, the effect of the extract on the probiotic bacteria originating from breast milk was tested to obtain the potential use of the extract together with probiotic bacteria for the pharmaceutical and food industries. Antimicrobial activity was obtained by performing micro-dilution and macro-dilution assays as well as disc diffusion. Among the food-borne and clinical test microorganisms, the highest inhibition zone diameter (22.39±1.92 mm) was detected on *Listeria monocytogenes*. The highest antibacterial activity on the fish pathogens was recorded as 17.67±0 mm against *Vibrio anguillarum* A4. It was determined that *P. terebinthus* fruit extract had higher antimicrobial activity against some test microorganisms than Amikacin and Gentamicin antibiotics. Antifungal activity on *Candida glabrata* was also investigated by counting viable cells. At a concentration of 20 mg mL^{-1}, no viable cells were determined after 24 hours. The extract inhibited all the tested LAB, however, with low MIC and MBC values. The sun protection factor (SPF) of the extract and the extract+cream mixture at 10 mL concentration was recorded as 9.36 and 7.51. The results of the study indicated that *P. terebinthus* fruit ethanol extract can be an alternative as a natural additive in various industries such as feed, food, pharmaceutical, and cosmetic industries.

Microbiology

Research Article

Article History
Received : 19.04.2023
Accepted : 19.12.2023

Keywords
Menengiç
Antimicrobial activity
Extract
Lactic acid bacteria
Solar protection

Menengiç (*Pistacia terebinthus*)'in Çeşitli Alanlar İçin Yeni Potansiyel Kullanımlarının Araştırılması

ÖZET

Pistacia terebinthus, alternatif tıpta yaygın olarak kullanılmaktadır. *P. terebinthus* meyvesinden elde edilen etanol meyve ekstresinin gıda kaynaklı ve klinik test mikroorganizmaları üzerindeki inhibitör aktivitesi, ekstrenin gıda ve ilaç endüstrilerinde doğal bir antimikrobiyal kaynağı olarak potansiyel kullanımını belirlemek için tespit edilmiştir. Etanol ekstresinin, balık orijinli patojen bakteriler üzerindeki antibakteriyel aktivitesi, yem endüstrisinde kullanım potansiyeli için belirlenmiştir. Ayrıca ekstrenin probiyotik bakterilerle birlikte ilaç ve gıda endüstrilerinde kullanım potansiyelini ortaya çıkarmak için ekstrenin anne sütü kaynaklı probiyotik bakteriler üzerindeki aktivitesi test edilmiştir. Antimikrobiyal aktivite, disk difüzyonunun yanı sıra mikro seyreltme ve makro seyreltme deneyleri yapılarak belirlenmiştir. Gıda kaynaklı ve klinik test mikroorganizmaları arasında en yüksek inhibisyon zon çapı (22,39±1,92 mm) *Listeria monocytogenes* üzerinde tespit edilmiştir. Balık patojenleri üzerinde en yüksek antibakteriyel aktivite *Vibrio anguillarum* A4'e karşı 17,67±0 mm olarak kaydedilmiştir. *P. terebinthus* meyve ekstresinin bazı test mikroorganizmalarına karşı Amikasin ve Gentamisin antibiyotiklerinden daha yüksek antimikrobiyal aktiviteye sahip olduğu belirlenmiştir. *Candida glabrata* üzerindeki antifungal aktivite, canlı

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 19.04.2023
Kabul Tarihi : 19.12.2023

Anahtar Kelimeler
Menengiç
Antimikrobiyal aktivite
Ekstre
Laktik asit bakterisi
Güneş koruyucu

hücre sayımı ile de araştırılmıştır. 20 mg mL^{-1}'lik konsantrasyonda, 24 saat sonra hiçbir canlı hücre belirlenmemiştir. Ekstre, test edilen tüm LAB'leri düşük MİK ve MBK değerleri ile inhibe etmiştir. Ekstre ve ekstre+krem karışımının 10 mL konsantrasyonundaki güneş koruma faktörü (GKF) 9,36 ve 7,51 olarak kaydedilmiştir. Çalışmanın sonuçları, *P. terebinthus* meyve etanol ekstresinin yem, gıda, ilaç ve kozmetik endüstrileri gibi çeşitli endüstrilerde doğal katkı maddesi olarak alternatif olabileceğini göstermiştir.

Atıf Şekli: Asan Ozusaglam, M. (2024) Menengiç (*Pistacia terebinthus*)'in Çeşitli Alanlar İçin Yeni Potansiyel Kullanımlarının Araştırılması. *KSÜ Tarım ve Doğa Derg 27* (4), 793-801. https://doi.org/10.18016/ksutarimdoga.vi.1285786

To Cite : Asan Ozusaglam, M. (2024) Investigation of New Potential Uses of Menengiç (*Pistacia terebinthus*) for Various Areas. *KSU J. Agric Nat 27* (4), 793-801. https://doi.org/10.18016/ksutarimdoga.vi.1285786

INTRODUCTION

Menengiç (*Pistacia terebinthus*) is an important plant species due to its biological activity and chemical properties (Köten, 2021). Menengiç (*Anacardiaceae*) is widely grown in the southern and western regions of Turkey (Özcan et al., 2020). In traditional remedies, menengiç fruits have been used in the treatment of many diseases such as eczema, throat infections, stomachache, rheumatism, asthma, and cough (Matthäus & Özcan, 2006). Recently, the medicinal use of compounds such as phenolic compounds, saponins, fatty acids, flavonoids, alkaloids, and terpenoids from *P. terebinthus* has gained attention (Rauf et al., 2017; Buriani et al., 2017; Özcan et al., 2020; Kaçar et al., 2022). *P. terebinthus* fruits have been involved in many studies due to their biological activities (Topçu et al., 2007; Naghmachi et al., 2022) and high oil content (Matthäus & Özcan, 2006).

Nowadays, clinical and food-borne microbial infections and gained antibiotic resistance are among the most important problems threatening the health of societies. Worldwide, microbial infections cause millions of deaths each year (Khameneh et al., 2019). The increased antibiotic resistance has led to a decrease in the effectiveness of antimicrobial drugs and even their ineffectiveness (WHO, 2014; Baym et al., 2016). Therefore, it is necessary to obtain new and natural antimicrobial agents from plants.

Aquaculture is one of the most important animal food sectors that can meet the protein needs of the World's population and compensate for the lack of food (Mabrok & Wahdan, 2018). The occurrence of infectious diseases in aquaculture causes important problems in the development of the sector and leads to significant economic losses (Direkbusarakom et al., 1998). Antibiotics are extensively used to treat diseases caused by microorganisms in aquaculture. The use of antibiotics adds to the extension of antibiotic-resistant bacteria and genes into other organisms (Watts et al., 2017). Antibiotic resistance has been reported in *Aeromonas hydrophila*, *V. anguillarum,* and *Yersinia ruckeri* as a result of antibiotics used in fish farms (Petersen et al., 2002). Herbal antimicrobials can be natural alternatives to these antibiotics used in the prevention and treatment of bacterial infections in fish.

Probiotics are live microorganisms that benefit the host when taken in adequate amounts (Hill et al., 2014). Probiotic microorganisms have been very popular in recent years due to their multi-faceted health-promoting benefits such as anticancer, antidiabetic, stimulating the immune system, and anti-inflammatory (Song et al., 2015; Andrabi et al., 2016; Bhat et al., 2017). Plant extracts can be used to improve probiotic effects as natural and safety additives (Noor, 2017; Cocetta et al., 2019).

Ultraviolet radiation (UV) is divided into three regions UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm) (Polonini et al., 2011). Exposure to UV radiation for a long time increases the risk of various skin diseases such as cancer and photoallergic reactions. Skin problems are mainly caused by UV-B (280-320 nm) radiation (Kim et al., 2022). The effectiveness of a sunscreen or sunscreen component to protect against UV-B radiation is measured by the sun protection factor (SPF) (Twilley et al., 2021). Sunscreens are chemicals that protect against the negative effects of the sun, especially UV radiation (Maske et al., 2013). Recently, natural substances have been recognized as potential sources due to their absorption of UV radiation and their sunscreen properties (Cefali et al., 2019; Al-Amoody et al. 2020; Kurzawa et al., 2022; Ibrahim et al., 2022).

In the present study, to determine the potential use of *P. terebinthus* fruit ethanol extract in various industries; (i) the antimicrobial activity of the extract on food-borne and clinical test microorganisms and fish-borne bacterial pathogens, (ii) the potential use of the extract together with the probiotic candidate lactic acid bacteria (LAB) originated from breast milk, and (iii) also SPF value of the ethanol extract and the ethanol extract+cream mixture were determined.

MATERIAL and METHOD

Preparation of Extracts

P. terebinthus fruits were purchased from a local

market in Adıyaman (Türkiye). The fruits were first washed under tap water to remove dust and then rinsed with pure water. After drying in an airy environment, the fruits were ground using a grinder (Waring, USA). The powdered fruit material (15 grams) was extracted with ethanol using the Soxhlet device for 24 hours. The sample was then filtered using the Whatman No. 1 paper. The extract was concentrated under a vacuum using a rotary evaporator (Heidolph, Germany) and stored in the dark at 4 °C until use. The crude extract was dissolved with dimethylsulfoxide (DMSO) and then sterilized by filtering through a 0.45 µm filter.

Determination of Antimicrobial Activity

The antimicrobial activity of *P. terebinthus* fruit ethanol extract was first determined by using a disc diffusion assay. Nutrient Broth (NB)-Agar (for *Escherichia coli* O157:H7, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* RSKK 863, *Shigella sonnei* Mu:57, *Salmonella enteritidis* ATCC 13076, *Yersinia enterocolitica* ATCC 11175, *Micrococcus luteus* B-4375, *Pseudomonas aueroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *A. hydrophila* ATCC 19570), Yeast Extract Peptone Dextrose (YPD)-Agar (for *C. albicans* ATCC 10231, *C. glabrata* RSKK 04019), Tryptic Soy Broth (TSB)-Agar (for, *Lactococcus garvieae, Y. ruckeri*, *Streptococcus agalactiae, L. monocytogenes* ATCC 7644), Tryptic Soy Broth-NaCl (for *Vibrio alginoliticus*, *V. anguillarum* A4 and M1 strains), De Man, Rogosa and Sharpe (MRS)-Agar (for *Lactobacillus gasseri* MA-2, *L. gasseri* MA-3, *L. gasseri* MA-4, *L. gasseri* MA-5, *Lactobacillus fermentum* MA-7, *L. fermentum* MA-8, *Lactobacillus delbrueckii* MA-9, *Lactobacillus vaginalis* MA-10, *Lactobacillus plantarum* RSKK 1062) were used as growth medium. The test microorganisms (0.5 McFarland) were inoculated onto solid media. Sterile discs were placed on the agar medium and then 20 µL (2000 µg disc^{-1}) of *P. terebinthus* fruit extract was dropped onto the discs. Amikacin (AK, 10 µg disc^{-1}) and Gentamicin (CN, 10 µg disc^{-1}) antibiotics were used as control groups in the disc diffusion test. After incubation for 24 h, the inhibition zones were measured. The experiments were repeated in triplicate.

MiCRO-dilution Method

MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) or MFC (Minimum Fungicidal Concentration) values of the extract were obtained against the test microorganisms using a micro-dilution assay. The test microorganisms (0.5 McFarland) were added to the mixture of extract and growth medium and then vortexed. After incubation for 24 h, the non-growth concentration of the extract in the broth medium was obtained as MIC values. Then, the mixture was inoculated on the agar media using spot dropping method. After the incubation period (24 h), the microbial growth-preventing extract concentrations on the agar media were recorded as MBC or MFC values.

Macro-dilution Method

Macro-dilution assay was also used to obtain the antimicrobial activity of the fruit ethanol extract against *C. glabrata* RSKK 04019 by counting viable cells using the method described by Sousa et al. (2006) with some modifications. *C. glabrata* RSKK 04019 suspension (0.5 McFarland) was added to the mixture of fruit ethanol extract (at concentrations of 5, 10, and 20 µg µL^{-1}) and growth medium. The cell suspension without the extract was used as a control group. Then, the control and test groups were incubated at 30 °C for 0, 24 and 48 h. The samples from the suspension were then diluted and inoculated onto YPD agar medium after each incubation time. The viable cell was counted and recorded as log10 CFU mL^{-1}.

Determination of in-vitro Solar Protection Factor of Extract and Extract+Cream Mixture

The SPF value of *P. terebinthus* fruit ethanol extract was determined in vitro. The extract (2 µg µL^{-1}) was prepared in ethanol (96%) and measured in a spectrophotometer (Beckman Coulter) in the wavelength range of 290 nm-320 nm (at 5 nm intervals). The experiments were done in triplicate. The Mansur equation (1) used to calculate the sun protection factor was below (Mansur et al., 1986).

The SPF value of extract and cream mixtures was determined using the developed modified method (Asan Ozusaglam & Celik, 2023). 1 g of cream and 0.5 g of *P. terebinthus* fruit extract was mixed made up to 10 g with distilled water. The mixture diluted with ethanol to various concentrations (2.5 mL, 5 mL, and 10 mL) was measured using the spectrophotometer and then calculated as mentioned above.

Mansur equation:

$$\text{Solar protection factor(SPF)} = CF_x \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda) \quad (1)$$

Statistical Analysis

The mean values of the analysis results obtained in three replicates were evaluated statistically (IBM, SPSS Statistics 25 software, USA). One-way ANOVA and Tukey tests were used at the 5% level to make comparisons between means.

RESULTS and DISCUSSION

The antimicrobial activity of *P. terebinthus* fruit ethanol extract was investigated against food, clinical, and fish-originated test microorganisms using a disc diffusion assay. MIC and MBC or MFC values of the extract were also determined. In the current research, ethanol was used as the extraction solvent because the

ethanol solvent has good solubility of active substances compared to other solvents such as methanol, chloroform, and ethyl acetate (De Boer et al., 2005). The inhibition zone diameters of the extract on food-borne and clinical test microorganisms were determined between 22.39±1.92 mm and 10.03±0.06 mm. The results showed that the *P. terebinthus* fruit ethanol extract had antimicrobial activity on all test microorganisms tested. The amikacin and gentamicin antibiotics were used as the control group. It was determined that the inhibition zone diameters of the fruit extract on food-borne and clinical microorganisms were generally higher than amikacin and gentamicin antibiotics. *P. terebinthus* fruit extract also showed an inhibition zone diameter of 10.42±0.35 mm and 17.67±0.26 mm on fish pathogens. It was obtained that the extract showed higher antimicrobial activity against fish pathogens *V. alginoliticus, V. anguillarum* M1, and A4 strains compared to the two commercially available antibiotics (AK and CN) (Table 1).

Table 1. Antimicrobial activity of *P. terebinthus* fruit ethanol extract
Çizelge 1. P. terebinthus meyve etanol ekstresinin antimikrobiyal aktivitesi

Test microorganisms	Inhibition zone diameter (mm)		
	Ethanol extract	AK	CN
Food-borne and clinical test microorganisms			
E. coli O157:H7	15.57±0.10 ^{a,h,I,s,u,w,z}	17.76±0	14.07±0.01
E. coli ATCC 35218	14.54±0.22^{c,h,I,q,s,u,z}	18.75±0.70	10.19±0.02
L. monocytogenes ATCC 7644	22.39±1.92$^{e,h,\ I,q,s,u,z}$	19.50±0.01	19.38±0.02
B. cereus RSKK 863	18.29±0.74^{b,d,f,g,k,m,o,q,w}	16.81±0.20	12.97±0.30
S. sonnei Mu:57	10.03±0.06$^{b,d,f,\ I,k,q}$	13.48±1.40	11.08±0.80
S. enteritidis ATCC 13076	12.75±0.88^{h,I,j,s,u,z}	13.87±0.01	10.51±0.02
Y. enterocolitica ATCC 11175	16.29±0.58^{h,l,q,z}	23.04±0.02	19.92±0.01
M. luteus B-4375	16.28±0.33^{h,n,q,z}	13.28±0.02	10.93±0.01
P. aueroginosa ATCC 27853	11.60±0.95^{d,f,h,I,m,o,p,s,u,w,z}	18.88±0.01	16.31±0.02
S. aureus ATCC 25923	15.13±2.09^{b,d,f,k,q,r}	17.34±0.01	13.05±0.02
E. faecalis ATCC 29212	17.29±0.35^{b,d,f,k,q,t}	24.02±0.30	13.48±1.44
C. albicans ATCC 10231	16.33±1.39^{b,h,q,v}	NA	NA
C. glabrata RSKK 04019	15.42±1.17^{b,d,f,k,m,o,q,y}	NA	NA
Fish pathogens			
A. hydrophila ATCC 19570	12.63±0.33$^{a,f,h,\ I,k,m}$	30.57±0.11	19.03±0.09
Y. Rucker	14.50±0.98$^{c,f,h,\ I,k,m}$	18.69±0.12	18.85±0.05
V. anguillarum M1	15.78±0.72^{b,d,e,h,k}	9.46 ±0.12	12.38±0.09
V. alginoliticus	16.25±0.24$^{b,d,f,g,\ I,k,m}$	15.03±0.03	15.06±0.07
V. anguillarum A4	17.67±0.26$^{b,d,h,\ I,k}$	12.07±0.13	15.13±0.15
L. garvieae	10.42±0.35$^{b,d,f,h,\ I,j,m}$	10.30±0.08	15.19±0.10
S. agalactia Pas.Ins. 55118	12.19±0.83^{b,d,h,k,l}	16.15±0.08	19.72±0.08

AK: Amikacin, CN: Gentamicin, NA: No activity
The different letters in the columns denote significant differences according to one-way ANOVA followed by Tukey's Multiple Comparison Test (P<0.05).

MIC, MBC, or MFC values of *P. terebinthus* fruit ethanol extract are given in Table 2. Among the food and clinical pathogens, the lowest MIC and MBC values of the extract were determined as 2.5 µg µL^{-1} on *C. glabrata* RSKK 04019. The lowest MIC and MBC values (2.5 µg µL^{-1}) for fish pathogens were obtained on *V. alginoliticus* and *V. angillarium* A4. Low MIC, MBC, and MFC values indicated that *P. terebinthus* fruit extract had high antimicrobial activity.

In a study, the antimicrobial activity of the methanol extract from *P. terebinthus* fruit collected from Elazığ (Turkey) was investigated by using a disc diffusion assay (Erecevit & Kırbağ, 2017). In the study, the inhibition zone diameter of the methanol extract was recorded as 16 mm on *E. coli* and 17 mm on *S. aureus* which is close to the results of the current study. Çoban et al. (2017) investigated the antimicrobial activity of the methanol, water, and ethyl acetate extracts from *P. terebinthus* fruit obtained from Aydın (Turkey) on various microorganisms. They reported the highest inhibition zone diameter on *E. coli* ATCC 35218 (13 mm) in fruit methanol extract and on *P. aueroginosa* (11 mm) in ethyl acetate extract. In the present study, nearly or slightly higher antimicrobial activity was obtained compared to the results of the study of Çoban et al. (2017). Plants secrete secondary metabolites as defense molecules, so factors such as the season in which the plant is harvested and the temperature of the environment can change the metabolites of the plant (Haliki et al., 2005). Therefore, the reason for the different results in biological activity may be due to the different environmental conditions, extraction methods as well as solvents used.

Table 2. MIC, MBC or MFC values of *P. terebinthus* fruit ethanol extract
Çizelge 2. P. terebinthus meyve etanol ekstresinin MİK, MBK veya MFK değerleri

	Ethanol extract	
Test microorganisms	**MIC (µg µL^{-1})**	**MBC or MFC (µg µL^{-1})**
Food-borne and clinical test microorganisms		
E. coli O157:H7	20	40
E. coli ATCC 35218	20	40
L. monocytogenes ATCC 7644	5	5
B. cereus RSKK 863	20	40
S. sonnei Mu:57	40	80
S. enteritidis ATCC 13076	40	40
Y. enterocolitica ATCC 11175	10	10
M. luteus B-4375	10	20
P. aueroginosa ATCC 27853	20	40
S. aureus ATCC 25923	5	5
E. faecalis ATCC 29212	40	80
C. albicans ATCC 10231	2.5	5
C. glabrata RSKK 04019	2.5	2.5
Fish pathogens		
A. hydrophila ATCC 19570	20	40
Y. Rucker	10	10
V. anguillarum M1	10	10
V. alginoliticus	2.5	2.5
V. anguillarum A4	2.5	2.5
L. garvieae	20	20
S. agalactia Pas.Ins. 55118	20	40

MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), MFC (Minimum Fungicidal Concentration)

There are only a few antifungal agents in the treatment of *Candida* species, which are opportunistic human fungal pathogens, and one of the biggest problems is that they gain resistance to these antifungals in a short time (Silva et al., 2020). Therefore, the development of new natural drugs with fewer side effects for the prevention or treatment of fungal pathogens such as *C. glabrata* is of great interest. The antifungal activity of the extract on *C. glabrata* RSKK 04019 was recorded as 15.42 mm zone diameter in the disc diffusion assay. *P. terebinthus* fruit extract against *C. glabrata* RSKK 04019 was determined to have the lowest MIC and MFC values (2.5 µg µL^{-1}) among the foodborne and clinically tested microorganisms tested. Therefore, the antifungal activity of the fruit extract on *C. glabrata* RSKK 04019 was also investigated by counting viable cells and the results were given in Figure 1. At 5 and 10 µg µL^{-1} concentrations of *P. terebinthus* fruit extract, there was a certain decrease in viable cell count compared to the control group after 24 h. No viable cell was observed after 24 h and 48 h incubation at 20 µg µL^{-1} *P. terebinthus* fruit ethanol extract concentration.

Fungi infect billions of people each year but are still largely underappreciated as human pathogens (Brown et al, 2012). *C. albicans* and *C. glabrata* can be found particularly in the oral cavity and in the gastrointestinal tract of most healthy people (Fidel et al., 1999; Cole et al., 1996). *C. glabrata* infections can be mucosal or systemic, and are more common in immunocompromised individuals or hosts with diabetes mellitus (Sinnott et al., 1987; Sobel, 1988; Geiger et al., 1995; Wingard, 1995). The antifungal activity of *P. terebinthus* fruit methanol extract collected from Elazig province on *C. glabrata* was investigated by Erecevit & Kırbağ (2017). In their study, they reported that the inhibition zone diameter of *P. terebinthus* fruit methanol extract on *C. glabrata* was 10 mm. A study showed that methanol, ethyl acetate, and boiled water extract obtained from *P. terebinthus* fruit had no antifungal activity against *C. glabrata* (Çoban et al., 2017). The higher antifungal activity obtained from this study may be due to the ethanol solvent used. Because ethanol has a better dissolving potential for active ingredients compared to other solvents.

LAB are microorganisms that are widely used as probiotic cultures in various processes that are generally considered safe (GRAS) and have therapeutic effects on the host (Gerez et al., 2013). The antimicrobial activity of *P. terebinthus* fruit extract against probiotic candidate LAB from breast milk, used at the concentration (2000 µg disc^{-1}) tested on pathogens is presented in Table 3. The results indicated that *P. terebinthus* fruit extract had inhibitory activity on all the tested LAB strains. The

disc diffusion assay results showed the lowest inhibition zone diameters as 11.91±0.28 mm on *L. gasseri* MA-3 and 11.92±0.37 mm on *L. fermentum* MA-2. The MIC and MBC values of the extract on the test LAB were determined as 10-40 µg µL^{-1} and 10-80 µg µL^{-1}, respectively. The high MIC and MBC values of the extract on LAB indicated that *P. terebinthus* fruit extract and LAB mixtures could have the potential to be used as natural preservatives in the food and pharmaceutical industries after adjusting the appropriate concentrations.

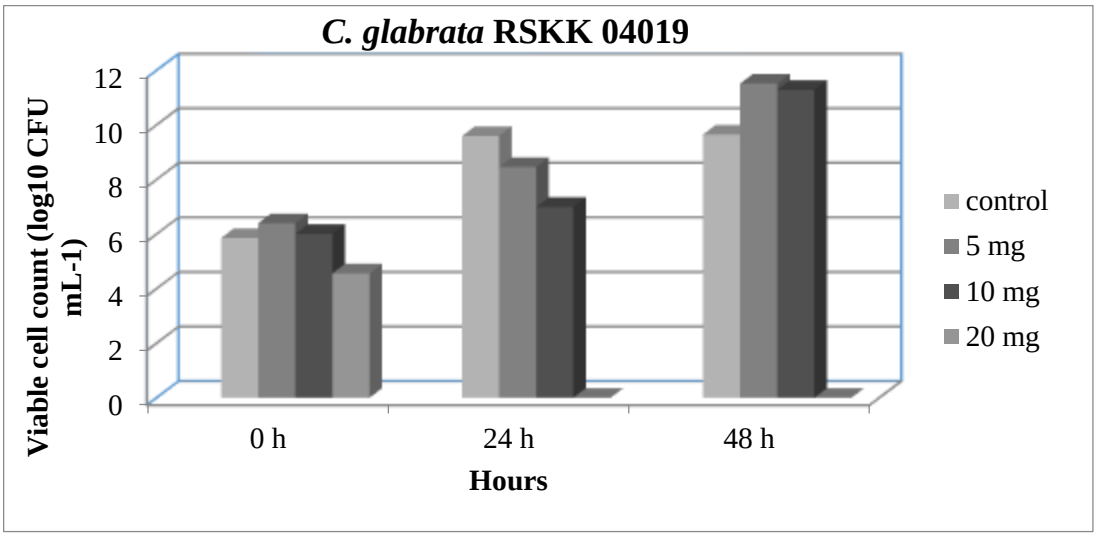


Figure 1. Antifungal activity of *P. terebinthus* fruit ethanol extract on *C. glabrata* RSKK 04019
Şekil 1. P. terebinthus meyve etanol ekstraktının C. glabrata RSKK 04019 üzerindeki antifungal aktivitesi

Table 3. Inhibitory activity of *P. terebinthus* fruit ethanol extract on probiotic candidate lactic acid bacteria originated from human milk
Çizelge 3. P. terebinthus meyve etanol ekstresinin insan sütü kaynaklı probiyotik aday laktik asit bakterileri üzerindeki inhibitör aktivitesi

Test microorganisms	Ethanol extract		
	Inhibition zone diameter (mm)	MIC (µg µL^{-1})	MBC (µg µL^{-1})
L. gasseri MA-2	11.92±0.37	10	10
L. gasseri MA-3	11.91±0.28	10	40
L. gasseri MA-4	13.18±0.81	10	40
L. gasseri MA-5	12.33±0.45^{a,f}	10	20
L. fermentum MA-7	12.72±0.05	40	80
L. fermentum MA-8	11.95±0.58^{c,f}	10	40
L. delbrueckii MA-9	12.44±0.34^{b,d,e}	10	40
L. vaginalis MA-10	12.82±0.37	10	20
L. plantarum RSKK 1062	13.18±0.03	20	80

MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration)
Values with the different superscript letters in the columns mean significantly different by one-way ANOVA followed by Tukey's post-hoc test (P<0.05).

The antimicrobial activity of the *P. terebinthus* ethanol extract on the test microorganisms was statistically evaluated, and the results showed a statistically significant difference between food-borne and clinical test microorganisms (P<0.05). The significant variation in inhibition zone diameter averages of the ethanol extract for fish pathogens or the LAB group was determined at a significance level of 0.05 (P<0.5). Multiple comparison analysis using by Tukey test was performed to determine differences between means for each group and presented in Table 1 and Table 3.

The SPF value of *P. terebinthus* fruit ethanol extract was found to be 9.36. Sunscreens with an SPF of over 2 are considered to have good sun protective activity (Alves-Rodrigues & Shao, 2004). As a result of the literature review, no study was found showing the sunscreen effect of *P. terebinthus* fruit ethanol extract. The SPF value of the *P. terebinthus* fruit ethanol extract used in the presented study was found to be quite high.

The SPF values of the cream+*P. terebinthus* fruit ethanol extract mixture of various concentrations is

presented in Table 4. The SPF values of the cream+extract mixture were found higher than cream (control) at all tested concentrations. Therefore, it can be said that the addition of *P. terebinthus* fruit extract increased the SPF value of commercial cream. The percentage of sun protection of *P. terebinthus* fruit ethanol extract at a concentration of 10 mL was found to be approximately 80% according to Imam et al. (2015).

Table 4. SPF values of *P. terebinthus* fruit ethanol extract+cream mixture
Çizelge 4. P. terebinthus meyve etanol ekstresi+krem karışımının SPF değerleri

Extract Concentration	SPF Values	
	Cream (Control)	Fruit Extract+Cream
2.5 mL	0.16	0.49
5 mL	0.47	1.03
10 mL	1.29	7.51

Recently, natural active compounds have been used in formulations to reduce the possible side effects of synthetic compounds and are accepted by consumers (Rodrigues et al., 2019). As a result of the literature review, no study was found to investigate the SPF value of cream mixtures of *P. terebinthus* fruit extracts.

CONCLUSION

The potential use of *P. terebinthus* fruit ethanol extract in various industries was investigated. The extract, with its good antimicrobial activity, can be used as a natural alternative for the food, feed, and pharmaceutical industries. Additionally, the extract and LAB mixtures, which combine antimicrobial activity and probiotic effects, can be used as natural additives in the pharmaceutical and food industries. In addition, the fruit ethanol extract of *P. terebinthus* showed good sun protection activity and even increased the SPF value of a commercial cream. Thus, the fruit ethanol extract could be an alternative as a natural additive for the cosmetic industry. *P. terebinthus* grows naturally in many regions but has limited use. This study revealed that *P. terebinthus* may be a new alternative for various industries and has the potential to be a natural resource to avoid synthetic additives.

ACKNOWLEDGMENTS

This work was supported by the Research Fund of the Aksaray University (Project number 2019-022). The author thanks Gulden KOC for her help in the study.

Conflict of interest

None.

REFERENCES

Al-Amoody, A. A., Yayman, D., Kaan, T., Özkök, E. A., Özcan, A., Özen, E., & Çobanoğlu Özyiğitoğlu, G. (2020). Role of lichen secondary metabolites and pigments UV-screening phenomenon in lichens. *Acta Biologica Turcica, 33*(1), 35–48. [DOI: 10.24411/1300-0152-1000]

Alves-Rodrigues, A., & Shao, A. (2004). The science behind lutein. *Toxicology Letters, 150*(1), 57–83. [DOI: 10.1016/j.toxlet.2003.07.001]

Andrabi, S. T., Bhat, B., Gupta, M., & Bajaj, B. K. (2016). Phytase-producing potential and other functional attributes of lactic acid bacteria isolates for prospective probiotic applications. *Probiotics and Antimicrobial Proteins, 8*(2), 121–129. [DOI: 10.1007/s12602-015-9186-5]

Asan-Ozusaglam, M., & Celik, I. (2023). White pitahaya as a natural additive: Potential usage in the cosmetic industry. *Foods and Raw Materials, 11*(1), 57–63. [DOI: 10.21603/2308-4057-2023-1-57-63]

Baym, M., Stone, L. K., & Kishony, R. (2016). Multidrug evolutionary strategies to reverse antibiotic resistance. *Science, 351*(6268), aad3292. [DOI: 10.1126/science.aad3292]

Bhat, B., Gupta, M., Andrabi, S. T., & Bajaj, B. K. (2017). Growth and viability of probiotic *Weissella kimchi* R-3 in fruit and vegetable beverages. *Indian Journal of Biochemistry & Biophysics, 54*, 191–199.

Brown, G. D., Denning, D. W., & Levitz, S. M. (2012). Tackling human fungal infections. *Science, 336*(6082), 647–647. [DOI: 10.1126/science. 1222236]

Buriani, A., Fortinguerra, S., Sorrenti, V., Dall'Acqua, S., Innocenti, G., Montopoli, M., Gabbia, D., & Carrara, M. (2017). Human adenocarcinoma cell line sensitivity to essential oil phytocomplexes from *Pistacia* species: A multivariate approach. *Molecules, 22*(8), 1336. [DOI: 10.3390/molecules 22081336]

Cefali, L. C., Ataide, J. A., Fernandes, A. R., Sousa, I. M. O., Gonçalves, F. C. S., Eberlin, S., Dávila, J. L., Jozala, A. F., Chaud, M. V., Sanchez-Lopez, E., Marto, J., d'Ávila, M. A., Ribeiro, H. M., Foglio, M. A., Souto, E. B., & Mazzola, P. G. (2019). Flavonoid-enriched plant-extract-loaded emulsion: A novel phytocosmetic sunscreen formulation with antioxidant properties. *Antioxidants, 8*(10), 443. [DOI: 10.3390/antiox8100443]

Çoban, E. P., Bıyık, H. H., & Törün, B. (2017). Investigation of antimicrobial effects in different solvents of *Pistacia terebenthus* L. fruits collected from Aydın. *Journal of Applied Biological Sciences, 11*(3), 20–22.

Cocetta, V., Catanzaro, D., Borgonetti, V., Ragazzi, E., Giron, M. C., Governa, P., Carnevali, I., Biagi, M., & Montopoli, M. (2019). A fixed combination of probiotics and herbal extracts attenuates intestinal barrier dysfunction from inflammatory stress in an in vitro model using Caco-2 cells. *Recent Patents on Food, Nutrition & Agriculture, 10*(1), 62–69. [DOI: 10.2174/2212798410666181221151452]

Cole, G. T., Halawa, A. A., & Anaissie, E. J. (1996). The role of the gastrointestinal tract in hematogenous candidiasis: From the laboratory to the bedside. *Clinical Infectious Diseases, 22*(Supplement-2), 73–88. [DOI: 10.1093/clinids/22.Supplement_2.S73]

De Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I., & Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology, 96*(3), 461–469. [DOI: 10.1016/j.jep.2004.09.012]

Direkbusarakom, S., Ruangpan, L., Ezura, Y., & Yoshimizu, M. (1998). Protective efficacy of *Clinacanthus nutans* on yellow-head disease in black tiger shrimp (*Penaeus monodon*). *Fish Pathology, 33*(4), 401–404. [DOI: 10.3147/ jsfp.33.401]

Erecevit, P., & Kırbağ, S. (2017). Antimicrobial activity of some plant species used for medical purposes in Turkey. *The Journal of Phytopharmacology, 6*(2), 93–97.

Fidel Jr, P. L., Vazquez, J. A., & Sobel, J. D. (1999). *Candida glabrata*: Review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. *Clinical Microbiology Reviews, 12*(1), 80–96. [DOI: 10.1128/CMR.12.1.80]

Geiger, A. M., Foxman, B., & Sobel, J. D. (1995). Chronic vulvovaginal candidiasis: Characteristics of women with *Candida albicans*, *C. glabrata* and no candida. *Genitourinary Medicine, 71*(5), 304–307. [DOI: 10.1136/sti.71.5.304]

Gerez, C. L., Torres, M. J., De Valdez, G. F., & Rollán, G. (2013). Control of spoilage fungi by lactic acid bacteria. *Biological Control, 64*(3), 231–237. [DOI: 10.1016/j.biocontrol.2012.11.012]

Haliki, A., Denizci, A. A., & Çetingül, V. (2005). Bazı Deniz Alglerinin (*Phaeophyta*, *Rhodophyta*) Antifungal Aktiviteleri Üzerine Bir Araştırma. *Ege University Journal of Fisheries and Aquatic Sciences, 22*(1–2), 13–15.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514. [DOI: 10.1038/nrgastro.2014.66]

Ibrahim, N. D., Seow, L. J., Sekar, M., Rani, N. N. I. M., & Lum, P. T. (2022). Ten commonly available medicinal plants in Malaysia with potential sun protection factor and antioxidant properties–A Review. *Pharmacognosy Journal, 14*(2), 444–445. [DOI: 10.5530/pj.2022.14.57]

Imam, S., Azhar, I., & Mahmood, Z. A. (2015). In-vitro evaluation of sun protection factor of a cream formulation prepared from extracts of *Musa acuminate* (L.), *Psidium guava* (L.), and *Pyrus communis* (L.). *In-Vitro, 8*(3), 234–237.

Kaçar, D., Bayraktar, O., Erdem, C., Alamri, A. S., & Galanakis, C. M. (2022). Antioxidant and antimicrobial activities of plants grown in the Mediterranean region. *JSFA Reports, 2*(9), 452–461. [DOI: 10.1002/jsfa.12479]

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrobial Resistance & Infection Control, 8*(1), 1–28. [DOI: 10.1186/s13756-018-0448-2]

Kim, H. M., Byun, K-A., Oh, S., Yang, J. Y., Park, H. J., Chung, M. S., Son, K. H., & Byun, K. (2022). A mixture of topical forms of polydeoxyribonucleotide, vitamin C, and niacinamide attenuated skin pigmentation and increased skin elasticity by modulating nuclear factor erythroid 2-like 2. *Molecules, 27*(4), 1276. [DOI: 10.3390/molecules 27041276]

Köten, M. (2021). Influence of raw/roasted terebinth (*Pistacia terebinthus* L.) on the selected quality characteristics of sponge cakes. *International Journal of Gastronomy and Food Science, 24*, article 100342. [DOI: 10.1016/j.ijgfs.2021.100342]

Kurzawa, M., Wilczyńska, E., Brudzyńska, P., & Sionkowska, A. (2022). Total phenolic content, antioxidant capacity, and UV radiation protection properties of Marigold (*Calendula officinalis*), Carrot (*Daucus carota*), Tomato (*Solanum lycopersicum*) and Hop (*Humulus lupulus*) extracts. *Cosmetics, 9*(6), 134. [DOI: 10.3390/cosmetics 9060134]

Mabrok, M. A. E., & Wahdan, A. (2018). The immune modulatory effect of oregano (*Origanum vulgare* L.) essential oil on *Tilapia zillii* following intraperitoneal infection with *Vibrio anguillarum*. *Aquaculture International, 26*, 1147–1160. [DOI: 10.1007/s10499-018-0210-y]

Mansur, J. S., Breder, M. N. R., Mansur, M. C. A., & Azulay, R. D. (1986). Determinação do fator de proteção solar por espectrofotometria. *Anais Brasileiros de Dermatologia, 61*, 121–124.

Maske, P. P., Lokapure, S. G., Nimbalkar, D., Malavi, S., & D'souza, J. I. (2013). In vitro determination of sun protection factor and chemical stability of *Rosa hordes* extract gel. *Journal of Pharmacy Research, 7*(6), 520–524.

Matthäus, B., & Ozcan, M. M. (2006). Quantitation of fatty acids, sterols, and tocopherols in Turpentine (*Pistacia terebinthus* Chia) growing wild in Turkey. *Journal of Agricultural and Food Chemistry, 54*(20), 7667–7671. [DOI: 10.1021/jf0610016]

Naghmachi, M., Raissi, A., Baziyar, P., Homayoonfar, F., Amirmahani, F., & Danaei, M. (2022). Green synthesis of silver nanoparticles (AgNPs) by *Pistacia terebinthus* extract: Comprehensive evaluation of antimicrobial, antioxidant and anticancer effects. *Biochemical and Biophysical Research Communications, 608*, 163–169. [DOI: 10.1016/j.bbrc.2021.12.113]

Noor, S. O. (2017). Screening of some commonly used plant extracts for their effects on some gut pathogens and probiotics. *Journal of Pure and Applied Microbiology, 11*(1), 163–171.

Özcan, M. M., Al Juhaimi, F., Uslu, N., Ahmed, I. A. M., Babiker, E. E., Osman, M. A., Gassem, M. A., Alqah, H. A. S., & Ghafoor, K. (2020). Effect of sonication process of terebinth (*Pistacia terebinthus* L.) fruits on antioxidant activity, phenolic compounds, fatty acids, and tocopherol contents. *Journal of Food Science and Technology, 57*, 2017–2025. [DOI: 10.1007/s13197-020-04518-y]

Petersen, A., Andersen, J. S., Kaewmak, T., Somsiri, T., & Dalsgaard, A. (2002). Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Applied and Environmental Microbiology, 68*(12), 6036–6042. [DOI: 10.1128/ AEM.68.12.6036-6042.2002]

Polonini, H. C., Raposo, N. R. B., & Brandão, M. A. F. (2011). Fotoprotetores naturais no contexto da saúde pública brasileira. *Revista de APS, 14*(2), 216–223.

Rauf, A., Patel, S., Uddin, G., Siddiqui, B. S., Ahmad, B., Muhammad, N., Mabkhod, Y. N., & Hadda, T. B. (2017). Phytochemical, ethnomedicinal uses and pharmacological profile of genus *Pistacia*. *Biomedicine & Pharmacotherapy, 86*, 393–404. [DOI: 10.1016/j.biopha.2016.12.057]

Rodrigues, M. J., Matkowski, A., Ślusarczyk, S., Magne, C., Poleze, T., Pereira, C., & Custodio, L. (2019). Sea knotgrass (*Polygonum maritimum* L.) is a potential source of innovative industrial products for skincare applications. *Industrial Crops and Products, 128*, 391–398. [DOI: 10.1016/j.indcrop. 2018.11.025]

Silva, F. D. S., Landell, M. F., Paulino, G. V. B., Coutinho, H. D. M., & Albuquerque, U. P. (2020). Antifungal activity of selected plant extracts based on an ethnodirected study. *Acta Botanica Brasilica, 34*, 442–448. [DOI: 10.1590/0102-33062019 abb0191]

Sinnott, J. T., Cullison, J. P., & Sweeney, M. P. (1987). *Candida* (*Torulopsis*) *glabrata*. *Infection Control & Hospital Epidemiology, 8*(8), 334–336. [DOI: 10.1017/s0195941700064898]

Sobel, J. D. (1988). Pathogenesis and epidemiology of vulvovaginal candidiasis. *Annals of the New York Academy of Sciences, 544*, 547–557. [DOI: 10.1111/j.1749-6632.1988.tb40406.x]

Song, M., Yun, B., Moon, J. H., Park, D. J., Lim, K., & Oh, S. (2015). Characterization of selected *Lactobacillus* strains for use as probiotics. *Korean Journal for Food Science of Animal Resources, 35*(4), 551–556. [DOI: 10.5851/kosfa.2015.35.4.551]

Sousa, A., Ferreira, I. C., Calhelha, R., Andrade, P. B., Valentão, P., Seabra, R., Estevinho, L., Bento, A., & Pereira, J. A. (2006). Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra'. *Bioorganic & Medicinal Chemistry, 14*(24), 8533–8538. [DOI: 10.1016/j.bmc.2006.08.015]

Topçu, G., Ay, M., Bilici, A., Sarıkürkcü, C., Öztürk, M., & Ulubelen, A. (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. *Food Chemistry, 103*(3), 816–822. [DOI: 10.1016/ j.foodchem.2006.08.048]

Twilley, D., Moodley, D., Rolfes, H., Moodley, I., McGaw, L. J., Madikizela, B., Summers, B., Raaff, L., Lategan, M., Kgatuke, L., Mabena, E. C., & Lall, N. (2021). Ethanolic extracts of South African plants, *Buddleja saligna* Willd. and *Helichrysum odoratissimum* (L.) Sweet, as multifunctional ingredients in sunscreen formulations. *South African Journal of Botany, 137*, 171–182. [DOI: 10.1016/j.sajb.2021.08.016]

Watts, J. E., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs, 15*(6), 158. [DOI: 10.3390/md15060158]

WHO. (2014). Antimicrobial resistance: Global report on surveillance. Geneva: WHO. Retrieved from https://apps.who.int/iris/handle/10665/112642. ISBN: 9789241564748

Wingard, J. R. (1995). Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clinical Infectious Diseases, 20*(1), 115–125. [DOI: 10.1093/clinids/20.1.115]

KSÜ Tarım ve Doğa Derg 27 (4), 802-816, 2024
KSU J. Agric Nat 27 (4), 802-816, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1373704

Sarıyar, Gökçekaya ve Yenice Baraj Göllerinin (Eskişehir, Ankara) Fitoplanktonunun ve Su Kalitesinin Değerlendirilmesi

Nurhayat DALKIRAN[1]✉, Belgin YOLDAŞ[2], Cafer BULUT[3]
[1]Bursa Uludağ Üniversitesi, Fen Edebiyat Fakültesi Biyoloji Bölümü, Görükle Kampüsü, Nilüfer, Bursa, [2]Adıyaman Sert Kabuklu Araştırma Enstitüsü Müdürlüğü, Adıyaman, [3]Eğirdir Su Ürünleri Araştırma Enstitüsü Müdürlüğü, Isparta
[1]https://orcid.org/0000-0002-1222-8809, [2]https://orcid.org/0000-0002-4104-3184, [3]https://orcid.org/ 0000-0002-5525-170X
✉: dalkiran@uludag.edu.tr

ÖZET

Bu çalışmada birbiri ile bağlantılı üç baraj gölünün fitoplanktonik alg florasının ve fitoplankton temelli indeks kullanarak trofik seviyesinin belirlenmesi amaçlanmıştır. Sarıyar Baraj gölünden beş, Gökçekaya ve Yenice baraj göllerinden ise birer istasyondan fitoplankton örnekleri toplanmıştır. Fitoplankton örneklemesi Ağustos ve Kasım 2016 tarihlerinde iki dönem olacak şekilde yapılmıştır. Bazı fizikokimyasal değişkenler ise Haziran 2016 - Mayıs 2017 tarihleri arasında aylık olarak örneklenmiştir. Elektriksel iletkenliğin yıllık ortalaması her üç baraj gölünde de 1000 µS/cm'in üzerinde olduğu belirlenmiştir. Kruskal-Wallis analizine göre amonyum azotu Sarıyar Barajı'ndan Yenice Barajı'na doğru anlamlı bir azalma gösterirken, nitrat azotu ise istatistiksel olarak anlamlı bir artış göstermiştir. Her üç baraj gölünde toplam 144 alg taksonu belirlenirken, Yenice baraj gölünde 73, Gökçekaya baraj gölünde 64 ve Sarıyar baraj gölünde 108 alg taksonu tespit edilmiştir. Eğilimsiz Uyum Analizi sonuçları takson sayısındaki mekânsal dağılımı belirgin olarak ortaya çıkarmıştır. Takson çeşitliliği en yüksek divizyo 69 taksonla Chlorophyta olarak belirlenmiş, bu divizyoyu 38 taksonla Bacillariophyta takip etmiştir. Fitoplanktonik alg florası daha önce yapılan çalışmalar ile karşılaştırıldığında 95 taksonun birbiri ile bağlantılı bu üç baraj gölü sistemi için yeni kayıt olduğu tespit edilmiştir. Fitoplankton trofik indeks sonuçları her üç baraj gölünde de biyolojik kalite oranının zayıf seviyede olduğunu göstermiştir. Baraj göllerinde yapılması planlanan alabalık yetiştiriciliği faaliyetleri için Sarıyar, Gökçekaya ve Yenice baraj göllerinin uygun değerlendirilemediği sonucuna varılmıştır.

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 10.10.2023
Kabul Tarihi : 24.01.2024

Anahtar Kelimeler
Fitoplankton
Baraj gölleri
Tür çeşitliliği
Fitoplankton trofik indeksi
Su kalitesi

Assessment of Phytoplankton and Water Quality of Sarıyar, Gökçekaya and Yenice Dam Lakes (Eskişehir, Ankara)

ABSTRACT

In this study, it was aimed to determine the phytoplanktonic algal flora and trophic level using a phytoplankton-based index of three interconnected dam lakes. Phytoplankton samples were collected from five study sites in the Sarıyar dam and one site each in the Gökçekaya and Yenice dams. Phytoplankton sampling was carried out in two periods in August and November 2016. Some physicochemical variables were sampled monthly between June 2016 and May 2017. The annual average electrical conductivity was determined to be over 1000 µS/cm in all three dam lakes. According to Kruskal-Wallis analysis, ammonium nitrogen significantly decreased from Sarıyar Dam to Yenice Dam, however, nitrate nitrogen showed a statistically significant increase. While a total of 144 algal taxa were identified, 73 taxa were detected in Yenice, 64 in Gökçekaya, and 108 in Sarıyar. Detrended correspondence analysis results revealed the spatial distribution of the phytoplanktonic taxa. The highest taxa number was determined as Chlorophyta with 69 taxa, followed by Bacillariophyta with 38 taxa. When the phytoplanktonic algal flora was compared with previous studies, 95 taxa were found to be new records for these three interconnected dam lake systems. The

Microbiology

Research Article

Article History
Received : 10.10.2023
Accepted : 24.01.2024

Keywords
Phytoplankton
Dam lakes
Species richness
Phytoplankton trophic index
Water quality

phytoplankton trophic index results showed that the biological quality range was poor status in all three dam lakes. It has been concluded that Sarıyar, Gökçekaya, and Yenice dam lakes cannot be evaluated as suitable for trout farming activities planned to be carried out in the dam lakes.

Atıf Şekli: Dalkıran, N., Yoldaş, B., & Bulut, C., (2024). Fitoplanktonunun ve Su Kalitesinin Değerlendirilmesi. *KSÜ Tarım ve Doğa Derg 27* (4), 802-816. https://doi.org/10.18016/ksutarimdoga.vi.1373704

To Cite : Dalkıran, N., Yoldaş, B., & Bulut, C., (2024). Manuscript Title. Manuscript Title. Assessment of Phytoplankton and Water Quality of Sarıyar, Gökçekaya and Yenice Dam Lakes (Eskişehir, Ankara). *KSU J. Agric Nat 27*(4), 802-816. https://doi.org/10.18016/ksutarimdoga.vi.1373704

GİRİŞ

Baraj gölleri içme ve kullanma suyu, sulama, enerji üretimi, rekreasyonel amaçlı kullanım gibi çeşitli insan aktiviteleri ve kullanımı için uzun yıllardır inşaa edilen su yapılarıdır. Bu nedenle düzenli olarak su kaliteleri takip edilmektedir. Türkiye'de son yıllarda barajlarda yapılan biyolojik ve kimyasal su kalitesinin belirlenmesi ile ilgili çalışmalar giderek önem kazanmıştır (Köse ve ark., 2020; Maraşlıoğlu & Gönülol, 2014; Sönmez ve ark., 2022; Varol, 2020; Varol ve ark., 2012; Yılmaz ve ark., 2022). Sarıyar, Gökçekaya ve Yenice baraj gölleri Sakarya Nehri üzerinde enerji üretmek amacı ile inşa edilmiş üç baraj gölüdür. Gökçekaya ve Yenice Baraj Gölleri'nde su ürünleri yetiştiriciliği yapılmaktadır. Ancak 2011 yılından bu yana özellikle alabalık kafeslerinde balık ölümleri meydana gelmektedir. Her üç baraj gölünde de son yıllarda organik ve inorganik kirlilik yükü giderek artış göstermektedir.

Fitoplanktonik algler doğal göller ve baraj göllerinin birincil üretiminde ilk basamağı oluşturan canlılardır. Ayrıca besin tuzlarına hızlı cevap verdikleri için ötrofikasyon seviyesinin belirlenmesinde oldukça önemlidirler. Daha önceki yıllarda baraj göllerinde yapılan çalışmalar değerlendirildiğinde Sarıyar ve Gökçekaya baraj göllerinde fitoplanktonik ya da bentik algler ile ilgili yapılmış çalışmalar mevcut iken Yenice baraj gölünde bir çalışmaya rastlanmamıştır. Sarıyar baraj gölünde 7 farklı örnekleme noktasında Mart 1996 – Nisan 1997 yılları arasında yapılan çalışmada 195 alg taksonu tespit edilmiştir (Atıcı 1999, 2002a, 2002b, 2002c, 2004; Atıcı & Obalı 2006; Atıcı ve ark., 2008). Gökçekaya baraj gölünde ise 2005 ve 2006 yıllarında yapılan çalışmalarda 28 alg taksonu tespit edilmiştir (Akın ve ark., 2008). Dokcan (2010) 2009 yılında Sarıyar baraj gölü bentik alglerini çalışmış ve 69 takson tespit etmiştir.

Bu çalışmanın amacı bir baraj gölü sistemi olan Sarıyar, Gökçekaya ve Yenice baraj göllerinin güncel fitoplanktonik alg florasını belirlemek, her üç baraj gölündeki alg florasının farklılıklarını ortaya koymaktır. Ayrıca baraj göllerinde balık yetiştiriciliğinde önemli olan birincil üretici alglerin çeşitliliğini ve bolluğunu belirlemek, fitoplankton tabanlı indeksler kullanarak baraj göllerinin ekolojik kalitesini tespit etmek ve balık yetiştiriciliğinde zararlı olabileceği düşünülen siyanotoksin üretebilme potansiyeline sahip Cyanobacteria türlerinin varlığını tespit etmek ise bu çalışmanın diğer amaçlarını oluşturmaktadır.

MATERYAL ve METOD

Çalışma alanı

Her üç baraj gölü de birincil olarak enerji üretmek amacı ile çeşitli dönemlerde Sakarya Nehri üzerinde inşa edilmiştir. Birbiri ile bağlantılı olan bu barajlardan Sarıyar Hasan Polatkan Barajı Ankara'nın Nallıhan ilçesi sınırlarında bulunmaktadır. 1951-1956 yılları arasında inşa edilmiş Türkiye'nin ilk büyük hidroelektrik santrali olma özelliğindedir. Barajın gövde hacmi 568.000 m^3, akarsu yatağından yüksekliği 80 m`dir. Normal su kotunda göl hacmi 1.900 $hm^3$, yüzey alanı ise 83 $km^2$`dir. Baraj gölünün uzunluğu 63 km, genişliği ise yer yer 1 km ile 200 m arasında değişmektedir. Eskişehir ili Alpu'da bulunan Gökçekaya Barajı Sarıyar barajı mansabında yer alır. 1962-1972 yılları arasında inşa edilmiştir. Barajın gövde hacmi 650.000 m^3, akarsu yatağından yüksekliği 115 m, normal su kotunda göl hacmi 910 hm^3 ve gölalanı 20 km^2'dir. Yenice Barajı, enerji üretmek amacıyla 1985-1999 yılları arasında Eskişehir ili Yenice'de inşa edilmiştir. Barajın akarsu yatağından yüksekliği 41 m ve yüzey alanı 3.6 km2'dir.

Baraj göllerinde fitoplanktonun tür kompozisyonunu ve nispi bolluklarını belirlemek için Yenice ve Gökçekaya baraj göllerinden birer örnekleme noktası, Sarıyar Baraj Gölü'nden ise 5 örnekleme noktası belirlenmiştir. Fitoplankton örneklemeleri Ağustos ve Kasım 2016 tarihlerinde iki mevsim olarak gerçekleştirilmiştir. Bu örnekleme tarihleri su sıcaklığı artışı, su seviyesinin düşmesi, ve besin tuzu derişiminin artması ve suyun bekleme süresinin artması gibi çevresel faktörlerin siyanobakterileryel çoğalmalar için en uygun dönemler olması nedeni ile belirlenmiştir. Fizikokimyasal analizler için su örnekleri yüzeyden Haziran 2016 - Mayıs 2017 tarihleri arasında (Ocak ve Şubat ayları hariç) aylık olarak Sarıyar Barajı'ndan 4, Yenice ve Gökçekaya barajlarından birer noktadan alınmıştır. Örnekleme noktaları Şekil 1, 2, 3 ve 4'te verilmiştir. Örnekleme noktaları barajların kirlilik yükünü en iyi temsil

edecek noktalardan seçilmiştir. Örnekleme noktalarına ait koordinatlar Çizelge 1'de verilmiştir.

Çizelge 1. Örnekleme noktalarına ait koordinatlar
Table 1. Coordinates of sampling sites

Sarıyar-1	40° 1'39.88"K - 31°27'0.41"D
Sarıyar-2	40° 1'10.88"K - 31°35'25.23"D
Sarıyar-3	40° 3'53.49"K - 31°39'58.90"D
Sarıyar-4	40° 1'27.30"K - 31°42'57.18"D
Sarıyar-5	40° 3'1.51"K - 31°45'52.67"D
Gökçekaya	40° 2'23.66"K - 31° 0'56.15"D
Yenice	40° 3'41.27"K - 30°51'53.65"D

Fizikokimyasal Analizler

Su sıcaklığı (T, °C), elektriksel iletkenlik (Eİ, µS/cm), pH ve çözünmüş oksijen (ÇO, mg/L), WTW marka 3430i multiplus arazi ölçüm seti ile arazi esnasında anlık ölçülmüştür. Amonyum azotu (NH_4-N, mg/L) indophenole blue metodu ile, Nitrit azotu (NO_2-N, mg/L) N-(1-Naphthyl)-ethylendiamine metodu ile, Nitrat azotu (NO_3-N, mg/L) 2,6-dimethylphenole metodu ile ve Fosfat fosforu (PO_4-P, mg/L) phosphomolybdenum blue metodu kullanılarak Optizen Pop V Model UV Visible Spektrofotometre ile standart yöntemlere göre gerçekleştirilmiştir (APHA, 1998). Bu analizler Eğirdir Su Ürünleri Enstitüsü Kimya Laboratuarları'nda analiz edilmiştir.

Şekil 1. Sarıyar, Gökçekaya ve Yenice Baraj göllerinin genel görünümü
Figure 1. Location of Sarıyar, Gökçekaya and Yenice Dam lakes.

Şekil 2. Sarıyar barajı örnekleme noktaları
Figure 2. Sarıyar Dam sampling sites

Şekil 3. Gökçekaya HES örnekleme noktaları
Figure 3. Gökçekaya HEPP sampling sites

Şekil 4. Yenice HES örnekleme noktası
Figure 4. Yenice HEPP sampling sites

Fitoplankton Örneklemesi, Sayım ve Tanımlama

Baraj göllerinde fitoplankton nispi bolluğunu ve tür çeşitliliğini belirlemek için, 55 mikron göz açıklığına sahip plankton kepçesi ile Ağustos 2016 tarihinde belirlenen örnekleme noktalarında yüzeyden yatay ve su kolonundan dikey örnekleme yapılmıştır. Yatay çekim tekne ile 0.5-1 m derinlikten, dikey çekim ise 5 m derinlikten yüzeye doğru, toplam çekim 5 dakika olacak şekilde yapılmıştır. Ağustos ayında alınan örneklerde yatay ve dikey örneklemede önemli bir fark gözlenmediği için Kasım fitoplankton örneklemesinde sadece dikey örnekleme yapılmıştır. Örnekler 250 ml poliüretan örnekleme kaplarına alınmış ve lügol (I-KI) çözeltisi ile fikse edilmiştir. Tür teşhisleri 1000x büyütmeli Euromex marka ışık mikroskobunda gerçekleştirilmiştir. Alglerin tür teşhisleri Huber-Pestalozzi (1961), Krammer & Lange-Bertalot (1991a, 1991b, 1997a, 1997b), Komárek & Anagnostidis (1999) ve John ve ark. (2003)'e göre gerçekleştirilmiştir.

Baraj göllerinde fitoplankton tür çeşitliliği ve bolluğuna göre Plankton Trofik İndeksi (PTI) (Phillips ve ark., 2013) biyolojik su kalitesini belirlemek için Eşitlik 1'de verilen formüle göre hesaplanmıştır. aj örnekteki j'inci taksonun oranı ve sj örnekteki j'inci taksonun optimumudur. Hesaplanan indeks sonuçları 1- (değer-min/maks-min) formülü kullanılarak 0 – 1 aralığına standardize edilmiş ve biyolojik kalite oranı belirlenmiştir.

$$PTI = \frac{\sum_{j=1}^{n} ajsj}{\sum_{j=1}^{n} aj} \qquad \text{(Eşitlik 1)}$$

İstatistik Analizler

Baraj göllerinde belirlenen fizikokimyasal değişkenlerin göllere göre farklılıklarını tespit etmek için parametrik olmayan testlerden Kruskal Wallis analizi uygulanmıştır. Kolmogorov Smirnov testine göre verilerin normal dağılım göstermemesi ve baraj

göllerinde belirlenen örnekleme miktarlarının eşit olmaması nedeni ile bu test seçilmiştir. Çoklu karşılaştırma testi olarak ise parametrik olmayan Dunn's testi uygulanmıştır. Her iki analiz de Past 4.03 paket programında uygulanmıştır. Baraj göllerindeki fitoplanktonun zamansal ve mekânsal değişimini belirlemek için bir ordinasyon metodu olan Eğilimsiz Uyum Analizi (Detrended Correspondance Analysis, DCA) uygulanmıştır. Bu analizde taksonların nispi bollukları kullanılmış ve nadir taksonlar analizden çıkarılmıştır. DCA analizinde CANOCO 4.5 paket programı kullanılmıştır.

BULGULAR ve TARTIŞMA

Çalışma dönemi boyunca örnekleme noktalarında ölçülen bazı fizikokimyasal değişkenlerin tanımlayıcı istatistikleri Çizelge 1'de verilmiştir. Her üç baraj gölünde de hafif alkaliden bazik karaktere doğru bir pH gözlenirken, ortalama Eİ değerlerinin 1000 µS/cm'in üzerinde olduğu belirlenmiştir. Kruskal-Wallis analizi sonuçları NO_3-N (*H*: 6.99; *p*: 0.030) ve NH_4-N'in (*H*: 8.65; *p*: 0.013) baraj göllerinde istatistiksel olarak anlamlı farklılık gösterdigi tespit etmiştir (Çizelge 2, Şekil 5). NH_4-N Sarıyar Barajı'ndan Yenice Barajı'na doğru anlamlı bir azalma gösterirken, NO_3-N ise istatistiksel olarak anlamlı bir artış göstermiştir. Yıllık ortalama NO_3-N değerleri Sarıyar Barajı'nda 2.66±1.13, Gökçekaya ve Yenice barajları ise sırasıyla 3.77±1.2 ve 3.18±1.2 mg/L olarak tespit edilmiştir. NH_4-N Sarıyar Barajı'nda 0.70±0.57, Gökçekeya'da 0.27±0.12 ve Yenice'de ise 0.35±0.22 mg/L olarak belirlenmiştir. NO_2-N Sarıyar'da 0.14±1.14,Gökçekeaya'da 0.1±0.53 Yenice ise 0.1±0.04 mg/L olarak tespit edilmiştir.. PO_4-P değerlerinin yıllık ortalaması her üç baraj gölünde de 0.45 mg/'nin üzerinde tespit edilmiştir. (Çizelge 2). Fitoplankton örneklemesinin yapıldığı Ağustos ayında ÇO konsantrasyonunun Sarıyar ve Yenice Baraj göllerinde 16 mg/L'nin üstüne çıktığı, her üç baraj gölünde de Ekim ve Aralık aylarında 3 mg/L'nin altına düştüğü belirlenmiştir. Ağustos ayında uygun su sıcaklığı ve besin tuzu varlığına bağlı olarak fitoplanktonun çoğalması oksijen doygunluğunun %200 seviyelerine çıkmasının nedeni olabilir. Ekim-Aralık 2106 tarihlerinde su seviyesinin düşmesi, azalan sıcaklık, fitoplankton fotosentez oranının düşmesi, yağışların azalması ve artan oksidasyonun ÇO konsantrasyonunun bazı noktalarda düşmesine neden olmuş olabilir. Mart 2017 tarihinde ise ÇO derişimi Sarıyar ve Gökçekaya barajlarında 15 mg/L'nin üzerine çıkmıştır. Sarıyar Barajı'na özellikle Ankara tarafından yoğun atık girdisi olmaktadır. Ankara ve Porsuk çayları Ankara ve Eskişehir illerinin kirlilik yükünü Sakarya nehrine, buradan da Sarıyar Barajına taşımaktadır. Sarıyar barajını besleyen önemli akarsulardan biri de Kirmir Çayı'dır. Bu akarsularda oluşan organik ve inorganik kirlilik birbiri ile bağlantılı bu üç baraj gölünde kirlilik yükünün yıllara bağlı olarak giderek artmasına neden olmaktadır.

Çizelge 2. Baraj göllerinde belirlenen bazı fizikokimyasal değişkenlerin tanımlayıcı istatistikleri (ort±SS (min-maks))

Table 2. Descriptive statistics of some physicochemical variables determined in dam lakes (mean±SD (min-max))

	n	T ºC	ÇO mg/L	pH	Eİ µS/cm
Sarıyar B.	40	18.1±6.97(4.3-29.9)	9.98±5.24 (1.3-23)	8.7±0.54(8-10.57)	1185.68±247 (802-1807)
Gökçekaya B.	10	16.38±6.36 (8.5-27)	8.58±3.02 (4.6-13.53)	8.41±0.37(7.9-9.01)	1032.80±141.57(765-1150)
Yenice B.	10	14.50±3.93 (8-20.1)	12.51±6.78 (1.6-19.9)	8.86±0.54(8.1-9.61)	1026.30±12.8(775-1148)
P(Sig)		0.226ÖD	0.230ÖD	0.142ÖD	0.158ÖD

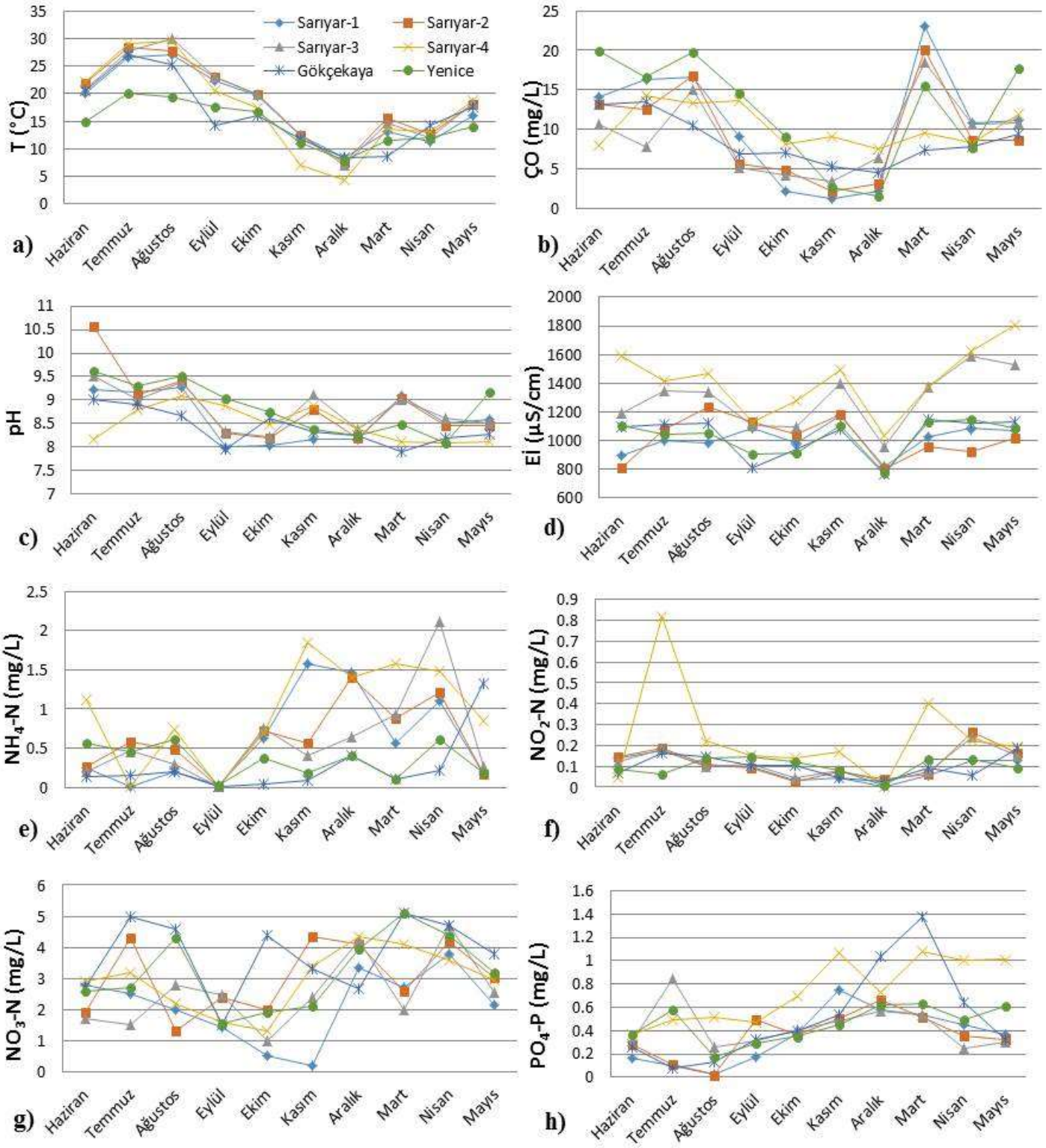
		NH_4-N mg/L	NO_2-N mg/L	NO_3-N mg/L	PO_4-P mg/L
Sarıyar B.	40	0.70^{a}±0.57 (0.005-2.12)	0.14±1.14 (0.004-0.82)	2.66^{a}±1.13 (0.2-4.7)	0.47±0.27 (0.02-1.07)
Gökçekaya B.	10	0.27^{b}±0.12 (0.03-1.33)	0.1±0.53 (0.027-0.186)	3.77^{b}±1.2 (1.5-5.1)	0.45±0.16 (0.17-0.63)
Yenice B.	10	0.35^{ab}±0.22 (0.03-0.61)	0.1±0.04 (0.01-0.14)	3.18^{ab}±1.2(1.54-5.1)	0.47±0.33 (0.08-1.04)
P(Sig)		0.015*	0.599ÖD	0.030*	0.944ÖD

*: P<0.05, ÖD: P>0.05 önemli değil (non-sig.); a ve b: Dunn's çoklu karşılaştırma testi sonuçları. Aynı harfle gösterilen veriler arasında fark yoktur.

Bu çalışmada Yenice baraj gölünde 73, Gökçekaya baraj gölünde 64 ve Sarıyar baraj gölünde 108 alg taksonu tespit edilmiştir. Üç baraj gölünde tespit edilen toplam takson sayısı ise 144'tür. Takson çeşitliliği en yüksek divizyo Chlorophyta olmuş ve üç baraj gölünde 69 takson tespit edilmiştir. İkinci en yüksek takson sayısı 38 taksonla Bacillariophyta'da tespit edilmiştir. Tespit edilen taksonlar ve kısaltmaları Çizelge 3'te verilmiştir.

Tespit edilen taksonlardan 28 tanesi her üç baraj gölünde de belirlenmiş olup büyük kısmı kozmopolit ve Türkiye'de yaygın olarak bulunan türlerdir. Baraj gölü sistemindeki 144 taksonun 41 tanesi sadece Sarıyar baraj gölünde, 16 tanesi sadece Gökçekaya baraj

gölünde ve 11 tanesi sadece Yenice Baraj gölünde tespit edilmiştir. Her üç baraj gölünde de Chlorophyta filumunun en yüksek takson sayısına sahip olduğu, bu divizyoyu Bacillariophyta'nın izlediği görülmektedir (Şekil 6).



Şekil 5. Ölçülen fizikokimyasal değişkenlerin örnekleme noktalarına göre değişimi
Figure 5. Variation of measured physicochemical variables according to sampling sites

Çizelge 3. Sarıyar, Gökçekaya ve Yenice baraj göllerinde tespit edilen alg taksonları ve kısaltmaları (*Daha önce yapılan çalışmalarda bildirilen taksonlar)

*Table 3. Algal taxa and their abbreviations detected in Sarıyar, Gökçekaya, and Yenice dam lakes (*Taxa reported in previous studies)*

		Kısaltma	Sarıyar	Gökçekaya	Yenice
Empire Prokaryota					
Kingdom Eubacteria					
Subkingdom Negibacteria					
Phylum Cyanobacteria					
Class Cyanophyceae					
Aphanizomenon sp.		*Apha sp*		+	
Chroococcus minutus (Kütz.) Nageli	*	*Chr min*	+		
Cyanobium diatomicola (Geitler) Komárek, Kopeck & Cepák		*Cya dia*	+		
Leptolyngbya angustissima (West & G.S.West) Anagn.& Komárek		*Lep ang*			+
Limnococcus limneticus (Lemmerm.) Komárková, Jezberová, O.Komárek & Zapomelová	*	*Limn li*	+		
Limnothrix redekei (Goor) Meffert		*Lim red*	+	+	+
Lyngbya sp.		*Lyng sp*	+		
Microcystis aeruginosa (Kützing) Kützing	*	*Mic aer*	+		
Oscillatoria tenuis C.Agardh ex Gomont	*	*Osc ten*	+		
Phormidium sp.		*Phor sp.*	+		
Planktothrix agardhii (Gomont) Anagn.&Komárek		*Pla aga*	+	+	+
Planktothrix isothrix (Skuja) Komárek & Komárková		*Pla iso*	+		
Plectonema sp.		*Plec sp*	+		
Pseudanabaena catenata Lauterborn		*Pse cat*			+
Pseudanabaena limnetica (Lemmerm.) Komárek	*	*Pse lim*	+		
Synechocystis aquatilis Sauvageau		*Syn aqua*	+		+
Empire Eukaryota					
Kingdom Chromista					
Phylum Bacillariophyta					
Subphylum Bacillariophytina					
Class Bacillariophyceae					
Subclass Bacillariophycidae					
Amphora ovalis (Kütz.) Kütz.	*	*Amp ova*		+	+
Amphora pediculus (Kütz.) Grunow ex A.Schmidt	*	*Amp ped*		+	
Cocconeis placentula Ehrenb.	*	*Coc pla*		+	
Craticula accommodate (Hustedt) D.G.Mann		*Cra acc*	+	+	
Craticula ambigua (Ehrenb.) D.G.Mann		*Cra amb*	+		
Fallacia pygmaea (Kütz.) A.J.Stickle&D.G.Mann		*Fal pyg*	+		
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski		*Hip hun*		+	
Navicula cryptocephala Kütz.	*	*Nav cry*		+	
Navicula sp.		*Nav sp*	+		+
Navicula tripunctata (O.F.Müller) Bory de Saint-Vincent	*	*Nav tri*		+	
Navicula veneta Kütz.	*	*Nav ven*	+	+	
Nitzschia acicularis (Kütz.) W.Sm.		*Nit aci*	+	+	+
Nitzschia amphibia Grunow		*Nit amp*	+	+	
Nitzschia dissipata (Kütz.) Grunow	*	*Nit dis*		+	
Nitzschia fonticola (Grunow) Grunow		*Nit fon*	+		
Nitzschia frustulum (Kützing) Grunow		*Nit fru*	+	+	+
Nitzschia gracilis Hanzsch		*Nit gra*	+		+
Nitzschia linearis (Agardh) W.Sm.	*	*Nit lin*		+	
Nitzschia palea (Kütz.) W.Sm.	*	*Nit pal*	+	+	+
Nitzschia paleacea (Grunow) Grunow		*Nit ple*	+	+	+
Nitzschia vermicularis (Kützing) Hantzsch	*	*Nit ver*	+		
Nitzschia umbonata (Ehrenberg) Lange-Bertalot		*Nit umb*	+		+

Pinnularia brebissonii (Kützing) Rabenhorst	*	*Pin bre*	+		
Tryblionella apiculata Gregory		*Try api*	+		
Subclass Fragilariophycidae					
Diatoma tenuis C. Agardh	*	*Dia ten*	+		+
Fragilaria capucina Desm.	*	*Fra cap*		+	
Fragilaria capucina var. *vaucheriae* (Kütz.) Lange-Bert.	*	*Fra va*			+
Fragilaria sp.		*Fra sp*	+		
Fragilaria tenera (W.Smith) Lange-Bert.		*Fra ten*			+
Fragilaria crotonensis Kitton		*Fra cro*	+		
Ulnaria acus (Kütz.) M.Aboal	*	*Uln acu*	+	+	+
Ulnaria danica (Kütz.) Compère & Bukhtiyarova		*Uln dan*	+	+	+
Ulnaria delicatissima (W.Smith) M.Aboal & P.C.Silva		*Uln del*	+	+	+
Ulnaria ulna (Nitzsch) P.Compère.	*	*Uln uln*	+	+	+
Class Mediophyceae					
Cyclotella meneghiniana Kütz.	*	*Cyc men*	+	+	+
Cyclotella sp.		*Cyc sp*	+	+	+
Stephanodiscus sp.		*Step sp*	+	+	+
Subphylum Coscinodiscophytina					
Class Coscinodiscophyceae					
Aulacoseira granulata (Ehrenb.) Simonsen	*	*Aul gra*	+	+	
Phylum Cryptophyta					
Class Cryptophyceae					
Cryptomonas ovata Ehrenb.		*Cry ova*	+	+	+
Cryptomonas erosa Ehrenberg		*Cry ero*	+	+	+
Phylum Miozoa					
Subphylum Myzozoa					
Infraphylum Dinozoa					
Superclass Dinoflagellata					
Class Dinophyceae					
Diplopsalis acuta (Apstein) Entz		*Dip acu*			+
Parvodinium inconspicuous (Lemmermann) S.Carty		*Par inc*	+		
Peridiniopsis cunningtonii Lemmermann		*Per cun*	+		
Peridinium aciculiferum Lemmermann		*Per aci*	+		+
Parvodinium pusillum (Penard) S.Carty		*Par pus*			+
Tripos furca (Ehrenb.) F.Gómez		*Tri fur*	+		
Phylum Ochrophyta					
Class Chrysophyceae					
Kephyrion littorale J.W.G.Lund		*Kep lit*		+	
Ochromonas sp.		*Ochr sp*	+		+
Class Synurophyceae					
*Mallomonas*sp.		*Mall sp*	+		
Kingdom Plantae					
Phylum Charophyta					
Class Conjugatophyceae					
Closterium acutum Brébisson		*Clo acu*	+		
Cosmarium sp.		*Cosm sp*	+		+
Staurastrum gracile Ralfs ex Ralfs	*	*Sta gra*	+	+	+
Phylum Chlorophyta					
Class Chlorophyceae					
Acutodesmus acuminatus (Lagerheim) P.M.Tsarenko	*	*Acu acu*	+	+	+
Ankyra Judayi (G.M.Smith) Fott	*	*Ank jud*		+	
Asterococcus superbus (Cienkowski) Scherffel		*Ast sub*			+
Carteria sp.		*Cart sp*			+
Chlamydomonas sp.	*	*Chla sp*	+		
Chlorococcum sp.		*Chlo sp*	+		+
Coelastrum astroideum De Not.		*Coe ast*	+	+	+
Coelastrum microporum Nägeli	*	*Coe mic*	+	+	+
Coenococcus planctonicus Korshikov		*Coen pl*		+	

Coenocystis planktonic var. *hercynica* (H.Henig) Fott		*Coe her*	+		
Coenocystis planctonica Korshikov		*Coe pla*	+	+	
Comasiella arcuata (Lemmermann) E.Hegewald, M.Wolf, Al.Keller, Friedl & Krienitz	*	*Com arc*	+	+	+
Comasiella arcuata var. *platydisca* (G.M.Smith) E.Hegewald & M.Wolf	*	*Com pla*	+	+	
Desmodesmus armatus (R.Chodat) E.Hegewald	*	Des arm		+	
Desmodesmus armatus var. longispina (Chodat) E.Hegewald		*Des lon*	+	+	+
Desmodesmus bicaudatus (Dedusenko) P.M.Tsarenko	*	*Des bic*			+
Desmodesmus communis (E.Hegewald) E.Hegewald	*	Des com	+		+
Desmodesmus intermedius (Chodat) E.Hegewald		*Des int*	+		+
Desmodesmus magnus (Meyen) Tsarenko		*Des mag*	+		+
Desmodesmus protuberans (F.E.Fritsch & M.F.Rich) E.Hegewald		*Des pro*	+		
Desmodesmus opoliensis var. *mononensis* (Chodat) E.Hegewald		*Des mon*	+		+
Dimorphococcus lunatus A.Braun	*	*Dim lun*	+	+	
Eudorina elegans Ehrenb.	*	*Eud ele*	+		
Fusola viridis J.W.Snow		*Fus vir*	+		
Golenkinia radiata Chodat		*Gol rad*	+		
Microspora sp.		*Micr sp*		+	
Monoraphidium arcuatum (Kors.) Hind.		*Mon arc*		+	+
Monoraphidium contortum (Thuret) Komárk.-Legn.	*	*Mon con*	+	+	+
Monoraphidium griffithii (Berkeley) Komárková-Legnerová		*Mon gri*	+	+	+
Monoraphidium komarkovae Nygaard		*Mon kom*	+	+	+
Monoraphidium minutum (Nägeli) Komárková-Legnerová		*Mon min*			+
Monoraphidium tortile (W.West&G.S.West) Komárk.-Legn.		*Mon tor*		+	
Mychonastes jurisii (Hindák) Krienitz, C.Bock, Dadheech & Proschold		*Myc jur*	+		+
Pandorina morum (O.F.Müll.) Bory	*	*Pan mor*	+	+	
Pediastrum boryanum (Turpin) Menegh.	*	*Ped bor*	+	+	+
Pediastrum duplex Meyen	*	*Ped dup*		+	
Pediastrum integrum Nägeli		*Ped int*	+		
Pseudoschroederia robusta (Korshikov) E.Hegewald & E.Schnepf		*Pse rob*		+	
*Pteromonas aequiciliata (*Gicklhorn) Chodat		*Pte aeq*	+	+	+
Quadrigula sp.		*Quad sp*		+	
Radiococcus planktonic J.W.G.Lund		*Rad pla*	+		+
Sphaerellopsis sp.		*Spha sp*	+		
Sphaerocystis schroeteri Chodat	*	*Sph sch*			+
Tetradesmus bernardii (G.M.Smith) M.J.Wynne	*	*Tet ber*	+	+	
Tetradesmus dimorphus (Turpin) M.J.Wynne		*Tet dim*	+	+	+
Tetraedron minimum (A.Braun) Hansg.	*	*Tetr mi*	+		+
Tetraëdron triangulare Korshikov	*	*Tetr tr*	+		+
Tetrastrum staurogeniiforme (Schröder) Lemmermann		*The stau*	+		+
Treubaria triappendiculata C.Bernard		*The tri*	+		+
Volvox sp.		*Volv sp*		+	
Westella botryoides (W.West) De Wildeman		*Wes bot*	+	+	+
Willea crucifer (Wolle) D.M.John, M.J.Wynne & P.M.Tsarenko		*Wil cru*	+		
Class Trebouxiophyceae					
Actinastrum hantzschii Lagerheim	*	*Act han*	+	+	+
Chlorella vulgaris Beij.	*	*Chl vul*	+	+	+

Closteriopsis acicularis (Chodat) J.H.Belcher & Swale		*Clos ac*	+		+
Crucigenia tetrapedia (Kirchner) Kuntze		*Cru ted*	+		
Dictyosphaerium pulchellum H.C.Wood	*	*Dic pul*	+	+	+
Lagerheimia subsalsa Lemmermann		*Lag sub*	+		+
Lemmermannia komarekii (Hindák) C.Bock & Krienitz in Bock et al.		*Lem kom*			+
Micractinium belenophorum (Korshikov) T.Proschold, C.Block, W.Luo & L.Kreinitz		*Mcr bel*			+
Oocystis borgei J.Snow	*	*Ooc bor*	+		+
Oocystis angelic A.Braun		*Ooc nae*		+	
Oocystis parva West & G.S.West	*	*Ooc par*	+		+
Oocystis solitary Wittrock	*	*Ooc sol*	+	+	+
Schizochlamydella solitaria (G.M.Smith) B.Fott		*Sch sol*	+		
Stichococcus bacillaris Nägeli		*Sti bac*	+		
Stichococcus variables West & G.S.West		*Sti var*	+		
Trochiscia reticularis (Reinsch) Hansgirg		*Tro ret*	+		+
Trochiscia sp.		*Tro sp*		+	+
Kingdom Protozoa					
Phylum Euglenozoa					
Class Euglenophyceae					
Euglena sp.		*Eugl sp*	+		
Euglenaformis proxima (Dangeard) M.S.Bennett & Triemer		*Eug pro*	+		
Lepocinclis fusiformis (H.J.Carter) Lemmermann		*Lep fus*	+		
Lepocinclis ovum (Ehr.) Lemm.		*Lep over*	+		
Phacus sp.		*Phac sp*	+		+
Trachelomonas hispida (Perty) F.Stein		*Tra his*	+		
Trachelomonas volvocina Ehrenb.		*Tra vol*	+		

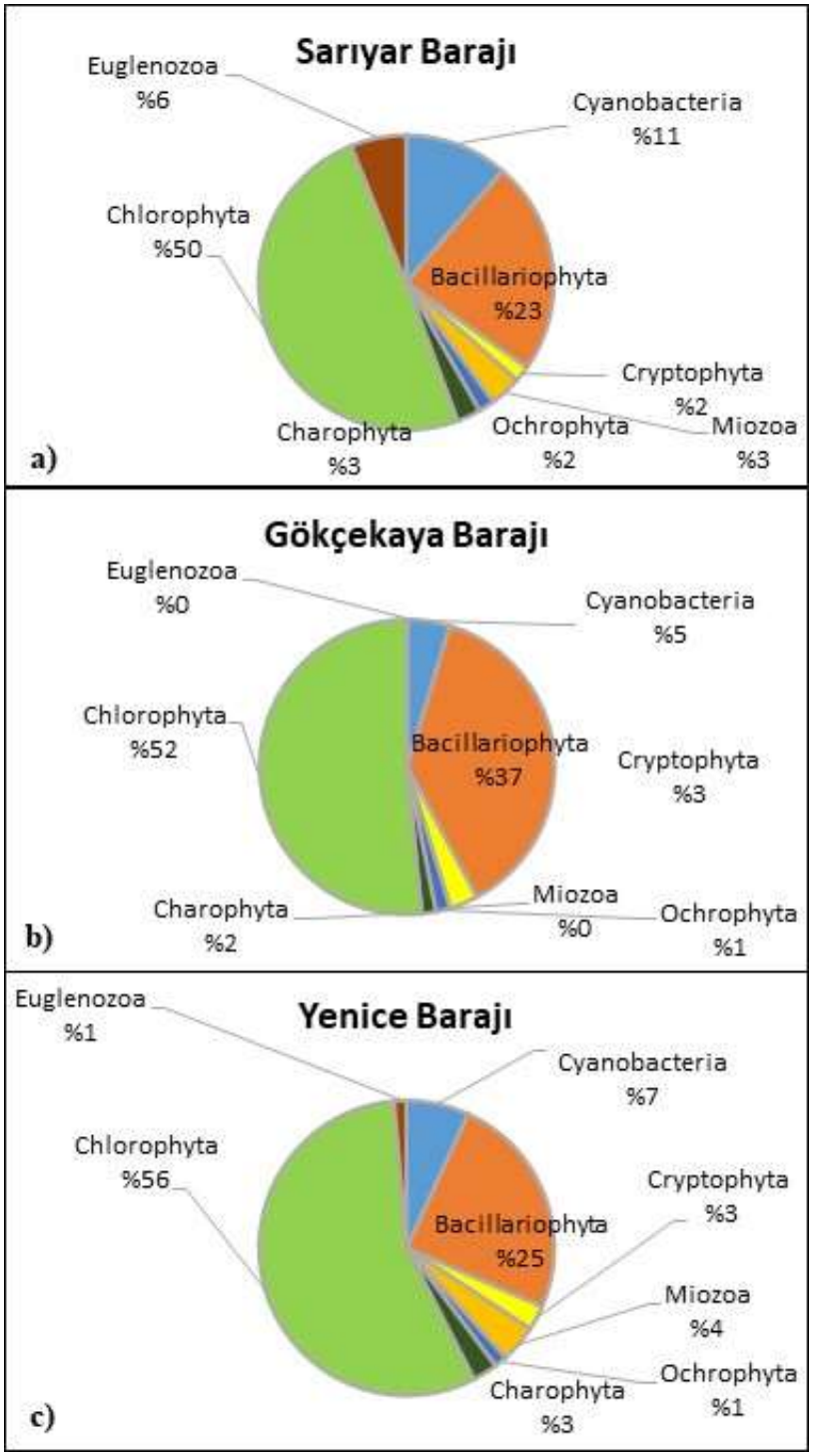
Chlorophyta, Charophyta ve Cyanobacteria, divizyosundan tespit edilen taksonların büyük kısmının planktonda yaygın olarak belirlenen taksonlardır. Ancak Bacillariophyta divizyosundan Bacillariophycidae altsınıfından tespit edilen taksonlar, fitoplanktondan çok genellikle akarsu ve göllerin bentik bölgeleri ile ilişkilendirilirler. Yapılan çeşitli çalışmalarda da tikoplanktonik diyatome türleri fitoplanktonda tespit edilmiştir (Gürbüz, 2020; Morkoyunlu Yüce & Aktaş, 2020; Aksoy & Soylu, 2023). Örnekleme noktalarından 5. istasyonun Kirmir Çayı girişine, 4. istasyonun ise Sakarya nehri girişine yakın olması bu akarsulardan sürüklenme yolu ile bentik diyatomelerin yoğun bir şekilde taşınmasına neden olmuş olabilir.

DCA analizi sonunda ilk iki eksenin gradient uzunlukları sırasıyla 5.304 ve 3.951 olarak belirlenmiştir. İlk iki eksenin gradient uzunluğunun >3'ten büyük olması (Ter Braak & Prentice, 1988) veri setinin unimodal metodlara uygun olduğunu işaret eder. DCA analizi sonucunda örnekleme noktalarının (Şekil 7) ve alg taksonlarının (Şekil 8) 4 farklı grupta toplandığı görülmektedir. DCA diagramına göre (Yenice baraj gölü Ağustos 2016 örnekleme noktaları A grubunda toplanmıştır (Şekil 7). Bu örnekleme noktalarını temsil eden taksonlar Şekil 8'de A grubunda görülmektedir. B grubunda ise Sarıyar Baraj gölünde Ağustos 2016 tarihinde örneklenen örnekleme noktalarının kümelendiği görülmektedir (Şekil 8). D grubunda Gökçekaya baraj gölünde Ağustos 2016 tarihinde örneklenen noktalar gruplanmıştır. C grubunda ise her üç baraj gölünün Kasım 2016 tarihinde örnekleme yapılan örnekleme noktaları kümelenmiştir. Bu bulgular bize her üç baraj gölünün ortak alg taksonları içermelerine rağmen özelikle Ağustos 2016 tarihinde her üç baraj gölünde takson çeşitliliğinin farklı olduğuna işaret etmektedir. Ancak Kasım 2016 tarihinde baraj göllerindeki alg çeşitliliğinin benzer olduğunu göstermektedir.

Şekil 8'e bakıldığında Şekil 7'den farklı olarak alg taksonlarının 3 ana grupta toplandığı görülmektedir. A grubu Yenice Baraj gölünde Ağustos 2016 tarihinde örneklenen örnekleme noktalarındaki baskın taksonları gösteriken D grubu da Gökçekaya baraj gölünde Ağustos 2016 tarihinde örneklenen noktalarda tespit edilen alg taksonları kümelenmiştir. B ve C grubu ise belirgin olarak ayrılmamakla birlikte alt bölümde Sarıyar baraj gölünde baskın olan taksonların kümelendiği, üst bölümde ise her üç baraj gölünde Kasım ayında baskın olan taksonların kümelendiği görülmektedir.

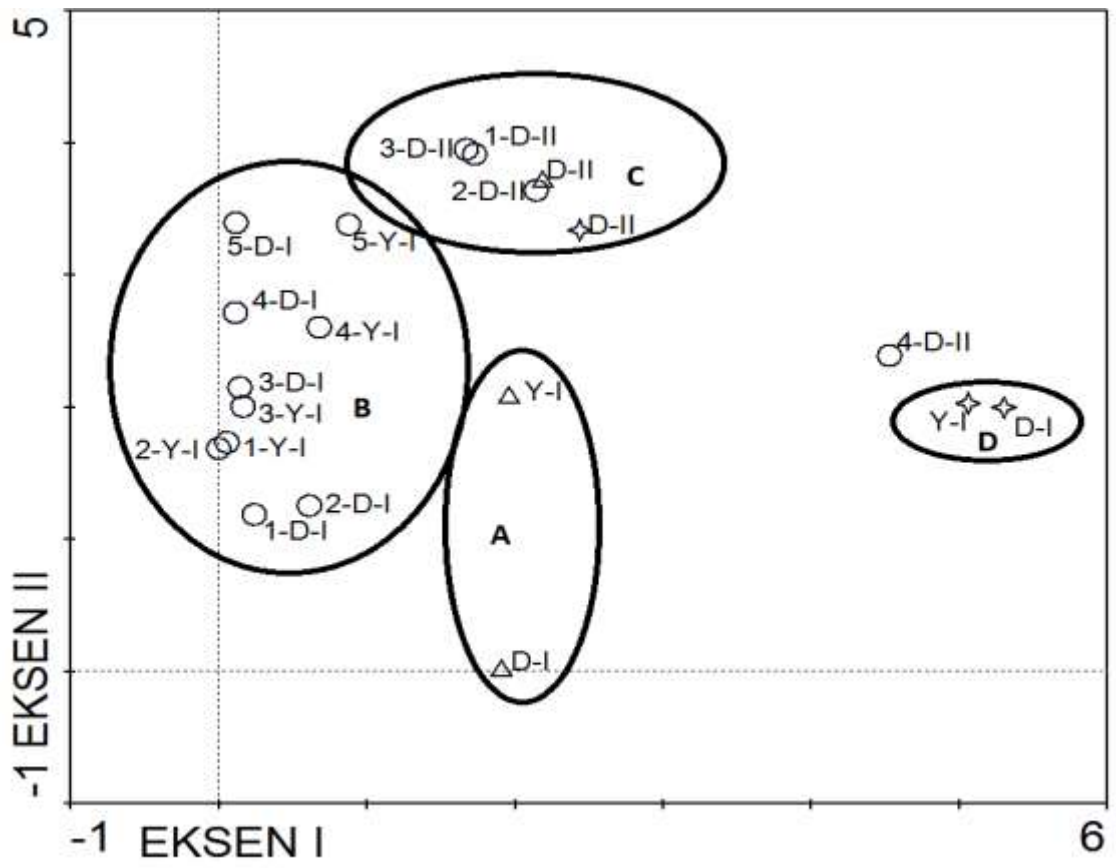
Baraj göllerinde tespit edilen fitoplankton taksonları daha önce yapılan çalışmalar ile karşılaştırıldığında (Atıcı 1999, 2002a, 2002b, 2002c, 2004; Atıcı & Obalı 2006; Atıcı ve ark., 2008; Akın ve ark., 2008, 2010; Atıcı & Katırcıoğlu, 2009; Dokcan, 2010) üç baraj gölünde tespit edilen taksonlardan 49'unun daha önce yapılan çalışmalarda tespit edildiği, 95 taksonun ise birbiri ile

bağlantılı bu üç baraj gölü sistemi için yeni kayıt olduğu görülmektedir (Çizelge 3). Tespit edilen bu taksonlardan bazıları ülkemizde yaygın olarak bulunan taksonlar olarak bilinirler.



Şekil 6. Baraj göllerinde tespit edilen taksonların filumlara göre dağılımları.
Figure 6. Distribution of taxa identified in dam lakes according to phyla.

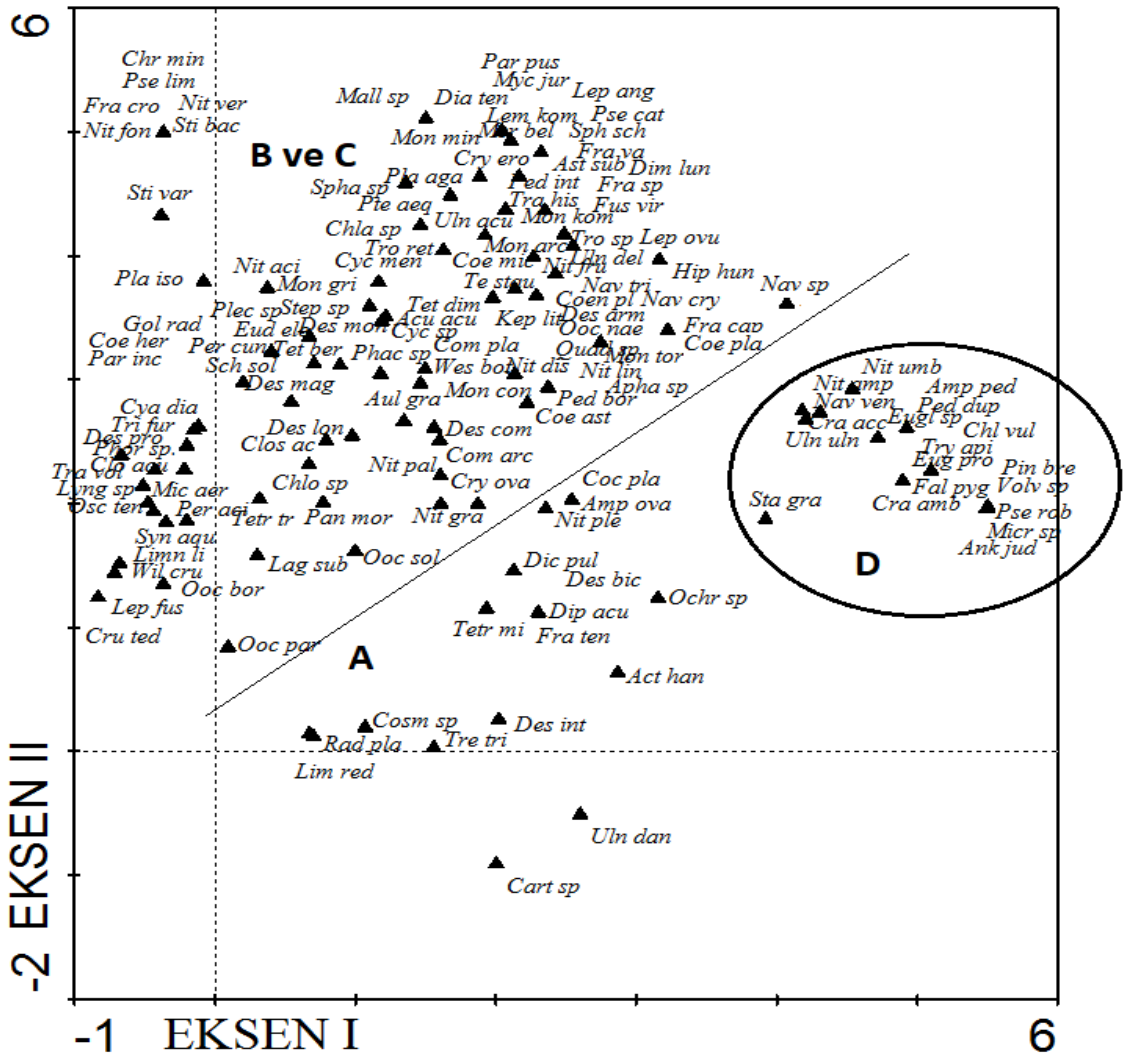
Fitoplankton lentik su kütlelerinde su kalitesinin biyolojik olarak belirlenmesinde kullanılan en önemli canlı gruplarından bir tanesidir. Standardize edilmiş PTI indeksi sonuçları (Şekil 9) incelendiğinde, her üç baraj gölünde de indeks sonuçlarının 0.2 - 0.4 aralığında olduğu tespit edilmiştir. Bu değerlerin su kalite sınıflarına göre karşılığı zayıf su kalitesidir. Çelekli ve Öztürk (2014) yaptıkları çalışmada PTI'nın biyolojik olarak su kalitesini belirlemek için uygun bir fitoplankton metriği olduğunu tespit etmişlerdir. Yine yapılan bazı çalışmalar PTI indeksinin barajların ekolojik kalitesinin belirlenmesi için uygun bir indeks olduğunu ortaya çıkarmıştır (Çelekli ve ark., 2018).



Şekil 7. Baraj Göllerinde tespit edilen alg taksonların örnekleme noktalarına göre dağılımı. (üçgen: Yenice Baraj gölü, daire: Sarıyar Baraj gölü, Yıldız: Gökçekaya baraj gölü, Y: Yüzey örnekleme, D: Dikey örnekleme, I: Birinci örnekleme dönemi (Ağustos 2016), II: İkinci örnekleme dönemi (Kasım 2016).

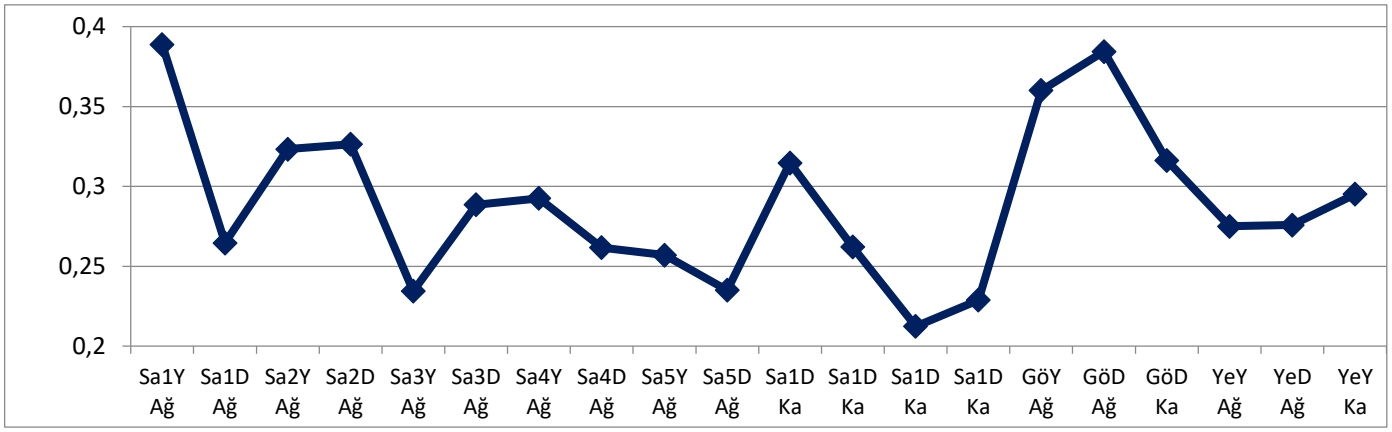
Figure 7. Distribution of algal taxa identified in dam lakes according to sampling sites (triangle: Yenice Dam reservoir, circle: Sarıyar Dam reservoir, Star: Gökçekaya reservoir, Y: Surface sampling, D: Vertical sampling, I: First sampling period (August 2016), II: Second sampling period (November 2016).

Kafes balıkçılığında balık ölümlerinin en çok gözlendiği dönemler su sıcaklığının yükseldiği, çözünmüş oksijenin düştüğü, azalan yağışlar nedeni ile su seviyesinin düşük seyrettiği ve buna bağlı olarak fizikokimyasal kirleticilerin derişiminin artış gösterdiği yaz ve sonbahar aylarıdır. Yaz aylarında gözlenen bu faktörler aynı zamanda siyanobakteriyel çoğalmalara çok iyi bir zemin oluşturmaktadır. Üç baraj gölünde de Cyanobacteria filumu'nun takson çeşitliliği yüksek bulunmamıştır (Şekil 4). Ancak bazı taksonların nispi bolluklarının yüksek olduğu görülmüştür. Bu Cyanobacteria taksonları siyanotoksin üretme potansiyeline sahip olan taksonlar olmaları (Boophati & Ki, 2014) sebebi ile önem arz etmektedir. Küçük boyuta sahip olan siyanobakter Synechocystis aquatilis, Sarıyar baraj gölünde Ağustos 2016 tarihinde tüm istasyonlarda gözlenmiş ve nispi bolluğu % 40'lara kadar çıkmıştır. Ayrıca *Limnothrix redekei* Sarıyar Baraj gölünde Ağustos 2016 tarihinde ilk üç istasyonda görülmüş ve nispi bolluğu % 40'lara kadar çıkmıştır. Oscillatoria tenuis ise yine Sarıyar baraj gölünde Ağustos 2016'da gözlenmiş ve nispi bolluğu % 20'lere kadar çıkmıştır. *Planktothrix agardhii* ve Sarıyar Barajı'nda Ağustos 2016'da sadece 4. ve 5. örnekleme noktasında tespit edilirken Kasım 2016'da her üç baraj gölünde de gözlenmiş ve bu ayda nispi bolluğu % 20'lere ulaşmıştır. *Planktothrix isotrix* ise sadece Sarıyar Baraj gölünde tespit edilmiş ve Ağustos 2016'da 4. ve 5. örnekleme noktalarında nispi bolluğu % 20'nin üzerine çıkmıştır. Sarıyar baraj gölünde *Microcystis aeruginosa* türünde tespit edilmiş Ağustos 2016'da gözlenen bu tür sadece birkaç örnekleme noktasında düşük sayılarda tespit edilmiştir. Daha önce yapılan çalışmalarda Sarıyar Baraj Gölü'nde bu türün varlığı tespit edilmiştir (Atıcı 1999, 2004). *Aphanizomenon* sp. ise sadece Gökçekaya baraj gölünde Kasım 2016'da az sayıda tespit edilmiştir. *Planktothrix agardhii*, *Microcystis aeruginosa* ve *Aphanizomenon* sp. Köker ve ark. (2017) tarafından hiperötrofik ve/veya ötrofik su kütlelerinde aşırı çoğalmalar yapan toksin üretme potansiyeline sahip taksonlar olarak tanımlanmıştır.



Şekil 8. Baraj göllerinde tespit edilen alg taksonlarının nispi bolluklarına göre ordinasyon grafiği (Taksonların kısaltmaları Çizelge 2'de verilmiştir).

Figure 8. Ordination graph according to the relative abundance of algal taxa identified in the dam lakes. (Abbreviations are given in Table 2).



Şekil 9. Standardize edilmiş PTI indeksi sonuçları (Sa: Sarıyar, Gö: Gökçekaya, Ye: Yenice, Y: yüzey, D: dikey, Ağ: Ağustos, Ka: Kasım)

Figure 9. Standardized PTI index results (Sa: Sarıyar, Go: Gökçekaya, Ye: Yenice, Y: surface, D: vertical, Ağ: August, Ka: November)

SONUÇ ve ÖNERİLER

Baraj göllerinde tespit edilen *M. aeruginosa*, mikrosistin adlı birçok varyantı bulunan hepatotoksin üretme potansiyeline sahip bir siyanobakteri türüdür (Boophati & Ki, 2014). *Synechocystis aquatilis* türünün de mikrosistin ürettiği (Magalhães ve ark., 2003) bilinmektedir. *Aphanizomenon*, *Dolichospermum* ve *Oscillatoria* cinslerine ait bazı türlerin mikrosistin, anatoksin, saksitoksin gibi birçok farklı toksini ürettikleri bildirilmektedir (Boophati & Ki, 2014). Yukarıda söz edilen Cyanobacteria taksonlarının toksin üretebilme potansiyeli olduğu için özellikle Sarıyar Baraj gölünün siyanotoksin ve toksin üreten taksonlar açısından izlenmesi gerektiği sonucuna varılmıştır.

Daha önceki su kalitesi ile ilgili yapılan çalışmalar (Atıcı ve ark., 2008; Akın ve ark., 2011) ve bu çalışmada elde edilen veriler göz önüne alındığında her üç baraj gölünün de Alabalık gibi kirliliğe tolerans göstermeyen balık yetiştiriciliği için uygun olmadığı sonucuna varılmıştır. PTI indeksi sonuçları da bu bulguyu desteklemektedir. Ancak sazan gibi daha toleranslı türlerin üretiminde baraj gölleri kullanılabilir. Baraj göllerinin rehabilitesi için özellikle Sarıyar barajına kesinlikle atık girişine engel olacak tedbirler alınmalıdır. Bunun için kirli suların arıtımı için gerekli arıtma faaliyetlerinin uygulanması gerekmektedir.

TEŞEKKÜR

Bu çalışma TAGEM Sarıyar, Gökçekaya ve Yenice Baraj Gölleri'nin (Eskişehir, Ankara) Trofik Seviyesi ve Alabalık Yetiştiriciliği Açısından İncelenmesi (Proje No: TAGEM/HAYSÜD/2017/A11/P-03/3) adlı proje kapsamında gerçekleştirilmiştir. Bu proje çalışmasının arazi ve laboratuvar çalışmalarında katkı sağlayan tüm arkadaşlara teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

Akın, B. S., Atıcı, T., Katırcıoğlu, H., & Keskin, F. (2011). Investigation of water quality on Gökcekaya Dam Lake using multivariate statistical analysis, in Eskişehir, Turkey. *Environmental Earth Sciences, 63*, 1251–1261. [DOI: 10.1007/s12665-010-0798-6]

Akın, B. S., Atıcı, T., & Katırcıoğlu, H. (2008). Physicochemical, toxicological, and ecological analysis of Gökçekaya Dam Lake. *African Journal of Biotechnology, 7*(4), 444–449.

Aksoy, A., & Soylu, E. N. (2023). Atasu Barajı (Trabzon, Türkiye) Alglerinin Mevsimsel Değişimi Üzerine Bir Araştırma. *Journal of Anatolian Environmental and Animal Sciences, 8*(2), 239–246. https://doi.org/10.35229/jaes.1274392

APHA. (1998). *Standards methods for the examination of water and wastewater* (20th ed.). American Public Health Association.

Atıcı, T. (1999). Sarıyar Baraj Gölü (Ankara) Fitoplanktonunun Floristik ve Ekolojik Yönden İncelenmesi (Tez no: 85928) [Doktora tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Atıcı, T. (2002a). Nineteen new records from Sariyar Dam Reservoir phytoplankton for Turkish freshwater algae. *Turkish Journal of Botany, 26*, 485–490.

Atıcı, T. (2002b). Sarıyar Barajı Planktonik Algleri Kısım: II- Chlorophyta. *SDÜ. Eğirdir Su Ürünleri Fakültesi Dergisi, 8*, 128–151.

Atıcı, T. (2002c). Sarıyar Barajı Planktonik Algleri Kısım: III- Bacillariophyta. *SDÜ. Eğirdir Su Ürünleri Fakültesi Dergisi, 8*, 1–25.

Atıcı, T. (2004). Sarıyar Barajı Planktonik Algleri Kısım: I- Cyanophyta. *SDÜ. Eğirdir Su Ürünleri Fakültesi Dergisi, 2*(12), 88–98.

Atıcı, T., & Katırcıoğlu, H. (2009). Sarıyar Barajının (Ankara) bazı fiziksel, kimyasal ve biyolojik özellikleri. *Hacettepe Üniversitesi Çevre Bilimleri Dergisi, 8*, 17–25.

Atıcı, T., & Obalı, O. (2006). Seasonal variation of phytoplankton value of chlorophyll a in the Sariyar Dam Reservoir (Ankara, Turkey). *Turkish Journal of Botany, 30*, 349–357.

Atıcı, T., Ahıska, S., Altındağ, A., & Aydın, D. (2010). Ecological effects of some heavy metals (Cd, Pb, Hg, Cr) pollution of phytoplanktonic algae and zooplanktonic organisms in Sarıyar Dam Reservoir in Turkey. *African Journal of Biotechnology, 7*(12), 1972–1977. [DOI: 10.5897/AJB2008.000-5044](DOI: 10.5897/AJB2008.000-5044)

Boopathi, T., & Ki, J. S. (2014). Impact of environmental factors on the regulation of cyanotoxin production. *Toxins, 6*(7), 1951–1978. doi:10.3390/toxins6071951

Celekli, A., & Öztürk, B. (2014). Determination of ecological status and ecological preferences of phytoplankton using multivariate approach in a Mediterranean reservoir. *Hydrobiologia, 740*, 115–135. [DOI: 10.1007/s10750-014-1948-8](DOI: 10.1007/s10750-014-1948-8)

Çelekli, A., Toudjani, A. A., Lekesiz, H. Ö., Kayhan, S., & Çetin, T. (2018). Bioassessment of ecological

status of three Aegean reservoirs based on phytoplankton metrics. *Turkish Journal of Water Science and Management, 2*(1), 76–99. https://doi.org/10.31807/tjwsm.363608

Dokcan, Ş. (2010). Ankara, Sarıyar Baraj Gölü Bentik Algleri(Tez no: 251382) [Yüksek lisans tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Gürbüz, O. A. (2020). Phytoplankton composition of Hirfanlı Dam Lake (Kırşehir-Turkey). *Eurasian Journal of Biological and Chemical Sciences, 3*(Suppl 1), 169–174. https://doi.org/10.46239/ejbcs.733774

Huber-Pestalozzi, G. (1961). *Das Phytoplankton des Sübwassers. 5. Teil. Band XVI. Chlorophyceae, Volvocales*. Stuttgart.

John, D. M., Whitton, B. A., & Brook, A. J. (2003). *The freshwater algal flora of the British Isles*. Cambridge University Press.

Komárek, J., & Anagnostidis, K. (1999). *Sübwasserflora von Mitteleuropa, Cyanoprokaryota 1. Teil: Chroococcales*. Gustav Fischer.

Köker, L., Akçaalan, R., Oğuz, A., Gaygusuz, Ö., Gurevin, C., Akat Kose, C., Gucver, S., Karaaslan, Y., Ertürk, A., Albay, M., & Kinaci, C. (2017). Distribution of toxic cyanobacteria and cyanotoxins in Turkish waterbodies. *Journal of Environmental Protection and Ecology, 18*(2), 425–432.

Köse, E., Emiroğlu, Ö., Çiçek, A., Aksu, S., Başkurt, S., Tokatlı, C., Şahin, M., & Uğurluoğlu, A. (2020). Assessment of ecologic quality in terms of heavy metal concentrations in sediment and fish on Sakarya River and Dam Lakes, Turkey. *Soil and Sediment Contamination: An International Journal, 29*(3), 292–303. DOI:10.1080/15320383.2019.1705755

Krammer, K., & Lange-Bertalot, H. (1991a). *Süßwasserflora von Mitteleuropa, Bacillariophyceae 3 Teil: Centrales, Fragilariaceae, Eunotiaceae*. Gustav Fischer.

Krammer, K., & Lange-Bertalot, H. (1991b). *Süßwasserflora von Mitteleuropa, Bacillariophyceae 4 Teil: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema*. Gesamtliteraturverzeichnis. Gustav Fischer.

Krammer, K., & Lange-Bertalot, H. (1997a). *Süßwasserflora von Mitteleuropa, Bacillariophyceae 1 Teil: Naviculaceae*. Spektrum Akademischer Verlag.

Krammer, K., & Lange-Bertalot, H. (1997b). *Süßwasserflora von Mitteleuropa, Bacillariophyceae 2 Teil: Bacillariaceae, Epithemiaceae, Surirellaceae*. Spektrum Akademischer Verlag.

Magalhães, V. F., Marinho, M. M., Domingos, P., Oliveira, A. C., Costa, S. M., Azevedo, L. O., & Azevedo. S. M. F. O. (2003). Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). *Toxicon, 42*(3), 289–295. doi:10.1016/S0041-0101(03)00144-2

Maraşlioğlu, F., & Gönülol, A. (2014). Phytoplankton community, functional classification, and trophic state indices of Yedikır Dam Lake (Amasya). *Journal of Biological and Environmental Sciences, 8*(24), 133–141.

Morkoyunlu Yüce, A., & Aktaş, M. (2020). Tahtalı, Davuldere ve Çayırköy Göletlerinin (Kocaeli) Algleri ve Su Kaliteleri Üzerine Bir Çalışma. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 10*(3), 1539–1550. https://doi.org/10.21597/jist.666823

Phillips, G., Lyche-Solheim, A., Skjelbred, B., Mischke, U., Drakare, S., Free, G., Ja¨rvinen, M., de Hoyos, C., Morabito, G., Poikane, S. et al. (2013). A phytoplankton trophic index to assess the status of lakes for the water framework directive. *Hydrobiologia, 704*, 75–95. DOI:10.1007/s10750-012-1390-8

Sönmez, F., Örnekçi, G. N., Koçer, M. A. T., & Uslu, A. A. (2022). A study on planktonic and epipelic algae occurring in Karakaya Dam Lake (Elazığ, Türkiye). *LimnoFish, 8*(3), 258–268. [doi: 10.17216/LimnoFish.873166]

Ter Braak, C. J. F., & Prentice, I. C. (1988). A theory of gradient analysis. *Advances in Ecological Research, 18*, 271–317. https://doi.org/10.1016/S0065-2504(08)60183-X

Varol, M. (2020). Spatio-temporal changes in surface water quality and sediment phosphorus content of a large reservoir in Turkey. *Environmental Pollution, 259*, 113860. https://doi.org/10.1016/j.envpol.2019.113860

Varol, M., Gökot, B., Bekleyen, A., & Şen, B. (2012). Spatial and temporal variations in surface water quality of the dam reservoirs in the Tigris River basin, Turkey. *Catena, 92*, 11–21. https://doi.org/10.1016/j.catena.2011.11.013

Yılmaz, N., Yardımcı, C. H., Yardımcı, R. E., & Elhag, M. (2022). An investigation on phytoplankton composition and bacterial load relationship in a drinking water resource: Büyükçekmece Dam Lake (Istanbul, Türkiye). *Desalination Water Treatment, 273*, 203–211. [doi: 10.5004/dwt.2022.28873]

KSÜ Tarım ve Doğa Derg 27 (4), 817-827, 2024
KSU J. Agric Nat 27 (4), 817-827, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1354060

In vitro Determination of Salt Stress Responses of OH×F 333 and OH×F 97 Pear Clonal Rootstocks

Elif UYDURAN[1], Bekir ŞAN[2]✉
[1,2] Isparta University of Applied Sciences, Agriculture Faculty, Horticulture Department, 32260, Isparta, Türkiye,
[1]https://orcid.org/0009-0003-3781-2205, [2]https://orcid.org/0000-0001-6483-8433
✉: bekirsan@isparta.edu.tr

ABSTRACT
In this study, the responses of OH×F 333 and OH×F 97 pear clonal rootstocks to salt stress were investigated. For this purpose, in vitro, plants of OH×F 333 and OH×F 97 pear clonal rootstocks were cultured in ½ MS medium containing different concentrations of salt (0, 50, 100, 150, and 200 mM NaCl). To adapt the plants to salt stress, the doses of NaCl added to the medium were gradually increased at weekly intervals. In the experiment, in parallel with the increasing salt stress, the regeneration rate and shoot number values decreased, while the degree of damage increased significantly. It was determined that most of the shoots died in the application of 200 mM NaCl. When the effects of different salt concentrations on biochemical parameters were examined, it was determined that total phenolics, total flavonoids, proline, and soluble protein contents decreased, while lipid peroxidation increased in parallel with salt concentrations. However, it was determined that there was an increase in the total phenolic and flavonoid contents in 150 mM NaCl application.

Horticulture

Research Article

Article History
Received : 01.09.2023
Accepted : 03.12.2023

Keywords
Pear
Proline
Salt stress
Total phenolic
Total flavonoid

OH×F 333 ve OH×F 97 Armut Klon Anaçlarının Tuz Stresine Tepkilerinin in vitro Koşullarda Belirlenmesi

ÖZET
Bu çalışmada OH×F 333 ve OH×F 97 armut klon anaçlarının tuz stresine gösterdiği tepkiler araştırılmıştır. Bu amaçla OH×F 333 ve OH×F 97 armut klon anaçlarının in vitro bitkileri kademeli olarak artırılan farklı konsantrasyonlarda tuz (0, 50, 100, 150 ve 200 mM NaCl) içeren ½ MS ortamında kültüre alınmıştır. Denemede in vitro koşullarda tuz konsantrasyonları arttıkça, rejenerasyon oranı ve sürgün sayısı değerleri azalmış, zararlanma derecesi ise önemli derecede artmıştır. 200 mM NaCl uygulamasında çoğu sürgünün canlılıklarını yitirdikleri tespit edilmiştir. Farklı tuz konsantrasyonlarının biyokimyasal parametreler üzerindeki etkisi incelendiğinde toplam fenolik madde, toplam flavonoid madde, prolin ve çözünebilir protein içeriklerinin azaldığı, lipid peroksidasyon içeriğinin ise tuz konsantrasyonlarına paralel şekilde arttığı belirlenmiştir. Ancak toplam fenolik ve flavonoid madde içeriklerinde 150 mM NaCl uygulamasında tekrar bir yükselişin olduğu tespit edilmiştir.

Bahçe Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 01.09.2023
Kabul Tarihi : 03.12.2023

Anahtar Kelimeler
Armut
Prolin
Tuz stresi
Toplam fenolik madde
Toplam flavonoid madde

Atıf Şekli: Uyduran, E., & Şan, B., (2024). OH×F 333 ve OH×F 97 Armut Klon Anaçlarının Tuz Stresine Tepkilerinin in vitro Koşullarda Belirlenmesi. *KSÜ Tarım ve Doğa Derg 27* (4), 817-827. https://doi.org/10.18016/ksutarimdoga.vi.1354060

To Cite : Uyduran, E., & Şan, B., (2024). In vitro Determination of Salt Stress Responses of OH×F 333 and OH×F 97 Pear Clonal Rootstocks. *KSU J. Agric Nat 27*(4), 817-827. https://doi.org/10.18016/ksutarimdoga.vi.1354060

INTRODUCTION
The decrease in natural resources in the world day by day reveals new searches in agriculture as in every field. The decrease in agricultural areas and product productivity due to the increasing world population and the effect of various stress factors shows that human beings will face a serious nutritional problem in the coming years. The efforts to obtain the highest efficiency from the existing production areas have accelerated depending on the increase in the world population. In addition, scientists have stated that deterioration in the climate system will cause negative consequences. Türkiye is among the countries that will

be most affected by climate change due to global warming. If the necessary precautions are not taken, the factors that contribute to the disruption of the natural balance will progressively intensify, ultimately resulting in climate changes caused by global warming. Because, due to human reasons, the increase in greenhouse gas accumulations and particles in the atmosphere, the destruction of the natural environment, and the depletion of the ozone layer, will cause a global temperature increase (Öztürk, 2002; Hayaloğlu, 2018). Temperature increases, on the other hand, will activate many stress factors such as decreased precipitation, drought, and salinization of the soil, and this will have negative consequences. In particular, these abiotic stressors have limiting effects on global food security, quality, and plant productivity (Hayaloğlu, 2018; Yıldırım et al., 2021). Soil quality and health are one of the most important factors for fruit cultivation (Koç and Yakupoğlu, 2022). Approximately 20% of the irrigable agricultural lands in the world are adversely affected by soil salinity. This problem has increased with the excessive use of fertilizers, inappropriate use of irrigation water, natural environmental conditions, and global climate change (Zhao et al., 2021). Salinity is one of the important environmental stress factors that negatively affect the development, quality, and yield of plants. In crop production, approximately 50% of crop losses occur due to abiotic stress factors. An area of 6% in total, 30% of the irrigable areas, is faced with soil salinity today. On the other hand, improper cultural practices (such as fertilization, and irrigation) cause an increase in salinity (Hasaruzzaman et al., 2013).

Salt stress is an abiotic stress factor that adversely affects all stages of the plant such as germination, development, flowering, and fruit set. High Na concentrations in saline soil limit plant water uptake and nutrient absorption. Lack of water and nutritional imbalance cause osmotic stress and ionic stress problems. Various physiological and molecular changes occur with salt stress and it causes problems in plant growth and development by limiting photosynthesis (Van Zelm et al., 2020; Gong, 2021). This shows that salt stress is an important abiotic stress factor. Studies have reported that damages occur in pear rootstocks under salt stress due to the transport of Na and Cl ions to the leaves (Matsumoto et al.,2006a).

To meet the increasing agricultural production need, it is necessary to carry out plant production in unsuitable areas. Although there are some differences in salt tolerance between plant species and cultivars, it is known that the majority of cultivated plants are generally sensitive to salinity (Kuşvuran et al., 2008). Studies on the development of salt-tolerant rootstocks or cultivars have gained importance in growing under salt-stress conditions in fruit growing. In addition, the tolerance levels of existing rootstocks and cultivars to salt stress should also be determined. In this regard, Asian (*Pyrus betulaefolia* Bunge., *P. pyrifolia* Nakai, and *P. xerophila* Yu) and Mediterranean pear species (*P. amygdaliformis* Vill. and *P. elaeagrifolia* Pall) were irrigated with 75 mM and 150 mM NaCl solutions for 30 days and salt tolerance levels were determined. According to the research, it was determined that Mediterranean pear species are more tolerant to salt than Asian species (Matsumoto et al., 2006b). Some studies have been conducted to determine the salt stress tolerance of some pear rootstocks (Aydınlı, 2021; Javadisaber et al., 2024). However, detailed studies are needed to determine tolerance mechanisms against stress conditions. There are significant differences according to genotypes, especially in the synthesis of biochemical substances such as phenolics, flavonoids, and proline.

In this study, it was aimed to determine the responses of OH×F 333 and OH×F 97 pear clonal rootstocks to salt stress at different concentrations in vitro. For this purpose, some morphological (shoot number, shoot length, degree of damage) and biochemical (total phenolic, total flavonoid, proline, soluble protein, lipid peroxidation) analyses were performed on rootstocks.

MATERIAL and METHOD

Material

In this study, shoot tips taken from OH×F 333 and OH×F 97 pear clonal rootstocks were used as material. The experiment was carried out at Isparta University of Applied Sciences, Faculty of Agriculture, Department of Horticulture, Tissue Culture Laboratory in 2020-2021. Autoclavable glass magentas were used for cultures in the study. Forceps, scalpels, and sterile filter papers were used as materials at all stages of in vitro culture. MS medium and agar used in the study were purchased from Duchefa Biochemie B.V. (Haarlem, The Netherlands). NaCl, BAP, IBA, sucrose, gallic acid, catechin, and proline were obtained from Merck (Merck KGaA, Germany).

Method

Sterilization

The prepared media were distributed in glass magentas with an internal volume of 100 ml, with approximately 25 ml of medium. Then, the media were sterilized by keeping them in an autoclave set at 121 ℃ for 15 min (Şan et al., 2015).

The 2-3 cm shoot tips used in the study were taken in May 2020 and first washed under tap water for 15 min. After being kept in 70% ethanol for a min., it was washed with sterile distilled water to remove alcohol. In the last stage, shoot tips were kept in a 20% sodium hypochlorite solution (15% Cl content) containing a few drops of Tween 20 for 18 min by shaking. At the end of

the period, the shoot tips taken into the laminar air flow cabinet were washed with sterile distilled water 3 times for 5 min each to remove sodium hypochlorite (Tuncel & Şan, 2023). The sterile shoot tips were left on sterile filter paper and their moisture was removed. Then, the shoot tips prepared in 0.5-1 cm length were planted in nutrient media.

Salt stress experiment

Sterilized shoot tips were prepared about 0.5-1 cm in length and planted in nutrient media in magenta containers. MS medium (Murashige & Skoog, 1962) was used in all stages of the experiment.

Microshoots were propagated by subcultures at 4-week intervals until sufficient shoot numbers were reached for the salt stress experiment. In the propagation step, 1.5 mg/L BAP, 0.1 mg/L IBA, and 30 g/L sucrose were added to the MS medium. After the pH of the medium was adjusted to 5.7, 7 g/L agar was added and sterilized. In vitro, cultures at the propagation stage were incubated in a climate chamber set at 22 ℃ and 16 hours of light and 8 hours of darkness. The experiment was initiated once the required number of shoots was reached in the research.

In the experiment, approximately 1-1.5 cm of in vitro shoots were planted in the medium. In the study, different concentrations of NaCl (0, 50, 100, 150, and 200 mM) were added to the ½ MS medium (Table 1). The pH of the nutrient media was adjusted to 5.7. The shoots were incubated in a climate chamber set at 22 ℃ and 16 hours of light/8 hours of darkness (Çalhan, 2020). The incubation process took a total of 8 weeks under salt stress conditions. The conclusion of the experiment involved the investigation of certain morphological traits, such as regeneration rate, shoot length, the number of shoots, and the degree of damage. At the same time, the experiment included the determination of biochemical parameters such as total phenolics, total flavonoids, lipid peroxidation, proline, and soluble protein contents.

Table 1. Applications of different NaCl concentrations on OH×F 333 and OH×F 97 pear clonal rootstock
Çizelge 1. OH×F 333 ve OH×F 97 armut klon anaçlarına farklı NaCl konsantrasyonları uygulamaları

Treatments*	Incubation conditions
Control (0 mM NaCl)	Incubation in ½ MS basal medium for 8 weeks
50 mM NaCl	Incubation in ½ MS medium without NaCl for a week, then in medium containing 50 mM NaCl for 7 weeks
100 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in the medium containing 50 mM NaCl for a week, and then in the medium containing 100 mM NaCl for 6 weeks
150 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in a medium containing 50 mM NaCl for a week, in a medium containing 100 mM NaCl for a week, and then in a medium containing 150 mM NaCl for 5 weeks
200 mM NaCl	Incubation in ½ MS medium without NaCl for a week, in a medium containing 50 mM NaCl for a week, in a medium containing 100 mM NaCl for a week, in a medium containing 150 mM NaCl for a week and then in a medium containing 200 mM NaCl for 4 weeks

* 30 g L^{-1} sucrose, 7 g L^{-1} agar, 1.5 mg L^{-1} BAP and 0.1 mg L^{-1} IBA were added to all nutrient media.

Measurement and Analysis

Regeneration rate: It was determined by the ratio of new shoot-forming explants to the total number of explants cultured and expressed as %.

Shoot length: Shoot lengths were measured with the help of a digital caliper and determined by calculating the average.

Number of shoots: The shoots that emerged on the main explant were counted averaged and determined as number/explant.

The degree of damage: The degree of damage seen in the explants in the experiment was scored according to the following scale (Sivritepe et al., 2008).

1: Very severe damage (complete drying of explants)

2: Severe damage (chlorosis and local drying of explants),

3: Moderate damage (severe chlorosis on explants and developmental arrest),

4: Less damage (chlorosis and poor growth of explants),

5: No damage (explants are healthy and growth is good.).

Total phenolic analysis: Total phenolic contents were made by modifying the Folin-Ciocalteau method specified by Singleton and Rossi (1965). The results were calculated according to the gallic acid standard and are expressed as mg GAE g^{-1} fresh weight (FW).

Total flavonoid analysis: Total flavonoid analysis was carried out by modifying the method specified by Zhishen et al. (1999). The results were calculated according to the catechin standard and expressed as mg CE g^{-1} FW.

Soluble protein analysis: Soluble protein analysis was performed by modifying the method specified by Hartree (1972). Results were calculated according to BSA (Bovine Serum Albumin) standardization and are expressed in mg g^{-1} FW.

Lipid peroxidation analysis: Lipid peroxidation was carried out by modifying the method determined by Jiang et al (2010). Results are expressed as nmol g^{-1} FW.

Proline analysis: Proline analysis was performed according to the method described by Li et al. (2012). The results were calculated according to the D-Proline standard and expressed as µmol g^{-1} FW.

Experimental Design and Data Analysis

The experiment was planned according to the factorial randomized plots experimental design with 3 replications and 10 explants in each replication. A total of 150 microshoots were used for each rootstock in the study. The obtained data were subjected to variance analysis in the Minitab package program (MINITAB 17 inc). The difference between the significant means was determined according to the Tukey test and shown with different letters (Mathews, 2004). The results were given as standard errors in the tables.

RESULTS

Morphological Features

In the study, the effects of salt stress on regeneration rate, shoot number, shoot length, and degree of damage were investigated in OH×F 333 and OH×F 97 pear clonal rootstocks. Analysis of variance was applied to the obtained data and the results are presented in Table 2.

Table 2. The effects of different NaCl applications on morphological values in OH×F 333 and OH×F 97 pear clonal rootstocks

Çizelge 2. OH×F 333 ve OH×F 97 armut klonal anaçlarında farklı NaCl uygulamalarının morfolojik değerler üzerine etkileri

Rootstocks	Salt stress applications	Regeneration rate (%)	Shoot length (mm)	Shoot number	Degree of damage (0-5 range)
OH×F 333	Control	90±6.1 ab*	15.3±1.48bc	3.94±0.44 ab	4.6±0.25 a
	50 mM NaCl	70±12.2 abcd	17.5±1.69 abc	2.70±0.29 cd	3.6±0.25 ab
	100 mM NaCl	80±6.1 abc	17.9±0.73 abc	2.20±0.20 cde	4.6±0.25 a
	150 mM NaCl	40±10.0 de	20.3±1.67 abc	1.50±0.16 e	4.2±0.37 a
	200 mM NaCl	20±12.2 e	17.6±1.62 abc	1.10±0.10 e	1.2±0.20 c
OH×F 97	Control	100±0.0 a	17.4±1.14 abc	4.15±0.29 a	4.6±0.25 a
	50 mM NaCl	80±9.3 abc	21.5±0.89 ab	2.90±0.23 bc	4.2±0.20 a
	100 mM NaCl	60±6.1 bcd	21.9±1.43 a	2.00±0.18 cde	3.4±0.51 ab
	150 mM NaCl	50±7.9 cde	20.9±1.96 abc	1.60±0.15 de	2.6±0.51 bc
	200 mM NaCl	15±6.1 e	14.5±0.63 c	1.15±0.06 e	1.2±0.20 c
Applications Mean	Control	95±3.3 a	16.4±0.93 b	4.04±0.25 a	4.6±0.16 a
	50 mM NaCl	75±7.4 a	19.5±1.12 ab	2.80±0.18 b	3.9±0.18 ab
	100 mM NaCl	73±5.8 a	19.9±1.00 ab	2.10±0.13 c	4.0±0.33 ab
	150 mM NaCl	45±6.2 b	20.6±1.22 a	1.55±0.10 cd	3.4±0.40 b
	200 mM NaCl	18±6.5 c	16.1±0.97 b	1.13±0.06 d	1.2±0.13 c
Rootstocks Mean	OH×F 333	61±6.8	17.8±0.69	2.29±0.23	3.64±0.28 a
	OH×F F 97	61±6.5	19.2±0.79	2.36±0.23	3.20±0.29 b

* The difference between the means shown with different letters for each rootstock is statistically significant. ($p \leq 0.05$)

In the evaluation of the regeneration rate, it was observed that the interaction between the rootstock and application had notable significance (Table 2). It was observed that the regeneration rates decreased as the salt concentration increased in both rootstocks. In the study, the highest regeneration rates were observed in both OH×F 333 and OH×F 97 rootstocks in the control application (90% and 100%, respectively). It was determined that the lowest regeneration rate was 20% in OH×F 333 rootstock and 15% in OH×F 97 rootstock in 200 mM NaCl application. When the application means were examined, the difference between the applications was found to be significant. The average regeneration rate was determined as 95% in the control application and 18% in the 200 mM NaCl application. There was no statistically significant difference between rootstocks in terms of regeneration rate.

Rootstock x application interaction was found to be statistically significant in terms of shoot length. While there was no difference between applications in OH×F 333 rootstock, 200 mM NaCl application in OH×F 97 rootstock significantly reduced shoot length compared to control. Considering the application averages, the difference was found to be statistically significant. The highest shoot length was determined as 20.6 mm in 150 mM NaCl concentration, and the lowest shoot length was 16.1 mm in 200 mM NaCl application. There was no statistical difference between rootstocks in terms of shoot length.

In the study, rootstock x application interaction was significant in terms of shoot number. The highest shoot number was observed in the control application in both OH×F 97 and OH×F 333 rootstocks (4.15 and 3.94, respectively). It was determined that there was a decrease in the number of shoots of the plants in parallel with the increase in the applied salt concentrations. It was determined that the lowest shoot number values were 1.10 in OH×F 333 rootstock and 1.15 in OH×F 97 rootstock in 200 mM NaCl application. Considering the application averages, the difference was found to be statistically significant. It was determined that the highest shoot number was 4.04 in the control application, and the lowest shoot number was 1.13 in the 200 mM NaCl application. There was no statistically significant difference between rootstocks in terms of the number of shoots.

In terms of the degree of damage, rootstock x application interaction was found to be statistically significant. The signs of damage to the plants were manifested as weak shoot development, chlorosis, and drying of the leaves. When the values in Table 2 were examined, it was determined that the highest damage was observed in 200 mM NaCl application with a value of 1.2 for both rootstocks (Figures 1 and 2). In this application, it was observed that although some plants were alive, most plants died. Considering the application averages, the difference was found to be statistically significant. In parallel with the increase in salt concentration, the damage status of the plants also increased. When the rootstocks were compared in terms of damage, it was seen that OH×F 333 rootstock was relatively more tolerant to salt stress conditions than OH×F 97 rootstock (3.64 and 3.20, respectively).

Fig. 1. Growth of OH×F 97 rootstock in ½ MS medium containing different concentrations of NaCl
Şekil 1. Farklı konsantrasyonlarda NaCl içeren ½ MS ortamında OH×F 97 anacının gelişmesi

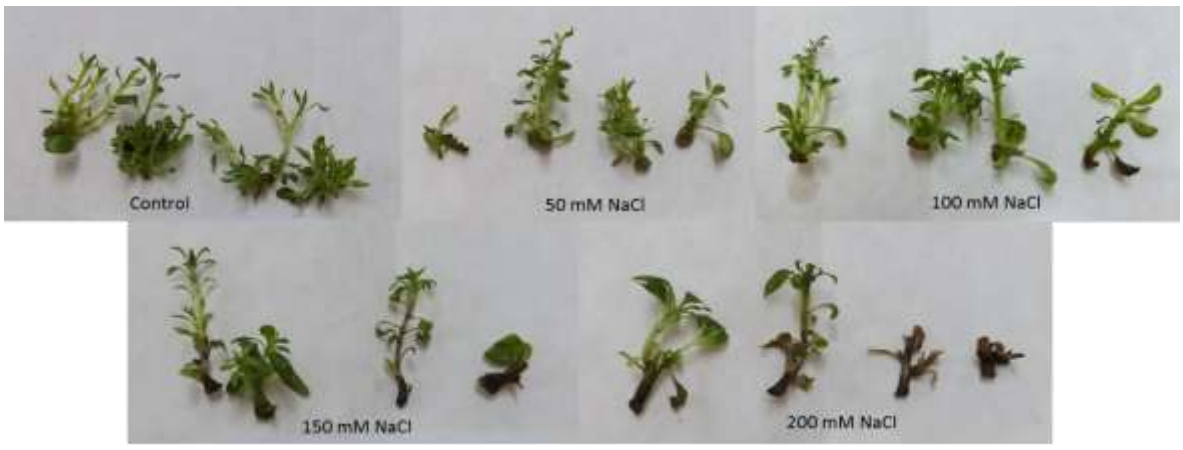


Fig. 2. Growth of OH×F 333 rootstock in ½ MS medium containing different concentrations of NaCl
Şekil 2. Farklı konsantrasyonlarda NaCl içeren ½ MS ortamında OH×F 333 anacının gelişmesi

Biochemical Properties

In the study, the effects of salt stress treatments on total phenolic, total flavonoid, lipid peroxidation, and proline contents of OH×F 333 and OH×F 97 pear clonal rootstocks were investigated. The results are given in Table 3.

In salt stress studies, because of high damage to plants in media containing 200 mM NaCl, sufficient plant samples could not be taken for biochemical analysis. Therefore, biochemical analyses were not performed in 200 mM NaCl application. In the study, rootstock x application interaction was found to be statistically significant in terms of total phenolic content. The

highest total phenolic was determined in OH×F 333 rootstock in the control application (4.66 mg g^{-1}). It was detected in 150 mM NaCl application (1.98 mg g^{-1}) in OH×F 97 rootstock. The lowest total phenolic contents were found in OH×F 333 and OH×F 97 rootstocks in 100 mM NaCl application (1.99 mg g^{-1} and 0.83 mg g^{-1}, respectively). In the study, as the salt concentrations increased, a decrease was observed in the total phenolic content. However, in the application of 150 mM NaCl, an increase was detected again in both rootstocks. The difference between the salt stress application averages was found to be statistically significant. It was determined that the highest total phenolic content was in the control application, while the lowest content was determined at 100 mM NaCl concentration. The difference between rootstocks was also significant. Accordingly, while the total phenolic content of OH×F 333 rootstock was 3.02 mg g^{-1}, this value was found to be 1.60 mg g^{-1} in OH×F 97 rootstock.

Table 3. The effects of NaCl applications on biochemical properties of OH×F 333 and OH×F 97 pear clonal rootstocks.

Çizelge 3. NaCl uygulamalarının OH×F 333 ve OH×F 97 klon armut anaçlarının biyokimyasal özelliklerine etkileri

Rootstocks	Salt stress applications	Total phenolics (mg g^{-1} FW)	Total flavonoids (mg g^{-1} FW)	Soluble protein (mg g^{-1} FW)	MDA (nmol g^{-1} FW)	Proline (µmol g^{-1} FW)
OH×F 333	Control	4.66±0.13 a*	0.53±0.01 ab	0.76±0.06 cd	23.0±1.41 ab	111.0±2.41 a
	50 mM NaCl	2.12±0.02 c	0.44±0.01 b	0.82±0.04 cd	26.9±1.19 a	48.4±1.48 b
	100 mM NaCl	1.99±0.10 cd	0.43±0.02 b	0.64±0.08 de	23.2±1.61 ab	13.9±0.10 b
	150 mM NaCl	3.22±0.09 b	0.55±0.02 ab	0.45±0.03 e	22.9±0.47 ab	40.5±0.90 b
OH×F 97	Control	1.87±0.06 cd	0.49±0.03 ab	1.38±0.02 a	16.1±0.70 c	23.1±1.16 b
	50 mM NaCl	1.66±0.10 d	0.67±0.14 ab	0.97±0.05 bc	20.7±1.19 bc	18.8±1.60 b
	100 mM NaCl	0.83±0.03 e	0.44±0.05 b	1.11±0.02 b	22.9±0.37 ab	21.7±1.81 b
	150 mM NaCl	1.98±0.05 cd	0.90±0.21 a	0.81±0.03 cd	21.3±0.90 b	24.2±2.89 b
Applications mean	Control	3.30±0.43 a	0.51±0.02 ab	1.07±0.10 a	19.5±1.14 b	67.3±19.80 a
	50 mM NaCl	1.20±0.10 c	0.55±0.07 ab	0.90±0.04 b	23.8±1.08 a	33.6±2.15 b
	100 mM NaCl	1.40±0.18 d	0.44±0.03 b	0.90±0.08 b	23.0±0.70 a	17.8±1.92 b
	150 mM NaCl	2.60±0.20 b	0.72±0.11 a	0.70±0.05 c	22.1±0.40 ab	32.4±3.89 b
Rootstocks mean	OH×F 333	3.02±0.23 a	0.50±0.01 b	0.70±0.04 b	24.0±0.49 a	53.6±11.40 a
	OH×F 97	1.60±0.10 b	0.62±0.07 a	1.10±0.05 a	20.2±0.75 b	22.0±1.04 b

* The difference between the means shown with different letters for each rootstock is statistically significant. ($p \leq 0.05$)

Rootstock x application interaction was found to be significant in terms of total flavonoid content. The highest total flavonoid content was observed in 150 mM NaCl application (0.55 mg g^{-1} in OH×F 333, 0.90 mg g^{-1} in OH×F 97) on both rootstocks. However, it was determined that salt stress applications did not significantly affect the total flavonoid content in OH×F 333 rootstock. The difference between the salt stress application averages was found to be statistically significant. The highest value was determined in the application of 150 mM NaCl, while the lowest value was observed in the application of 100 mM NaCl. The difference between rootstocks in terms of total flavonoid content was statistically significant. In this respect, the total flavonoid content of OH×F 97 rootstock was higher than that of OH×F 333 rootstock (0.62 mg g^{-1} and 0.50 mg g^{-1}, respectively).

Rootstock x application interaction was found to be statistically significant in terms of soluble protein content. In the study, the highest soluble protein content was found in 50 mM NaCl application for OH×F 333 (0.82 mg g^{-1}) and control (1.38 mg g^{-1}) for OH×F 97. The lowest soluble protein content was detected in the application of 150 mM NaCl on both OH×F 333 and OH×F 97 rootstocks (0.45 mg g^{-1} and 0.81 mg g^{-1}, respectively). The difference between the salt stress application averages in terms of soluble protein was found to be statistically significant. It was determined that the highest soluble protein content was in the control application. The difference between rootstocks in terms of soluble protein content was found to be statistically significant. Accordingly, while the soluble protein content was 1.10 mg g^{-1} in OH×F 97 rootstock, this value was determined as 0.70 mg g^{-1} in OH×F 333 rootstock.

Rootstock x application interaction was found to be statistically significant in terms of lipid peroxidation. The highest lipid peroxidation content was observed in OH×F 333 rootstock with a value of 26.9 nmol g^{-1} in 50 mM NaCl application. In OH×F 97 rootstock, the highest lipid peroxidation was determined in 100 mM NaCl application (22.9 nmol g^{-1}). The difference between the salt stress application averages in terms of lipid peroxidation was found to be statistically significant. The highest lipid peroxidation was detected in the application of 50 and 100 mM NaCl (23.8 and 23.0 nmol g^{-1}, respectively). The lowest value was determined to be 19.5 nmol g^{-1} in the control

application. When the rootstocks were compared, higher values were obtained in OH×F 333 rootstock (24.0 nmol g^{-1}) compared to OH×F 97 rootstock (20.2 nmol g^{-1}) in terms of lipid peroxidation.

When the proline contents of the rootstocks were examined, the rootstock x application interaction was found to be significant. In the study, the highest proline content was observed in the control application (111.0 µmol g^{-1}) in OH×F 333 rootstock. In OH×F 97 rootstock, there was no statistical difference between applications. When the salt stress application averages were evaluated, it was determined that the highest proline content was (67.3 µmol g^{-1}) in the control application. The difference between rootstocks in terms of proline content was found to be statistically significant, and the proline content of OH×F 333 rootstock (53.6 µmol g^{-1}) was found to be higher than that of OH×F 97 rootstock (22.0 µmol g^{-1}).

DISCUSSION

In this study, we determined the tolerance or sensitivity levels of OH×F 333 and OH×F 97 pear clonal rootstocks, which are widely used in pear cultivation, to salt stress at different concentrations in vitro.

Morphological Features

Plants are generally sensitive to saline conditions. Therefore, salt stress negatively affects the vegetative development of plants in many ways. In the study, when the effects of different salt concentrations on the regeneration rate under in vitro conditions were evaluated, it was observed that there was a statistically significant decrease in the regeneration rates in parallel with the increase in salt concentrations. In some studies, similar findings to the results were obtained. Krasensky and Jonak (2012) found that there was a decrease in the fresh and dry weight of onion plants under increased salt stress conditions. Similarly, Zambia (2019) stated that the development slowed down in parallel with the increase in salt concentration in peas.

In the study, it was determined that the number of shoots decreased significantly in parallel with the increase in the salt stress level. Supporting the results, Rahman et al. (2007) reported that the application of salt stress in vitro conditions reduced the number of shoots in *P. communis* rootstocks compared to the control. Similarly, Shiyab et al. (2003) stated that salt stress applications (control, 50, 100, 150, 200, and 300 mM NaCl) decreased shoot growth in sour orange. The researchers stated that at 200 and 300 mM NaCl concentrations, the growth parameters of the plants were greatly affected and the shoots lost their vitality.

In the study, it was observed that shoot lengths increased as NaCl concentrations increased in both rootstocks. However, it was determined that shoot length was shorter in the 200 mM NaCl application than in the control application because growth and development completely stopped. It is thought that the increase in shoot length values in parallel with the salt concentration may be a result of the decrease in the number of shoots. In parallel with the study, Javadisaber et al. (2024) and Sotiropoulos et al. (2006) reported that they obtained the longest shoots from high salt concentrations. However, it is reported that there is a decrease in shoot length in general under salt stress. Studies conducted on pear (Sotiropoulos et al. 2006) and quince (Sotiropoulos et al. 2007) reported that the length of micro shoots decreased with increasing salt concentrations. Dajic (2006) reported that photosynthesis slowed down with the decrease in shoot growth of plants exposed to salt stress. It was also stated in the study that turgor decreased and mineral transport was difficult. In addition, it has been reported that the increased Na concentration in plants under salt stress causes damage to the shoots (Uyar, 2016).

In the study, it was determined that the damage increased in direct proportion to the increase in salt concentrations. It was observed that chlorosis, browning, drying, and shortening of shoot length occurred in the shoots of plants grown in in vitro conditions where different salt concentrations were applied. Javadisaber et al. (2024), in their study on pear genotypes, stated that the level of damage in micro shoots increased in parallel with the increase in salt concentration. In another study, it was determined that Asian wild pear species (*P. betulaefolia* Bunge, *P. pyrifolia* Nakai, and *P. xerophila* Yu) were more affected by salt stress than Mediterranean wild pear species (*P. amygdaliformis* Vill. and *P. elaeagrifolia* Pall.) (Matsumoto et al., 2006b).

Biochemical Properties

Plants make changes in their metabolism to adapt to different environmental conditions. These changes are symptoms such as chlorosis of leaves, early flowering, shedding of leaves, or curling of leaves. Phenolic compounds, which play an active role in this process, are effective in signaling stress factors in the plant and synthesizing chemical substances. It also plays an active role in the opening and closing of stomata in leaves, early maturation, and changes in respiratory activity (Hacıkamiloğlu, 2023). In this study, the total phenolic content decreased in parallel with the salt stress level in both rootstocks. However, it was determined that there was an increase again in the application of 150 mM NaCl. Supporting the results, Çalhan (2020) reported that the total phenolic content decreased in parallel with the increasing salt concentration in myrtle genotypes, while the total phenolic content increased again at high salt

concentrations. In other studies, it has been reported that there is an increase of up to three times in the total phenolic content of sugar cane and olive with the increase in salt stress (Wahid & Ghazanfar, 2006; Petridis et al., 2012). However, it has been stated in the studies that the total phenolic content decreases or is not affected under salt stress conditions and that these compounds may differ according to the salt concentration. Bourgou et al (2010), in their study on the *Nigella sativa* plant, reported that the total phenolic content decreased as a result of salt stress applications. Similarly, Shaheen et al. (2012) reported that the total phenolic content of the eggplant decreased under salt stress. The results we obtained in this study were found to be compatible with the literature.

Flavonoids, on the other hand, are a subgroup of phenolic compounds and participate in the plant defense system against different stress conditions (Harborne & Williams, 2000). In the findings obtained in this study, it was determined that the total flavonoid content did not show a statistically significant difference according to salt concentrations. However, an increase was detected in 150 mM NaCl application only in OH×F 97 rootstock compared to the control. In the study conducted by Gengmao et al. (2015), it was reported that the total flavonoid content of sunflowers increased under salt stress conditions compared to the control application. However, there are studies reporting reductions in the total flavonoid content of plants under salt stress. Petropoulos et al (2017) reported that the total flavonoid content of high salt concentrations increased little or had no stress effect. In a study conducted on marigolds, it was determined that the total flavonoid content decreased in salt stress application compared to the control (Khalid et al., 2010). In this study, a partial decrease was observed in the total flavonoid content of OH×F 333 and OH×F 97 pear clonal rootstocks under salt stress conditions, and an increase was observed in 150 mM NaCl application.

Since plants activate oxidative stress under salt stress, membrane lipid peroxidation causes damage (Ye et al., 2000). With this damage, lipid peroxidation occurs. The damage to the membrane with lipid peroxidation is irreversible and becomes malondialdehyde, the most important product. In this study, it was determined that lipid peroxidation was not statistically affected in OH×F 333 rootstocks in salt stress applications, while it increased in OH×F 97 rootstocks in 100 and 150 mM NaCl applications compared to the control. Ertürk et al., (2007) applied salt stress to cherry rootstocks in vitro and stated that both lipid peroxidation and antioxidative enzyme activities increased under salt stress. Again, supporting the results, it has been reported that lipid peroxidation increases under salt stress conditions in strawberry and *Pyrus betulafolia* species (Tanou et al., 2009; Wu & Zou, 2009). Researchers have reported that oxidative stress occurs, necrotic lesions occur on leaves, H_2O_2/O_2 accumulation occurs in tissues, and lipid peroxidation increases, especially in 200 mM NaCl application.

When plants are under stress, they synthesize and accumulate various osmotic regulators to protect themselves from stress.

Proline, which is one of these osmotic regulators, is an amino acid that has different functions such as being an energy source as well as having antioxidant properties as well as its osmotic effect (Ben Ahmed et al., 2008). As a result of this research, it was seen that salt stress applications did not affect the proline content of OH×F 97 rootstocks statistically. On the other hand, it was determined that the proline content of OH×F 333 rootstock decreased significantly with salt stress applications. In the studies, it was stated that the accumulation of proline increased with the increase in the stress level in plants under salt stress. As a result of examining the tolerance effect of proline under stress conditions, it has been argued that this situation may not be valid in some plant species under salt stress (Mansour & Ali, 2017). As a matter of fact, in the salt stress study conducted on 46 genotypes of *Panicum virgatum*, it was determined that the proline content increased 5000 times in some salt-sensitive genotypes, while this increase was at a small level in tolerant lines (Kim et al., 2016).

It is known that plants protect themselves from reactive oxygen species (ROS) by stabilizing their protein structure with the help of osmolytes under salt-stress conditions (Zhu, 2001). Considering the soluble protein content in this study, it was observed that there were significant decreases in parallel with the increase in salt concentration. Studies have reported that stress factors cause increases or decreases in soluble protein content. It has been reported that soluble protein contents increase in onions (Bekheet et al., 2006) and decrease in sorghum (Parlak & Özaslan Parlak, 2006) under increasing salt stress conditions. The response of plants to stress conditions may vary according to the tolerance levels of genotypes.

As a result, both OH×F 97 and OH×F 333 clonal rootstocks were found to be sensitive to high doses of salt stress. However, it was determined that they showed little improvement under salt stress, which can be considered as high as 100 mM NaCl. When the rootstocks are compared with each other, although there is no statistical difference according to the morphological and biochemical analysis results, it has been determined that the OH×F 333 rootstock is slightly more prominent than the OH×F 97 rootstock. Especially in terms of damage degree, OH×F 333 clonal rootstock gave better values than OH×F 97 clonal rootstock. However, more detailed studies should be done in vitro and in vivo conditions to obtain more precise results.

ACKNOWLEDGEMENTS

This research was supported by the Scientific Research Projects Coordination Unit of Isparta University of Applied Sciences within the scope of the master's project numbered 2021-YL1-0123.

Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The author declares no conflicts of interest.

REFERENCES

Aydınlı, M. (2021). *Armut Yetiştiriciliğinde Kullanılan Farklı Anaçların Tuzluluğa Toleranslarının Morfolojik, Fizyolojik ve Biyokimyasal Parametreler ile İncelenmesi (Tez no 700258).* [Doktora Tezi, Isparta Uygulamalı Bilimler Üniversitesi Lisansüstü Eğitim Enstitüsü Bahçe Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Bekheet, S. A., Taha, H. S., & Solliman, M. E. (2006). Salt tolerance in tissue culture of onion (*Allium cepa* L.). *Arab Journal Biotechnology, 9*(3), 467-476.

Ben Ahmed, C., Rouina, B. B., & Boukhris, M. (2008). Changes in water relations, photosynthetic activity, and proline accumulation in one-year-old olive trees (*Olea europaea* L. cv. Chemlali) in response to NaCl salinity. *Acta Physiologiae Plantarum, 30*(4), 553-560. DOI: 10.1007/s11738-008-0154-6

Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H., & Marzouk, B. (2008). Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Comptes, Rendus Biologies, 331*(1), 48-55. https://doi.org/10.1016/j.crvi.2007.11.001

Çalhan, G. (2020). *Mersin (Myrtus communis L.) Genotiplerinin Tuz Stresine Tolerans Seviyelerinin In Vitro Koşullarda Belirlenmesi (Tez no 633153).* [Yüksek Lisans Tezi, Isparta Uygulamalı Bilimler Üniversitesi Lisansüstü Eğitim Enstitüsü Bahçe Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Dajic, Z. (2006). *Salt stress*. In K.V Madhava Rao, A.S. Raghavendra, K. Janardhan Reddy (Eds.), *Physiology and Molecular Biology of Stress Tolerance in Plants*, Springer Link Publishing.

Ertürk, U., Sivritepe, N., Yerlikaya, C., Bor, M., Özdemir, F., & Türkan, İ. (2007). Responses of the cherry rootstock to salinity in vitro. *Biologia Plantarum, 51*(3), 597-600. https://doi.org/10.1007/s10535-007-0132-7

Gengmao, Z., Yu, H., Xing, S., Shihui, L., Quanmei, S., & Changhai, W. (2015). Salinity stress increases secondary metabolites and enzyme activity in safflower. *Industrial Crops and Products, 64*, 175-181. https://doi.org/10.1016/j.indcrop.2014.10.058

Gong, Z. (2021). Plant abiotic stress: New insights into the factors that activate and modulate plant responses. *Journal Integrative Plant Biology., 63*, 429. https://doi.org/10.1111/jipb.13079

Hacıkamiloğlu, M. S. (2023). *Karadeniz Bölgesindeki Bazı Aspir (Carthamus spp.) Türlerinin Taksonomik, Fenolojik, Ekolojik ve Tarımsal Özelliklerinin Belirlenmesi (Tez no 798386).* [Doktora Tezi, Ondokuz Mayıs Üniversitesi Lisansüstü Eğitim Enstitüsü Tarla Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481-504. https://doi.org/10.1016/S0031-9422(00)00235-1

Hartree, E. F. (1972). Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry, 48*(2), 422-427. https://doi.org/10.1016/0003-2697 (72) 90094-2

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences, 14*(5), 9643- 9684. https://doi.org/10.3390/ijms14059643

Hayaloğlu, P. (2018). İklim değişikliğinin tarım sektörü ve ekonomik büyüme üzerindeki etkileri. *Gümüşhane Üniversitesi Sosyal Bilimler Dergisi, 9*(25), 51-62.

Javadisaber, J., Dumanoğlu, H., Şahin, Ö., Sarıkamış, G., Ergül, A., Çakır Aydemir, B. (2024). Salt stress tolerance of *Pyrus spp.* and *Cydonia oblonga* genotypes assessed by morphological, biochemical, and dehydrin gene expression analysis. *Journal of Plant Growth Regulation*, 43, 165–177. https://doi.org/10.1007/s00344-023-11071-3

Jiang, T., Jahangir, M. M., Jiang, Z., Lu, X., & Ying, T. (2010). Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity, and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage. *Postharvest Biology and Technology, 56*(3), 209-215. https://doi.org/10.1016/j.postharvbio.2010.01.011

Khalid, K., Jaime, A., & Teixeira da, S. (2010). Yield, essential oil, and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. *Scientia Horticulturae, 126*(2), 297-305. https://doi.org/10.1016/j.scienta.2010.07.023

Kim, J., Liu, Y., Zhang, X., Zhao, X., & Childs, K. L. (2016). Analysis of salt-induced physiological and proline changes in 46 switchgrass (*Panicum virgatum*) lines indicates multiple response modes. *Plant Physiology and Biochemistry, 105*, 203-212. https://doi.org/10.1016/j.plaphy.2016.04.020

Koç, A., & Yakupoğlu, T. (2022). The effect of farm manure on yield and some soil properties in a pear garden in Yozgat. *KSU J. Agric Nat 25 (Suppl 1)*, 180-191. https://doi.org/10.18016/ksutarimdoga.vi.959149

Krasensky, J. & Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of experimental botany, 63*(4), 1593-1608. https://doi.org/10.1093/jxb/err460

Kuşvuran, S., Yasar, F., Abak, K., & Ellialtıoğlu, S. (2008). Tuz stresi altında yetiştirilen tuza tolerant ve duyarlı *Cucumis sp.*'nin bazı genotiplerinde lipid peroksidasyonu, klorofil ve iyon miktarlarında meydana gelen değişimler. *Yüzüncü Yıl Üniversitesi Ziraat Fakültesi, Tarım Bilimleri Dergisi, 18* (1), 13-20.

Li, Z. Zhou, M. Hu, Q. Reighard, S. Yuan, S., Yuan, N., San, B., Li, D. Jia, H., & Luo, H. (2012). *Manipulating expression of tonoplast transporters*. In S. Shabala & T. A. Cuin (Eds.*), Plant Salt Tolerance: Methods and Protocols, Methods in Molecular Biology*, vol. 913. Humana Press, Totowa, New Jersey, USA

Mansour, M. M. F., & Ali, E. F. (2017). Evaluation of proline functions in saline conditions. *Phytochemistry, 140*, 52-68. https://doi.org/10.1016/j.phytochem.2017.04.016

Mathews, P. G. (2004). *Design of experiments with MINITAB. American Society for Quality*, Quality Press, Milwaukee, Wisconsin, USA

Matsumoto, K., Chun, J. P., Tamura, F., Kamamoto, Y., & Tanabe, K. (2006a). Salt tolerance in *Pyrus* species is linked to levels of Na and Cl translocation from roots to leaves. *Journal of the Japanese Society for Horticultural Science, 75* (5): 385–391.

Matsumoto, K., Tamura, F., Chun, J. P., & Tanabe, K. (2006b). Native Mediterranean *Pyrus* rootstock, *P. amygdaliformis,* and *P. elaeagrifolia* present higher tolerance to salinity stress compared with Asian natives. *Journal of the Japanese Society for Horticultural Science, 75* (6), 450–457.

Öztürk, K. (2002). Küresel iklim değişikliği ve Türkiye'ye olası etkileri. *Gazi Üniversitesi Gazi Eğitim Fakültesi Dergisi, 22*(1), 47-65

Parlak, M. & Özaslan Parlak, A. (2006), Sulama suyu tuzluluk düzeylerinin silajlık sorgumun (*Sorghum bicolor* L. Moench) verimine ve toprak tuzluluğuna etkisi. *Tarım Bilimleri Dergisi, 12*(1), 8-13. https://doi.org/10.1501/Tarimbil_0000000428

Petridis, A., Therios, I., Samouris, G., & Tananaki, C. (2012). Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environmental and Experimental Botany*, 79, 37-43. https://doi.org/10.1016/j.envexpbot.2012.01.007

Petropoulos, S. A., Levizou, E., Ntatsi, G., Fernandes, Â., Petrotos, K., Akoumianakis, K., Barros, L., & Ferreira, I. C. F. R. (2017). Salinity effect on nutritional value, chemical composition, and bioactive compounds content of *Cichorium spinosum* L. *Food Chemistry, 214*, 129-136. https://doi.org/10.1016/j.foodchem.2016.07.080

Rahman, A. E. M. F., Ansary, A. L., Rizkalla, A. A., & Badr-Elden, A. M. B. (2007). Micropropagation and biochemical genetic markers detection for drought and salt tolerance of pear rootstock. *Australian Journal of Basic and Applied Sciences, 1*(4), 625-636.

Shaheen, S., Naseer, S., Ashraf, M., & Akram, N. A. (2012). Salt stress affects water relations, photosynthesis, and oxidative defense mechanisms in *Solanum melongena* L. *Journal of Plant Interactions, 8*(1), 85-96. https://doi.org/10.1080/17429145.2012.718376

Shiyab, S.M., Shibli, R.A., & Mohammad, M.M. (2003). Influence of sodium chloride salt stress on growth and nutrient acquisition of sour orange in vitro. *Journal of Plant Nutrition, 26*(5), 985–996. https://doi.org/10.1081/PLN-120020070

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158. DOI: 10.5344/ajev.1965.16.3.144

Sivritepe, N., Ertürk, U., Yerlikaya, C., Türkan, İ., Bor, M., & Özdemir, F. (2008). Response of the cherry rootstocks to water stress ınduced in vitro. *Biologia Plantarum, 52* (3), 573-576. https://doi.org/10.1007/s10535-008-0114-4

Sotiropoulos, T.E., Fotopoulos, S., Dimassi, K.N., & Tsirakoglou, V. (2006). Response of the pear rootstock to boron and salinity in vitro. *Biologia Plantarum, 50*, 779-781. https://doi.org/10.1007/s10535-006-0130-1

Sotiropoulos, T.E., Therios, I.N., Tsirakoglou, V., & Dimassi, K.N. (2007). Response of the quince genotypes BA 29 and EMA used as pear rootstocks to boron and salinity. *International Journal of Fruit Science, 6*; 93-101. https://doi.org/10.1300/J492v06n04_09

Şan, B., Karakurt, Y. & Dönmez, F. (2015). Effects of thidiazuron and activated charcoal on in vitro shoot proliferation and rooting of myrtle (*Myrtus communis* L.). *Tarım Bilimleri Dergisi–Journal of Agricultural Sciences, 21*, 177-183. https://doi.org/10.1501/Tarimbil_0000001319

Tanou, G., Molassiotis, A., & Diamantidis, G. (2009). Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. *Environmental and Experimental Botany, 65*, 270–281. https://doi.org/10.1016/j.envexpbot.2008.09.005

Tuncel, T.E. & Şan, B. (2023). Micropropagation of pear clone rootstocks OHxF 97 and OHxF 333.

Anadolu Journal of Agricultural Sciences, 38 (2), 315-330. https://doi.org/10.7161/omuanajas. 125 2461

Uyar, H. (2016). *Hamburg misketi (V. vinifera L.) ve Isabella (V. labrusca) Üzüm Çeşitlerinin Tuz Stresine Toleranslarının Belirlenmesi (Tez no 446993).* [Yüksek Lisans Tezi, Ordu Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol., 71*, 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

Wahid, A. & Ghazanfar, A. (2006). Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *Journal of Plant Physiology*, 163(7), 723-730. https://doi.org/10.1016/j.jplph. 2005.07.007

Wu, Q. S. & Zou, Y. N. (2009). Adaptive Responses of Birch-Leaved Pear (Pyrus betulaefolia) Seedlings to Salinity Stress. *Notulae Botanicae Horti Agrobotanici ClujNapoca, 37*(1), 133-138. https://doi.org/10.15835/nbha3713109

Ye, Z., Rodriguez, R., Tran, A., Hoanh, H., Los Santos, D. D., Brown, S., & Vellanoweth. (2000). The developmental transition to flowering represses ascorbate peroxidation activity and reduces enzymatic lipid peroxidation in leaf tissue in *Arabidopsis thaliana*. *Plant Science, 158*, 115-127. https://doi.org/10.1016/S0168-9452(00)00316-2

Yıldırım, A.N., Şan, B., Yıldırım, F., Çelik, C., Bayar, B., & Karakurt, Y. (2021). Physiological and biochemical responses of almond rootstocks to drought stress, *Turkish Journal of Agriculture and Forestry, 45*(4), 12. https://doi.org/10.3906/tar-2010-47

Zambi, H. (2019). *Farklı NaCl Konsantrasyonlarının Bazı Bezelye (Pisum sativum) Çeşit ve Genotiplerinin Bitki Gelişimine Etkisi (Tez no 596269).* [Yüksek Lisans Tezi, Ordu Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., & Wang, P. (2021). Regulation of plant responses to salt stress. *International Journal of Molecular Sciences, 22*(9), 4609. https://doi.org/10.3390/ijms22094609

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zhu, J. K. (2001). Plant salt tolerance. *Trends in Plant Science, 6*(2), 66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

KSÜ Tarım ve Doğa Derg 27 (4), 828-838, 2024
KSU J. Agric Nat 27 (4), 828-838, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1333244

Identification of Root-knot Nematode (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) Species in Celery (*Apium graveolens* L.) (Apiaceae) Growing Areas of Çanakkale Province

Şerifali YAĞCIKÖSE[1], Gülsüm UYSAL[2], Ayşenur YILMAZ[3], Zübeyir DEVRAN[4], Uğur GÖZEL[5]✍
[1,3,5]Department of Plant Protection, Faculty of Agriculture, University of Çanakkale Onsekiz Mart, Çanakkale, Türkiye, [2]Batı Akdeniz Agricultural Research Institute, Department of Plant Health Department, 07100-Antalya, Türkiye, [4]Department of Plant Protection, Faculty of Agriculture, University of Akdeniz 07070-Antalya, Türkiye
[1]https://orcid.org/0000-0003-4575-1150, [2]https://orcid.org/0000-0003-1722-2518, [3]https://orcid.org/0000-0002-3873-6537
[4]https://orcid.org/0000-0001- 7150-284X, [5]https://orcid.org/0000-0003-1363-1189
✉: ugozel@comu.edu.tr

ABSTRACT
In this study, the species and extensiveness of root-knot nematodes (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) causing yield loss in the celery (*Apium graveolens* L.) (Apiaceae) production areas of Çanakkale were determined. For this purpose, celery plant roots and soil samples were taken by making non-periodical surveys of a total of 75 different celery growing areas in Çanakkale province and districts in 2020-2021. Females of root-knot nematodes and second-stage juveniles (J2s) from egg masses were obtained from celery samples brought to the laboratory. Morphological identification of root-knot nematode species was done by making sections obtained from perineal regions of female individuals and morphometric measurements were performed from J2s for each population. For molecular identification, DNA was then extracted from samples and analyzed by species-specific primers referring to the most common *Meloidogyne* spp. *Meloidogyne javanica* (Treub, 1885) Chitwood,1949 in 9 samples, and *M. arenaria* (Neal, 1889) in 5 samples were determined based on morphologic -morphometric and molecular methods. The result of the study indicated that the rate of root-knot nematode infestation in celery cultivation areas in Çanakkale was 18,6%. The infestation rates of *M. javanica* and *M. arenaria* determined in celery growing areas in the province were 12% and 6.6%, respectively. In this study, *M. javanica* and *M. arenaria* species were detected for the first time in the celery fields of Çanakkale province.

Plant Protection

Research Article

Article History
Received : 27.07.2023
Accepted : 16.10.2023

Keywords
Celery
Çanakkale
Prevalence
Root-knot nematode

Çanakkale İli Kereviz (*Apium graveolens* L.) Yetiştirilen Alanlardaki Kök-ur Nematod (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) Türlerinin Tanılanması

ABSTRACT
Bu çalışmada Çanakkale ili kereviz (*Apium graveolens* L.) (Apiaceae) üretim alanlarında verim kaybına neden olan kök-ur nematodlarının (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) türleri ve yaygınlıkları belirlenmiştir. Bu amaçla, 2020-2021 yıllarında Çanakkale il ve ilçelerinde bulunan toplam 75 farklı kereviz üretim alanlarına periyodik olmayan arazi çıkışları yapılarak kereviz bitki kök ve toprak örnekleri alınmıştır. Laboratuvara getirilen kereviz örneklerinden kök-ur nematodlarının dişi bireyleri ve yumurta paketlerinden ikinci dönem juveniller (J2s) elde edilmiştir. Kök-ur nematodlarının morfolojik tür teşhisleri her popülasyon için dişi bireylerin perineal bölgelerinden elde edilen kesitler ve ikinci dönem larvaların morfometrik ölçümleri yapılarak belirlenmiştir. Daha sonra moleküler tanılama için örneklerden DNA elde edilmiştir ve en yaygın *Meloidogyne* spp. türlerine istinaden türe özgü primerler ile analiz edilmiştir. Yapılan morfolojik ve moleküler tanılama çalışmalarına göre 9 örnekte *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 türü, 5 örnekte ise *M. arenaria* (Neal, 1889) Chitwood, 1949 türü belirlenmiştir. Çalışma sonucunda Çanakkale ili kereviz yetiştiriciliği yapılan alanlarda kök-ur nematodu bulaşıklık oranı

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 27.07.2023
Kabul Tarihi : 16.10.2023

Anahtar Kelimeler
Kereviz
Çanakkale
Yaygınlık
Kök-ur nematodu

%18,6 olarak belirlenmiştir. İlde kereviz yetiştirilen alanlarda belirlenen *M. javanica* ve *M. arenaria*'nın bulaşıklık oranı sırası ile %12 ve %6,6 olarak tespit edilmiştir. Bu çalışma ile Çanakkale ili kereviz alanlarında *M. javanica* ve *M. arenaria* türleri ilk defa tespit edilmiştir.

Atıf Şekli: Yılmaz, A., Çakmak, T., & Gözel, U. (2024). Çanakkale İli Kereviz (*Apium graveolens* L.) Alanlarındaki Kök-ur Nematodu (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) Türlerinin Tanılanması. *KSÜ Tarım ve Doğa Derg 27* (4), 828-838. https://doi.org/10.18016/ksutarimdoga.vi.1333244

To Cite : Yılmaz, A., Çakmak, T., & Gözel, U. (2024). Identification of Root-knot Nematode (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) Species in Celery (*Apium graveolens* L.) (Apiaceae) Areas of Çanakkale Province. *KSU J. Agric Nat 27* (4), 828-838. https://doi.org/10.18016/ksutarimdoga.vi.1333244

INTRODUCTION

The main winter vegetables grown in Çanakkale province are cabbage (*Brassica oleraceae* L. var. *capital*), lettuce (*Lactuca sativa* L.), cauliflower (*Brassica oleraceae* L. var. *botrytis*), spinach (*Spinacia oleracea* L.), celery (*Apium graveolens* L.) and leek (*Allium porrum* L.). In 2020, 2.750.000 tonnes of winter vegetables were grown in 1,700.000 decares cultivation areas of Türkiye. Among the winter vegetables with the highest production rate, celery belonging to the Apiaceae family, comes to the forefront with 230 tonnes in Çanakkale (TUIK, 2020).

It has been reported that the celery plant is known as a host of many plant-parasitic nematodes, such as *Pratylenchus penetrans* Cobb, 1917 (Townsend & Wolynetz, 1991); *P. hamatus* Thorne & Allen (Townshend, 1962); *Hemicycliophora Arenaria* Raski, 1958 (Franklin & Stone, 1974); *Longidorus plus* Lamberti & Zacheo, 1977 (Bleve-Zacheo et al., 1979; Wyss, 1980); *Cactodera cacti* Filipjev & Schuurmans Stekhoven, 1941 (Esser, 1992); *Paratylenchus* sp. Micoletzky, 1922, (Lownsbery et al., 1952); *Nacobbus aberrans* (Thorne) Thorne & Allen,1944 (Doucet, 1999); *Ditylenchus dipsaci* Kühn, 1857 (Di Benedetto, 2005) and *H. poranga* Monteiro & Lordello, 1978 (Emilse et al., 2011). Root-knot nematodes (RKNs) have a wide host range, and it was reported that they feed on 5500 different plant varieties, including vegetables, fruit trees, ornamental plants, weeds, and medicinal plants (Trudgill & Block, 2001; Karssen et al., 2013; Ataş et al., 2021). Hitherto 105 root-knot nematodes species (*Meloidogyne* spp. Goeldi, 1887) (Tylenchida: Meloidogynidae) were identified all over the world (Ghaderi & Karssen, 2020; Maleita et al., 2021), and the most common species are *Meloidogyne incognita* (Kofoid & White, 1919), *M. javanica* (Treub, 1885) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. Chitwood* (Golden et al., 1980), *M. fallax* (Karssen, 1996), and *M. hapla* Chitwood, 1949 (Adam et al., 2007). In the studies conducted in Türkiye, 10 root-knot nematodes species were detected; *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. Chitwood*, *M. Thames*, *M. Martinelli*, *M. exiqua*, *M. luci* and *M. graminis* species (Yüksel, 1974; Elekçioğlu & Uygun, 1994; Elekçioğlu et al., 1994; Mennan & Ecevit, 1996; Söğüt & Elekçioğlu, 2000; Devran & Söğüt 2009; Özarslandan et al., 2009; Akyazı & Ecevit, 2010; Özarslandan & Elekçioğlu, 2010; İmren et al., 2014; Kepenekçi et al., 2014; Çetintaş & Çakmak, 2016; Devran et al., 2017; Aydınlı, 2018; Uysal et al., 2023).

Meloidogyne species cause significant yield losses and more serious damage to celery compared to other plant-parasitic nematodes. Some studies indicated that the most dominant root-knot species infesting celery are *M. incognita*, *M. incognita* race-1, *M. javanica*, *M. hapla*, and *M. arenaria* in the world (Incer & Lopez, 1979; Doucet, 1999; Chaves, 2002; Vovlas et al., 2008; Malakeberhan et al., 2012). There is also, a study on the presence of root-knot nematodes in celery growing areas of Türkiye. *Meloidogyne incognita* and *M. arenaria* were reported on the celery plant in the Black Sea Region of Türkiye (Yüksel 1974). This present study aimed to determine root-knot nematode species collected from Çanakkale celery cultivation areas by using morphometric-morphologic and molecular methods.

MATERIAL and METHOD

Survey

Overall, 75 root samples were collected from celery cultivated fields of Çanakkale in 2020 and 2021. The sampling locations of the celery cultivation areas of Çanakkale are given in Figure 1. Celery plants infested with root-knot nematodes in an ice box were brought to the laboratory. In both years, adult females, egg masses, and second-stage juveniles (J2s) of root-knot nematodes were extracted from samples.

Culture of the root-knot nematodes

To obtain pure cultures, egg masses on infected celery roots were collected by using a small needle. Each root-knot nematode isolate was cultured from a single egg mass taken from galled celery roots and multiplied on the susceptible tomato cv. Çanakkale F1 in a growth chamber at 25±1 □C and 65% RH with a 16:8 L:D photoperiod.

Morphologic-morphometric identification

Perineal patterns: Adult root-knot nematode females were dissected from the roots of the celery plants with a needle and scalpel under the binocular microscope.

Perineal patterns of the extracted females were cut in 45% lactic acid and their preparations were made in glycerin (Hooper, 1986). Morphological identification of Meloidogyne species was made according to Jepson (1987) and Karssen (2002).

Figure 1. Sampling locations of the celery cultivation areas in Çanakkale.
Şekil 1. Çanakkale kereviz ekim alanlarındaki örnekleme noktaları.

Morphometric characters of second-stage juveniles: J2s hatched from the egg masses multiplied as pure cultures were fixed in TAF fixative and permanent preparations were done according to Seinhorst's (1959) method. Measurements of approximately 25 J2s were made according to Karssen (2002) under the Leica DM1000 stereomicroscope.

Molecular identification

DNA isolation

DNA was extracted from pure cultures of J2s by using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) according to the manufacturer's guidelines.

PCR analyses

The PCR reactions were conducted on the SimpliAmp™ Thermal Cycler (Applied Biosystems, CA, USA) using the reaction conditions in a total volume of 25 µ L: 20 ng of DNA, 2 mM MgCl2, 2.5 µ L 10X PCR buffer, 200 µ M dNTPs, 0.4 µ M of each primer, 1 U Taq DNA Polymerase (ABM), and molecular double distilled water. To screen samples; Inc-K14F/Inc-K14R primers (Randig et al., 2002) and MincF1/MincR1 (Devran et al., 2018) for *M. incognita*; Far/Rar primers (Zijlstra et al., 2000) for *M. arenaria*; Fjav/Rjav primers (Zijlstra et al., 2000) for *M. javanica* and JMV primers for *M. hapla* (Wishart et al., 2002) were used in the PCR reactions. Then, PCR products were electrophoresed on a 1.5% agarose gel in 1X TAE and visualized under UV light with Xpert Green DNA Stain by using the Gel iX Imager (Intas Science, Germany).

RESULTS and DISCUSSION

Morphologic-morphometric identification

Root samples taken from celery cultivation areas revealed that there was an 18.6% prevalence of root-knot nematodes in Çanakkale. In other words, root-knot nematode was detected in 14 of 75 celery samples collected, and galled symptoms of plant roots were shown in Figure 2.

Figure 2. Celery plant roots infested with root-knot nematode.
Şekil 2. Kök-ur nematodları ile infekteli kereviz kökleri.

From a total of 14 root-knot nematode populations, 9 samples were identified as *M. javanica*, while 5 samples were identified as *M. arenaria* by perineal pattern and morphometric measurement of J2s. Locations and coordinates of root-knot nematode species in infested areas recorded with Global Positioning System (GPS) were given in Table 1 and Figure 3.

Meloidogyne javanica

Similar to the findings obtained in this study, distinct lateral ridges that divide the dorsal and ventral striae, and lateral lines extended on both sides of the tail terminus are typically as clear as the perineal patterns of *M. javanica (Figure 4).*

Table 1. Location, Root-knot nematodes species, and coordinates of celery samples collected from Çanakkale
Tablo 1. Çanakkale'den toplanan kereviz örneklerine ait konum Kök-ur nematodu türleri ve koordinatları

Sample No	Location	Species*	Latitude (N)	Longitude (E)
1	Kepez	*Mj*	40° 5′ 31"	26° 23′ 2"
3	Kepez	*Mj*	40° 5′ 41"	26° 22′ 8"
7	Kepez	*Mj*	40° 5′ 21"	26° 22′ 42 "
9	Saraycık	*Mj*	40° 8′ 17 "	26° 28′ 6 "
12	Çıplak	*Ma*	39° 57′ 31 "	26° 16′ 16 "
23	Halileli	*Ma*	39° 58′ 13 "	26° 16′ 55 "
27	Umurbey	*Ma*	40° 14′ 30 "	26° 38′ 26 "
30	Çardak	*Ma*	40° 24′ 40 "	26° 45′ 17 "
44	Halileli	*Mj*	39° 57′ 47"	26° 17′ 59"
62	Umurbey	*Mj*	40° 14′ 27"	26° 39′ 35"
63	Umurbey	*Mj*	40° 14′ 41"	26° 39′ 14"
65	Biga[a]	*Mj*	40° 17′ 10"	27° 17′ 7"
69	Biga[a]	*Mj*	40° 14′ 50"	27° 12′ 34"
72	Eceabat[a]	*Ma*	40° 15′ 44"	26° 23′ 24"

*Ma: *Meloidogyne arenaria*, Mj: *Meloidogyne javanica*, a: Distinct of Çanakkale

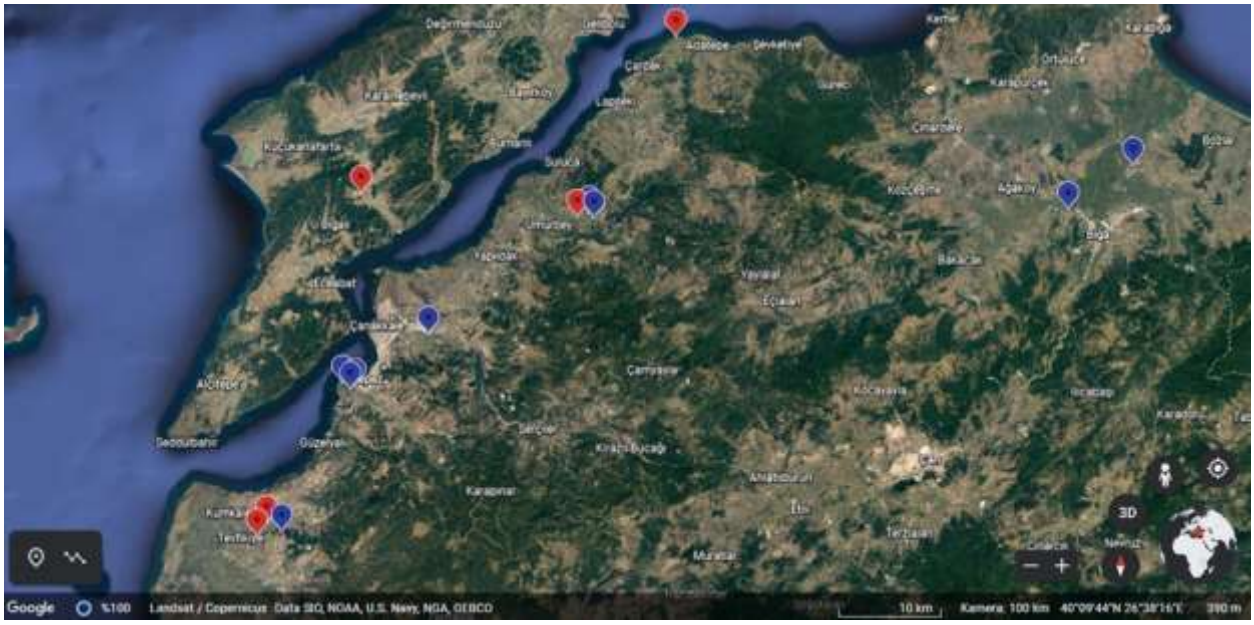


Figure 3. Locations of Root-knot nematode populations collected from celery growing areas in Çanakkale.
Şekil 3. Çanakkale kereviz alanlarından toplanan Kök-ur nematodu popülasyonlarının lokasyonları.
(Places where *Meloidogyne arenaria* was identified on celery- red color)
(Places where *Meloidogyne javanica* was identified on celery- blue color)

In the study, stylet lengths in all populations were long, and their DGO was relatively shorter than those reported by Whitehead (1968). Conversely, the ratios of body length to body width (a) in all populations were relatively short. All other lengths aligned with the findings of Whitehead (1968) (Table 2).

Meloidogyne arenaria

These patterns showed a typically rounded to flattened low dorsal arch near the lateral field with irregular forks (Figure 5).

The body length of all populations of *M. arenaria* was shorter than those reported by Whitehead (1968). The style length and the ratio of body length to tail length (c) of all populations were relatively long according to the Whitehead (1968) (Table 3).

Molecular identification

Molecular identification of a total of 14 DNA samples was done by using four different species-specific primer sets. *Meloidogyne arenaria-specific* Far/Rar and *M. javanica-specific* Fjav/Rjav primers were produced in expected amplicons of approximately 420 bp and 670 bp, respectively (Figure 6, 7).

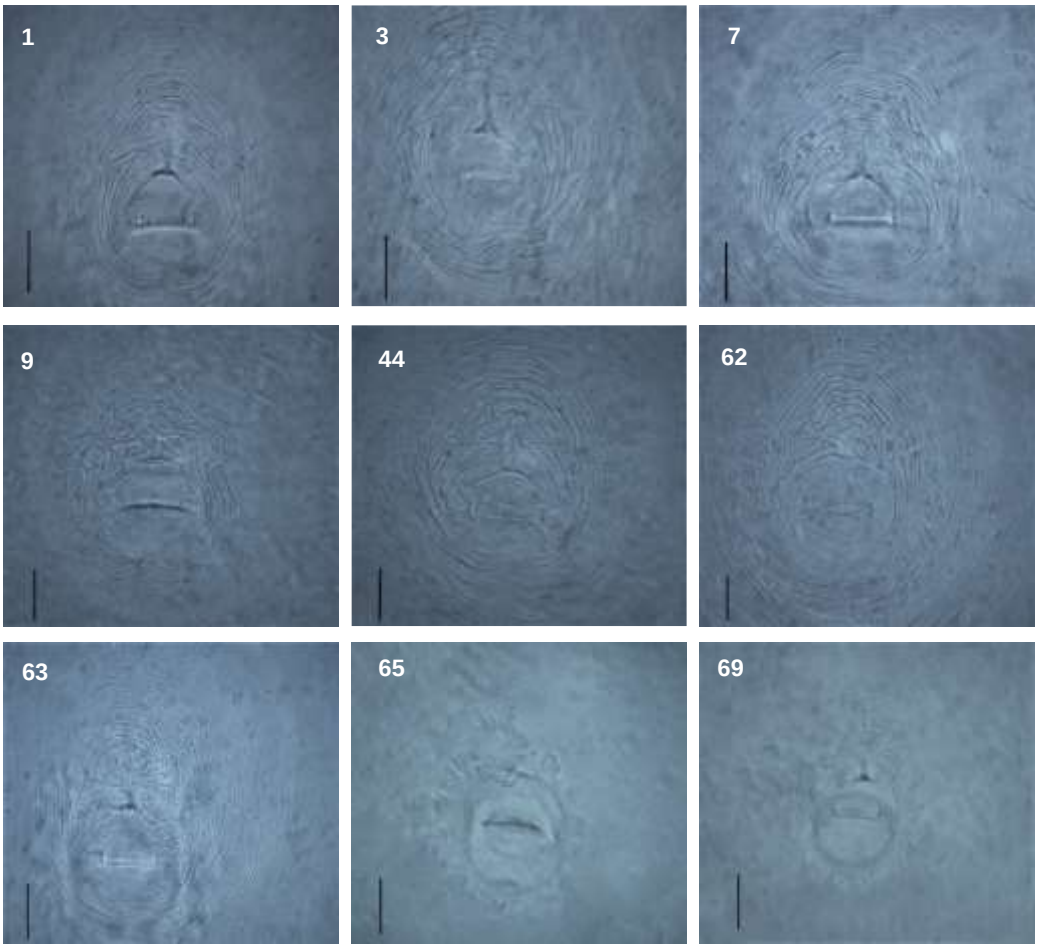


Figure 4. Perineal patterns of *Meloidogyne javanica* isolates collected from the celery fields of Çanakkale. Scale bar: 20 µm.
Şekil 4. Çanakkale'de kereviz alanlarından toplanan Meloidogyne javanica izolatlarının perineal bölge kesitleri. Ölçek çubuğu 20 µm.

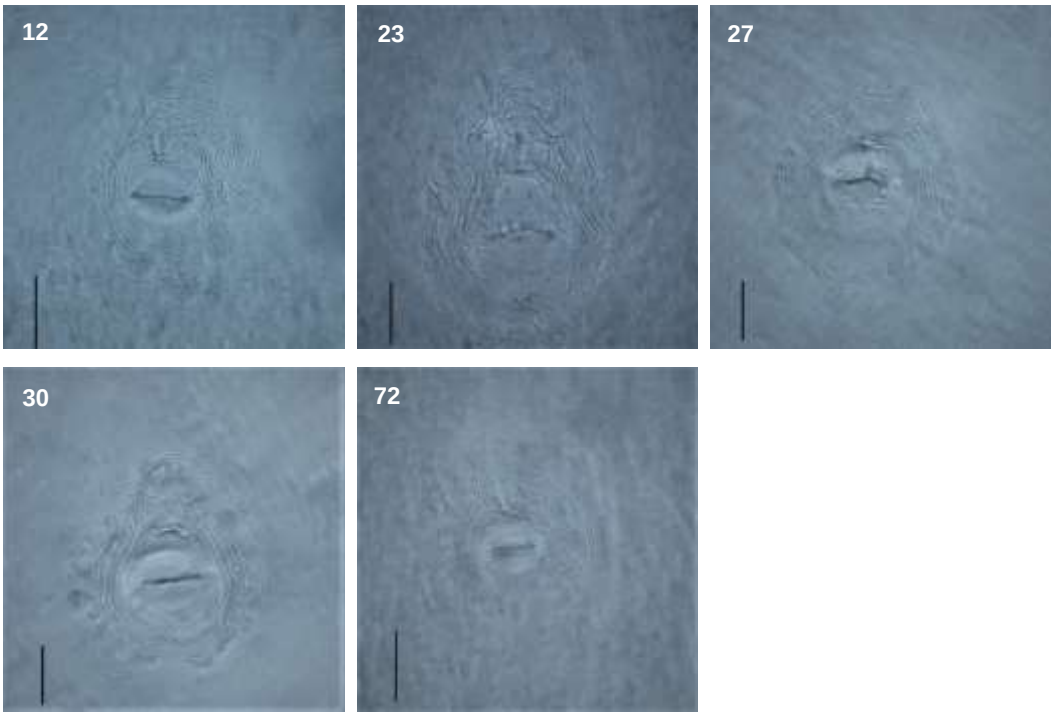


Figure 5. Perineal patterns of *Meloidogyne arenaria* isolates collected from the celery fields of Çanakkale. Scale bar: 20 µm.
Şekil 5. Çanakkale'de kereviz alanlarından toplanan Meloidogyne arenaria izolatlarının perineal bölge kesitleri. Ölçek çubuğu 20 µm.

Table 2. Diagnostic characters of second stage juveniles (J2) of *Meloidogyne javanica* on celery
Tablo 2. Kereviz üzerindeki Meloidogyne javanica'nın ikinci dönem juvenillerinin (J2s) teşhis karakterleri

Diagnostic characters	1	3	7	9	44	Whitehead (1968)
Body length	411.9±17.8 (385.1-445.7)	395.1±14.8 (371.7-419.9)	404.3±12.4 (380.6-431.3)	402.0±14.1 (378.8-429.8)	414.9±15.3 (380.2-449.7)	387-459
Body width	14.7±0.6 (13.3-15.8)	14.9±0.5 (14.1-16.0)	15.4±0.4 (14.7-16.2)	15.7±0.5 (14.6-16.6)	15.7±0.6 (14.8-16.9)	
Body width at stylet base	8.5±0.6 (7.0-9.8)	8.6±0.5 (7.9-9.8)	8.6±0.4 (7.0-10.0)	8.8±0.4 (7.9-10.8)	8.7±0.5 (7.8-10.0)	
Body width at the anus	9.2±0.6 (8.2-10.4)	9.7±0.6 (8.9-11.0)	9.8±0.6 (8.8-11.0)	9.8±0.7 (8.7-11.7)	9.3±0.7 (7.9-10.8)	
Stylet length	13.0±0.7 (11.6-14.2)	12.6±0.5 (10.7-12.8)	11.7±0.7 (10.0-13.6)	12.6±0.7 (11.1-13.6)	12.7±1.0 (10.8-14.1)	9.4-11.4
DGO	3.6±0.3 (2.7-4.1)	3.3±0.4 (2.3-4.0)	3.3±0.4 (2.4-4.0)	3.3±0.4 (2.5-4.0)	3.4±0.5 (2.5-4.1)	4
Tail length	50.3±1.8 (47.9-55.9)	47.8±2.0 (44.4-51.0)	50.9±3.2 (45.9-58.0)	50.0±2.3 (45.6-55.0)	50.7±2.8 (45.4-55.5)	36-56
Excretory pore to head end	79.6±3.3 (74.4-86.2)	77.2±3.9 (67.7-87.7)	76.1±4.2 (68.7-84.1)	81.4±3.9 (74.4-88.2)	80.1±4.8 (70.5-88.3)	
Body width at the excretory pore	11.6±0.7 (10.4-13.0)	11.8±0.5 (10.7-12.8)	11.0±1.1 (9.3-13.2)	11.2±0.9 (9.2-13.7)	11.9±2.8 (45.4-55.5)	
a	26.9±0.7 (26.5-29.2)	26.4±1.2 (24.9-29.6)	26.1±0.8 (24.6-27.9)	25.5±0.8 (24.0-27.0)	26.4±0.9 (24.6-27.8)	27.1-35.9
b	4.3±0.2 (3.9-4.8)	4.0±0.2 (3.7-4.5)	4.4±0.2 (4.0-4.8)	4.4±0.3 (3.8-5.0)	4.5±0.2 (4.0-5.2)	
c	8.1±0.4 (7.4-9.1)	8.2±0.5 (7.3-9.3)	7.9±0.6 (6.6-8.9)	8.0±0.4 (7.2-8.8)	8.2±0.6 (6.8-9.2)	7.3-11.1
c'	5.4±0.3 (4.8-6.2)	4.9±0.3 (4.3-5.4)	5.1±0.4 (4.4-6.05)	5.1±0.4 (4.2-5.7)	5.4±0.4 (4.9-6.4)	

Diagnostic characters	62	63	65	69	Whitehead (1968)
Body length	410.8±17.2 (378.0-440.8)	400.5±13.3 (375.7-426.8)	399.7±13.4 (375.6-426.7)	402.9±11.6 (377.6-421.6)	387-459
Body width	15.6±0.6 (14.5-16.9)	15.5±0.5 (14.4-16.8)	15.3±0.6 (14.1-16.8)	15.5±0.5 (14.5-16.5)	
Body width at stylet base	9.1±0.9 (7.4-11.0)	8.7±0.6 (7.6-10.0)	8.8±0.6 (7.4-10.0)	8.5±0.5 (7.6-9.6)	
Body width at the anus	9.3±0.7 (8.1-10.8)	9.5±0.7 (8.2-10.8)	9.7±0.8 (8.2-11.7)	9.7±0.7 (8.4-11.2)	
Stylet length	11.2±0.7 (10.7-11.6)	11.7±1.0 (10.4-11.8)	11.1±0.7 (10.6-11.4)	11.6±0.6 (10.9-11.9)	9.4-11.4
DGO	3.4±0.4 (2.6-4.1)	3.3±0.4 (2.6-4.1)	3.1±0.4 (2.4-4.0)	3.2±0.4 (2.4-4.0)	4
Tail length	52.1±3.7 (43.7-61.0)	50.3±3.0 (44.6-56.1)	50.9±2.7 (45.9-56.4)	49.6±2.9 (44.5-55.8)	36-56
Excretory pore to head end	80.3±3.2 (75.6-86.9)	73.5±5.0 (64.3-86.9)	79.0±3.6 (70.5-85.1)	74.9±5.8 (64.1-85.8)	
Body width at the excretory pore	12.2±0.9 (10.7-13.6)	12.0±0.8 (10.8-13.9)	12.0±0.8 (10.7-13.2)	11.9±0.5 (10.9-13.0)	
a	26.2±0.9 (24.3-27.7)	25.7±1.0 (24.2-27.9)	26.0±0.8 (25.0-27.6)	26.0±0.9 (24.3-27.9)	27.1-35.9
b	4.5±0.3 (3.9-5.3)	4.6±0.3 (3.9-5.5)	4.3±0.3 (3.9-5.1)	4.6±0.4 (3.7-5.4)	
c	7.9±0.6 (6.8-9.5)	7.9±0.5 (6.9-9.2)	7.8±0.4 (6.9-8.7)	8.1±0.4 (7.3-8.9)	7.3-11.1
c'	5.5±0.4 (4.5-6.4)	5.2±0.3 (4.6-5.9)	5.2±0.4 (4.4-6.1)	5.1±0.4 (4.4-6.0)	

Note: Note: All measurements are in µm Body length/Body width, b: Body length/ Intestine to the head end, c: Body length/Tail length, c': Tail length/Body width at the anus) and in the form: mean ± s.d. (range) n: 25

Table 3. Diagnostic characters of second-stage juveniles (J2) of *Meloidogyne arenaria* on celery
Tablo 3. Kereviz üzerindeki Meloidogyne arenaria'nın ikinci dönem juvenillerinin (J2) teşhis karakterleri

Diagnostic characters	12	23	27	30	72	Whitehead (1968)
Body length	449.5±16.3 (423.8-454.7)	442.6±9.8 (431.7-452.9)	441.2±17.0 (433.7-452.8)	442.5±14.2 (429.5-450.7)	448.9±12.0 (437.3-453.5)	450-490
Body width	15.8±0.4 (14.7-16.9)	15.7±0.5 (14.9-17.0)	15.7±0.6 (14.1-17.0)	15.0±0.6 (13.7-16.0)	15.6±0.4 (14.8-16.4)	
Body width at stylet base	8.5±0.6 (7.7-9.7)	8.8±0.5 (8.0-10.0)	8.8±0.7 (7.6-10.0)	9.1±0.8 (7.5-10.9)	8.7±0.5 (8.0-9.6)	
Body width at the anus	10.1±0.7 (8.7-11.1)	9.8±0.7 (8.5-11.2)	10.4±0.5 (9.0-11.7)	10.0±0.7 (8.5-11.1)	9.8±0.5 (8.5-10.9)	
Stylet length	10.5±0.7 (10.1-12.0)	11.2±1.0 (10.8.12.7)	11.2±0.6 (10.8-12.2)	11.0±1.0 (10.3-12.0)	11.0±0.7 (10.4-12.4)	10
DGO	3.1±0.3 (2.5-4.0)	3.1±0.4 (2.1-4.0)	3.2±0.4 (2.4-4.0)	3.2±0.4 (2.5-4.0)	3.1±0.4 (2.3-3.8)	3
Tail length	48.6±3.3 (44.3-58.5)	49.1±2.9 (42.9-54.3)	49.0±2.5 (44.7-53.7)	50.1±2.7 (45.6-55.3)	49.6±2.4 (45.6-54.5)	
Excretory pore to head end	78.0±4.9 (65.1-96.1)	77.6±4.7 (67.6-85.7)	77.8±6.5 (67.5-89.8)	76.9±4.4 (69.6-83.7)	78.5±3.7 (67.9-85.2)	
Body width at the excretory pore	12.2±0.7 (10.9-13.1)	11.6±0.9 (9.2-13.1)	12.7±0.7 (11.0-12.8)	11.5±0.8 (9.8-13.6)	12.2±0.6 (10.5-13.7)	
a	25.8±0.7 (24.6-27.4)	24.9±0.8 (23.6-26.8)	26.1±0.8 (24.5-27.6)	25.4±0.7 (24.2-26.6)	26.0±0.8 (24.4-27.3)	26-32
b	4.4±0.3 (3.8-5.1)	4.1±0.3 (3.8-4.9)	4.4±0.3 (3.9-5.0)	4.1±0.2 (3.7-4.7)	4.5±0.2 (4.1-5.0)	
c	7.6±0.5 (7.1-8.4)	7.8±0.5 (7.1-8.4)	7.7±0.1 (6.6-8.2)	7.6±0.4 (6.7-8.8)	7.9±0.4 (7.3-8.4)	6-7.5
c'	4.8±0.4 (4.2-5.9)	5.0±0.4 (4.2-6.1)	4.0±0.4 (4.0-5.5)	5.0±0.5 (4.1-6.3)	5.0±0.4 (4.5-6.1)	

Note: Note: All measurements are in µm Body length/Body width, b: Body length/ Intestine to the head end, c: Body length/Tail length, c': Tail length/Body width at the anus) and in the form: mean ± s.d. (range) n: 25

However, both *M. incognita* primer sets and JMV primers did not give any DNA bants in analyzed samples. As a result of PCR studies, *M. arenaria* and *M. javanica* were found in 5 and 9 samples, respectively. These results show that this is the first report of root-knot nematode infecting celery in Çanakkale of Türkiye.

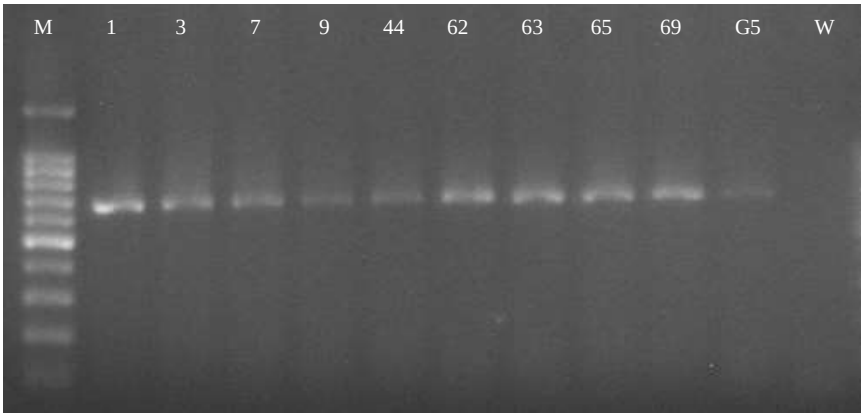


Figure 6. PCR products of amplified DNA using *Meloidogyne javanica*-specific primer Fjav/Rjav, M: 100 bpDNA Ladder (Hibrigen); Samples:1, 3, 7, 9, 44, 63, 65 and 69; G5: *M. javanica* (positive control); W: Water.
Şekil 6. Meloidogyne javanica'ya özgü primer Fjav/Rjav primer kullanılarak coğaltılmış DNA ya ait PCR ürünleri, M: 100 bpDNA Ladder (Hibrigen); Örnekler:1, 3, 7, 9, 44, 63, 65, ve 69; G5: M. javanica (pozitif kontrol); W: Su.

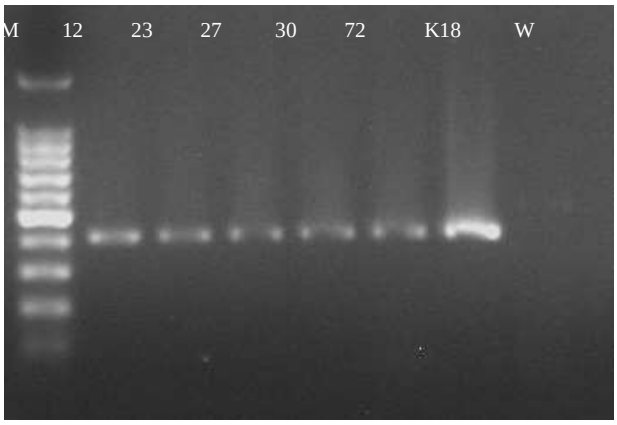


Figure 7. PCR products of amplified DNA of using *Meloidogyne arenaria*-specific primer Far/Rar, M: 100 bp DNA Ladder (Hibrigen); Samples: 12, 23, 27, 30 and72; K18: *M. arenaria* (positive control); W: Water.

Şekil 7. Meloidogyne arenaria'ya özgü primer Fjav/Rjav primeri kullanılarak coğaltılmış DNA'ya ait PCR ürünleri, M: 100 bp DNA Ladder (Hibrigen); Örnekler: 12-72r; K18: M. arenaria (pozitif kontrol); W: Su.

Morphologic-morphometric and molecular data were evaluated together. In the provincial center, 46 samples were taken from celery growing areas and it was determined that 5 of them were infected with *M. javanica* and 2 of them were infected with *M. arenaria*. Ten samples were taken from the fields in Lapseki and they were infected with *M. javanica* (2) and *M. arenaria* (2) respectively. Twelve samples were taken from the fields in Biga and 2 of them were found to be infected with *M. javanica*. Five samples were taken from the fields in Eceabat and one of them was found to be infected with *M. arenaria*. Single samples taken from each of Ezine and Bayramiç district's cultivation areas were not infected with RKNs. It has been determined that 12% and 6.6% of the celery-growing areas of Çanakkale are infested with *M. javanica* and *M. arenaria*, respectively (Table 4).

As an important edible vegetable, celery plants ought to be protected from soil-borne pests including plant parasitic nematodes. Especially RKNs have a wide host range, and their complete control is quite costly and difficult. Root-knot nematode infestation has been reported in many other important plant cultivars with edible tubers, such as carrot (Singh, 2009; Evlice et al., 2020), potato (Özarslandan et al., 2009; Maleita et al., 2018), sugar beet (Yu, 1995; Maareg et al., 1998), sweet potato (Rutter et al., 2019; Yigezu, 2021). Accurate identification is of great importance in the control of RKNs. Therefore, control methods such as the use of resistant cultivars and crop rotation can significantly reduce the population density of the pest.

Table 4. Rates of finding of root-knot nematode species in Çanakkale provincial center and districts

Tablo 4. Çanakkale il merkezi ve ilçelerinde kök-ur nematodu türlerinin bulunma oranları

Districts	Number of Samples	Number of Infected Samples and Finding Rate			
		Meloidogyne javanica	Finding Rate (%)	*Meloidogyne arenaria*	Finding Rate (%)
Center	46.0	5.0	10.9	2.0	4.4
Ezine	1.0	0.0	0.0	0.0	0.0
Bayramiç	1.0	0.0	0.0	0.0	0.0
Lapseki	10.0	2.0	20.0	2.0	20.0
Biga	12.0	2.0	16.6	0.0	0.0
Eceabat	5.0	0.0	0.0	1.0	20.0
Total	75.0	9.0	12.0	5.0	6.6

In the present study, in total, 14 of 75 celery samples (18,6%) collected from celery cultivation areas in the Çanakkale were found to be infested with RKNs. It was determined that *M. javanica* is the most dominant species with a 12% infestation rate in the region. In the international *Meloidogyne* project in Malawi, Saka (1981) reported the presence of *M. javanica* on celery. *Meloidogyne javanica* has been found also in different vegetable-growing areas of Türkiye, (Devran & Söğüt, 2009; Aydınlı & Mennan, 2016; Devran et al., 2017; Uysal et al., 2017; Gürkan et al., 2019; Aslan & Elekçioğlu, 2022).

In the present study, the infestation rate of *M. arenaria* in celery cultivation areas was found to be 6.6%. *Meloidogyne arenaria* was identified in some districts. Celery cultivation areas in Halileli and Umurbey regions were found to be infested with both RKN species. In Türkiye, the first and only study on the presence of RKNs in celery cultivation areas in the Black Sea Region was conducted and *M. arenaria* and *M. incognita* species were reported (Yüksel, 1974). Previous studies also reported that *M. arenaria* has been found on cultivated plants (Devran & Söğüt, 2009; Aydınlı & Mennan, 2016; Devran et al., 2017; Uysal et al., 2017; Gürkan et al., 2019; Aslan & Elekçioğlu, 2022). However, it was reported that *Meloidogyne hapla* is the most prevalent RKN species

in celery cultivation areas in some parts of the world (Anita, 2012; Malakeberhan & Wei, 2012). In this study, *M. hapla* was not detected in the survey areas.

In conclusion, the survey was carried out for the identification of RKNs in celery-growing areas of Çanakkale. *Meloidogyne javanica* and *M. arenaria* were identified on celery in the region. This study is the first report of *M. javanica* in celery cultivation areas in Türkiye.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Adam, M.A.M., Phillips, M.S. & Blok, V.C. (2007). Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathology, 56*(1), 190-197. https://doi.org/10.1111/j.1365-3059.2006.01455.x

Akyazı, F. & Ecevit, O. (2010). Determination of *Meloidogyne incognita* (Kofoid & White, 1919) (Nemata: Meloidogynidae) races in vegetable fields in Erbaa and Niksar plains in Tokat. Journal of *Journal of Agricultural Faculty of Gaziosmanpaşa University*, *28*(2), 25-30. https://doi.org/10.7161/ANAJAS.2011.26.1.1-9

Anita, B. (2012). Crucifer vegetable leaf wastes as biofumigants for the management of root-knot nematode (*Meloidogyne hapla* Chitwood) in celery (*Apium graveolens* L.). *Journal of Biopesticides*, 5, 111-114.

Aslan, A., & Elekcioğlu, İ.H. (2022). Biochemical and molecular identification of root-knot nematodes in greenhouse vegetable areas of Eastern Mediterranean Region (Turkey). *Turkish Journal of Entomology, 46*(1), 115-127. https://doi.org/10.16970/entoted.1055181

Ataş, H., Uysal, G., Gözel, Ç., Özalp, T., Gözel, U. & Devran Z. (2021). First report of root-knot nematode, *Meloidogyne incognita* on calendula in Turkey. *Journal of Nematology, 53*(1), 1-5. https://doi.org/10.21307/jofnem-2021-041

Aydınlı, G. & Mennan, S. (2016). Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. *Turkish Journal of Zoology, 40*(5), 675-685. http://dx.doi.org/10.3906/zoo-1508-19

Aydınlı, G. (2018). Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province, Turkey. *Turkish Journal of Entomology*, *42*(3), 229-237. https://doi.org/10.16970/entoted.409941

Bird, G. W., & Warner, F. (2018). Nematodes and Nematologists of Michigan. *Plant Parasitic Nematodes in Sustainable Agriculture of North America: Vol. 2-Northeastern, Midwestern and Southern USA*, 57-85. http://dx.doi.org/10.1007/978-3-319-99588-5_3

Bleve-Zacheo, T., Zacheo, G., Lambertı, F. & Arrıgonı, O. (1979). Cell wall protrusion and associated membranes in roots parasitized by *Longidorus apulus*. *Nematologica 25*(11), 62-66.

Chaves, E. (2002). Nematodos causantes de daños en cultivos hortícolas del sudeste bonaerense. (On line) Instituto Nacional de Tecnología Agropecuaria (INTA). Estación Experimental Agropecuaria Balcarce. Buenos Aires, Argentina.

Cobb, N. A. (1917). A new parasitic nema was found infesting cotton and potatoes. *Journal of Agricultural Research, 11*(2733), 317329.

Cetintas, R., & Cakmak, B. (2016). *Meloidogyne* species infesting tomatoes, cucumbers, and eggplants grown in Kahramanmaraş Province, Turkey. *Turkish Journal of Entomology, 40*(4), 355-364. http://dx.doi.org/10.16970/ted.40839

Devran, Z., Mıstanoğlu, İ., & Özalp, T. (2017). Occurrence of mixed populations of root-knot nematodes in vegetable greenhouses in Turkey, as determined by PCR screening. *Journal of Plant Diseases and Protection, 124*, 617-630. https://doi.org/10.1007/s41348-017-0113-9

Devran, Z., Polat, İ., Mıstanoğlu, İ., & Baysal, Ö. (2018). A novel multiplex PCR tool for simultaneous detection of three root-knot nematodes. *Australasian Plant Pathology, 47*, 389-392. https://doi.org/10.1007/s13313-018-0570-z

Devran, Z., & Söğüt, M. A. (2009). Distribution and identification of root-knot nematodes from Turkey. *Journal of nematology, 41*(2), 128-133.

Di Benedetto, A. (2005). Manejo de cultivos hortícolas. Bases ecofisiológicas y tecnológicas. Ed. Orientación Gráfica Editora. pp.61-77. Buenos Aires.

Doucet, M. E. (1999). Nematodos del suelo asociados con vegetales en la República Argentina. Academia Nacional de Agronomía y Veterinaria. Serie de la Academia Nacional de Agronomía y Veterinaria No 24.

Elekcioglu, İ.H., Ohnesorge, B., Lung G. & Uygun, N. (1994). Plant parasitic nematodes in the east Mediterranean region of Turkey. *Nematologia Mediterranea, 22*(1), 59-63.

Elekçioğlu, İ. H., & Uygun, N. (1994). Occurrence and distribution of plant parasitic nematodes in cash crop in Eastern Mediterranean region of Turkey. *In Proceedings of 9th Congress of the Mediterranean Phytopathological Union, KuşGadası-Aydın-Türkiye* (pp. 409-410).

Emilse, C., Oggero, A., Tordable, M. D. C., Lax, P., De Ley, I. T., & Doucet, M. (2011). Anatomical and histological alterations induced by *Hemicycliophora sporangia* Monteiro & Lordello, 1978 in celery (*Apium graveolens* L.) roots. *Russian Journal of Nematology, 19*(1), 75-81.

Esser, R. P. (1992). Cactus cyst nematode, Cactodera cacti. Fla. Department Agric. & Consumer Serv., Division of Plant Industry.

Evlice, E., Erdoğuş D. & Yatkın, G. (2020). Reaction of some carrot varieties against root-knot nematode species (*Meloidogyne* spp. Goeldi, 1982). *Bitki Koruma Bülteni, 60*(1), 25-30. https://doi.org/10.16955/bitkorb.599172

Filipjev, I. N., & Schuurmans Stekhoven Jr, J. H. (1941). A Manual of Agricultural Helminthology Brill, Leiden. Filipjev IN and Schuurmans Stekhoven, JH Jr.

Franklin, M.T & Stone, A.R. (1974). *Hemicycliophora arenaria*. C.I.H. Descriptions of Plant-parasitic nematodes. Set 3, No 43. Commonwealth Institute of Parasitology. C.A.B. International. 3pp.

Ghaderi, R. & Karssen, G. (2020). An updated checklist of *Meloidogyne* Goeldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. *Journal of Crop Protection, 9*(2), 183-193.

Gürkan, B., Çetintaş, R., & Gürkan, T. (2019). Gaziantep ve Osmaniye sebze alanlarında bulunan Kök-ur nematodu türleri (*Meloidogyne* spp.)'nin teşhisi ile bazı nematod popülasyon irklarının belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22*(Ek sayı 1), 114-125. https://doi.org/10.18016/ksutarimdoga.v22i49073.551240

Hooper, D.J. (1986). "Extraction of free-living stages from soil, 5-30". In: Laboratory methods for work with plant soil nematodes. (Ed. J. F. Southey), Her Majesty's Stationary Office, London, 402 pp.

Incer, A & López, R. (1979). Evaluación de prácticas selectas para el combate integrado de *Meloidogyne incognita* en apio. *Nematropica 9*, 140-146.

Imren, M., Özarslandan, A., Kasapoğlu, E. B., Toktay, H., & Elekcioğlu, İ. H. (2014). Morphological and molecular identification of a new species *Meloidogyne artiellia* (Franklin) on wheat fauna in Turkey. *Turkish Journal of Entomology, 38*(2), 189-196. https://doi.org/10.16970/ted.69266

Jepson, S.B. (1987). Identification of Root-knot Nematodes *Meloidogyne* species. CABI Wallingford, UK, 265 pp.

Karssen, G. (2002). The plant parasitic nematode genus *Meloidogyne* Goeldi, 1892 (Tylenchida) in Europe. Leiden, The Netherlands: Brill Academic Publishers.

Karssen, G., Wesemael W. & Moens, M. (2013). Root-knot nematodes. *Plant Nematology*, (Ed. 2), 73-108. https://doi.org/10.1079/9781780641515.0073

Kepenekçi, İ., Evlice, E. & Öztürk, G. (2014). Taxonomic characteristics of *Meloidogyne exigua* Goeldi which is a new root-knot nematode for Turkey and other root-knot nematode species. *Plant Protection Bulletin, 54*(1), 1-9.

Kühn, J. (1857). Über das vorkommen von Anguillulen in erkrankten Blüthenköpfen von *Dipsacus fullonum* L. Zeitschr. wissenschaftl. *Zoologie, 9*(1), 129-137.

Lamberti, F., & Zacheo, T. B. (1977). Two new species of *Longidorus* (nematoda: Longidoridae) from Italy. *Nematologia mediterranea*.

Lownsbery, B. F., Stoddard, E. M., & Lownsbery, J. W. (1952). *Paratylenchus hamatus* is pathogenic to celery. *Phytopathology, 42*, 651-653.

Maareg, M. F., Hassanein, M. A., Allam, A. I., & Oteifa, B. A. (1998). Susceptibility of twenty-six sugarbeet varieties to root-knot nematodes, *Meloidogyne* spp. in the newly reclaimed sandy soils of the Al-Bostan region. *Egyptian Journal of Agronematology, 2*(1), 11-125.

Maleita, C., Esteves, I., Cardoso, J. M. S., Cunha, M. J., Carneiro, R. M. D. G., & Abrantes, I. (2018). *Meloidogyne luci*, a new root-knot nematode parasitizing potato in Portugal. *Plant Pathology, 67*(2), 366-376. https://doi.org/10.1111/ppa.12755

Maleita, C., Cardoso, J., Rusinque, L., Esteves, I. & Abrantes, I. (2021). Species-Specific molecular detection of the root-knot nematode *Meloidogyne luci. Biology, 10*(8), 775. https://doi.org/10.3390%2Fbiology10080775

Melakeberhan, H. & Wei, W. (2012). Suitability of celery cultivars to infection by populations of *Meloidogyne hapla. Nematology 14*(5), 623-629.

Mennan, S., & Ecevit, O. (1996). Bafra ve Çarşamba Ovaları yazlık sebze ekim alanlarındaki kök-ur nematodları (*Meloidogyne* spp.)'nın biyolojisi, yayılışı ve bulaşıklık oranları üzerine araştırmalar. Türkiye III. Entomoloji Kongresi, 24(28), 700-705.

Micoletzky, H. (1922). Die freilebenden Erd-nematoden. *Archiv für Naturgeschichte A*, 87, 1-650.

Monteiro, A. R., & Lordello, L. G. E. (1978). Description of *Hemicycliophora poranga* n. sp. from Brazil (Nemata). Revista brasileira de biologia.

Özarslandan, A. & Elekcioğlu, İ.H. (2010). Identification of the root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) collected from different parts of Turkey by molecular and morphological methods. *Turkish Journal of Entomology, 34*(3), 323-335. http://dx.doi.org/10.16969/teb.89808

Ozarslandan, A., Devran, Z., Mutlu, N., & Elekcioglu, I. H. (2009). First report of Columbia root-knot nematode (*Meloidogyne chitwoodi*) in potato in Turkey. *Plant Disease*, *93*(3), 316-316. http://dx.doi.org/10.1094/PDIS-93-3-0316C

Randig, O., Bongiovanni, M., Carneiro, R. M., & Castagnone-Sereno, P. (2002). Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. *Genome*, *45*(5), 862-870. https://doi.org/10.1139/g02-054

Raski, D. J. (1958). Four new species of *Hemicycliophora* de Man, 1921, with further observations on *H. brevis* Thorne, 1955 (Nematoda: Criconematidae). *Proceedings of the Helminthological Society of Washington, 25*(2), 125-131.

Rutter, W. B., Skantar, A. M., Handoo, Z. A., Mueller, J. D., Aultman, S. P., & Agudelo, P. (2019). *Meloidogyne enterococci* found infecting root-knot nematode-resistant sweet potato in South Carolina, United States. *Plant Disease, 103*(775), 2019. https://doi.org/10.1094/PDIS-08-18-1388-PDN

Saka, V.W. (1981). Bvumbwe research station box 5748, Limbe mal awl. In the third research planning conference on root-knot nematodes, *Meloidogyne* spp. (p. 31).

Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica, 4*(1), 67-69. https://doi.org/10.1163/187529259X00381

Singh, V.K. (2009). Occurrence of root-knot nematode, *Meloidogyne incognita* on carrot in Jammu. *Indian Journal of Nematology, 39*(2), 245-245.

Söğüt, M.A. & Elekcioğlu, İ.H. (2000). Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetable growing areas of the Mediterranean Region of Turkey. *Turkish Journal of Entomology, 24*(1), 33-40.

Thorne, G., & Allen, M. W. (1944). Nacobbus dorsalis, nov. gen. nov. spec. (Nematoda: Tylenchidae) producing galls on the roots of alfileria, *Erodium cicutarium* (L.) L'Hér. *Proceedings of the Helminthological Society of Washington, 11*(1), 27-31.

Townshend, J. L. (1962). The root-lesion nematode, *Pratylenchus* penetrans (Cobb, 1917) Filip. & Stek. 1941, in celery. *Canadian Journal of Plant Science, 42*(2), 314-322. https://doi.org/10.4141/cjps62-046

Townsend, J. L. & Wolynetz, M. S. (1991). Penetration of celery and alfalfa roots by *Pratylenchus penetrans* as affected by temperature. *Journal of Nematology, 23*, 194-197.

Trudgill, D. L., & Blok, V. C. (2001). Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual review of phytopathology, 39*(1), 53-77. https://doi.org/10.1146/annurev.phyto.39.1.53

TUIK, 2020. Turkish Statistical Institute, Çanakkale crop production statistics (Web page: http://tuikrapor.tuik.gov.tr), (Date accessed: November 2020).

Uysal, G., Mıstanoğlu, I., & Devran, Z. (2023). Genetic variation and identification of *Meloidogyne graminis* populations from golf fields in Antalya province of Türkiye. *Journal of Phytopathology*, *171*(7-8), 291-299. https://doi.org/10.1111/jph.13182

Uysal, G., Söğüt M.A. & Elekçioğlu, İ. H. (2017). Identification and distribution of root-knot nematode species (*Meloidogyne* spp.) in vegetable growing areas of Lakes Region in Turkey. *Turkish Journal of Entomology, 41*(1), 105-122. http://dx.doi.org/10.16970/ted.91225

Vovlas, N., Lucarelli, G., Sasanelli, N., Troccoli, A., Papajova, I., Palomares-Rius, J. E., & Castillo, P. (2008). Pathogenicity and host-parasite relationships of the root-knot nematode *Meloidogyne incognita* on celery. *Plant Pathology, 57*(5), 981-987. https://doi.org/10.1111/j.1365-3059.2008.01843.x

Whitehead, A.G. (1968). Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. *Transactions of Zoological Society of London, 31*(3), 263-401. https://doi.org/10.1111/j.1096-3642.1968.tb00368.x

Wishart, J., Phillips, M. & Blok, V. (2002). Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne Chitwood, M. fallax*, and *M. hapla*. *Phytopathology 92*, 884-892. https://doi.org/10.1094/phyto.2002.92.8.884

Wyss, U. (1980). Ultrastructure of modified root-tip cells in Ficus carica, induced by the ectoparasitic nematode *Xiphinema index*. *Journal of Cell Science 41*(1), 193-208. https://doi.org/10.1242/jcs.41.1.193

Yigezu Wendimu, G. (2021). Biology, taxonomy, and management of the root-knot nematode (*Meloidogyne incognita*) in sweet potato. *Advances in Agriculture*, 1-13. https://doi.org/10.1155/2021/8820211

Yu, M. H. (1995). Root-Knot Nematode Development and Root Gall Formation in Sugarbeett. *Journal of Sugar Beel Research Vol, 32*, 0-1.

Yüksel, H. (1974). Kök-ur nematodlarının (Melaidogyne Spp.) Türkiye'deki durumu ve bunların popülasyon problemleri üzerinde düşünceler. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 5*(1), 83-105.

Zijlstra, C., Donkers-Venne, D. T., & Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. *Nematology, 2*(8), 847-853. https://doi.org/10.1163/156854100750112798

KSÜ Tarım ve Doğa Derg 27 (4), 839-855, 2024
KSU J. Agric Nat 27 (4), 839-855, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1383042

Determination of Fungal and Bacterial Disease Agents on Significant Brassicaceous Vegetable Species Grown in Hatay Province

Soner SOYLU[1✉], Merve KARA[2], Aysun UYSAL[3], Yusuf GÜMÜŞ[4], Emine Mine SOYLU[5], Şener KURT[6]
İlhan ÜREMİŞ[7], Erdal SERTKAYA[8]
[1,2,4,5,6,7,8]Faculty of Agriculture, Department of Plant Protection, Hatay Mustafa Kemal University, 31034 Antakya, Hatay, Türkiye, [3]Centre for Implementation and Research of Plant Health Clinic, Hatay Mustafa Kemal University, 31034 Antakya, Hatay, Türkiye
[1]https://orcid.org/0000-0003-1002-8958, [2]https://orcid.org/0000-0001-7320-3376, [3]https://orcid.org/0000-0002-9067-285X
[4]https://orcid.org/0000-0001-7818-1831, [5]https://orcid.org/0000-0001-5961-0848, [6]https://orcid.org/0000-0003-4545-5968
[7]https://orcid.org/0000-0001-5937-9244, [8]https://orcid.org/ 0000-0001-9956-943X
✉: soylu@mku.edu.tr

ABSTRACT

The *Brassicaceae* family, commonly known as the *Cruciferae* or mustard family, encompasses plant species of global economic significance, including oilseed crops, vegetables, as well as condiment purposes. Hatay is one of the most important provinces in Turkey where *Brassicaceous* vegetable crops are grown. This study aimed to identify the causative disease agents affecting *Brassicaceous* vegetables including cabbage (red and white varieties), broccoli, cauliflower, garden cress, rocket, and radish in the districts of Hatay Province where vegetable cultivation took place during the 2020-2021 growing seasons. Isolations from suspicious cabbage, broccoli, cauliflower, and radish plants exhibiting disease symptoms in the surveyed areas revealed the presence of various fungal disease agents, including *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium solani, Fusarium oxysporum, Fusarium exquisite, Alternaria alternata, Botrytis cinerea*, and *Stemphylum* sp. Furthermore, obligate oomycete pathogens such as *Albugo lepidii, Albugo candida*, and *Perofascia lepidii* were detected with varying prevalence and incidence rates on leaves and stems of water cress, rocket, and radish plants. In addition to fungal pathogens, primary bacterial pathogens, including *Xanthomonas campestris* pv. *campestris*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri, Pseudomonas corrugata*, and *Pseudomonas victoria*, were identified through morphological, biochemical, and pathogenicity tests, and MALDI-TOF analyses. *Pantoea agglomerans, Pseudomonas marginalis, Pseudomonas fluorescens, Enterobacter cloacae,* and *Bacillus pumilus* were also identified as opportunistic soft rot bacterial pathogens. To the best of our knowledge, this is the first report of *F. solani, F. oxysporum, F. exquisite, A. alternata, B. cinerea, Stemphylum sp.,* as fungal disease agents; *P. parmentieri, P. corrugata,* and *P. victoria* as primary soft rot bacterial disease agents; *P. agglomerans, P. marginalis, P. fluorescens, E. cloacae,* and *B. pumilus* as opportunistic secondary soft rot bacterial disease agents affecting different minor vegetables belong to *Brassica* spp, such as cabbage (red and white varieties), broccoli, cauliflower, radish, rocket, and garden cress, grown in Turkey.

Plant Protection

Research Article

Article History
Received : 30.10.2023
Accepted : 13.12.2023

Keywords
Cruciferae
Brassica
Fungal disease
Bacterial disease
Oomycete

Hatay İlinde Yetiştirilen Önemli Brassicaceous Sebze Türlerinde Fungal ve Bakteriyel Hastalık Etmenlerinin Belirlenmesi

ÖZET

Genellikle lahanagiller, *Cruciferae* veya hardal ailesi olarak bilinen *Brassicaceae* familyası, dünya çapında ekonomik açıdan tarımsal öneme sahip yağlı tohum, sebze ve çeşni amaçlı kullanılan bitki türleri içerir. Hatay, Türkiye'nin *Brassicaceous* sebze türlerinin yetiştiriciliğinin yapıldığı en önemli illerinden biridir. Bu çalışmada 2020-2021 yetiştirme sezonlarında Hatay ilinin sebze yetiştiriciliğinin yapıldığı ilçelerde *Brassicaceous* sebzelerden lahana (kırmızı ve beyaz baş), brokoli,

Bitki Koruma

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 30.10.2023
Kabul Tarihi : 13.12.2023

karnabahar, tere, roka ve turp bitkilerinde sorun olan fungal, oomycet ve bakteriyel hastalık etmenlerinin tanılanması ve yaygınlık durumlarının belirlenmesini amaçlanmıştır. Sürvey yapılan alanlarda hastalık belirtisi gösteren şüpheli lahana, brokoli, karnabahar ve turp bitkilerin kök, gövde ve yapraklarından yapılan izolasyonlarda *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium solani, Fusarium oxysporum, Fusarium equiseti, Alternaria alternata, Botrytis cinerea* ve *Stemphylum* sp., ait fungal hastalık etmenlerin yanısıra yanısıra *Albugo lepidii*, *Albugo candida ve Perofascia lepidii* gibi obligat parazit oomycet patojenler tere, roka ve turp bitkilerinin yaprak ve gövdelerinde değişen yaygınlık ve rastlama sıklıklarında tespit edilmiştir. Fungal etmenlerin yanısıra yapılan morfolojik, biyokimyasal, patojenisite testler ve MALDI-TOF analizleri sonucunda *Xanthomonas campestris* pv. *campestris*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium parmentieri*, *Pseudomonas corrugata* ve *Pseudomonas cichorii* primer bakteriyel hastalık etmenleri olarak belirlenmiştir. *Pantoea agglomerans, Pseudomonas marginalis, Pseudomonas fluorescens, Enterobacter cloacae* ve *Bacillus pumilus* ise fırsatçı sekonder bakteriyel yumuşak çürüklük etmenler olarak tanılanmışlardır.Bilindiği kadarıyla *F. solani, F. oxysporum, F. equiseti, A. alternata, B. cinerea*, *Stemphylum* sp., fungal hastalık etmenleri; *P. parmentieri*, *P. corrugata* ve *P. cichorii* primer bakteriyel yumuşak çürüklük hastalık etmenleri; *P. agglomerans, P. marginalis, P. fluorescens, E. cloacae* ve *B. pumilus* ise sekonder fırsatçı bakteriyel yumuşak çürüklük hastalık etmenleri olarak Türkiye'de yetiştirilen farklı *Brassica* spp ait lahana (kırmızı ve beyaz baş), brokoli, karnabahar, tere, roka ve turp gibi minör sebzelerde hastalıklara neden olduğu ilk kez bu çalışma ile tespit edilmiştir.

Anahtar Kelimeler
Cruciferae
Brassica
Fungal hastalık
Bakteriyel hastalık
Oomycete

Atıf Şekli: Soylu, S., Kara, M., Uysal, A., Gümüş, Y., Soylu, E.M., Kurt, Ş., Üremiş, İ., & Sertkaya, E., (2024). Hatay İlinde Yetiştirilen Önemli *Brassicaceous* Sebze Türlerinde Fungal ve Bakteriyel Hastalık Etmenlerinin Belirlenmesi. *KSÜ Tarım ve Doğa Derg 27*(4), 839-855. DOI: 10.18016/ksutarimdoga.vi.1383042.

To Cite : Soylu, S., Kara, M., Uysal, A., Gümüş, Y., Soylu, E.M., Kurt, Ş., Üremiş, İ., & Sertkaya, E., (2024). Determination of Fungal and Bacterial Disease Agents on Significant *Brassicaceous* Vegetable Species Grown in Hatay Province. *KSU J. Agric Nat 27* (4), 839-855. DOI: 10.18016/ksutarimdoga.vi.1383042.

INTRODUCTION

The *Brassicaceae* family is commonly known as the Cruciferae or mustard family. This family encompasses a diverse array of plant species with significant global economic importance (Raza et al., 2020). Within the *Brassicaceae* family, there are 338 genera and more than 3700 plant species, each serving various purposes (Shankar et al., 2019). The *Brassica* genus is widely recognized as the most significant genus within the *Brassicaceae* family, encompassing several vital crops. This genus includes oilseeds like canola and mustard, as well as a variety of vegetables suitable for consumption in raw, cooked, or salad form, such as cabbage, Brussels sprouts, broccoli, cauliflower, radish, rocket, and Chinese cabbage. Additionally, it comprises plants used for seasoning purposes, including mustard, wasabi, and wild radish (Rakow, 2004; Chen et al., 2013; Gupta, 2016). Among the most extensively cultivated and utilized *Brassicaceous* vegetables, virtually all are edible, *Brassica oleracea* and *B. rapa* are prominent examples, while the seeds of *B. nigra, B. carinata*, and *B. juncea* find application as seasonings in kitchens. According to data from the Food and Agriculture Organization (FAO), *B. napus, B. rapa, B. juncea*, and *B. carinata*, which also serve as leaf and root vegetables, collectively contribute to 12% of the world's supply of edible vegetable oils (Anonymous, 2021a).

In recent decades, there has been a notable 33.8% increase in the global production of *Brassicaceous* vegetable species. As of 2020, China, India, and Russia emerged as the top producers (Anonymous, 2021b). Within Hatay Province, located in Turkey, the cultivation of minor *Brassicaceous* vegetables is widespread across various districts, including Antakya, Kırıkhan, Hassa, Reyhanlı, Kumlu, Altınözü, Samandağı, Arsuz, İskenderun, Erzin, and Dörtyol. These minor vegetables include white and red cabbage, cauliflower, radish, and rocket. In Hatay Province, white cabbage was cultivated on 727 da, resulting in a total production of 1,315 tons, while red cabbage covered 130 da, yielding 260 tons. Additionally, radish, rocket, and watercress were cultivated on 255, 530, and 814 days, respectively (Anonymous, 2020).

Brassicaceous vegetable plants, encompassing vital vegetable species such as white and red cabbage, cauliflower, broccoli, radish, garden cress, and rocket, are susceptible to a range of disease agents, including

bacteria and fungi, which can adversely affect both yield and quality (Smith et al., 1988; Koike et al., 2007; Srivastava et al., 2011). Among the factors that can limit yield and quality in *Brassicaceous* vegetables, soil and leaf-borne fungal, oomycete, and bacterial diseases play a prominent role. These disease agents have the potential to significantly influence crop yield and quality, manifesting at various stages of growth, from the seedling stage to later developmental phases (Bruehl, 1987). Fungal and oomycete diseases pose significant threats to *Brassicaceous* vegetables, including Alternaria leaf spot and blight (*Alternaria* spp.), anthracnose (*Colletotrichum* spp.), downy mildew (*Hyaloperonospora parasitica* [Syn. *Peronospora parasitica*], *Perofascia lepidic*), powdery mildew (*Erysiphe cruciferous*), Sclerotinia stem and head rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida, Albugo lepidic*), wilt (*Fusarium* spp.), leaf spot (*Leptosphaerulina brassicas*, *Cercospora chianti*), damping-off (*Pythium* spp.), clubroot (*Plasmodiophora brassica*e), and blackleg (*Leptosphaeria maculans*) (Koike, 2007; Srivastava et al., 2011; Kumar et al., 2017; Al-Lami et al., 2019; Shaw et al., 2021; Kiran et al., 2022; Greer et al., 2023; Mourou et al., 2023).

In addition to fungal and oomycete pathogens, bacterial diseases are significant biotic factors that can detrimentally affect the production and yield of vegetable species within this genus. Primary bacterial diseases include soft rot, caused by *Pectobacterium carotovorum subsp. carotovorum* (=*Erwinia carotovora* subsp. *carotovora*), *Pseudomonas viridiflava, Pseudomonas corrugata*, leaf spot disease caused by *Pseudomonas syringae* pv. *maculicola*, and black rot, caused by *Xanthomonas campestris* pv. *campestris*. Furthermore, species such as *Pseudomonas marginalis* and *Pseudomonas fluorescens* are known as opportunistic bacterial diseases (Koike et al., 2007; Rimmer et al. 2007).

While studies examining fungal and bacterial disease agents affecting key *Brassicaceous* vegetable species are conducted in regions around the world (Mourou et al., 2023), research in Turkey on these disease agents within such plants remains notably scarce. Several disease agents have been identified for the first time in Turkey, specifically within *Brassicaceous* vegetable species, including cabbage, cauliflower, broccoli, and Brussels sprouts, grown in various regions. Notably, *X. campestris* pv. *campestris*, the causative agent of bacterial black spot disease, along with soft rot agents *P. carotovorum* subsp. *carotovorum* and *P. viridiflava*, have been reported as the first disease records for *Brassicaceous* vegetable species (Mirik et al., 2008; Aksoy et al., 2017a, b; Aksoy & Öztürk, 2018; Öztürk et al., 2019; Öztürk & Soylu, 2022; Meral et al., 2022). Similarly, *R. solani*, responsible for root and crown rot, has been reported in red and white cabbage plants cultivated in various regions of Turkey (Saygi et al., 2020; Benli & Türkkan, 2020; Erper et al., 2021). In the case of cabbage, head rot disease caused by *S. sclerotiorum* has also been previously reported (Tozlu et al., 2016). Furthermore, oomycete disease agents such as *P. lepidii* were found to cause downy mildew, while *A. lepidii* was associated with white rust disease in garden cress (*Lepidium sativum*) plants growing in Hatay province (Soylu et al., 2017; Soylu et al., 2019).

In recent years, farmers in the districts of Hatay Province have been confronted with significant challenges related to economic losses stemming from fungal and bacterial diseases that afflict *Brassicaceous* vegetables. While some information is available regarding diseases caused by *P. lepidii* and *A. lepidii* in garden cress plants, there remains a dearth of knowledge regarding disease agents responsible for other ailments and their prevalence in production areas. This study is aimed at addressing this gap by identifying and assessing the prevalence of fungal, oomycete, and bacterial disease agents on significant *Brassicaceous* vegetables, including white and red cabbage, cauliflower, broccoli, radish, garden cress, and rocket growing in districts of Hatay Province.

MATERIAL and METHODS

Determination of the Prevalence of Fungal and Bacterial Disease Agents.

Disease survey investigations were conducted during the 2020-2021 cultivation season, taking into account data provided by the Hatay Provincial Agriculture Directorate for the year 2019. These surveys encompassed approximately 10% of the total cultivated area in Hatay Province, covering various districts including Antakya, Kırıkhan, Hassa, Reyhanlı, Kumlu, Altınözü, Samandağı, Arsuz, İskenderun, Erzin, and Dörtyol. These districts are known for the cultivation of different minor vegetable crops belonging to *Brassica* species, spanning a total area of 6181 da.

The survey studies were conducted at different growth stages of the plant, including the seedling (September-October 2020), before and after heading stages (November 2020-April 2021). In the surveyed fields, a systematic zigzag pattern was followed to inspect the entire area, and any observed symptoms were recorded (Bora & Karaca, 1970). Suspicious plant samples were assigned unique codes and subsequently transported to the laboratory for isolation and identification. The prevalence of disease agents was determined by calculating the number of fields in which the disease was observed out of the total surveyed fields. Additionally, the incidence rate for each field was calculated by relating the number of diseased samples to every 100 plants within that field.

Isolation of Fungal Disease Agents

Fungal pathogens were isolated from various parts of the host plants displaying characteristic disease symptoms. General and selective nutrient media, including Potato Sucrose Agar (PSA; Merck, Darmstadt, Germany), Czapek Dox Agar (CPA; Merck, Darmstadt, Germany), and Carnation Leaf Piece Agar (CLA) were used for the isolation process. Plant tissues, such as roots, stems, heads, and leaves from affected plants, were first washed under tap water. Subsequently, necrotic tissues resulting from the infection were cut into 5 mm pieces using a sterile scalpel. Tissue pieces were surface disinfected in 75% ethanol for 1 minute, followed by a 5-minute exposure to 3% hypochlorite. After disinfection, the tissue pieces were rinsed three times in sterile distilled water and left to air dry for 1-2 hours on sterile blotting papers. Once they were completely dried, the tissue pieces were transferred to 90 mm diameter Petri dishes containing general and selective culture media, supplemented with 50 µg ml^{-1} streptomycin sulfate. Four pieces were placed in each petri dish, which was then incubated at 25°C for 5-7 days. Following incubation, mycelial disks were taken from actively growing colonies of each isolate and transferred to 60 mm diameter PDA Petri dishes containing the 50 µg ml^{-1} streptomycin sulfate, and incubated at 25°C for 5 days. Subsequently, single spore cultures were prepared for all fungal isolates and preserved at -20°C in envelopes on filter paper for further studies.

Morphological Identification of Fungal Disease Agents

All single-spored cultures of fungal isolates were evaluated for their colony morphologies, pigment formations, presence of overwintering structures (sclerotic), mycelium, conidiophores, and conidial developments on general (V8 Juice Agar) and selective media (CLA) kept at 25°C with 12-hour alternating fluorescent/UV light and darkness. To measure the dimensions of the conidia produced by each isolate, conidial suspensions were prepared from 7-10-day-old cultures grown on PDA using sterile distilled water, and the shape and size measurements of their conidia were determined using a trinocular light microscope (Olympus BX51, Japan). Identification was performed using diagnostic keys based on morphocultural characteristics published by Ellis (1971), Sneh et al. (1991), Leslie & Summerell (2006), and Simmons (2007). The identification of oomycete pathogens was made based on the conidia and conidiophore structures taken directly from the diseased tissues, as previously described (Constantinescu & Fatehi, 2002; Choi et al., 2007).

Pathogenicity Tests of Fungal and Oomycete Pathogens

The pathogenicity of the fungal and oomycete isolates was confirmed on different parts (leaves, root collar, etc.) of 8-week-old seedlings of host plants, including white head cabbage (cv. Lades F1), red cabbage (cv. Karmen F1), cauliflower (cv. Bahara F1), broccoli (cv. Vole), garden cress (cv. Arzuman), rocket (cv. Arzuman), and radish (cv. Kadirli). For leaf pathogens such as *A. lepidii*, *A. candida*, and *P. lepidii*, conidial suspensions were prepared at a concentration of 10^6 conidia ml^{-1} in sterile water and sprayed onto the leaf surface (Soylu et al., 2019). Isolates of *A. alternata*, *B. cinerea*, and *Stemphylum* sp. were inoculated by applying 20 µl of conidial suspensions, at a concentration of 10^6 conidia ml^{-1} in sterile water, onto wounds made on the leaf surface using a sterile syringe needle (Blagojević et al., 2020). Isolates of *R. solani*, *S. sclerotiorum*, *F. solani*, *F. oxysporum*, and *F. equiseti* were inoculated by placing mycelial pieces obtained from 5-day-old mycelial cultures into wounds at the base of stems of 8-week-old healthy cabbage seedlings. In the pathogenicity tests, ten seedlings for each representative isolate were used. Following the onset of disease symptoms at the inoculation points, the fungal pathogens were re-isolated and compared with the original isolates.

Molecular Identification of Fungal Disease Agents

To confirm the morphological identification of the fungal pathogens from infected plants, molecular identification studies were conducted. Amplification of internal transcribed spacer (ITS) rDNA locus was performed using universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Fungal genomic DNA was extracted from 5-day-old aerial mycelia of pure cultures of representative isolates grown on PDA. Fungal mycelia were homogenized in 2 ml Eppendorf tubes, and their DNA was extracted by using the genomic DNA isolation kit (DNeasy Plant Mini Kit, Qiagen Inc., Valencia, CA) following the manufacturer's recommendations. The quantity and quality of the extracted genomic DNA were assessed using the Qubit 3.0 fluorometer. The PCR procedure was carried out in a thermal cycler (Applied Biosystems, Singapore), with each mixture consisting of 1x PCR buffer, 0.2 µM dNTP, 0.5 µM of each primer, 1 U Taq polymerase (Invitrogen), and 2 µl of genomic DNA. PCR condition was set to initiate denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds, concluding with a final extension step at 72°C for 10 minutes (White et al., 1990). The quality of PCR products was visualized by capillary electrophoresis (QIAxcel Advanced, Qiagen, Germany) as previously described (Uysal et al., 2022). Molecular identification of fungal isolates, relying on sequencing results, involved using the BLAST program at the

National Center for Biotechnology Information (NCBI) to compare the sequences with known species.

Isolation and Pathogenicity of Bacterial Disease Agents

Bacterial pathogens were isolated directly from infection sites and cultured on general (Tryptic Soy Agar, TSA) and selective media (King B Agar, KB). The plates were then incubated at 25°C for 24-48 hours in incubators. Bacterial isolates with varying morphological structures that grew on the plates were subcultured from single colonies onto TSA following the procedures outlined by Lelliot & Stead (1987). Each bacterial colony isolated from a different plant was treated as a separate isolate and preserved at -80°C in a 40% glycerol solution for use in future studies. Biochemical tests, such as colony color, colony form, cell morphology, gram reaction, oxidase and catalase production, as well, and levan formation, were employed to make a preliminary selection of isolates by the methods outlined by Lelliott & Stead (1987). All isolates originating from single colonies were initially tested for Hypersensitive Reaction (HR) on tobacco leaves and subsequently, rotting tests on various parts of host plants, including leaves, stems, flowers, and potato slices. Pathogenicity tests of representative isolates were conducted on their original host plants. A bacterial suspensions of *Xanthomonas campestris pv. campestris* at 10^6 cfu ml^{-1} concentration was injected into the leaves of 8-week-old white cabbage seedlings (cv. Yalova). Pathogenicity tests of soft rot bacterial disease agents were conducted on cabbage leaves, broccoli, cauliflower, and radish. Two-day-old bacterial colonies were taken with a sterile toothpick and directly inoculated on the stem of their host plants. The inoculation site was covered with parafilm. The inoculated plant parts were placed into a transparent storage box and incubated at 25 C for 3 days and the formations of soft rot lesions at the inoculation site were examined (Öztürk & Soylu, 2022).

Identification of Bacterial and Fungal Disease Agents by MALDI-TOF

Morphological and biochemical identification of fungal and bacterial isolates were also confirmed by using the MALDI-TOF (Bruker Daltonics GmbH, Bremen, Germany) Biotyper identification system, (Pavlovic et al., 2012). Bacterial isolates on TSA and fungal isolates in Potato Dextrose Broth were cultivated for 24-48 hours. The ethanol-formic acid method was utilized for protein extraction (Soylu et al., 2022). Specific protein spectra were obtained through the MALDI-TOF Biotyper. Obtained spectra were then compared with the protein spectra of reference bacterial isolates available in the device's microbial library, employing the Maldi Biotyper Real-Time Classification (RTC) software (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) for species-level identification, as detailed by Soylu et al. (2023).

RESULTS and DISCUSSIONS

The initial disease survey was conducted in cultivation areas of cabbage (white and red), cauliflower, broccoli, rocket, and radish in September 2020, coinciding with the emergence of the first seedlings. No signs of disease were detected on the seedlings when they were transplanted into the fields. Based on communication with local farmers, sporadic reports mentioned a slight drying of some seedlings approximately one week after planting. Since farmers promptly replaced the affected seedlings with healthy ones, the surveys did not reveal any notable instances of such drying.

Subsequent 3 surveys were carried out in October and November 2020, aligning with the growth of the seedlings and the commencement of the heading stage (for white and red cabbage and cauliflower). These surveys collectively covered a total of 77 fields located in the districts of Antakya, Kırıkhan, Kumlu, Reyhanlı, Hassa, and Arsuz. The presence of root rot was identified at the root and crown of certain red cabbage seedlings (Figure 1).

In cabbage fields, only one field exhibited signs of mild wilting, affecting a minimal percentage of plants, approximately 2-3% (see Figure 2A-E). In the third and fourth surveys conducted in October and November 2020, the prevalence of pathogens exhibited considerable variation across the surveyed fields. A cross-section of the stem of the plant displaying such symptoms revealed darkening in the vascular bundles, reminiscent of the thinning observed at the root collar in red cabbage seedlings. Subsequent isolations from symptomatic plants collected during all surveys yielded 76 different fungal isolates. Based on morphological characteristics of the fungal structures of each isolate, 6 different fungal species tentatively identified as *R. solani* (Sneh et al., 1991), *S. sclerotiorum* (Kohn, 1979), *Fusarium* spp. (Leslie & Summerell, 2006), *Alternaria* spp (Simmons, 2007) and *Stemphylum* spp. (Woudenberg et al., 2017).

The last 3 surveys, which occurred just before harvest, were conducted in March and April 2021, encompassing 130 fields of cabbage (white and red), garden cress, rocket, radish, and cauliflower plants that were either harvested or at a stage of delayed harvest (Figure 3). These fields were spread across the districts of Antakya, Arsuz, Payas, İskenderun, Dörtyol, Erzin, Hassa, Kırıkhan, Reyhanlı, Kumlu, and Altınözü. Subsequent isolations from symptomatic plants collected during these surveys yielded 38 different fungal isolates White-headed cabbage plants exhibited symptoms of head rot disease, caused by *S. sclerotiorum* (with a prevalence ranging from 6% to 11%), leaf spot disease caused by *Alternaria spp.* (with a prevalence of 2% to 7%), and gray mold diseases

associated with *B. cinerea* (with a prevalence of 1% to 5%).

During all surveys, a total of 114 fungal isolates were obtained, 32 isolates were identified as *Fusarium* spp., 25 isolates as *R. solani*, 21 isolates as *S. sclerotiorum,* 19 isolates as *Alternaria* spp., 9 isolates as *B. cinerea*, and 8 isolates as *Stemphylum* spp.. According to observations and identification results, prevalence rates range from 3% to 11%, 1% to 8%, 1% to 7%, 1% to 4%, 2% to 7%, 1% to 3%, 1% to 2%, and <1% were recorded for *R. solani, S. sclerotiorum, F. solani, F. oxysporum, F. exquisite, Alternaria* spp., *B. cinerea*, and *Stemphylum* spp., respectively. Morphological identification of these isolates was confirmed by MALDI-TOF analysis results.

Figure 1. General wilting symptoms (arrows) associated with root and root collar rot on some seedlings, indicating the presence of fungal disease agents in *Brassica* planting areas in Hatay Province during the October 2020 surveys.

Şekil 1. Hatay ili önemli Brassica ekim alanlarında Ekim 2020 tarihinde yapılan sörveylerde bazı fidelerin köklerinde kök ve kökboğazı çürüklüğüne (ok) bağlı genel solgunluk belirtileri.

The isolates, initially characterized based on morphological features and MALDI-TOF, were conclusively identified using molecular methods employing universal primer pairs ITS1-4. Molecular analyses demonstrated that the morphologically identified isolates exhibited a remarkable similarity of 99.83-100% with sequences available in the NCBI database. Notably, among the obtained isolates, the following fungal species were molecularly identified and their sequence data deposited in GenBank: *Alternaria alternata* LAa21 (OM854806, 100% matched with the sequence of KU360613), *Sclerotinia sclerotiorum* LRs11 (OM867578, 100% matched with the sequence of MG516658), *Fusarium exquisite* LFe33 (OM883923, 100% matched with the sequence of MT601958), *Rhizoctonia solani* LRs5 (OM883982, 99.83% matched with the sequence of HF912172), *Fusarium solani* KFs9 (OM883985, 100% matched

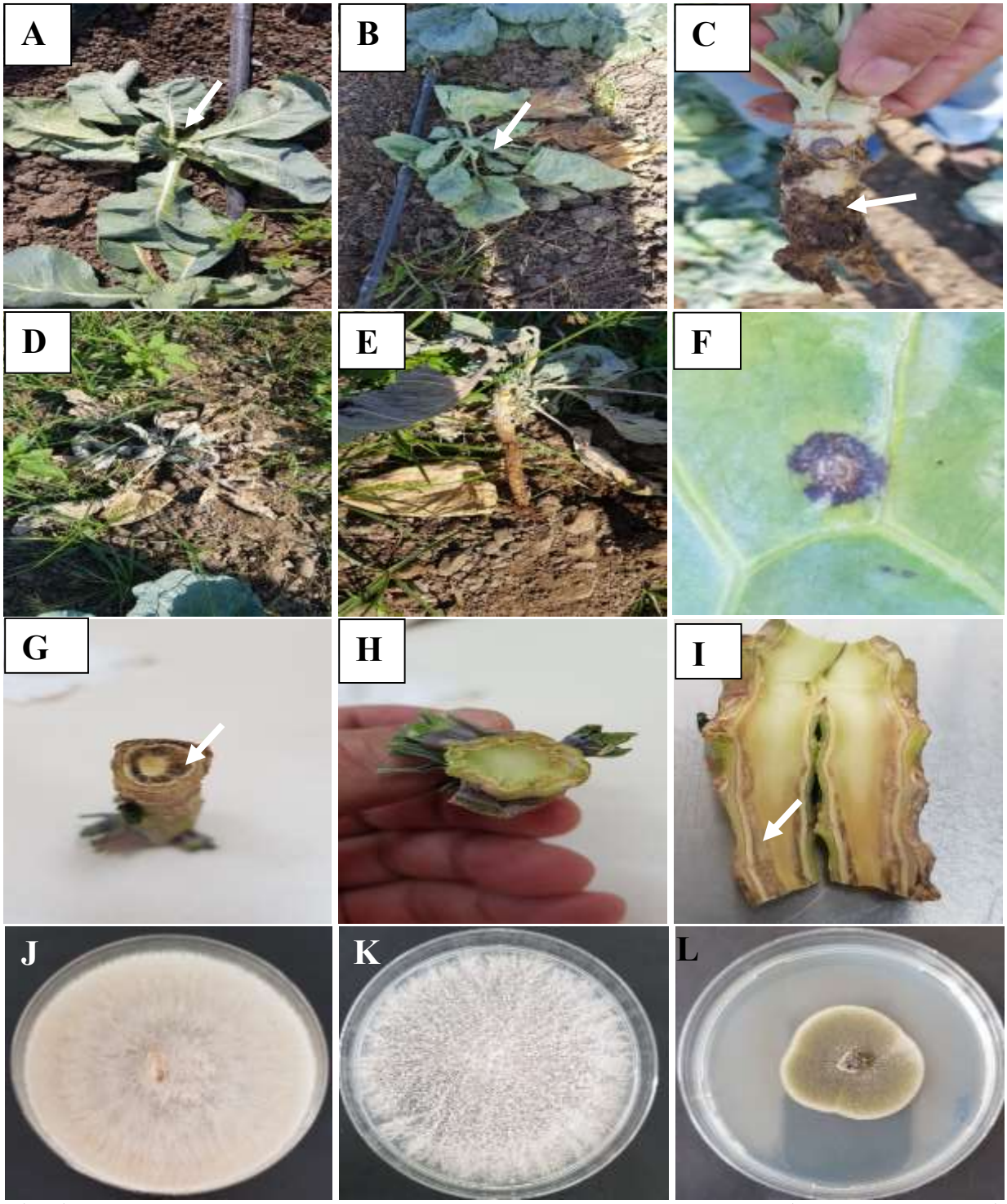


Figure 2. (A-E) Typical wilting symptoms associated with root thinning and root rot (indicated by arrows) were observed during surveys in significant *Brassica* cultivation areas in Hatay Province in November 2020. (F) Notable necrotic lesion symptoms were observed on leaves. (G-I) Darkening of the vascular bundles in stem cross-sections of plants displaying wilting symptoms (indicated by arrows). Petri dish images of isolates of *Rhizoctonia solani* (J), *Fusarium solani* (K), and *Alternaria alternata* (L) were obtained from tissues exhibiting disease symptoms.

*Şekil 2. (A-E) Hatay ili önemli Brassica ekim alanlarında Kasım 2020 tarihlerinde yapılan sörveylerde gözlenen bitkilerin köklerinde incelme ve kök çürüklüğüne bağlı genel solgunluk belirtileri (ok); F, Yapraklarda gözlenen nekrotik leke belirtileri. **G-I**, solgunluk belirtisi gösteren bitkilerin gövde kesitlerinde iletim demetlerindeki kararma belirtileri (ok). Hastalık belirtileri gösteren dokulardan elde edilen Rhizoctonia solani (J), Fusarium solani (K) ve Alternaria alternata (L) izolatlarına ait petri görüntüleri.*

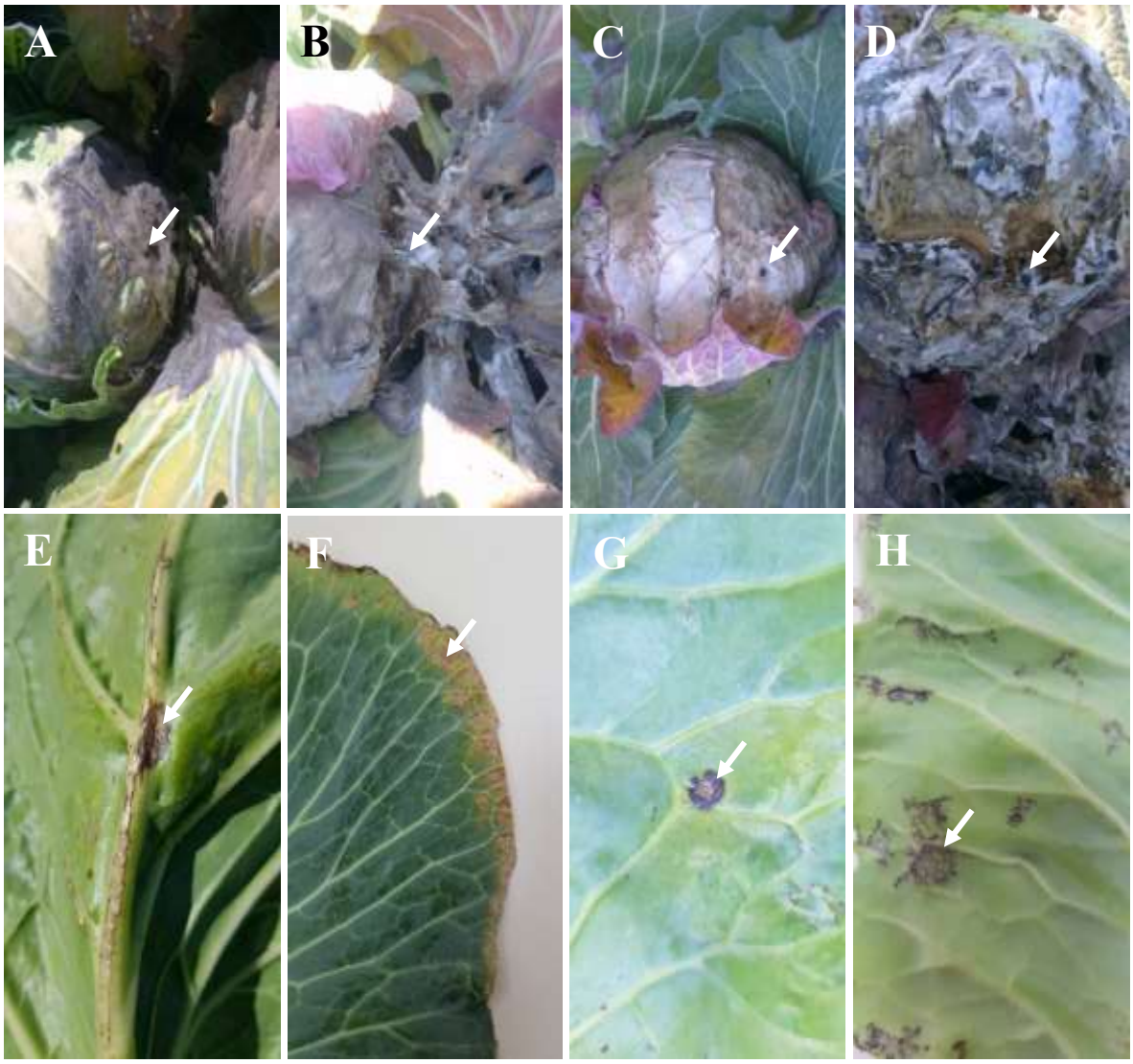


Figure 3. Diseases caused by different fungal pathogens identified in *Brassica* spp. cultivation areas of Hatay Province. (A) Abundant sporulation of gray mold disease caused by *Botrytis cinerea* on main heads and leaves (indicated by arrows). (B-D) Formation of sclerotia due to severe infection on cabbage heads and leaves, resulting from head rot disease caused by *Sclerotinia sclerotiorum* (indicated by arrows). (E-H) Disease symptoms induced by fungal pathogens, including Stemphylim sp. (E and F), *Alternaria alternata* (G), and *Rhizoctonia solani* (H), were observed on cabbage leaves (indicated by arrows).

Şekil 3. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde tespit edilen hastalıklar. (A) Botrytis cinerea tarafından neden olunan gri küf hastalığının ana baş ve yapraklardaki yoğun sporulasyonu (ok). (B-D) Sclerotinia sclerotiorum tarafından neden olunan beyaz çürüklük hastalığının lahana baş ve yapraklardaki yoğun enfeksiyon sonucu oluşturduğu sklerotlar (ok). (E-H) Lahana yapraklarında Stemphylim sp., (E ve F) Alternaria alternata (G) ve Rhizoctonia solani (H) gibi fungal hastalık etmenleri tarafından neden olunan hastalık belirtileri (ok).

with the sequence of MG991246), *Fusarium oxysporum* KFo27 (OM883986, 100% matched with the sequence of KU528846), and *Stemphylum* sp. LSt33 (OM884463, 100% matched with the sequence of OK560128). The sequence of the *Stemphylum* sp. LSt33 isolate exhibited a 100% match with the sequence of *Stemphylium eturmiunum*, which is the causal agent of black spot disease in apples (OK560128), as confirmed by BLAST analysis.

During the last surveys conducted from March to April 2021, a distinct pattern emerged, highlighting variations in the prevalence of diseases caused by oomycete pathogens in leafy vegetables, such as rocket and garden cress, in comparison to the fungal and bacterial diseases observed in cabbage and cauliflower, affecting their leaves, shoots, and stems (Figure 4). The prevalence of white rust disease, caused by *A. lepidii*, was notably higher in garden cress plants, ranging from 14% to 55% (see Figure 4A). In contrast, downy mildew disease, caused by *P. lepidii*, exhibited relatively lower prevalence rates, ranging from 4% to

11% (see Figure 4C). This trend was also observed in rocket and radish plants, which share similar small planting areas with garden cress (Figure 4B and Figure 5). In rocket and radish plant cultivated areas, white rust disease, caused by *A. candida*, was common (Figure 4B and Figure 5). The prevalence rate of *A. candida* ranged from 25% to 70%, whereas downy mildew disease, caused by *P. lepidii* agent, exhibited a relatively lower prevalence, ranging from 9% to 18% in garden cress plants. Importantly, powdery mildew disease caused by *Erysiphe cruciferarum* was not detected during any of the surveys.

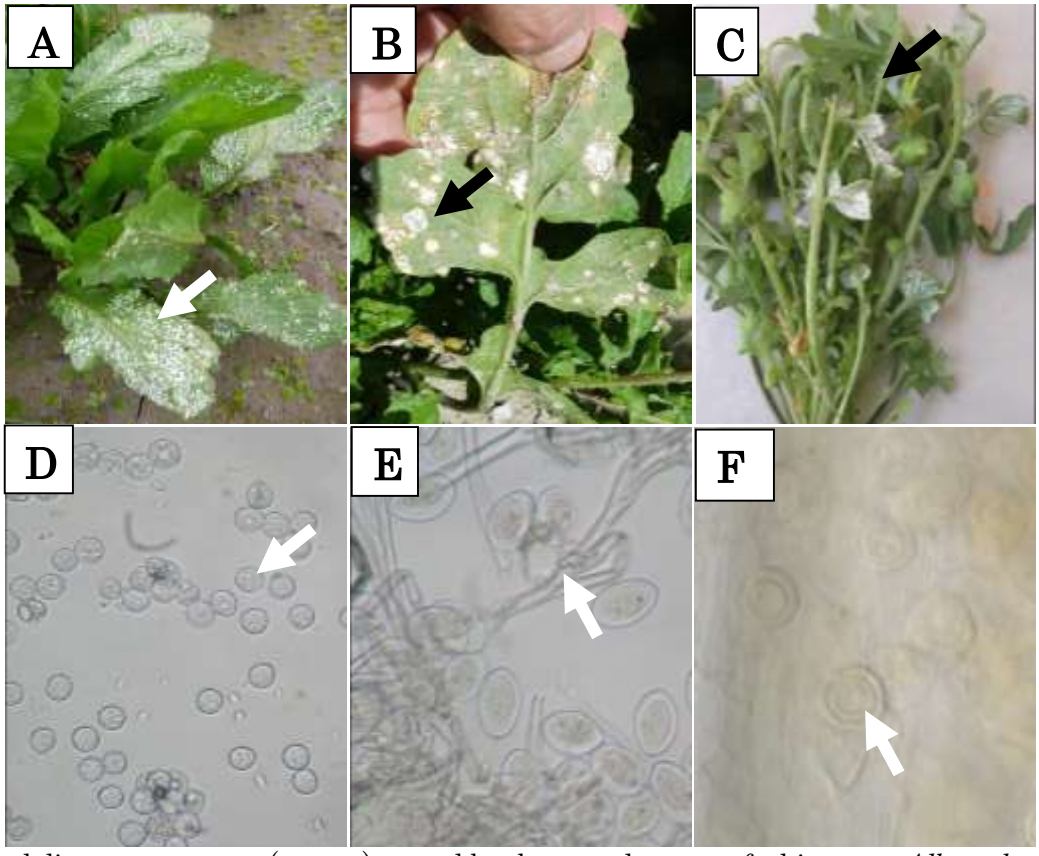


Figure 4. Typical disease symptoms (arrows) caused by the causal agents of white rust, *Albugo lepidii* (A), Albugo candida (B), and downy mildew, *Perofascia lepidii* (C), on leaves of garden cress (A, C) and rocket (B) plant surveyed in Hatay province. (D) Dense sporangia (arrow) of *Albugo*. (E) Conidiophores (arrow) and ellipsoidal-oval conidia are produced by the downy mildew pathogen, *Perofascia lepidii*. (F) Thick-walled oospores (arrows) produced by *Albugo lepidic* on leaf surfaces

Şekil 4. Hatay ilinde yapılan sörveylerde tere (A, C) ve roka (B) bitkilerinde beyaz pas hastalık etmeni Albugo lepidii (A), Albugo candida (B) ve (mildiyö hastalık etmeni Perofascia lepidii C) tarafından neden olunan tipik hastalık belirtileri (ok). (D) Albugo lepidii nin yoğun sporangiumları (ok). (E) Mildiyö hastalık etmeni Perofascia lepidii konidioforu (ok) ve elipsik-oval yapılı konidileri. (F) Albugo lepidii'nin yaprak üzerinde oluşturduğu kalın duvarlı oosporlar (ok)

During the surveys, unusual physiological disorders, characterized as abiotic stress-induced edema (Oedema), were also observed on cabbage leaves in only three fields in the Arsuz and Kırıkhan districts (Figure 6). Edema represents a physiological anomaly that arises in conditions where air temperatures are cooler than the soil, and there is a high relative humidity discrepancy between the soil and the atmosphere. This phenomenon heightens cell turgor by promoting water uptake, diminishes transpiration rates, leads to the rupture of epidermal cells, facilitates the expansion of underlying cells, and ultimately culminates in the formation of wart-like outgrowths. These outgrowths, although mimicking symptoms induced by parasitic organisms, ultimately result in cellular necrosis and discoloration.

In the surveyed fields, beyond the presence of fungal diseases, symptoms indicative of black rot, caused by *X. campestris* pv. *campestris* (with a prevalence ranging from 1% to 7%), as well as soft rot disease caused by diverse bacterial species (ranging from 1% to 13%), were observed on the leaves, stems, heads, and tubers of cabbage, broccoli, cauliflower, and radish plants (Figure 7 and Figure 8). Black rot symptoms were typically characterized by V-shaped wilting along the margins of the cabbage leaves, encircled by a yellow discoloration around the affected area, and the presence of darkened veins (Figure 7A, B).

Subsequently, 17 isolates were obtained from infected plant leaves of white-headed cabbage and identified using MALDI-TOF.

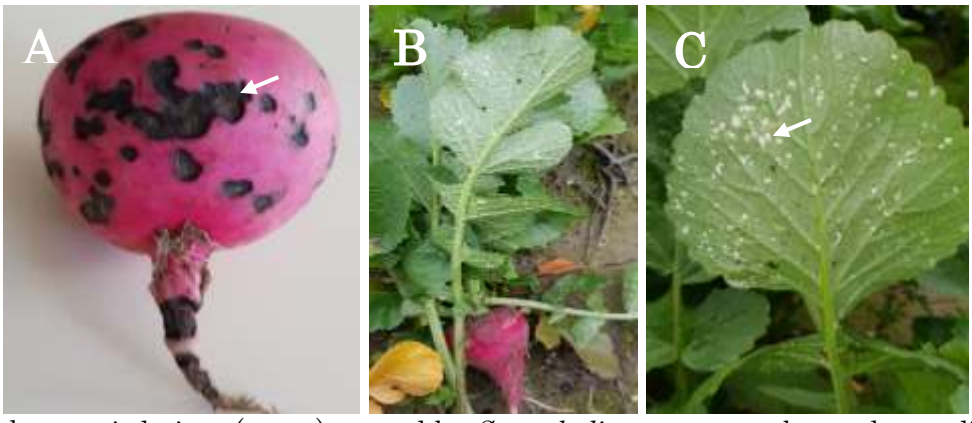


Figure 5. (A) Black necrotic lesions (arrow), caused by *Stemphylium* sp., were observed on radish tubers during surveys conducted in the significant *Brassica* cultivation regions of Hatay Province. (B-C) Distinctive disease symptoms manifest on radish plant leaves caused by *Albugo candida* (arrow).

Şekil 5. (A) Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde turp yumrularında Stemphylium sp. tarafından neden olunan siyah nekrotik çöküntüler (ok). (B-C) Turp bitki yapraklarında beyaz pas hastalık etmeni Albugo candida tarafından neden olunan tipik hastalık belirtileri (ok)

Figure 6. Typical edema (Oedema) symptoms, caused by abiotic factors, in cabbage leaves grown in the *Brassica* cultivation regions of Hatay Province.

Şekil 6. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde lahana yapraklarında abiyotik etkenlerden kaynaklanan ödem (Oedema) belirtileri (ok)

Using MALDI-TOF analysis, the identification of 56 pure colonies, isolated from the root collars, stems, and heads of white cabbage and cauliflower plants exhibiting soft rot symptoms, confirmed the prevalence of primary causative agents responsible for soft rot disease, including *P. caratovorum* subsp. *caratovorum* (15 isolates), *P. parmentieri* (8 isolates), *P. victoria* (7 isolates), and *P. corrugata* (6 isolates), which were recorded at a prevalence of 4% to 13%. Secondary opportunistic bacterial agents, such as *P. agglomerans* (6 isolates), *P. fluorescens* (4 isolates), *P. marginalis* (3 isolates), *E. cloacae* (4 isolates), and *B. pumilus* (3 isolates), were detected at a prevalence rate of less than 1%.

All bacterial isolates were subjected to pathogenicity tests on their respective host plants. *P. caratovorum, P. parmentieri, P. victoria,* and *P. corrugata* caused typical soft rot disease symptoms on artificially inoculated cabbage, broccoli, cauliflower, and radish plants (Figure 9). Opportunistic secondary soft rot disease agents *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilus*, however, did not cause typical soft rot disease symptoms at the inoculation sites (Figure 10).

According to literature records, there is limited research focusing on the identification of fungal diseases, primarily affecting *Brassicaceous* vegetables such as cabbage (white and red), cauliflower, and

broccoli in Turkey. The results obtained in this study revealed the prevalence of various fungal pathogens in different districts of Hatay Province, where cabbage,

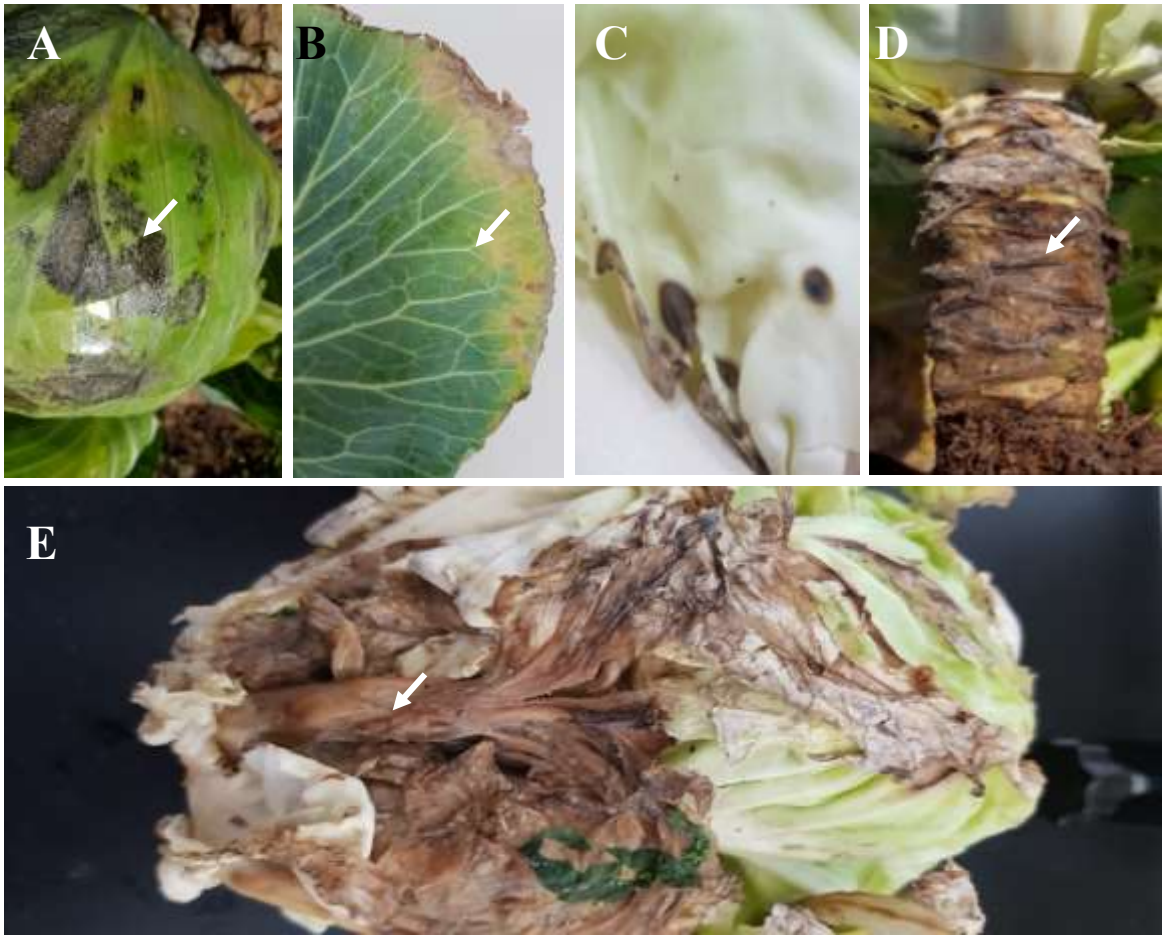


Figure 7. Disease Symptoms on cabbage leaves from surveyed *Brassica* cultivation areas in Hatay Province. (A-B) Symptoms caused by *Xanthomonas campestris* pv. *campestris*, (C) Symptoms associated with *Pantoaea agglomerans*. Typical symptoms of soft rot disease caused by *Pectobacterium* spp. and *Pseudomonas* spp. on cabbage stems (D) and heads (E) are indicated by arrows.

Şekil 7. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde lahana yapraklarında (A-B) Xanthomonas campestris pv. campestris ve Pantoaea agglomerans (C) tarafından neden olunan hastalık belirtileri. Lahana kök boğazı (D) ve başlarda (E) farklı Pectobacterium spp., Pseudomonas spp., türleri tarafından neden olunan yumuşak çürüklük hastalık belirtileri (ok)

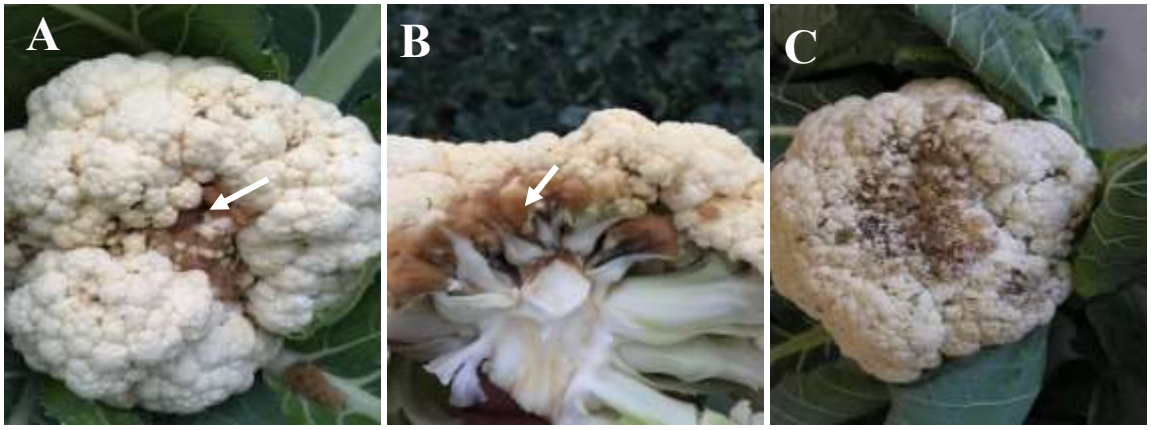


Figure 8. Soft rot disease symptoms (arrows) caused by different *Pectobacterium* spp. in cauliflower heads in the surveyed *Brassica* cultivation areas of Hatay Province.

Şekil 8. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde Karnabahar başlarında farklı Pectobacterium spp. tarafından neden olunan yumuşak çürüklük hastalık belirtileri (ok)

Figure 9. Typical soft rot disease symptoms caused by different *Pectobacterium* spp. and *Pseudomonas* spp. on various *Brassica* spp. during pathogenicity tests.

Şekil 9. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde farklı Pectobacterium spp. ve Pseudomonas spp. tarafından farklı Brassica türleri üzerinde yapılan patojenite testlerinde oluşturdukları tipik yumuşak çürüklük hastalık belirtileri (ok)

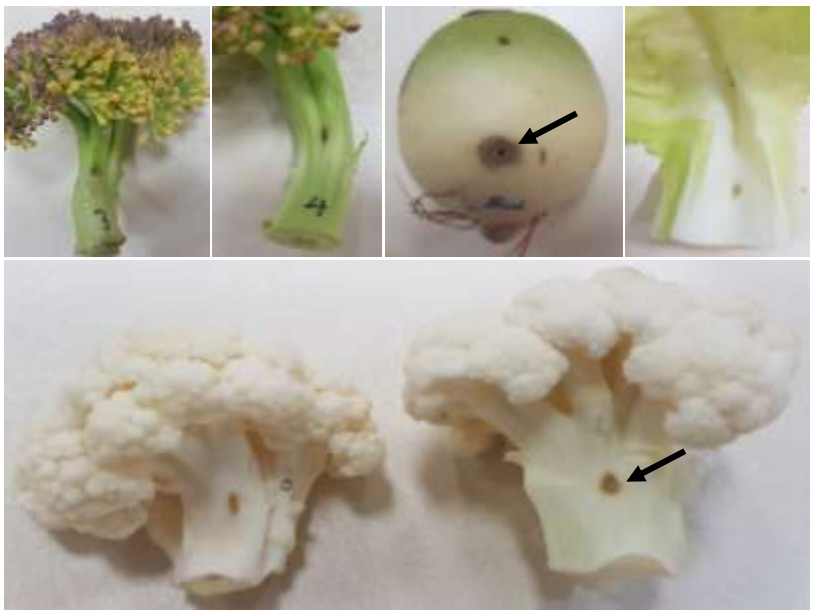


Figure 10. Atypical disease symptoms (arrow) caused by opportunistic bacterial isolates of *Pantoae agglomerans, Pseudomonas fluorescens, Pseudomonas marginalis, Enterobacter cloacae,* and *Bacillus pumilis* on various *Brassica* spp. during pathogenicity tests.

Şekil 10. Hatay ili önemli Brassica ekim alanlarında yapılan sörveylerde yumuşak çürüklük belirtilerinden izole edilmiş Pantoae agglomerans, Pseudomonas fluorescens, Pseudomonas marginalis, Enterobacter cloaceae ve Bacillus pumilis izolatları tarafından farklı Brassica türleri üzerinde yapılan patojenite testlerinde oluşturdukları atipik hastalık belirtileri (ok)

brccoli, and cauliflower are cultivated. Morphological and molecular results confirmed that soil-borne fungal pathogens such as *R. solani*, *S. sclerotiorum*, *F. solani*, *F. oxysporum*, *F. equiseti*, and *A. alternata* were major causal disease agents on various plants. Notably, oomycete white rust disease agents *A. candida* and *A. lepidii*, along with the downy mildew pathogen *P. lepidii*, were identified as major pathogens affecting leafy *Brassicaceous* vegetables such as rocket, garden cress, and radish. Previous studies have reported the existence of different anastomosis groups of *R. solani* in cabbage cultivation areas in the Black Sea region

(Erper et al., 2017; Türkkan et al., 2020, Saygı et al., 2020; Benli et al., 2021; Erper et al., 2021). *S. sclerotiorum* has been identified as the causative agent of head rot in cabbage crops in Erzurum Province (Tozlu et al., 2016). Recent studies have also reported the presence of *A. candida* on rocket and garden cress in Ankara and Eskişehir (Canpolat & Tülek, 2019), *A. lepidii* and *P. lepidii* on garden cress plants in Hatay Province (Soylu et al., 2017; Soylu et al., 2019).

To the best of our knowledge, *A. alternata* LAa21 (OM854806), *F. equiseti* LFe33 (OM883923), *F. solani* KFs9 (OM883985), *F. oxysporum* KFo27 (OM883986), and *Stemphylium* sp. LSt33 (OM884463) have been identified as causal agents in different *Brassicaceous* vegetables cultivated in Turkey for the first time. The presence of *Fusarium equiseti* in cabbage plants grown in Korea (Afroz et al., 2021), *F. oxysporum* and *F. solani* in cabbage plants grown in Egypt (Khafagi et al., 2018) and China (Yan et al., 2018) and *S. sclerotiorum* in cabbage and canola crops in Sri Lanka and Iran (Yousefdoost & Ghosta, 2013; Mahalingam et al., 2017; Khan et al., 2022) has been previously reported during routine disease surveys.

In addition to fungal diseases, *Brassicaceous* plants are also susceptible to various bacterial pathogens. Among the bacterial pathogens known to affect *Brassicaceous* plants, *Pectobacterium* spp. and *Pseudomonas* spp. are associated with soft rot disease, *P. syringae* pv. *maculicola* causes leaf spot disease and *X. campestris* pv. *campestris* is responsible for black rot disease (Rimmer et al., 2007). The present study also investigated the presence of bacterial diseases in different *Brassicaceous* vegetable crops cultivated in Hatay Province, Turkey. The identification of these bacterial species was accomplished through a combination of biochemical analyses and MALDI-TOF analyses. *X. campestris* pv. *campestris* was isolated from the leaves of cabbage plants. This pathogen was more prevalent in fields with high groundwater levels, especially in late-harvested fields. Isolates of *Pectobacterium* spp., *Pseudomonas* spp., *P. agglomerans, E. cloacae,* and *B. pumulis* were identified from white cabbage, cauliflower, broccoli, and radish plants displaying symptoms of soft rot disease. To assess the pathogenicity of all bacterial isolates obtained from soft rot symptoms, experiments were conducted on both the plant species from which they were isolated and on potato slices. This comprehensive approach allowed us to determine the virulence of these isolates. Following the inoculation process and subsequent re-isolation from *Brassicaceous* vegetable crops and potato slices, pure colonies were examined and their species were reconfirmed. This rigorous methodology ensured the accuracy of our findings regarding the pathogenicity of these bacterial isolates.

Previous studies have documented the presence of *X. campestris pv. campestris* (Mirik et al., 2008; Aksoy et al., 2018; Öztürk et al., 2019; Öztürk & Soylu, 2021) and *P. caratovorum* (Aksoy et al., 2017; Öztürk & Soylu, 2022) on *Brassica* spp growing in various provinces of Turkey, including Adana, Samsun, and Yozgat. This study represents a significant contribution as it is the first report of primary soft rot pathogens such as *P. parmentieri, P. victoria*, and *P. corrugata*. Additionally, this is also the first report of opportunistic (secondary) soft rot pathogens, including *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilis* on *Brassica* spp growing in Turkey. *P. parmentieri, P. victoria,* and *P. corrugata* have been previously documented as primary soft rot disease agents in various vegetables in Turkey (Mirik et al., 2011; İmriz and Çınar, 2015; Öztürk et al., 2018; Soylu et al., 2022). Similarly, *P. agglomerans* has been reported to cause soft rot diseases in Napa cabbage in China (Guo et al., 2019). Furthermore, *P. fluorescens, P. marginalis,* and *E. cloacae* have been reported in various vegetables displaying soft rot symptoms worldwide, including Turkey (Godfrey & Marshall, 2002; Koike et al., 2007; Mikiciński et al., 2010; Hausdorf et al., 2011; Achbani et al., 2014; Dadaşoğlu & Kotan, 2017; García-González et al., 2018; Soylu et al., 2022).

CONCLUSION

This study conducted a comprehensive investigation into the identification, prevalence, and incidence of fungal, oomycete, and bacterial pathogens in various *Brassicaceous* plants, which hold significance as minor vegetables cultivated in the Hatay province of Turkey. Among the fungal pathogens examined, it is noteworthy that *A. alternata* LAa21 (OM854806), *F. equiseti* LFe33 (OM883923), *F. solani* KFs9 (OM883985), *F. oxysporum* KFo27 (OM883986), and *Stemphylum* sp. LSt33 (OM884463) were identified as the causal agents of diseases on *Brassicaceous* species grown in Turkey for the first time. This expands our knowledge of the diversity of pathogens affecting these crops. In addition to the fungal agents, the results of this study also revealed the presence of bacterial pathogens. Among the bacterial pathogens examined, *P. parmentieri, P. cichorii,* and *P. corrugata,* along with opportunistic (secondary) soft rot pathogens like *P. agglomerans, P. fluorescens, P. marginalis, E. cloacae,* and *B. pumilis*, were determined as causative bacterial disease agents on *Brassicaceous* vegetable plants for the first time in Turkey. The identification of these previously unreported disease agents is of significant importance. Neglecting to implement necessary precautions against these pathogens could potentially result in substantial yield and quality losses in the region in the forthcoming periods.

ACKNOWLEDGEMENTS

This study was financially supported by the Scientific Research Projects Commission of Hatay Mustafa Kemal University (Project No: 20.M.021)

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Achbani, E. H., Sadik, S., El Kahkahi, R., Benbouazza, A., & Mazouz, H. (2014). First report on *Pseudomonas marginalis* bacterium causing soft rot of onion in Morocco. *Atlas Journal of Biology, 3,* 218-223. https://doi.org/10.5147/ajb.v3i2.33

Afroz, T., Jee, S., Choi, H. W., Kim, J. H., Assefa, A. D., Aktaruzzaman, Md., Hahn, B. S., & Lee, H. S. (2021). First report of fusarium wilt caused by *Fusarium equiseti* on cabbage (*Brassica oleracea* var. *capitate*) in Korea. *Plant Disease, 105*(4), 1198. https://doi.org/10.1094/PDIS-06-20-1278-PDN

Aksoy, H. M., & Öztürk, M., & Tufan, S. (2018). First report on *Xanthomonas campestris* pv. *campestris* causing bacterial black rot disease of cauliflower in Turkey. *Journal of Plant Pathology, 100,* 141. https://doi.org/10.1007/s42161-018-0030-1

Aksoy, H. M., Ozturk, M. & Aktas, A. (2017b). First report of *Pectobacterium carotovorum* subsp. *carotovorum* causing soft rot on white head cabbage in Turkey. *Journal of Plant Pathology*, 99, 810.

Aksoy, H. M., Ozturk, M., & Aktas, A. (2017a). First report of *Pseudomonas viridiflava* causing cabbage bacterial leaf spot in Turkey. *Journal of Plant Pathology*, *99*, 801-801. https://doi.org/10.4454/JPP.V99I3.3929

Al-Lami, H. F. D., You, M. P., & Barbetti, M. J. (2019). Incidence, pathogenicity, and diversity of *Alternaria* spp. associated with Alternaria leaf spot of canola (*Brassica napus*) in Australia. *Plant Pathology, 68,* 492-503. https://doi.org/10.1111/ppa.12955

Anonymous (2020). TUİK Bitkisel Üretim İstatistikleri. Available at: https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul (Accessed September 25, 2023).

Anonymous (2021a). Crop information on cabbage. Available at: https://www.fao.org/land-water/databases-and-software/crop-information/cabbage/en/ (Accessed September 25, 2023).

Anonymous (2021b3). FAOSTAT – Worldwide production and yield of cabbage, cauliflower, broccoli and rape seed. Available at: https://www.fao.org/faostat/en/#data/QCL (Accessed September 25, 2023).

Ayadi, J., Debouba, M., Rahmani, R., & Bouajila, J. (2022). Brassica genus seeds: A review on phytochemical screening and pharmacological properties. *Molecules, 27*(18), 6008. https://doi.org/10.3390/molecules27186008

Benli, H. İ., Türkkan, M., Ozer, G., & Erper, I. (2021). First report of *Rhizoctonia solani* AG 1-IB causing root and stem rot of kale (*Brassica oleracea* var. *acephala*) in Turkey. *Journal of Plant Pathology, 103,* 663. https://doi.org/10.1007/s42161-021-00750-3

Benli, H.İ., & Türkkan, M. (2020). Ordu ili yaprak lahana ekim alanlarından izole edilen *Rhizoctonia* grubu fungusların anastomozis gruplarının, karakteristik özelliklerinin ve patojenisitelerinin belirlenmesi. *Akademik Ziraat Dergisi*, *9*(2), 229-238.

Blagojević, J., Vukojević, J., Ivanović, B., & Ivanović, Ž. (2020). Characterization of *Alternaria* species associated with leaf spot disease of *Armoracia rusticana* in Serbia. *Plant Disease, 104*(5),1378-1389. https://doi.org/10.1094/PDIS-02-19-0289-RE

Bora, T., & Karaca, İ. (1970). Kültür Bitkilerinde Hastalık ve Zararlıların Ölçülmesi. Ege Üniversitesi, Ziraat Fakültesi Yardımcı Ders Kitabı Yayın No: 167, 43s., İzmir.

Bruehl, G. W. (1987). Soilborne Plant Pathogens. Macmillan, New York.

Campbell, B., Han, D. Y., Triggs, C. M., Fraser, A. G., & Ferguson, L. R. (2012). Brassicaceae: Nutrient analysis and investigation of tolerability in people with Crohn's disease in a New Zealand study. *Functional Foods in Health and Disease, 2*(11), 460-486. https://doi.org/10.31989/ffhd.v2i11.70

Canpolat S., & Tülek S., (2019). Orta Anadolu Bölgesi'nde yaprağı yenen sebzelerde görülen fungal hastalıkların belirlenmesi, *Bitki Koruma Bülteni*, *59*-39-46. https://doi.org/10.16955/bitkorb.527754

Chen, J., Jing, J., Zhan, Z., Zhang, T., Zhang, C., & Piao, Z. (2013). Identification of novel QTLs for isolate-specific partial resistance to *Plasmodiophora brassicae* in *Brassica rapa*. *PLoS ONE, 8*(12), e85307. https://doi.org/10.1371/journal. pone.0085307

Choi, Y. J., Shin, H. D., Hong, S. B., & Thines, M. (2007). Morphological and molecular discrimination among *Albugo candida* materials infecting *Capsella bursa-pastoris* worldwide. *Fungal Diversity, 27,* 11-34.

Cicio, A., Serio, R., & Zizzo, M. G. (2022) Anti-inflammatory potential of Brassicaceae-derived phytochemicals: In vitro and in vivo evidence for a putative role in the prevention and treatment of IBD. *Nutrients, 15*(1), 31. https://doi.org/10.3390/nu15010031

Constantinescu, O., & Fatehi, J. (2002). Peronospora-like fungi (Chromista, Peronosporales) are parasitic

on Brassicaceae and related hosts. Nova Hedwigia, 74, 291-338. https://doi.org/10.1127/0029-5035/2002/ 0074-0291

Dadaşoğlu, F., & Kotan, R. (2017). Bazı sebze ve meyvelerde yumuşak çürüklük oluşturan pektolitik bakterilerin tanı ve karakterizasyonu. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi 7*(1), 155-161. https://doi.org/10.21597/jist.2017127428

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, U.K.

Erper, I., Ozer, G., Kalendar, R., Avci, S., Yildirim, E., Alkan, M., & Turkkan, M. (2021). Genetic diversity and pathogenicity of *Rhizoctonia* spp. isolates associated with red cabbage in Samsun (Turkey). *Journal of Fungi, 7,* Article Number 234. https://doi.org/10.3390/jof7030234

Erper, I., Türkkan, M., Ozgen., T., & Balkaya, A. (2017). Determination of reactions of some *Brassica* spp. against three subgroups of *Rhizoctonia solani* AG 4. The 69th International Symposium on Crop Protection, 23 May, Ghent, Belgium.

García-González, T., Sáenz-Hidalgo, H.K., Silva-Rojas, H.V., Morales-Nieto, C., Vancheva, T., Koebnik, R., & Ávila-Quezada, G.D. (2018). *Enterobacter cloacae*, an emerging plant-pathogenic bacterium affecting chili pepper seedlings. *Plant Pathology Journal, 34*(1), 1-10. https://doi.org/10.5423/ PPJ.OA.06.2017.0128

Godfrey, S., & Marshall, J. (2002). Identification of cold-tolerant *Pseudomonas viridiflava* and *P. marginalis* causing severe carrot postharvest bacterial soft rot during refrigerated export from New Zealand. *Plant Pathology, 51,* 155-162. https://doi.org/10.1046/j.1365-3059.2002.00679.x

Greer, F. S., Surendran, A., Grant, M., & Lillywhite, R. (2023). The current status, challenges, and future perspectives for managing diseases of brassicas. *Frontiers in Microbiology, 14,* 1209258. https://www.frontiersin.org/articles/10.3389/fmicb. 2023.1209258

Guo, M., Liu, Y., Liu, S. N., Qu, Q. Z., Cui, T. F., & Zhang, Y. W. (2019). First report of bacterial soft rot caused by *Pantoea agglomerans* on Chinese Cabbage in China. *Plant Disease 104*(1), 277. https://doi.org/10.1094/PDIS-04-19-0746-PDN

Gupta, S.R. (2016). Brassicas. In: Gupta, SK, (Ed)., Breeding Oilseed Crops for Sustainable Production, Academic Press. pp 33-53. https://doi.org/10.1016/ B978-0-12-801309-0.00003-3.

Hausdorf, L., Fröhling, A., Schlüter, O., & Klocke, M. (2011). Analysis of the bacterial community within carrot wash water. *Canadian Journal of Microbiology, 57,* 447-452. https://doi.org/ 10.1139/w11-013

İmriz, G., & Çınar, Ö. (2015). ELISA-based and traditional diagnosis methods for identification of *Pseudomonas victoria* and *Pseudomonas corrugata* causing pith necrosis on tomato plants. *Bahri Dağdaş Bitkisel Araştırma Dergisi, 3*(1), 7-13.

Jabeen, N. (2020). Agricultural, Economic and Societal Importance of Brassicaceae Plants. In The Plant Family Brassicaceae; Hassanuzzaman, M., Ed.; Springer: Singapore, pp. 45-128.

Jahangir, M., Kim, H., Choi, Y., & Verpoorte, R. (2009). Health-Affecting Compounds in Brassicaceae. *Comprehensive Reviews in Food Science and Food Safety, 8,* 31-43. https://doi.org/10.1111/j.1541-4337.2008.00065.x

Khafagi, A. A., Ghamery, A. A., Ghaly, O., & Ragab, O. G. (2018). Fruit and seed morphology of some species of Solanaceae. *Taeckholmia, 38,* 123-140.

Khan, M. A., Cowling, W., Banga, S. S., You, M. P., Tyagi, V., Bharti, B., & Barbetti, M. J. (2022). Quantitative inheritance of Sclerotinia stem rot resistance in *Brassica napus* and relationship to cotyledon and leaf resistances. *Plant Disease, 106*(1), 127-136. https://doi.org/10.1094/PDIS-04-21-0885-RE

Kiran, R., Kumar, P., Akhtar, J., Nair, K., & Dubey, S. C. (2022). Development of multiplex PCR assay for detection of *Alternaria brassicae, A. brassicicola,* and *Xanthomonas campestris* pv. *campestris* in crucifers. *Archives of Microbiology, 204,* 224. https://doi.org/10.1007/s00203-022-02846-5

Kohn, L. M. (1979) Delimitation of the economically important plant pathogenic *Sclerotinia* species. *Phytopathology, 69,* 881-886. http://dx.doi.org/10.1094/Phyto-69-881

Koike, S. T., Gladders, P., & Paulus, A. O. (2007). Vegetable Diseases: A Colour Handbook. Manson Publishing, London, pp. 95-115.

Kumar, K., Tiwari, Y., & Trivedi, L. (2017). Major fungal diseases of oilseed brassica and their preventive measures: A brief review. *International Journal of Botany Studies, 2,* 105-112.

Lelliot, R.A., & Stead, D.E. (1987). Methods for the diagnosis of bacterial diseases of plants. In: Preece TF (ed). Methods in Plant Pathology, Vol 2, Oxford: Blackwell Scientific Publications. pp. 176-177.

Leslie, J. F., & Summerell, B. A. (2006). The Fusarium Laboratory Manual. Blackwell Publishing, Hoboken, 1-2. https://doi.org/10.1002/9780470278376

Mahalingam, T., Guruge, B. M. A., Somachandra, K. P., Rajapakse, C. S., & Attanayake, R. N. (20176). First report of white mold caused by *Sclerotinia sclerotiorum* on cabbage in Sri Lanka. *Plant Disease, 101*(1), 249. https://doi.org/10.1094/PDIS-05-16-0693-PDN

Meral, S.E., Bibi, S., Rodríguez, C. A. D., Menkovic, J., Bernal, A. J., & Koebnik, R. (2022). Complete genome sequence of *Xanthomonas campestris* pv. campestris SB80, a race 4 strain isolated from whitehead cabbage in Turkey. *Microbiology Resource Announcements, 11*(3), e0002222. https://doi.org/10.1128/mra.00022-22

Mikiciński, A., Sobiczewski, P., Sulikowska, M., Puławska, J., & Treder, J. (2010). Pectolytic bacteria associated with soft rot of Calla Lily (*Zantedeschia* spp.) tubers. *Journal of Phytopathology, 158,* 201-209. https://doi.org/10.1111/j.1439-0434.2009.01597.x

Mirik, M., Aysan, Y., & Şahin, F. (2011). Characterization of *Pseudomonas victoria* isolated from different hosts in Turkey. *International Journal of Agriculture and Biology, 13*(2), 203-209.

Mirik, M., Selcuk, F., Aysan, Y., & Şahin, F. (2008). The first outbreak of bacterial black rot on cabbage, broccoli, and Brussels sprouts was caused by *Xanthomonas campestris* pv. *campestris* in the Mediterranean Region of Turkey. *Plant Disease, 92*(1), 176. https://doi.org/10.1094/PDIS-92-1-0176C

Mourou, M., Raimondo, M., Lops, F., & Carlucci, A. (2023). Brassicaceae fungi and chromista diseases: Molecular detection and host–plant interaction. *Plants, 12*(5), 1033. https://doi.org/10.3390/plants12051033

Murillo, G., & Mehta, R. (2001). Cruciferous Vegetables and Cancer Prevention. *Nutrition and Cancer, 41*(1-2), 17-28.

Öztürk, M., & Soylu, S. (2021). Identification of *Xanthomonas campestris* pv. *campestris* isolates causing black rot disease on cabbage in Niğde Province of Turkey. Proceedings of the XII International Scientific Agricultural Symposium "Agrosym 2021", 647-652.

Öztürk, M., & Soylu, S. (2022). Yozgat ili beyaz baş lahana üretim alanlarında bakteriyel yumuşak çürüklük hastalığına neden olan *Pectobacterium* izolatlarının tanılanması. *KSU Tarım ve Doğa Dergisi, 25,* 495-503. https://doi.org/10.18016/ksutarimdoga.vi.943765

Öztürk, M., Aksoy, H. M., Potrykus, M., & Lojkowska, E. (2018). Genotypic and phenotypic variability of *Pectobacterium* strains causing blackleg and soft rot on potato in Turkey. *European Journal of Plant Pathology, 152*(1), 143-155. https://doi.org/10.1007/s10658-018-1459-y

Öztürk, M., Eroglu, Z., & Secgin, Z. (2019). First report of *Xanthomonas campestris* pv. *campestris* as the causal agent of black rot disease on red cabbage in Turkey. *Journal of Plant Pathology, 101*(3), 795. https://doi.org/10.1007/s42161-019-00262-1

Pavlovic, M., Konrad, R., Iwobi, A. N., Sing, A., Busch, U., & Huber, I. (2012). A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the *Enterobacter cloacae* complex. *FEMS Microbiology Letters, 328*(1), 46-53. https://doi.org/10.1111/j.1574-6968.2011.02479.x

Rakow, G. (2004). Species Origin and Economic Importance of Brassica. In: Pua EC., Douglas C.J. (eds) Brassica. Biotechnology in Agriculture and Forestry, vol 54. Springer, Berlin, Heidelberg.

Ranjan, A., Ramachandran, S., Gupta, N., Kaushik, I., Wright, S., Srivastava, S., Das, H., Srivastava, S., Prasad, S., & Srivastava, S. K. (2019). Role of phytochemicals in cancer prevention. International *Journal of Molecular Sciences, 20*(20), 4981. https://doi.org/10.3390/ijms20204981

Raza, A., Hafeez, M. B., Zahra, N., Shaukat, K., Umbreen, S., Tabassum, J., Charagh, S., Khan, R. S. A., & Hasanuzzaman, M. (2020). The Plant Family Brassicaceae: Introduction, Biology, and Importance. In The Plant Family Brassicaceae; Springer: Berlin/Heidelberg, Germany, pp. 1-43.

Rimmer, S. R., Shattuck, V. I., & Buchwaldt, L. (2007). Compendium of Brassica Diseases. American Phytopathological Society (APS Press).

Šamec, D., Piljac Žegarac, J., Bogović, M., Habjanič, K., & Grúz, J. (2011). The antioxidant potency of white (*Brassica oleracea* L. var. *capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. *Scientia Horticulturae, 128,* 78-83. https://doi.org/10.1016/j.scienta.2011.01.009

Saygi, S., Erper, I., Ozer, G., & Turkkan, M. (2020). The first report of *Rhizoctonia solani* AG 4 HG-I caused damping off and wire stem of white cabbage in Turkey. *Journal of Plant Pathology, 102,* 587-587. https://doi.org/10.1007/s42161-019-00472-7

Shankar, S., Segaran, G., Sundar, R. D. V., Settu, S., & Sathiavelu, M. (2019). Brassicaceae classical review on its pharmacological activities. *International Journal of Pharmaceutical Sciences Review and Research, 55,* 107-113.

Shaw, R. K., Shen, Y., Zhao, Z., Sheng, X., Wang, J., Yu, H., & Gu, H. (2021). Molecular breeding strategy and challenges towards improvement of downy mildew resistance in cauliflower (*Brassica oleracea* var. *botrytis* L.). *Frontiers in Plant Science, 12,* 667757. https://doi.org/10.3389/fpls.2021.667757

Simmons, E. G. (2007). *Alternaria*: An Identification Manual. CBS Fungal Biodiversity Center, Utrecht, Netherlands.

Smith, I. M., Dunez, J., Lellioth, R. A., Phillips, P. H., & Archer, S.A. (eds)., 1988. European Handbook of Plant Disease. Blackwell Scientific Publications, Oxford.

Sneh, B., Burpee, L., & Ogoshi, A. (19914). Identification of *Rhizoctonia* species. APS Press, Minnesota.

Soylu, E. M., Kara, M., Kurt, Ş., Uysal, A., Shin, H. D., Choi, Y. J., & Soylu, S. (2017). First report of downy mildew disease caused by *Perofascia lepidii* on garden cress *Lepidium sativum* in Turkey. *Plant Disease, 101*(10), 1827. https://doi.org/10.1094/PDIS-05-17-0624-PDN

Soylu, E. M., Kara, M., Kurt, Ş., Uysal, A., Shin, H. D., Choi, Y. J., & Soylu, S. (2019). First report of *Albugo lepidii* causing white blister rust on broadleaved garden cress (*Lepidium sativum*) in Turkey. *Plant*

Disease, 103(10), 2698. https://doi.org/10.1094/PDIS-12-18-2179-PDN

Soylu, S., Atay, M., Kara, M., Uysal, A., Soylu, E. M., & Kurt, Ş. (2023). Morphological and molecular characterization of *Fusarium incarnatum* as a causal disease agent of pepper (*Capsicum annuum*) fruit rot. *Journal of Phytopathology*, in press. https://doi.org/10.1111/jph.13228

Soylu, S., Kara, M., Uysal, A., Kurt, Ş., Soylu, E. M., Üremiş, İ., Sertkaya, E., Bozkurt, İ. A., & Öztürk, M. (2022). Amik ovası havuç ekim alanlarında sorun olan fungal ve bakteriyel hastalık etmenlerin belirlenmesi. *KSÜ Tarım ve Doğa Dergisi, 25*(6), 1326-1340. https://doi.org/10.18016/ksutarimdoga.vi.1015936

Srivastava, M., Gupta, S. K., Saxena, A. P., Shittu L. A. J., & Gupta, S. K. (2011). A review of the occurrence of fungal pathogens on significant Brassicaceous vegetable crops and their control measures. *Asian Journal of Agricultural Sciences, 3*(2), 70-79.

Tozlu, E., Mohammadi, P., Kotan, M. S., Nadaroglu, H., & Kotan, R. (2016). Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold disease in red cabbage, by some bacteria. *Plant Protection Science, 52,* 188-198. https://doi.org/10.17221/96/2015-PPS

Türkkan, M., Kılıçoğlu, M. Ç., & Erper, İ. (2020). Characterization and pathogenicity of *Rhizoctonia* isolates collected from *Brassica oleracea* var. *acephala* in Ordu, Turkey. *Phytoparasitica*, *48*, 273-286. https://doi.org/10.1007/s12600-020-00793-9

Uysal, A., Kurt, Ş., Soylu, S., Kara, M., & Soylu, E.M. (2022). Hatay ilinde yer alan turunçgil paketleme tesislerinde meyve ve hava kökenli mikrobiyata içerisindeki fungal ve bakteriyel türler ile yoğunluklarının belirlenmesi. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, *27*(2), 340-351. https://doi.org/10.37908/mkutbd.1095692

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) In PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp. 315-322.

Woudenberg, J. H. C., Hanse, B., van Leeuwen, G. C. M., Groenewald, J. Z., & Crouse, P. W. (2017). *Stemphylium* revisited. *Study Mycology, 87,* 77-103. https://doi.org/10.1016/j.simyco.2017.06.001

Yan, W., Shi, Y., & Li, P. (2018). Preliminary study on *Fusarium* species causing Chinese cabbage wilt [J]. *Acta Phytopathologica Sinica*, *48*(5), 587-593. https://doi.org/10.13926/j.cnki.apps.000141

Yousefdoost, V., & Ghosta, Y. (2013). First report of *Sclerotinia* head rot of cabbage in Iran. *Iranian Journal of Plant Pathology, 49,* 361-362

KSÜ Tarım ve Doğa Derg 27 (4), 856-867, 2024
KSU J. Agric Nat 27 (4), 856-867, 2024
DOI:10.18016/ksutarimdoga.vi.1364180

Insecticidal Effect of Some Essential Oils on Larval Survival of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Laboratory Conditions

Hanife YANDAYAN GENÇ[1], Ceren SARAN[2]✉, Sevim AKÇURA[3]
[1,2]Department of Agricultural Biotechnology, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale 17100, Türkiye, [3]Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale 17100, Türkiye
[1]https://orcid.org/0000-0001-8180-4307, [1]https://orcid.org/0000-0001-9598-8856, [2]https://orcid.org/0000-0002-7699-748X
✉: hgenc@comu.edu.tr

ABSTRACT
The Mediterranean fruit fly, medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a serious pest of many fruits and vegetables. This study was conducted to determine the antifeeding and insecticidal activities of some essential oils extracted from *Pelargonium graveolens* (Geraniaceae), geranium, and *Lavandula intermedia* Mill. (Lamiaceae), lavender, *Nigella sativa* L. (Ranunculaceae) black cumin, and *Laurus nobilis* L. (Lauraceae), laurel, against second instars of the Medfly on an artificial diet. The essential oils were obtained by Clevenger-type water distillation and a laboratory-reared medfly colony was used in the study. The doses of each tested essential oil were determined by multiplying their specific gravities by applying amounts into the diet and then distributing oil over the diet in a Petri dish having 20 larvae. All experiments were performed under laboratory conditions of 23±1°C, 50% RH, and 16: 8 (L:D) photoperiods. Probit MsChart was used to estimate the LC_{50} and LC_{90} values of the tested essential oils. GGE Biplot analyses were created with the larval mortality based on the different essential oil doses. As a result, the highest larval mortality was determined with the addition of laurel and black cumin oils into the diet. The highest mortality was detected in black cumin oil at the lowest concentrations. Based on GGE Biplot analyses, the essential oil of black cumin had larvicidal properties. The results provided fundamental information about the insecticidal and antifeeding properties of the medfly in the laboratory. Further studies are needed to integrate sustainable management programs with natural insecticides against the medfly larvae.

Entomology

Research Article

Article History
Received : 21.09.2023
Accepted : 15.01.2024

Keywords
Ceratitis capitata
Insecticidal activity
Essential oil
Black cumin
Laurel

Bazı Uçucu Yağların Kimyasal Yapısı ve Laboratuvar Koşullarında *Ceratitis capitata* 'nın (Wiedemann) (Diptera: Tephritidae) Larva Canlılığı Üzerindeki İnsektisidal Etkisi

ÖZET
Akdeniz meyve sineği, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) birçok meyve ve sebzenin ciddi bir zararlısıdır. Bu çalışma, *Pelargonium graveolens* (Geraniaceae), ıtır, *Lavandula intermedia* Mill. (Lamiaceae), lavanta, *Nigella sativa* L. (Ranunculaceae) çörek otu ve *Laurus nobilis* L. (Lauraceae), defneden izole edilen bazı uçucu yağların yapay besiyeri üzerinde yetiştirilen Akdeniz meyve sineğinin ikinci dönem larvalarına karşı beslenmeyi önleyici ve insektisidal aktivitelerini belirlemek amacıyla yapılmıştır. Uçucu yağlar Clevenger tipi su distilasyon yoluyla elde edildi ve bu çalışmada laboratuvarda yetiştirilen Akdeniz meyve sineği kolonisi kullanıldı. Test edilen her bir uçucu yağın dozu, özgül ağırlıklarının diyete uygulanan miktarı ile çarpılarak belirlendi ve 20 larva içeren Petri kabındaki diyet üzerine dağıtıldı. Tüm deneyler 23±1°C, %50 bağıl nem ve 16:8 (L:D) fotoperiyotlu laboratuvar koşullarında gerçekleştirildi. Test edilen uçucu yağların LC_{50} ve LC_{90} değerlerini tahmin etmek için Probit MsChart kullanıldı. GGE Biplot analizleri, farklı uçucu yağ dozlarına dayalı olarak larva ölümleri ile oluşturuldu. Sonuç olarak, en yüksek larva ölümleri, yapay besiyerine defne ve çörek otu yağı ilave edilen grupta belirlendi. En yüksek ölüm,

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 21.09.2023
Kabul Tarihi : 15.01.2024

Anahtar Kelimeler
Ceratitis capitata,
İnsektisidal aktivite
Uçucu yağ
Çörek otu
Defne

en düşük konsantrasyonlarda çörek otu yağında tespit edildi. GGE Biplot analizlerine göre, çörek otu uçucu yağının larvalarda öldürücü özellikleri bulunmaktadır. Çalışma sonuçları Akdeniz meyve sineğinin laboratuvardaki böcek öldürücü ve beslenmeyi önleyici özellikleri hakkında temel bilgiler sağladı. Akdeniz meyve sineği larvalarına karşı sürdürülebilir yönetim programlarını doğal insektisitlerle entegre etmek için daha fazla çalışmaya ihtiyaç bulunmaktadır.

To Cite: Genç, H.Y., Saran, C. & Akçura, S., (2024). Insecticidal Effect of Some Essential Oils on Larval Survival of *Ceratitis Capitata* (Wiedemann) (Diptera: Tephritidae) in Laboratory Conditions. *KSÜ Tarım ve Doğa Derg 27* (4), 856-867. DOI: 10.18016/ksutarimdoga.vi.1364180.

Atıf Şekli: Genç, H.Y., Saran, C. & Akçura, S., (2024). Bazı Uçucu Yağların Kimyasal Yapısı ve Laboratuvar Koşullarında *Ceratitis capitata* 'nın (Wiedemann) (Diptera: Tephritidae) Larva Canlılığı Üzerindeki İnsekitisidal Etkisi. *KSU J. Agric Nat 27* (4), 856-867. DOI: 10.18016/ksutarimdoga.vi.1364180.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is an important pest. It is usually known as a medfly damaging more than 350 different host plants including fruits, subtropical fruits, vegetables, ornamental plants, and nuts (Weems,1981; Mau & Kessing, 2007; Genc & Yücel, 2017). It evolved in sub-Saharan Africa and is now well-established worldwide (Gasperi et al., 1991; Liquido et al., 1991; Malacrida et al., 1992).

Medfly is well well-recognized pest in Turkey and mainly attacks Citrus however, in 2016 it became established in Çanakkale (Genç & Yücel, 2017). Several studies have been conducted on biology and laboratory rearing (Carey et al., 2008; Genç & Yücel, 2017), biological control (Cunningham, 1989; Gözel & Genç, 2021), mass rearing and survival (Economopoulos & Bruzzone, 1989; Fletcher, 1989), host preferences (Aluja & Mangan, 2008) and toxic effects of essential oils (Bazzoni et al., 1997).

Essential oils are secondary metabolic products of plants, having strong aromatic components that affect aroma and taste (Koul et al., 2008). Moreover, they have some toxic activities through direct contact, ingestion, and inhalation (Lee et al., 2003; 2004; Yıkınç & Tunaz, 2023). There have been some studies examining the insecticidal activity of essential oils on the *Ceratitis capitata* (Moretti et al., 1998; Miguel et al., 2010; Lopez et al., 2011; Benelli et al., 2012; Luu-Dam et al., 2021; Ouarhach et al., 2022). However, no such study has been found in Turkey on local medfly populations which were previously collected from infested fruits and then adapted and reared in the laboratory.

The present study aimed to determine the insecticidal activities of the essential oils extracted from *Pelargonium graveolens*, *Lavandula officinalis*, *Nigella sativa,* and *Laurus nobilis* against medfly larvae collected previously in Çanakkale province. The toxicity was tested for different doses. The chemical composition of the tested plants was characterized to determine putative responsible compounds caused larval mortality in the laboratory conditions.

MATERIALS and METHOD

Medfly Colony

The medfly larvae were reared on a cellulose-based artificial diet, previously used to rear olive fruit fly larvae (Genc, 2008; Genç & Yücel, 2017). Wild medfly colonies were maintained in the laboratory (Genc, 2008) on different fruit hosts collected in Çanakkale province (Genç & Yücel, 2017) and held under laboratory rearing conditions continuously since 2017. The laboratory-adapted medfly colony was used in this study. For bioassay studies, eggs were collected from oviposition domes and incubated for 3 days in an environmental chamber at 23±1ºC, 50% RH, and 16: 8 (L:D) photoperiods. The first instars were transferred to the artificial diet and reared for 72 hours. The 2nd instars were used for the bioassays.

Plant Materials

The leaves of a laurel tree in the landscaping area of Çanakkale Onsekiz Mart University Faculty of Agriculture were used to extract essential oil. The leaves were picked before midday in the first week of February 2021. The collected fresh leaves were immediately ground with a plant grinder without drying. Shade-dried flowers of Lavandula x intermedia Emeric ex Loisel hybrid Super (Lamiales: Lamiaceae) and *Pelargonium graveolens* 'Bourbon (Geraniales: Geraniaceae)' variety grown in Balıkesir conditions in 2021 were used to obtain essential oils in lavender and geranium, respectively. The essential oil in black cumin was obtained from the seeds of the Çameli (Ranunculales: Ranunculaceae) variety of *Nigella sativa* L. grown in Balıkesir in 2021.

Isolation of Essential oils and Gas Chromatography (GS) Analyses

The plant parts were extracted with S-H Clevenger equipment (Figure 1) using 300 g of weighed materials ground in a mill. The materials were divided into three parts then transferred into ballons with a volume of 2000 ml each, filled with samples, and added 1200 ml of distilled water then placed in Clevenger apparatus.

It was boiled for 3 hours (Figure 1A). The accumulated essential oil was separated with distilled water (Figure 1B). The extractions were performed over 8 hours until all essential oils were obtained then transferred to a Falcon tube, and stored at 4 °C in dark conditions until used (Figure 1C).

Figure 1. S-H Clevenger equipment (A) collection of black cumin oil (B) and black cumin oil transferred into the falcon tube (C)

Şekil 1. S-H Clevenger ekipmanı (A), çörek otu yağının toplanması (B) ve çörek otu yağının falcon tüpe transfer edilmesi (C)

The chemical composition of the essential oils was identified with gas chromatography and mass spectrometry analysis (GC-MS) Shimadzu GC-MS QP2020 NX system with an inner Restek Rxi-MS column (30 m x 0.25 mm x 0.25 µm). Helium was used as a carrier gas. At the beginning temperature of the column was 40 °C held for 3 min. The column was heated to 240 °C at a rate of 5 °C/min and waited for 10 min. Then heated to 275 °C at a rate of 4°C/min and waited for 10 min. The injector temperature was 250°C. The results were calculated as the percentage of the area, taken up by each compound, and represented as the average in each plant extract. The components were characterized by the comparison of their retention index (RI) with those of pure commercial standards and by comparing mass spectra using electronic libraries (FFNSC 3, W9N11, NIST11) (Akçura, 2023).

Experimental Conditions

The 2nd instar medfly larvae were used to test the effects of the essential oils (Genc & Yücel, 2017). Four grams of the cellulose-based artificial diet (Genc, 2008) were placed into Petri dishes (6 cm in diameter) then different doses of essential oils (w/v) were added with the help of a micropipette (Figure 2A and 2B) and mixed thoroughly. Tested essential oil doses were obtained by multiplying the specific gravity (weight) of each essential oil. They were determined as 0.887 g/ml for geranium oil, 0.894 g/ml for lavender oil, 0.919 g/ml for black cumin oil, and 0.960 g/ml for laurel oil (Anonymous, 2023a; Anonymous, 2023b). Tested essential oil amounts were 1 ml, 0.75 ml, 0.50 ml, 0.25 ml, 0.125 ml, 0.0625 ml and 0.0312 ml. Three dishes of each tested oil dose were used and considered as 3 replications. Twenty 2nd instars were used for each Petri dish/replicate. The Petri dish having larvae was placed in a larger plastic container (8 cm in diameter, 0.33 ml volume) which was used for monitoring larval movement. The lid was secured. Distilled water was used as a control. Larval survival was observed for 24 hours under an Olympus SZX9 stereo-zoom microscope. Monitoring continued until death. Mortality was confirmed by examining any movement in reaction to touch with soft forceps. Mortality data were corrected based on control treatment. Probit analysis was performed to estimate LC_{50} and LC_{90} and slopes.

Statistical Analyses

The data were analyzed with SAS software (Version 9.1.3; SAS Institute, 1990). The data for larval mortality were corrected using Abbott's formula (Abbott, 1925) and probit analyses with Probit MSChart (Chi, 2020). The means were compared with the LSD test (SAS Institute, 2000). Furthermore, the insecticidal activity of tested essential oils on the 2nd instar medfly was evaluated using two-way data analysis by GGE Biplot Sofware Version 8 (Yan, 2000). The tested essential oils were accepted as genotypes and different treatments were examined as the

environment (Kang & Gauch, 1996; Yan et al., 2000). When larval mortality was considered as environment, GGE Biplot analysis was created to evaluate the highest larval mortality based on the highest insecticidal activities of the essential oils. Kaplan Meier analysis was used to reveal the differences in larval survival period between the essential oils. A life distribution test was conducted to determine the relationship between the survival of medfly larvae with the time (hours). In addition, the Log-Rank test was applied to evaluate the significance of the differences between essential oils. Statistical analyses were conducted with the SAS JMP (version 16.1; SAS Institute, Cary, NC) statistical program.

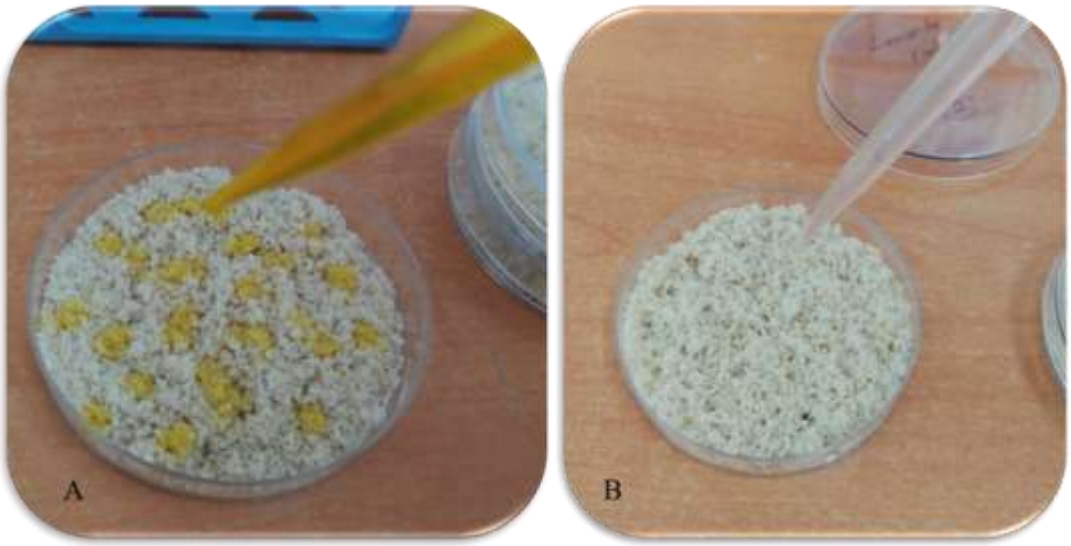


Figure 2. Applying essential oils by micropipette to the artificial diet. A) Black cumin oil, and B) Lavender oil
Şekil 2. Esansiyel yağların mikropipet ile yapay diyete uygulanması. A) Çörek otu yağı ve B) Lavanta yağı

RESULTS and DISCUSSION

Chemical Characterization of the Tested Plant Extracts

The components and chemical analyses of essential oils are indicated in Table 1. The main constituents (more than 1% of the total area) of the extract of geranium were citronellol, neryl formate, iso-Menthone, linalool, cis-rose oxide, geranyl formate, citronellyl butanoate, geranyl butanoate, 2-phenylethyl tiglate, and geraniol. The lavender extract was characterized by ß-myrcene, 1,8-cineole, cis-ocimene, α-terpineol, camphor, borneol, Lavandula, linalyl acetate, linalyl propionate, neryl acetate, geranyl formate, linalyl propionate, neryl acetate, geranyl acetate, caryophyllene, ß-farnesene, caryophyllene oxide, hexyl 2-methylbutanoate and α-bisabolol. The extract of black cumin consisted of α-pinene, sabinene, ß-pinene, p-cymene, limonene, γ -terpinene, α-thujene, thymoquinone, and longifolene. The major components of the laurel were sabinene, myrcene, eucalyptol, γ -terpinene, α-terpineol, 3-allyl-6-methoxyphenol, α-terpinylacetate, ß-element, benzene, 1,2-dimethoxy-4-(2-propenyl), t-muurolol, ß-eudesmol and squalene. Based on GC-MS analyses, the most abundant components of essential oil were citronellol (42.53±4.47) in *P. graveolens* (geranium), linalyl propionate (26.09±4.44) in *L. intermedia* (lavender), p-Cymene (52.13±0.879) in *N. sativa* (black cumin), and eucalyptol (36.44±4.97) in *L. nobilis* (laurel).

Insecticidal activities of essential oils

The insecticidal activities of four essential oils on the 2nd instars of medfly were investigated. Mortalities of the medfly larvae are shown in Table 2. The highest insecticidal effects were reported after 24 h exposure to 0.120 g/ml of laurel oil and 0.115 g/ml of black cumin oil having 100% and 90% mortality respectively (Figure 3, Table 2). Overall, the mortalities were caused by black cumin oils at 78.30% (15.66±0.57 larvae) and 25% (5±1.00 larvae) at 0.057 g/ml and 0.029 g/ml doses on diet respectively. Because of the limited amounts of geranium and lavender oils, four doses were tested and the mortalities were caused by 100% at 0.887 g/ml geranium oil and 0.894 g/ml lavender oil. The larval mortality was not observed in the control trial.

The medfly larvae were shown in Figure 3 after being exposed to essential oils at the highest dose. They were immobile and brown (Figure 3A). There were no signs of larval movement in response to touching by soft forceps (Figure 3B) so mortality was verified as 100%.

The estimated lethal concentration (LC_{50} and LC_{90}) values obtained for essential oils were reported in Table 3. As calculated by probit analysis, LC_{50} values were 0.313, 0.105, 0.038, and 0.070 g/ml for geranium, lavender, black cumin, and laurel oils, respectively. The highest insecticidal toxicity was observed for black cumin oil (Table 3). LC_{90} values revealed that medfly larvae were more susceptible to black cumin and laurel

oils (0.144 and 0.172 g/ml, respectively) than geranium and lavender oils (0.635 and 0.511 g/ml, respectively) (Table 3).

Table 1. Chemical compositions of *Pelargonium graveolens*, *Lavandula intermedia*, *Nigella sativa*, and *Laurus nobilis* oils and their relative proportions (% Area)

Çizelge 1. Pelargonium graveolens, Lavandula intermedia, Nigella sativa ve Laurus nobilis yağlarının kimyasal bileşimleri ve bunlara ilişkin oranları (% Alan)

No	Compound	RI[a]	Content (Mean±SE)[b]			
			P. graveolens (Geranium)	*L. intermedia* (Lavander)	*N. sativa* (Black cumin)	*L. nobilis* (Laurel)
1	Butanoic acid	761	-	-	-	0.02±0.00
2	Isopropyl Isobutyrate	788	-	-	-	0.03±0.00
3	isopropyl 2-methyl butyrate	878	-	-	-	0.06±0.01
4	α-Pinene	936	0.34±0.12	0.05±0.01	3.22±0.116	-
5	Camphene	944		0.07±0.01	-	0.10±0.01
6	Methyl heptenone	961	0.08±0.04	-	-	-
7	Sabinene	967		0.02±0.00	1.42±0.077	10.36±1.41
8	ß-Pinene	974	-	0.06±0.01	3.12±0.074	-
9	Myrcene	986	-	-	-	1.66±0.23
10	ß-Myrcene	988	0.13±0.04	1.19±0.20	0.14±0.029	-
11	α-Phellandrene	1000		-	-	0.65±0.09
12	δ-2-Carene	1002	-	0.04±0.01	-	-
13	α-Terpinene	1012	-	-	0.71±0.119	0.52±0.07
14	o-Cymene	1020	-	-	-	0.22±0.03
15	p-Cymene	1020	0.05±0.02	-	52.13±0.879	-
16	Eucalyptol	1024	-	-	0.05±0.046	36.44±4.97
17	α-Limonene	1024	-	0.63±0.11	-	-
18	Limonene	1024	0.11±0.03	-	2.10±0.047	-
19	1,8-Cineole	1026	-	3.63±0.62	-	-
20	(E)- ß-Ocimene	1030	-	-	-	0.07±0.01
21	(Z)-ß-Ocimene	1032	0.06±0.02	-	-	-
22	cis-Ocimene	1032	-	1.08±0.18	-	-
23	Phenyl acetaldehyde	1051	0.08±0.04	-	-	-
24	γ -Terpinene	1052	-	0.05±0.01	2.49±0.777	1.02±0.14
25	trans Sabinene hydrate	1052	-	0.03±0.01	-	0.81±0.11
26	cis- Linalool oxide	1067	0.10±0.04	-	-	-
27	α-Terpinolene	1084	-	-	-	0.34±0.05
28	trans-Linalool oxide	1084	0.17±0.08	-	-	-
29	Linalool	1088	3.30±1.40	-	0.96±0.054	-
30	cis- Rose oxide	1106	1.45±1.10	-	-	-
31	trans-Rose oxide	1122	0.63±0.50	-	-	-
32	α-Terpineol	1128	0.31±0.14	4.89±0.83	-	2.81±0.38
33	Camphor	1141	-	4.48±0.76	-	-
34	neo-Isopulegol	1144	0.07±0.02	-	-	-
35	Menthone	1148	0.26±0.08	-	-	-
36	iso-Menthone	1149	6.50±1.53	-	-	-
37	Borneol	1165	-	6.48±1.10	-	-
38	Lavandula	1167	-	1.15±0.20	-	-
39	Unidentified		0.07±0.05	-	-	-
40	Terpinen-4-ol	1174	-	-	0.93±0.062	-
41	iso-Menthol	1184	0.20±0.08	-	-	-
42	3-Allyl-6-methoxyphenyl	1187	-	-	-	5.07±0.69
43	ß-Cyclocitral	1217	-	-	0.20±0.061	-
44	Citronellol	1223	42.53±4.47	-	-	-
45	Nerol	1225	-	-	-	0.44±0.06
46	Linalyl Acetate	1231	-	19.46±3.31	-	-

47	Carvone	1239	-	0.31±0.05	-	-
48	Geraniol	1247	8.15±3.02	0.11±0.02	-	0.22±0.03
49	α-Thujene	1248	-	-	16.49±0.623	0.17±0.02
50	Thymoquinone	1248	-	-	8.07±0.760	-
51	l-Phellandrene	1250	-	0.12±0.02	-	-
52	Bornyl acetate	1257	-	-	0.11±0.013	0.19±0.03
53	α-Terpinylacetate	1262	-	-	-	16.69±2.28
54	Geranial	1264	0.40±0.18	-	-	-
55	Neryl formate	1271	10.25±1.43	-	-	-
56	Thymol	1289	-	-	0.72±0.159	-
57	Geranyl formate	1298	1.59±0.89	-	-	-
58	Benzaldehyde	1313	-	0.42±0.07	-	-
59	Z-Citral	1316	0.23±0.09	-	-	-
60	Linalyl propionate	1334	-	26.09±4.44	-	-
61	α-Cubebene	1345	0.08±0.04	0.12±0.02	-	-
62	Citronellyl acetate	1350	0.45±0.13	-	-	-
63	α-Longipinene	1350	-	-	0.17±0.014	-
64	Neryl acetate	1356	-	1.73±0.29	-	0.24±0.03
65	α-terpinyl acetate	1360	-	0.05±0.01	-	-
66	α-Copaene	1371	0.23±0.06	-	-	0.08±0.01
67	Geranyl acetate	1376	0.50±0.15	3.24±0.55	-	0.09±0.01
68	(E) -Cinnamyl acetate	1385	-	-	-	0.08±0.01
69	β-Elemene	1386	0.05±0.02	-	0.09±0.145	1.44±0.20
70	β-Bourbonene	1387	0.09±0.14	-	-	-
71	7-epi-Sesquithujene	1390	-	0.30±0.05	-	-
72	Benzene, 1,2-dimethoxy-4-(2-propenyl)	1391	-	-	-	1.50±0.18
73	trans-Isoeugenol	1403	-	-	-	0.08±0.01
74	Caryophyllene	1405	-	2.55±0.43	-	0.58±0.08
75	Longifolene	1407	-		1.03±0.106	-
76	trans-Caryophyllene	1408	0.97±0.19		-	-
77	α-Gurjunene	1406	0.15±0.04	0.03±0.01	-	0.29±0.04
78	trans-α-Bergamotene	1408	-		-	0.45±0.06
79	β-Cedrene	1409	-	0.14±0.02	-	-
80	α-Cedrene	1410	0.14±0.04		-	-
81	α-Bergamotene	1411	-	0.23±0.04	-	-
82	α-Guaiene	1434	-		-	0.12±0.02
83	Allo-aromadendrene	1436	-	-	-	0.11±0.02
84	β-Farnesene	1440	-	1.95±0.33	-	-
85	Citronellyl propionate	1444	0.63±0.17	-	-	-
86	α-Humulene	1449	0.31±0.06	-	-	0.14±0.02
87	Alloaromadendrene	1458	0.21±0.08	-	-	-
88	α-Muurolene	1475	-	-	-	0.17±0.02
89	Geranyl propanoate	1476	0.75±0.16	-	-	-
90	Germacrene-D	1481	0.64±0.19	0.98±0.17	-	0.15±0.02
91	Bicyclogermacrene	1497	-	-	-	0.79±0.11
92	α-Muurolene	1500	0.13±0.04	-	-	-
93	γ-Cadinene	1510	0.14±0.15	-	-	0.28±0.04
94	endo-1-Bourbonanol	1515	-	-	-	0.43±0.06
95	δ-Cadinene	1519	0.50±0.12	-	-	0.78±0.11
96	β-Sesquiphellandrene	1520	-	0.09±0.02	-	-
97	Citronellyl butanoate	1530	1.21±0.35	-	-	-
98	1.10-di-epi-Cubenol	1533	-	0.04±0.01	-	-
99	α-Elemol	1545	-	-	-	0.22±0.03
100	Geranyl butanoate	1562	1.25±0.48	-	-	-
101	Spathulenol	1577	0.35±0.23	-	-	-
102	Caryophyllene oxide	1579	-	1.22±0.21	-	0.28±0.04

103	2-Phenylethyl Tiglate	1584	1.92±0.43	-	-	-
104	Viridiflorol	1589	0.91±0.37	0.04±0.01	-	0.63±0.05
105	Guaiol	1597	-		-	0.45±0.06
106	Hexyl 2-methylbutanoate	1601	-	1.01±0.17	-	-
107	Ledol	1602	0.14±0.04	-	-	-
108	Humulene Oxide	1608	0.19±0.10	-	-	-
109	Cubenol	1618	0.24±0.12	-	-	-
110	Citronellyl valerate	1624	0.16±0.08	-	-	-
111	8-epi-γ-eudesmol	1627		-	-	0.19±0.03
112	α-Cadinol	1635	0.32±0.20	-	-	0.61±0.08
113	α-Muurolol	1640	-	0.42±0.07	-	-
114	t-Muurolol	1641	-	-	-	1.29±0.18
115	Agarospirol	1643	-	-	-	0.08±0.01
116	ß-Eudesmol	1646	-	-	-	1.09±0.15
117	Geranyl hexanoate	1650	-	0.07±0.01	-	-
118	α-Bisabolol oxide	1656	-	0.22±0.04	-	-
119	E-Citronellyl tiglate	1666	0.53±0.16	-	-	-
120	α-Bisabolol	1685	-	4.29±0.73	-	-
121	6-epi-Shyobunol	1685	-	-	-	0.63±0.09
122	Junpier camphor	1686	-	-	-	0.35±0.05
123	Farnesene	1688	-	0.03±0.01	-	-
124	Geranyl tiglate	1696	1.44±0.60	-	-	-
125	Neryl butyrate	1783	0.15±0.08	-	-	-
126	Geranyl hexanoate	1795	0.20±0.17	-	-	-
127	Octadecane	1800	-	0.03±0.01	-	-
128	γ-Cadinene	1803	-	0.24±0.04	-	-
129	Squalene	2764	-	-	-	2.73±0.37
130	Unidentified		-	-	5.85±0.530	-

[a] Retention index on a Restek Rxi-MS column relative to a homologous series of n- n-alkanes,
[b] Mean ± SE, -, undetected

Table 2. Mortality rates (%) of medfly larvae exposed to essential oils at various doses in 24 h (N=20, Mean±SE)*
Çizelge 2. 24 saatte çeşitli dozlarda uçucu yağlara maruz kalan Akdeniz meyve sineği larvalarının mortalite oranları (%) (N=20, Ortalama±SE)

Essential Oils	Mortality (%)						
Amount of used oil (ml)	1	0.75	0.50	0.25	0.125	0.0625	0.0312
Geranium oil (g/ml)	0.887	0.665	0.443	0.222			
%	100	81.65	88.33	21.65	*	*	*
Lavender oil (g/ml)	0.894	0.67	0.447	0.223			
%	100	91.65	85	83.3	*	*	*
Black cumin oil (g/ml)	0.919	0.689	0.459	0.23	0.115	0.057	0.029
%	100	100	100	98.3	90	78.3	25
Laurel oil (g/ml)	0.96	0.72	0.48	0.24	0.12	0.06	0.03
%	100	100	100	100	100	35	3.3
Control (g/ml)	1	0.75	0.50	0.25	0.125	0.0625	0.0312
%	0	0	0	0	0	0	0

*Means followed by different letters within a column are statistically different (P<0.05) from each other

GGE Biplot analysis was performed to determine the highest insecticidal activity of the essential oils. The graphs showed that black cumin and laurel oils were the most suitable or the highest insecticidal activities (Figure 4). When they were compared to each other at the lowest application dose, black cumin oil resulted in higher larval mortality than laurel oil. Therefore, black cumin oil has the highest insecticidal activity among the four tested essential oils (Figure 4).

Kaplan-Meier statistical analysis showed significant differences in the duration of larval survival at doses of 0.887 g/ml of geranium oil, 0.894 g/ml of lavender oil, 0.919 g/ml of black cumin oil, and 0.960 g/ml of laurel oil (Log-Rank test, Chi-square=124.1443; df=3; P<0.001). The average length of survival duration was 20.70±2.85 hours, 21.90±4.27 hours, 3.00±0.01 hours,

and 3.00±0.01 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

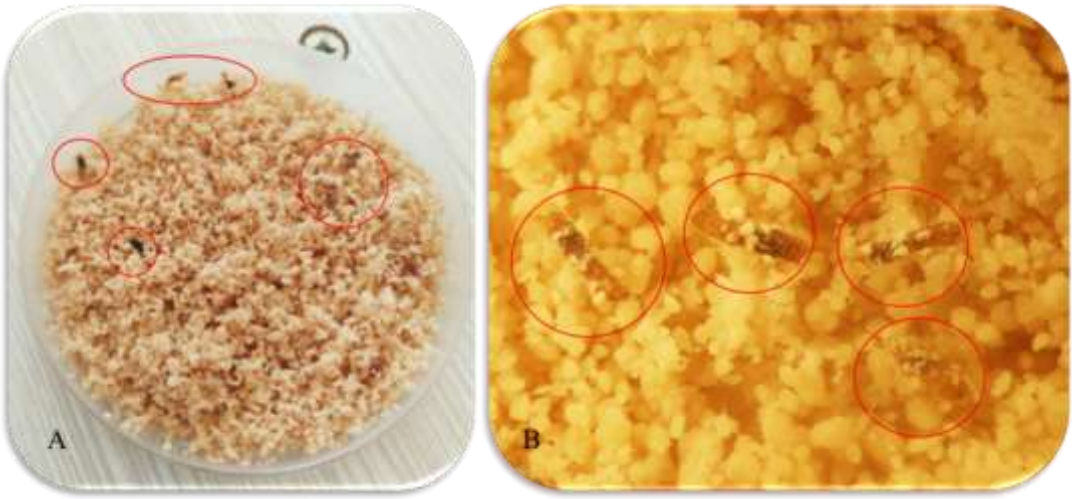


Figure 3. A view of medfly larvae after application of essential oil (A) and a close-up view of dead medfly larvae (B). Red circles indicate medfly larvae

Şekil 3. Esansiyel yağın (A) uygulanmasından sonra Akdeniz meyve sineği larvalarının görünümü ve ölü Akdeniz meyve sineği larvalarının (B) yakından görünümü. Kırmızı daireler Akdeniz meyve sineği larvalarını gösterir

Table 3. Toxicity of the essential oils against to 2nd instars of medfly

Çizelge 3. Esansiyel yağların Akdeniz meyve sineğinin 2.dönem larvalarına karşı toksisitesi

Essential Oils	Slope (Mean±SE)	LC_{50} (g/ml)	LC_{90} (g/ml)	Log (LC_{50})	Fiducial Limits (%95)	Chi square ($\chi 2$)
Geranium oil	0.232±0.482	0.313	0.635	-0.504	0.089-0.753	15.002
Lavender oil	0.224±0.473	0.105	0.511	-0.978	0.080-0.487	1.821
Black cumin oil	0.066±0.257	0.038	0.144	-1.419	0.017-0.080	29.73
Laurel oil	0.107±0.327	0.070	0.172	-1.151	0.015-0.304	267.41

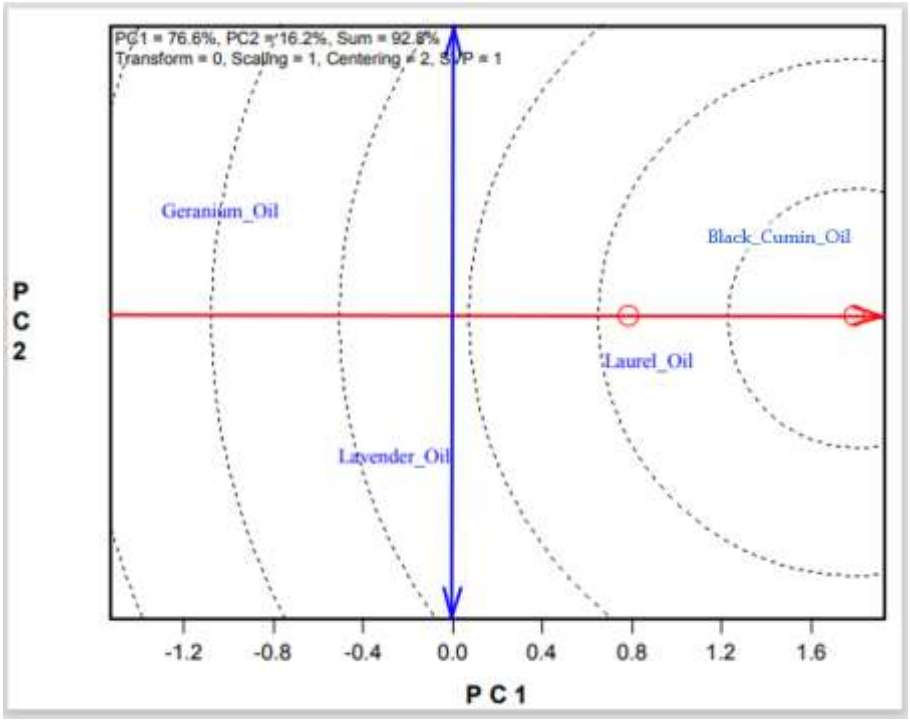


Figure 4. The effects of four essential oils on the larval mortality of medfly based on GGE biplot

Şekil 4. GGE biplot'a dayalı olarak dört uçucu yağın Akdeniz meyve sineğinin larval mortalitesi üzerindeki etkileri

KSÜ Tarım ve Doğa Derg 27 (4), 856-867, 2024
KSU J. Agric Nat 27 (4), 856-867, 2024
DOI:10.18016/ksutarimdoga.vi.1364180

At the dose of 0.665 g/ml of geranium, 0.670 g/ml of lavender, 0.689 g/ml of black cumin, and 0.720 g/ml of laurel oils, there was a significant difference between the survival durations (Log-Rank test, Chi-square=179.6337; df=3; P<0.001). The average length of survival duration was 57.45±6.53 hours, 45.66±7.27 hours, 3.00±0.01 hours, and 3.00±0.01 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

At the dose of 0.443 g/ml of geranium, 0.447 g/ml of lavender, 0.459 g/ml of black cumin, and 0.480 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=240.3168; df=3; P<0.001). The average length of survival duration was 34.10±2.94 hours, 109.66±12.10 hours, 3.00±0.01 hours, and 3.95±0.28 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

At the dose of 0.222 g/ml of geranium, 0.223 g/ml of lavender, 0.230 g/ml of black cumin, and 0.240 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=256.0147; df=3; P<0.001). The average length of survival duration was 424.70±19.28 hours, 136.84±13.53 hours, 17.65±4.05 hours, and 6.20±0.66 hours for geranium, lavender, black cumin, and laurel oils respectively (Figure 5).

In the tested doses of essential oils described above, black cumin and laurel oils had higher insecticidal activity and/or mortality in a shorter time than geranium and lavender oils.

At the dose of 0.115 g/ml of black cumin and 0.120 g/ml of laurel oils, the differences between the survival durations were not significant (Log-Rank test, Chi-square=0.04792; df=1; P>0.05). The average length of survival duration was 67.65±16.05 hours and 30.10±2.72 hours for black cumin and laurel oils respectively (Figure 5).

At the dose of 0.057 g/ml of black cumin and 0.060 g/ml of laurel oils, the differences between the survival durations were significant (Log-Rank test, Chi-square=9.119407; df=1; P<0.05). The average length of survival duration was 251.73±27.10 hours and 365.30±30.76 hours for black cumin and laurel oils respectively (Figure 5).

At the dose of 0.029 g/ml of black cumin and 0.030 g/ml of laurel oils, the differences between the survival durations were not significant (Log-Rank test, Chi-square=3.334939; df=1; P>0.05). The average lengths of survival duration were 296.49±21.06 hours and 23.65±0.49 hours for black cumin and laurel oils respectively (Figure 5).

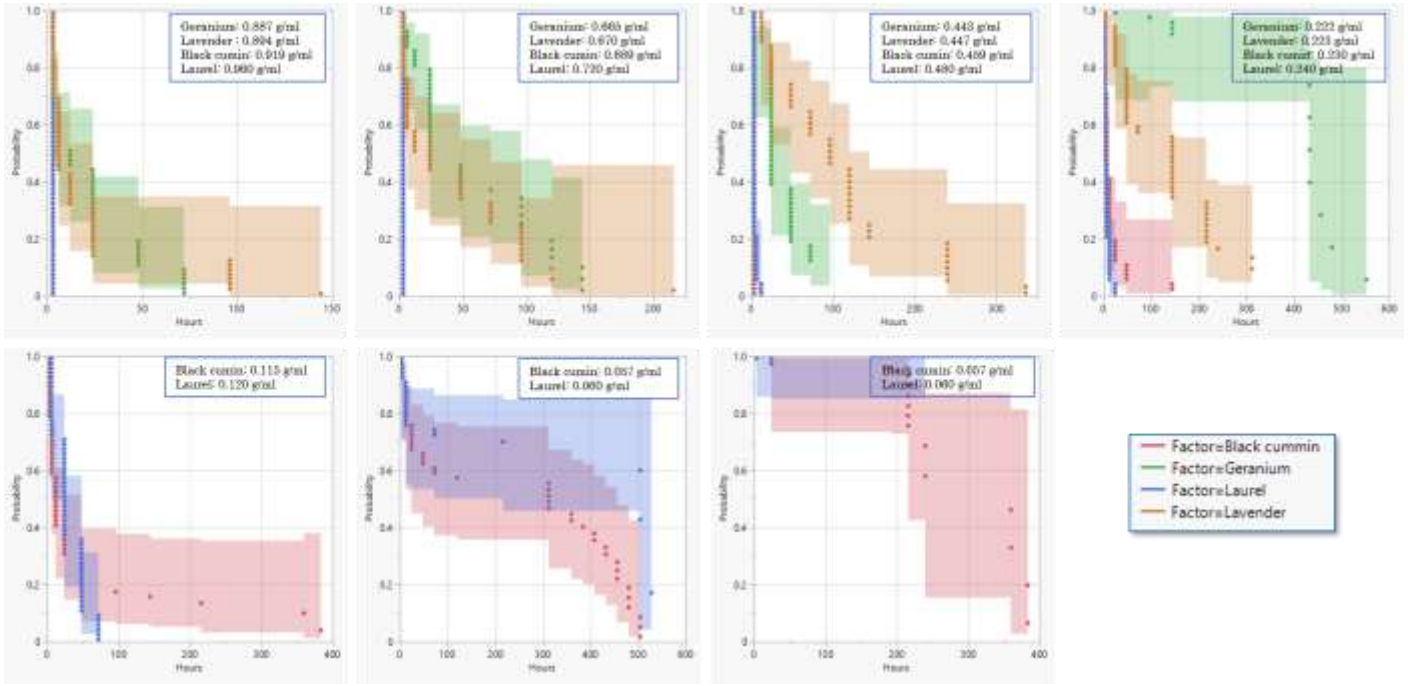


Figure 5 Kaplan-Meier survival curves of medfly larvae on tested essential oils
Şekil 5. Test edilen uçucu yağlar üzerinde Akdeniz meyve sineği larvalarının Kaplan-Meier canlılık eğrileri

The toxic effects of essential oils are likely due to the presence of several toxic constituents. There are several studies on plant essential oils and their components tested against different pest insects (Tayoub et al., 2012; Adil et al., 2015). Papachristos & Stamopoulos (2002) investigated that laurel oil has a repellent effect against, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae) by reducing fertility and

egg hatching and increasing larval mortality. Bouzi et al. (2020) reported the chemical composition and bioactivity of laurel oil against the larvae of *Culiseta longiareolata* (Macquart) (Diptera: Culicidae). They stated that the lethal effect of laurel oil decreased after the first exposure, but its larvicidal activity continued.

In our study, black cumin and laurel oils had the highest insecticidal activity at the lowest applied dose. The toxicities of the components were different and may depend on the stage evaluated. Additionally, we dispensed the compounds in the larval diet so some compounds possibly interacted with the artificial diet. We did not explore if the extracts were toxic to other biological stages or the chemical resistance mechanisms. It has also been stated that plant essential oils affect egg hatching rate and the sex ratio. Aissaoui et al. (2022) studied the insecticidal activities of laurel oil against the 3rd and 4th instars of *Culex pipiens* (Diptera: Culicidae). The toxicity of laurel oil was tested at different concentrations ranging from 5 to 35 µl/l. The result of the study showed laurel oil could be used as a biopesticide for vector insects. Saleem et al. (2014) conducted studies to determine the insecticidal activity of *Datura stramonium, Eucalyptus camaldulensis, Moringa oleifera,* and *Nigella sativa* essential oils against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae). Among investigated essential oils, the highest (20.06%) fumigant effect against *Tribolium castaneum* was black cumin oil (*Nigella sativa*). Adil et al. (2015) reported the insecticidal activities of black cumin (*Nigella sativa*) oil on *Tuta absoluta* (Lepidoptera: Gelechiidae). After being exposed to black cumin oil for 4 hours, 100% larval mortality was recorded at the dose of 0.203µl/cm². Raj et al. (2015) conducted a study on the larvicidal activity of black cumin oil against *Aedes aegypti, Anopheles stephensi,* and *Culex quinquefasciatus* (Diptera: Culicidae). As a result, black cumin oil showed a high larvicidal activity in 24 hours and was reported as a natural larvicidal agents. In conclusion, the insecticidal activity of the essential oils against medfly larvae seems to be worth more research for its use as a biopesticide in the control of this pest. The insecticidal activity is caused by one or more of the components of the essential oil hydrodistilled from the tested plants. It could be due to certain major or minor constituents or a synergistic effect of several components (Adil et al., 2015). The studies showed that especially monoterpenoids affected insect mortality by inhibiting the acetyl-cholinesterase enzyme activity (Houghton et al., 2006). The essential oils of *Pelargonium graveolens*, *Lavandula officinalis, Nigella sativa,* and *Laurus nobilis* showed insecticidal activity against the medfly larvae. The potential use of essential oils could be considered as an alternative control approach for the medfly larvae with further studies. The development of a device that enables the mixing of essential oils in a food bait or a spray could provide a new approach to medfly management. More studies are needed to explain the role of essential oils on the larval mortality of medflies.

CONCLUSION

In the study, different concentrations of essential oils from *Pelargonium graveolens*, *Lavandula intermedia*, *Nigella sativa*, and *Laurus nobilis* were applied against 2nd instars of the medfly on an artificial diet. Out of the tested essential oils, the laurel and black cumin oils were more effective against medfly larvae, and the highest mortality rate was observed in addition to black cumin oil at the lowest concentration. As a result, laurel and black cumin oils may have a potential use for medfly management. Further studies are needed to determine the effectiveness of essential oils under field conditions.

ACKNOWLEDGEMENT

The authors thank Emeritus Prof. Dr. James L. Nation for useful suggestions.

Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

REFERENCES

Abbott, W. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*, 265–267.

Adil, B., Tarik, A., Kribii, A., & Ounine, K. (2015). The Study Of The Insecticidal Effect Of *Nigella sativa* Essential Oil Against *Tuta absoluta* Larvae. *International Journal of Scientific&Technology Research, 4*(10), 88-90.

Aissaoui, L., Bouaziz, A., Boudjelida, H., & Nazlı, A. (2022). Phytochemical screening and biological effects of *Laurus nobilis* (Lauraceae) essential oil against mosquito larvae, *Culex pipiens* (Linnaeus, 1758) (Diptera: Culicidae) species. *Applied Ecology and Environmental Research, 21*(1), 287-300.

Akçura, S. (2023). Biplot analysis of monthly variations in essential oil concentration and chemical composition of *Pittosporum tobira* leaves in Mediterranean conditions. *Biochemical Systematics and Ecology, 110*, 1-7 (Article, 104712).

Aluja, M., & Mangan, R. L. (2008). Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. *Annual Review of Entomology, 53*, 473-502.

Anonymus. (2023a). Chemical Book. Retrieved from https://www.chemicalbook.com/ProductCatalog_EN/1524.htm (Access date: 06.01.2024).

Anonymus. (2023b). NHR Organic Oils. Retrieved from https://www.nhrorganicoils.com/certs.php?id=12229&type=CofA (Access date: 06.01.2024).

Bazzoni, E., Passino, G. S., Moretti, M. D. L., & Prota, R. (1997). Toxicity of anethole and its effects on egg production of *Ceratitis capitata* Wied. (Diptera, Tephritidae). *Ann Appl Biol, 131*, 369–374.

Bouzidi, O., Tine, S., Hamaidia, K., Djebbar, F. T., & Soltani, N. (2020). Chemical Composition and Bioefficacy of Essential Oil from Bay Laurel Shrub (Laurales: Lauraceae) against *Culiseta longiareolata* (Macquart) (Diptera: Culicidae) Larvae. *Journal of Entomological Science, 55*(2), 262–272.

Benelli, G., Flamini, G., Canale, A., Cioni, P. L., & Conti, B. (2012). Toxicity of some essential oil formulations against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). *Crop Protection, 42*, 223-229.

Carey, J. R., Papadopoulos, N. T., Müller, H. G., Katsoyannos, B. I., Kouloussis, N. A., Wang, J. L., K. Wachter., Yu, W., Liedo, P. (2008). Age structure changes and extraordinary lifespan in wild medfly populations. *Aging Cell, 7*(3), 426-437.

Chi, H. (2020). Probit-MSChart: a computer program for probit analysis.

Cunningham, R. T. (1989). Parapheromones. In: A. S. Robinson & G. Hooper (Eds.), Fruit Flies, Their Biology, Natural Enemies and Control (pp. 221–230). Elsevier, Amsterdam.

Economopoulos, A. P., & Bruzzone, N. D. (1989). Mass Rearing of the Mediterranean Fruit Fly (Diptera: Tephritidae): Continuous Light in the Adult Stage. *Journal of Econ Entomol, 82*(5), 1482–1489.

Fletcher, B. S. (1989). Movements of tephritid fruit flies. In A. S. Robinson & G. H. Hooper (Eds.), Fruit Flies: Their Biology, Natural Enemies and Control, Vol. 3B (pp. 209–219). Elsevier Science Publ., Amsterdam, Netherlands.

Gasperi, G., Guglielmino, C. R., Malacrida, A., & Milani, R. (1991). Genetic variability and gene flow in geographical populations of *Ceratitis capitata* (Wied.) (medfly). *Heredity, 67*, 347–356.

Genc, H., & Nation, J. L. (2008). Maintaining of *Bactrocera oleae* (Gmelin.) (Diptera: Tephritidae) colony on its natural host in the laboratory. *J. Pest Sci, 81*(3), 167-174.

Genç, H., & Yücel, S. (2017). Determination of Host Preferences of Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in the Laboratory. *Türk Tarım ve Doğa Bilimleri Dergisi, 4*(3), 292–301.

Gözel, Ç., & Genç, H. (2021). Implementing local entomopathogenic nematodes to control Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae). *Türk. Entomol. Derg., 45*(3), 389-396.

Houghton, P. J., Ren, Y., & Howes, M. J. (2006). Acetyl-cholinesterase inhibitors from plants and fungi. *Nat Prod Rep, 23*(2), 181-199.

Kang, M. S., & Gauch, H. G. (1996). AMMI analysis of yield trials. In M. S. Kang & H. G. Gauch (Eds.), Genotype-by-environment interaction (pp. 1–40). CRC Press, Boca Raton, FL.

Koul, O., Walia, S., & Dhaiwal, G. S. (2008). Essential oils as green pesticides: potential and constraints. *Biopestic Int, 4*(1), 63–84.

Lee, S., Peterson, C. J., & Coats, J. R. (2003). Fumigation toxicity of monoterpenoids to several stored product insects. *Journal of Stored Products Research, 39*, 77–85.

Lee, B. H., Annis, P. C., Tumaalii, F., & Choi, W. S. (2004). Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *Journal of Stored Products Research, 40*, 553–564.

Liquido, N.J., Shinoda L.A. & Cunningham, R.T. (1991). Host plants of the Mediterranean fruit fly (Diptera: Tephritidae). An annotated world list. *Ann. Entomol. Soc. Am., 77*, 1-57.

Lopez, SB., Lopez, M.L., Aragon, L.M., Tereschuk, M.L., Slanis, A.C., Feresin, G.E., Zygadlo, J.A., Tapia, A.A. (2011). Composition and Anti-insect Activity of Essential Oils from *Tagetes L.* Species (Asteraceae, Helenieae) on *Ceratitis capitata* Wiedemann and *Triatoma infestans* Klug. *J. Agric. Food Chem., 59*, 5286–5292.

Luu-Dam, N.A.; Tabanca, N.; Estep, A.S.; Nguyen, D.H.; Kendra, P.E. (2021). Insecticidal and Attractant Activities of *Magnolia citrata* Leaf Essential Oil against Two Major Pests from Diptera: *Aedes aegypti* (Culicidae) and *Ceratitis capitata* (Tephritidae). *Molecules, 26*, 2311.

Malacrida, A.R., Guglielmino, C.R., Gasperi, G., Baruffi, L., Milani, R. (1992). Spatial and temporal differentiation in colonizing populations of *Ceratitis capitata*. *Heredity, 69*, 101–111.

Mau, R.F.L. & Kessing, J.L.M. (2007). *Ceratitis capitata* (Wied.) http://www.extento.hawaii.edu/Kbase/crop/type/cera titi.htm, (Accessed: 20.12. 2015).

Miguel, M.G., Almeida, M.L., Gonçalves, M.A., Figueiredo, A.C., Barroso, J.G., Pedro, L.M. (2010). Toxic Effects of Three Essential Oils on *Ceratitis*

Capitata. *Journal of Essential Oil Bearing Plants, 13*(2), 191-199.

Moretti, M.D.L., Bazzoni, E., Passino, G.S. & Prota, R. (1998). Antifeedant effects of some essential oils on *Ceratitis capitata* Wied. (Diptera, Tephritidae). *J. Essent. Oil Research, 10*, 405-412.

Ouarhach, A., Said, L.A., Aboussaid, H., Ghalbane, I., Messoussi, S.E., Romane, A. (2022). Evaluation of the insecticidal activity of *Lavandula coronopifolia* essential oil against the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *South African Journal of Botany, 149*, 748-753.

Papachristos D. & Stamopoulos D. (2002). Repellent, toxic, and reproduction inhibitory effects of essential oil vapors on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J Stored Prod Res, 38*, 117–128.

Raj, G.A., Chandrasekaran, M., Krishnamoorthy, S., Jayaraman, M. & Venkatesalu, V. (2015). Phytochemical profile and larvicidal properties of seed essential oil from *Nigella sativa* L. (Ranunculaceae), against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research, 114*, 3385–3391.

Saleem, S., Hasan, M., Sagheer, M. & Sahi, S.T. (2014). Insecticidal Activity of Essential Oils of Four Medicinal Plants Against Different Stored Grain Insect Pests. *Pakistan J. Zool., 46*(5), 1407-1414.

Tayoub, G., Odeh, A. & Ghanem, I. (2012). Chemical composition and fumigation toxicity of *Laurus nobilis* L. and *Salvia Officinalis* L. essential oils on larvae of Khapra beetle (*Trogoderme granarium* Everts). *Herla polonica Vo:58 (2)*, 26-38.

Weems, H.V. (1981) Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Plant Industry, Entomology Circular, Florida Department of Agriculture, 230, pp. 8.

Weems, H.V. (1981) Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Plant Industry, Entomology Circular, *Florida Department of Agriculture*. 230, pp. 8.

Yan, W., Hunt, L A., Sheng, Q. & Szlavnins., Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*. 40, 597-605.

Yıkınç, M. & Tunaz, H. (2023). Bazı Bitkisel Kökenli Uçucu Yağların Amerikan Hamamböceği (*Periplaneta americana* L.) Erginlerine Karşı Ölüm Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 26 (3),* 579 – 587. https://10.18016/ksutarimdoga.vi.1168874

KSÜ Tarım ve Doğa Derg 27 (4), 868-880, 2024
KSU J. Agric Nat 27 (4), 868-880, 2024
DOI:10.18016/ksutarimdoga.vi.1310514

Monitoring adult populations of *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura), and *Zaprionus Indians* Gupta (Diptera: Drosophilidae) at different altitudes in fruit orchards of Adana Province in Türkiye

Asime Filiz ÇALIŞKAN KEÇE[1], Burcu ÖZBEK ÇATAL[2]✉, Zhanerke AMANGELDİ[3], Mehmet Rifat ULUSOY[4]
[1,3,4] Çukurova University, Department of Plant Protection, Faculty of Agriculture, Adana, Turkey, [2] Çukurova University, Pozantı Vocational School, Department of Plant and Animal Production, Adana, Turkey
[1]https://orcid.org/ 0000-0002-9330-1958, [2]https://orcid.org/ 0000-0003-0029-6190, [3]https://orcid.org/ 0000-0002-9017-8106
[4]https://orcid.org/ 0000-0001-6610-1398
✉: bozbek@cu.edu.tr

ABSTRACT

In recent years, fruit flies (Diptera: Drosophilidae and Tephritidae) have become an increasingly severe problem in fruit production in Turkey. *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura), and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) are the leading pests that cause worms in fruits. Using adult trapping, the population dynamics of these four pests were investigated in the orchards of Adana at different altitudes in 2019 and 2020. *Rhagoletis cerasi* adults emerged after the second week of May, and with the end of the harvest, the last adult emergence was seen in the first week of July. Adult population density was found at low levels. *Drosophila suzukii* adults appeared at 113 m (Balcalı) in April, and their emergence times were observed to be a little later as the altitude increased. It was determined that they continued their existence in nature until the end of December. It has the highest populations in late June - mid-July and late October - November. The occurrence of *C. capitata* and *Z. indianus* adults in nature in both years was after cherry fruits were harvested. It has been observed that different orchards appear in direct proportion as the altitude increases (from 113 m to 1510 m), the earliest in a low altitude place and the longer their duration in nature.

Entomology

Research Article

Article History
Received : 14.06.2023
Accepted : 30.11.2023

Keywords
Rhagoletis cerasi
Ceratitis capitata
Drosophila suzukii
Zaprionus indianus
Population dynamics

Türkiye'de Adana İli Meyve Bahçelerinde Farklı Yüksekliklerde *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura) ve *Zaprionus indianus* Gupta (Diptera: Drosophilidae)'un Ergin Popülasyonlarının Takibi

ÖZET

Son yıllarda Meyve sinekleri (Diptera: Drosophilidae ve Tephritidae) Türkiye'de meyve üretimini azaltan ciddi bir sorun haline gelmiştir. *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura) ve *Zaprionus indianus* Gupta (Diptera: Drosophilidae) meyvelerde kurtlanma yapan zararlıların başında gelmektedir. Ergin tuzağı kullanarak, 2019 ve 2020 yıllarında Adana'nın farklı rakımlarındaki meyve bahçelerinde dört türün populasyon dinamikleri incelenmiştir. *Rhagoletis cerasi* erginleri mayısın ikinci haftasından sonra ortaya çıkmış, hasatın tamamlanmasıyla birlikte temmuz ayının ilk haftası son ergin çıkışları görülmüştür. Ergin popülasyon yoğunluğu düşük seviyelerde bulunmuştur. *Drosophila suzukii* erginlerinin 113 m'de (Balcalı) nisan ayında çıkmaya başladığı rakım yükseldikçe ortaya çıkış zamanlarının biraz daha geç olduğu belirlenmiş ve aralık ayının sonuna kadar doğada varlıklarını sürdürdükleri tespit edilmiştir. En yüksek popülasyonlarını haziran sonu- temmuz ayı ortasında ve ekim sonu–kasım ayında yapmıştır. *Ceratitis capitata* ve *Z. indianus* erginlerinin her iki yılda da doğada görülme zamanları kiraz meyveleri hasat edildikten sonra da devam etmiştir. Farklı meyve bahçelerinde, rakım yükseldikçe (113 m'den 1510 m'ye) doğru orantılı

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 14.06.2023
Kabul Tarihi : 30.11.2023

Anahtar Kelimeler
Rhagoletis cerasi
Ceratitis capitata
Drosophila suzukii
Zaprionus indianus
Popülasyon takibi

olarak bu türlerin sırasıyla ortaya çıktıkları, düşük rakımlı yerde en erken çıktığı ve doğada görülme süresinin daha uzun olduğu belirlenmiştir.

Atıf İçin : Çalışkan Keçe, A. F., Özbek Çatal, B., Amangeldi, Z., & Ulusoy, M. R. (2024). Türkiye'de Adana İli Meyve Bahçelerinde Farklı Yüksekliklerde *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura) ve *Zaprionus indianus* Gupta (Diptera: Drosophilidae)'un Ergin Popülasyonlarının Takibi. *KSÜ Tarım ve Doğa Dergisi, 27*(4), 868-880. DOI: 10.18016/ksutarimdoga.vi.1310514.

To Cite: Çalışkan Keçe, A. F., Özbek Çatal, B., Amangeldi, Z., & Ulusoy, M. R. (2024). Monitoring adult populations of *Ceratitis capitata* (Wied.), *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura), and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) at different altitudes in fruit orchards of Adana Province in Türkiye. *KSU J. Agric Nat 27* (4), 868-880. DOI: 10.18016/ksutarimdoga.vi.1310514.

INTRODUCTION

Fruit flies (Diptera: Drosophilidae and Tephritidae) have become an increasingly severe problem for fruit production. The Eastern Mediterranean Region of Turkey has experienced a surge in cherry and fruit infestations due to pests such as *Ceratitis capitata* Wied., *Rhagoletis cerasi* L. (Diptera: Tephritidae), *Drosophila suzukii* (Matsumura), and *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae). These pests are the root cause of fruit worming. These pests lay their eggs on ripening fruits, causing them to decay and reducing the overall yield.

The Mediterranean Fruit Fly, *C. capitata*, is a significant insect pest that attacks a variety of hosts in subtropical and tropical regions around the globe, making it economically important. It belongs to the family Tephritidae and attacks a variety of hosts in subtropical and tropical regions across the world. This pest, which generally prefers ripe and thin-skinned fruits, causes them to fall because it directly damages the fruit during harvest. Buğday & Keçeci (2020) emphasized that it is crucial to follow the current situation of the pest in the Central Anatolia and Eastern Anatolia regions of our country together with the climatic changes.

Drosophila suzukii fruit flies were first recorded on the cherry of Yamanashi, Japan in 1916 (Kanzawa,1939). The spotted wing drosophila (SWD), a member of the Drosophilidae family, is widely known for its destructive nature as it inflicts severe damage on small fruit crops across the world (Walsh et al., 2011; Cini et al., 2012; Santos, 2014; Asplen et al., 2015). This is attributed to its high polyphagia (Dreves et al., 2009), dispersion capacity (Walsh et al., 2011; Cini et al., 2012), and rapid population growth (Tochen et al., 2014). It was first recorded on strawberries in Erzurum, Eastern Turkey in 2014 (Orhan et al., 2016). Later, this problem was felt in Turkey's fruit industry, causing significant economic harm to various types of fruits (Çatal et al., 2018; Ögür et al., 2018; Kasap & Özdamar, 2019; Zengin & Karaca, 2019).

The *R. cerasi*, commonly known as the European cherry fruit fly, is the most important pest of sweet cherries in Europe and causes severe yearly damage. Without the use of insecticide treatment, all fruits can become infested. Cherry growers face a challenge with *R. cerasi* due to the market's low tolerance for damaged fruit (maximum 2% of infested fruits).

Z. indianus is a common pest that affects over 80 types of fruit crops, such as figs, apples, and cherries (Yassin & David, 2010). It is known to cause significant harm to various kinds of fruit crops. It can cause severe damage to many fruit crops. Gupta (1970) was the first to collect and describe this species in India. Although initially believed to have originated in tropical Africa (Chassagnard & Kraaijveld, 1996), this fly has now spread to numerous countries worldwide, including old and new world nations (Commar et al., 2012). The species *Z. indianus* is known for being highly successful at colonizing (Chassagnard & Tsacas, 1993). According to Parkash & Yadav (1993), populations of this species have habitat-generalist or general niche-width characteristics, i.e., they utilize diverse food resources and display adaptation to variable climatic conditions. In 2017, *Z. indianus* was first reported on fruits such as fig, Trabzon persimmon, mulberry, blackberry, peach, cherry, and plum in Turkey's Eastern Mediterranean region. (Çatal et al., 2019).

Although the pest species is widespread, there is limited knowledge about population trends at varying altitudes. This research aimed to explore how altitude affects the seasonal flight patterns of *C. capitata*, *D. suzukii*, *R. cerasi*, and *Z. indianus*. Based on changing climate conditions, the study also aimed to distinguish any differences in adult emergence between nearby locations at varying altitudes.

MATERIAL and METHODS

The study examined natural populations of four fruit fly species, including *C. capitata*, *R. cerasi*, *D. suzukii*, and *Z. indianus*, in orchards where mid-season and late cherry varieties are grown. In addition, population monitoring of *C. capitata*, *D. suzukii,* and *Z. indianus* at different altitudes was carried out.

To capture *R. cerasi*, yellow sticky traps measuring 13.5x22.5 cm with ammonia capsules (Trece-Pherocon® AM No-Bait trap with Dual-Pak™ Supercharger™) were utilized. For *D. suzukii* and *Z. indianus*, traps were baited with 50% apple cider vinegar. Finally, a delta trap (KAPAR®AMS) was

employed for capturing *C. capitata*.

Selection and Planning of Trial Locations

Research has been conducted on the orchards listed in Table 1. To study the flight dynamics of adult specimens, we utilized traps that are typical of their species. The trial orchards' trees have consistently yielded fruit for at least five years.

Table 1. Geographic locations, orchards, and hosts allocated for monitoring the flight activity of *Ceratitis capitata*, *Rhagoletis cerasi*, *Drosophila suzukii,* and *Zaprionus indianus* adults in different locations Adana between 2019 and 2020.

Çizelge 1. 2019-2020 yılları arasında Adana'nın farklı lokasyonlarında Ceratitis capitata, Rhagoletis cerasi, Drosophila suzukii ve Zaprionus indianus erginlerinin uçuş aktivitesinin izlendiği coğrafi konumlar, meyve bahçeleri ve konukçuları.

Location	Orchards	Altitude	Lat. (North)	Long. (East)	Treatment
Balcalı	1	113m	37°01'44"	35°21'37"	Grapefruit
Belemedik	1	700m	37°20'49"	34°54'38"	Cherry
Alpu	1	1070m	37°28'34"	34°54'05"	Cherry
Aşçıbekirli	1	1180m	37°36'56.5"	34°57'54.4"	Apple
Hamidiye	1	1170m	37°34'01.5"	34°57'06.5"	Apple
	2	1300m	37°33'25.9"	34°57'32.0"	Cherry
Karakışlakçı	1	1300m	37°39'03.3"	34°54'32.9"	Apple
	2	1430m	37°39'50.3"	34°54'03.7"	Strawberry Apricot
	3	1510m	37°40'00"	34°53'46"	Cherry

Flight Activity

To monitor the flight activity of *C. capitata*, *R. cerasi*, *D. suzukii,* and *Z. indianus* adults, we placed traps at the locations given in Table 1 for the 2019-2020 years. The *Rhagoletis cerasi* population was monitored only in Alpu and Belemedik. Traps were set in February for *D. suzukii* and *Z. indianus* and in April for *C. capitata* and *R. cerasi* before their adult flight started.

Yellow sticky traps with ammonia capsules and delta traps were used to monitor the adult flight dynamics for *R. cerasi* and *C. capitata*. For *D. suzukii* and *Z. Indians*, traps were filled with apple cider vinegar and were placed at a height of approximately 1.5 m on cherry trees. To monitor adult fruit flies, two traps were hung around the orchard and checked on a weekly. The attractant was replaced weekly. The identification of species was realized by the authors.

Climatic Data

The climatic data, which includes the mean daily temperature and precipitation for the two experimental areas, has been provided by the Turkish State Meteorological Service. Mean daily temperatures and rain for the two areas from February 2019 to December 2020 (Figure 6).

RESULTS and DISCUSSION

Flight Activity

Populations of four types of fruit flies were evaluated in two cherry orchards (Alpu and Belemedik) in the Pozantı region (Adana) (Table 2; Figure 1, 2) according to the coexistence period of fruit fly species in nature, taking into account harvest times and general seasonal activity. Also, the performance of fruit flies and vinegar flies at different altitudes is illustrated in Tables 3, and 4 and Figures 3, 4, and 5.

For adults to hatch from pupae of fruit flies overwintering in the soil, the soil temperature must reach to certain level. Because the cherry fly is a heterodynamic insect species, it survives unfavorable climatic conditions in diapause (Kansu, 2000). To awaken from diapause, there must be long daylight hours, warmth, soil wetness (humidity), and matching plant phenology (Boller and Prokopy, 1976; Anonymous, 2011). Multivoltine, tropical species such as the Mediterranean fruit fly are homodynamic species and become active at ambient temperatures above their developmental threshold. As each insect species has a different developmental threshold temperature, developmental thresholds of the other biological periods of each species may also differ. The developmental threshold of the Mediterranean fruit fly is 12.4°C, and it cannot overwinter outside the fruit above 400 m and above 700 m when ambient conditions fall below zero degrees. The fig vinegar fly, *Z. indianus*, native to the tropics, is also a homodynamic species and cannot survive in environments below zero degrees Celsius. Winter forms of the adult cherry vinegar fly, *D. suzukii*, can survive winters as low as -35°C without going into diapause. Poikilothermal insects can become active when the ambient temperature rises above the growth threshold. With this general information in mind, the performance of fruit flies and vinegar flies at different altitudes is illustrated in Tables 3 and 4.

The first emergence of *R. cerasi* as an adult in both years took place in the third week of May in Belemedik (elevation 700 m) and Alpu (elevation 1070 m), with the highest number of adult flies emerging in the first week of June, and the final emergence of adults taking place in late June (Table 2; Figure 1, 2). No adults of *R. cerasi*, the primary pest of cherry, were found in traps after harvest. *Rhagoletis cerasi*, which produces one offspring per year, was found to be compatible with cherry phenology as well as duration of illumination, temperature, and wetness (humidity), beginning to appear when fruit entered the yellow ripening period and going into diapause after the harvest period (Table 2). In addition, adult population densities were low in both years. The cherry fruit fly usually pupates in the soil, occupying the area corresponding to the canopy projection of the cherry tree. Depending on soil moisture and ambient temperature in the spring, adults fly to the nearest tree. Since the adult cherry fruit flies are not very mobile, they usually feed on the nectar-containing substances that form in the fresh shoots of the tree on which the ripening food is growing. After mating, they first lay eggs on the fruit of the tree they are on, and when they are forced to do so, they go to the nearest trees and lay eggs on the fruit there. It has been reported that the movement of the cherry fruit fly over long distances is exceedingly small and that these movements are associated with activities such as oviposition, mating, and feeding (Katsoyannos et al., 1986; Wiesmann, 1933).

Table 2. First, maximum, and last capture, and harvest dates and duration of capture of adults at Pozantı (Alpu and Belemedik) in 2019 and 2020 (RC: *Rhagoletis cerasi*, CC: *Ceratitis capitata*, DS: *Drosophila suzukii* and Zİ: *Zaprionus indianus*).

Çizelge 2. Pozantı'da (Alpu ve Belemedik) 2019 ve 2020 yıllarında ergin bireylerin ilk, maksimum, son yakalanma ve hasat tarihleri ile doğada görülme süreleri (RC: Rhagoletis cerasi, CC: Ceratitis capitata, DS: Drosophila suzukii ve Zİ: Zaprionus indianus).

	Belemedik (703m)				Alpu (1077m)			
Year/2019	**RC**	**CC**	**DS**	**Zİ**	**RC**	**CC**	**DS**	**Zİ**
First capture	22 May	12 June	22 May	12 Sept.	21 May	5 July	7 June	23 Sept.
Maximum capture	22 May 05 June	28 Aug. 11 Sept. 16 Oct.	10 July 30 Oct. 27 Nov.	23 Oct. 13 Nov.	05 June 12 June	31 July	10 July 06 Nov.	none
Last capture	26 June	5 Dec.	25 Dec.	27 Nov.	5 July	16 Nov.	25 Dec.	27 Nov.
Harvest dates	17 June-26 June				10 June-05 July			
Duration of capture (d)	37	178	219	78	47	105	203	68
Year/2020	**RC**	**CC**	**DS**	**Zİ**	**RC**	**CC**	**DS**	**Zİ**
First capture	19 May	7 July	7 Apr.	2 June	12 May	14 July	26 May	1 Sep.
Maximum capture	9 June	19 Aug. 29 Sep.	14 July 10 Nov.	06 Oct. 17 Nov.	19 May	18 Aug. 20 Oct.	30 June	29 Sep. 20 Oct.
Last capture	30 June	10 Nov.	22 Dec.	29 Dec.	30 June	27 Oct.	10 Nov.	10 Nov.
Harvest dates	16 June - 30 June				23 June - 30 June			
Duration of capture (d)	43	127	260	211	50	106	169	71

In recent years, the first adults of the Mediterranean fruit fly, a pest of stone and pome fruits, especially cherries, were caught in the cool climate zone in pheromone and pole traps in Belemedik on 12 June 2019, when the cherry harvest began in the region. The pest survived in the wild until the first week of December. In the first year, the density of the adult population of *C. capitata* reached its highest values on 28 Agust, 11 September, and 16 October (Figure 2a). In the second year, the first adults were trapped three weeks after the cherry harvest, and the pest remained in the wild between the first week of July and the first week of November. The highest number of adults between these dates was observed on 19 Agust and 29 September (Figure 2b). In Alpu, the pest was trapped the first year after the cherry harvest in the first week of July, reaching its highest adult population on 31 July, and not trapped after 16 October (Figure 1a). The pest was trapped during the second week of July in the second year and was present in the wild until late October. The adult population of *C. capitata* reached high levels twice, on 18 Agust and 20 September 2020 (Figure 1b). In the other altitudinal regions where the experiment was conducted, *C. capitata* was detected on 15 May and 2 June in Sarıçam-Balcalı (altitude 113 m), 10 July and 28 July in Pozantı-Hamidiye (1170 m), on 7 and 4 August in Pozantı-Aşçıbekirli (1180 m), on 4 and 22 September in Pozantı-Karakışlakçı (1300 m), caught in traps in 2019 and 2020, respectively. On the other hand, the pest did not get caught in the traps in Pozantı-Hamidiye (1300 m), Pozantı-Karakışlakçı (1430 m), and Pozantı-Karakışlakçı (1510 m) (Table 3,

Table 3. First, maximum, and last capture dates and duration of capture of adults at different altitudes in Adana in 2019 (RC: *Rhagoletis cerasi*, CC: *Ceratitis capitata*, DS: *Drosophila suzukii* and Zİ: *Zaprionus indianus*).

Çizelge 3. 2019 yılında Adana'da farklı yüksekliklerde erginlerin ilk, maksimum, son yakalanma tarihleri ve doğada görülme süreleri (RC: Rhagoletis cerasi, CC: Ceratitis capitata, DS: Drosophila suzukii ve Zİ: Zaprionus indianus).

Karakışlakçı (1510m)	Karakışlakçı (1430m)	Karakışlakçı (1300m)	Hamidiye (1300m)	Hamidiye (1170m)	Aşçıbekirli (1180m)	Alpu (1070m)	Belemedik (700m)	Balcalı (113m)	**Location**	**2019**
*	*	*	*	*	*	21 May	22 May	*	First capture	**RC**
*	*	*	*	*	*	05 Jun 12 Jun	22 May 05 Jun	*	Maximum capture	
*	*	*	*	*	*	5 Jul	26 Jun	*	Last capture	
*	*	*	*	*	*	47	37	*	Duration of capture (d)	
4 Sept	*	4 Sept	*	10 Jul	7 Aug	5 Jul	12 Jun	15 May	First capture	**CC**
4 Sept	*	25 Sept 23 Oct	*	31 Jul 23 Oct	9 Oct	31 Jul	28 Aug 11 Sept 16 Oct	17 Jul 23 Oct 27 Nov	Maximum capture	
4 Sept	*	13 Nov	*	13 Nov	13 Nov	16 Nov	5 Dec	15 Dec	Last capture	
1	*	71	*	127	99	105	178	215	Duration of capture (d)	
17 Jul	17 Jul	17 Jul	17 Jul	17 Jul	17 Jul	7 Jun	22 May	3 Apr	First capture	**DS**
13 Nov	17 Jul 13 Nov	13 Nov	17 Jul 13 Nov	30 Oct 13 Nov	30 Oct	10 Jul 06 Nov	10 Jul 30 Oct 27 Nov	17 Apr 15 May 10 Jul	Maximum capture	
27 Nov	13 Nov	20 Nov	13 Nov	25 Dec	27 Nov	25 Dec	25 Dec	30 Oct	Last capture	
134	120	127	120	162	134	203	219	211	Duration of capture (d)	
None	6 Nov	23 Oct	6 Nov	4 Sept	23 Oct	23 Sept	12 Sept	28 Aug	First capture	**Zİ**
None	None	13 Nov	None	13 Nov	None	None	23 Oct 13 Nov	20 Nov	Maximum capture	
None	13 Nov	13 Nov	13 Nov	13 Nov	13 Nov	27 Nov	27 Nov	11 Dec	Last capture	
None	8	22	8	71	22	68	78	106	Duration of capture (d)	

Table 4. First, maximum, and last capture dates and duration of capture of adults at different altitudes in Adana in 2020 (RC: *Rhagoletis cerasi*, CC: *Ceratitis capitata*, DS: *Drosophila suzukii* and Zİ: *Zaprionus indianus*).
Çizelge 4. 2020 yılında Adana'da farklı yüksekliklerde erginlerin ilk, maksimum, son yakalanma tarihleri ve doğada görülme süreleri (RC: *Rhagoletis cerasi*, CC: *Ceratitis capitata*, DS: *Drosophila suzukii ve* Zİ: *Zaprionus indianus*).

Karakışlakçı (1510m)	Karakışlakçı (1430m)	Karakışlakçı (1300m)	Hamidiye (1300m)	Hamidiye (1170m)	Aşçıbekirli (1180m)	Alpu (1070m)	Belemedik (700m)	Balcalı (113m)	**Location**	**2020**
*	*	*	*	*	*	12 May	19 May	*	First capture	**RC**
*	*	*	*	*	*	19 May	9 Jun	*	Maximum capture	
*	*	*	*	*	*	30 Jun	30 Jun	*	Last capture	
*	*	*	*	*	*	50	43	*	Duration of capture (d)	
None	*	22 Sep	*	28 Jul	4 Aug	14 Jul	7 Jul	2 Jun	First capture	**CC**
None	*	27 Oct	*	11 Aug 27 Oct	29 Sep	18 Aug 20 Oct	19 Aug 29 Sep	30 Jun 18 Aug 17 Nov	Maximum capture	
None	*	27 Oct	*	27 Oct	27 Oct	27 Oct	10 Nov	15 Dec	Last capture	
None	*	36	*	92	85	106	127	197	Duration of capture (d)	
8 Sep	28 Jul	8 Sep	23 Jun	23 Jun	21 Apr	26 May	7 Apr	7 Apr	First capture	**DS**
none	13 Oct	8 Sep 20 Oct	7 Jul 27 Oct	8 Sep 20 Oct	3 Nov	30 Jun	14 Jul 10 Nov	26 May 30 Jun	Maximum capture	
27 Oct	27 Oct	3 Nov	27 Oct	27 Oct	3 Nov	10 Nov	22 Dec	10 Nov	Last capture	
50	92	57	127	127	197	169	260	218	Duration of capture (d)	
8 Sep	25 Aug	8 Sep	None	8 Sep	22 Sep	1 Sep	2 Jun	29 Sep	First capture	**Zİ**
8 Sep 29 Sep	8 Sep 27 Oct	8 Sep 20 Oct	None	8 Sep 20 Oct	29 Sep 27 Oct	29 Sep 20 Oct	06 Oct 17 Nov	17 Nov	Maximum capture	
29 Sep	10 Nov	27 Oct	None	10 Nov	10 Nov	10 Nov	29 Dec	1 Dec	Last capture	
22	78	50	None	64	50	71	211	64	Duration of capture (d)	

4; Figure 3). During this study, the duration of the appearance of adults in traps was recorded as 178 and 127 days in Belemedik, and 105 and 106 days in Alpu in the first and second years, respectively. Regarding the other altitudinal regions, the pest lasted 215-197 days in Balcalı, 99-85 days in Aşçıbekirli, 127-92 days in Hamidiye (1170 m), and 71-36 days in Karakışlakıçı (1300 m) (Tables 3 and 4).

Many studies show that *C. capitata*, whose distribution and abundance in temperate regions are determined by the lowest winter and highest summer temperatures, exhibits seasonal variations in different parts of the world (Escudero-Colomar et al., 2008). Accordingly, *C. capitata*, abundant in spring and summer, may not be detected in winter (Mansour & Mohamad, 2016). Also, it was reported that with increasing elevation, both the duration of appearance of *C. capitata* adults in the wild shortened and the number of adults was lower than at sites with lower elevation. This is thought to be related to the optimal temperature required for the species to develop. Although the optimal temperature for its development is 26°C, survival is high between 15 and 30°C. Duyck & Quilici (2002) reported that the development of the Mediterranean fruit fly showed a linear relationship with temperature, while Escudero-Colomar et al., (2008) said that the lowest temperature threshold required for its development varied according to developmental stages and that these thresholds were generally between 9 and 11 °C. *Ceratitis capitata* cannot continue its development at temperatures below 5 °C, and absolute death occurs at temperatures below zero (Ulusoy et al., 2022).

Adult specimens of the cherry vinegar fly *D. suzukii*, which has invaded our country recently, were detected in traps in Belemedik in the third week of May in the first year and in the first week of April in the second year. The pest could be trapped in both years until the last week of December. The highest population density of the pest in the cherry orchard was observed three times in the first week of July and the last week of October and November in the first year, and similarly in early July and late November in the second year (Table 2; Figure 2). In the Alpu cherry orchard, the *D. suzukii* was detected in the traps during the first week of June, when the fruit was ripe and about to be harvested. In contrast, in the second year, it was detected during the last week of June, towards the end of the fruit harvest. The pest remained in the wild from the first detection date until the last week of December. The highest adult population was detected in July and early November in the first year and not until late June in the second year (Table 2; Figure 1). In other altitudinal regions, the first capture of the pest in traps occurred on 03 and 07 April in Balcalı in 2019 and 2020, respectively, and the duration of appearance in the wild was determined to be 211 and 218 days (Table 3 and 4). It was detected in Aşçıbekirli on 21 June 2020, and in general, the first captures in traps were in July, and the last in September and October (Table 3 and 4; Figure 4). In studies monitoring the population movements of *D. suzukii*, researchers reported that after capturing a few specimens in early spring, no adults were found until June, and the population increased in late summer. They reported that their population reached higher peaks in October and November, while their numbers declined dramatically in winter, though a few adults were still captured (Mazzetto et al., 2015). According to Briem et al. (2018), the number of adult *D. suzukii* in the summer decreased because temperatures rose above 30°C. They found that the density of flies in the traps increased in September when the temperature started to drop.

Another vinegar fly, *Z. indianus*, was detected in Belemedik during the second week of September in the first year, and adults were caught in traps until late November. In the second year, adults were detected in traps in early June, and the pest was active in the wild until the last weeks of December. The highest adult population was observed in the third week of October and the second week of November in the first year, and in the first week of October and the third week of November in the second year (Figure 2). In the Alpu cherry orchard, the pest was detected in the last week of September in the first year, and in the first week of September in the second year. In both years, the pest was caught in traps from its first emergence until mid-November. The highest populations of adults were observed in the second week of October and November in the first year, the last week of September, in the third week of October in the second year (Table 2; Figure 1). In respect of other regions, the pest first appeared in September and later, as was the case in Belemedik and Alpu, with the last capture of adults seen in the traps in October and November, and the shortest adult time in nature was 8 days in 2019 and the longest was 106 days in Balcalı (Table 3 and 4; Figure 5). In contrast to other species, *Z. indianus* was observed to remain for shorter periods in the wild. Araripe et al., (2004) reported that *Z. indianus* is a tropical species and is not likely to colonize in cold temperate areas due to its low cold tolerance, just like other species.

CONCLUSION

The results of this study show that *R. cerasi* adults appear after the second week of May when fruits start to ripen and that the last adults emerge after the harvest is complete. In both years, adults were caught in the traps, consistent with cherry phenology, and adult population densities were low.

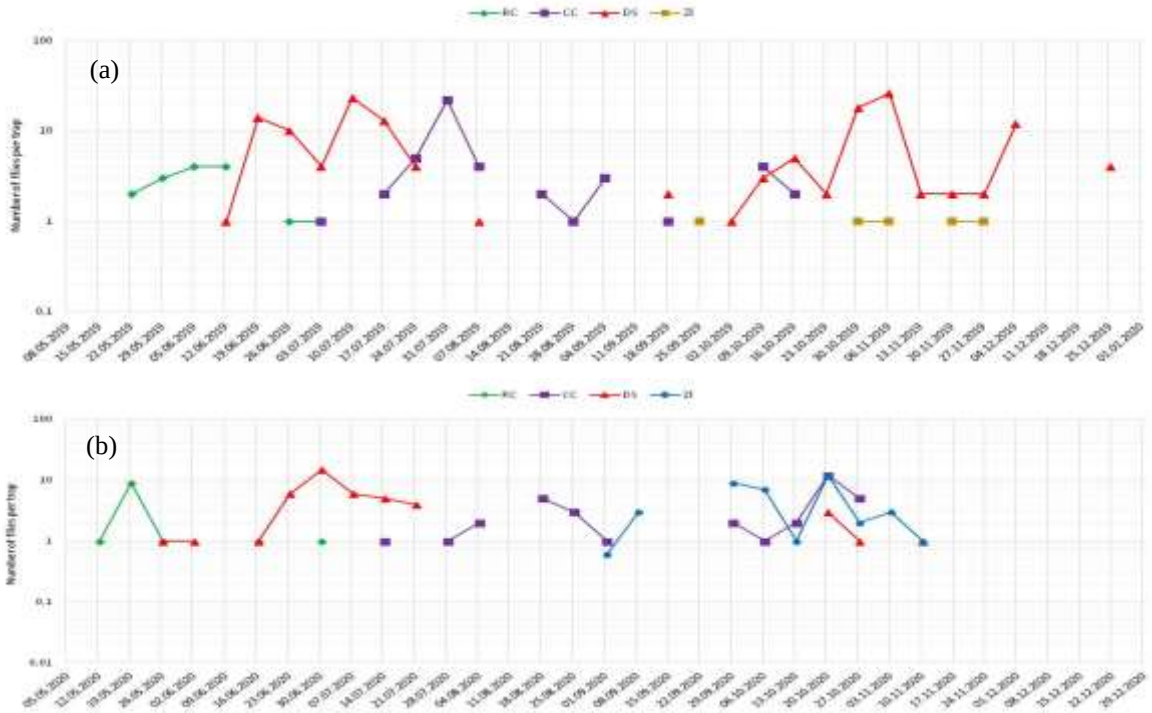


Figure1. Flight activity of *Rhagoletis cerasi* (RC), *Ceratitis capitata* (CC), *Drosophila suzukii* (DS), and, *Zaprionus indianus* (Zİ) in Alpu in 2019 (a) and 2020 (b).

Şekil 1. Rhagoletis cerasi (RC), Ceratitis capitata (CC), Drosophila suzukii (DS) ve Zaprionus indianus' un (Zİ) 2019 (a) ve 2020 (b) yıllarında Alpu'daki uçuş aktivitesi.

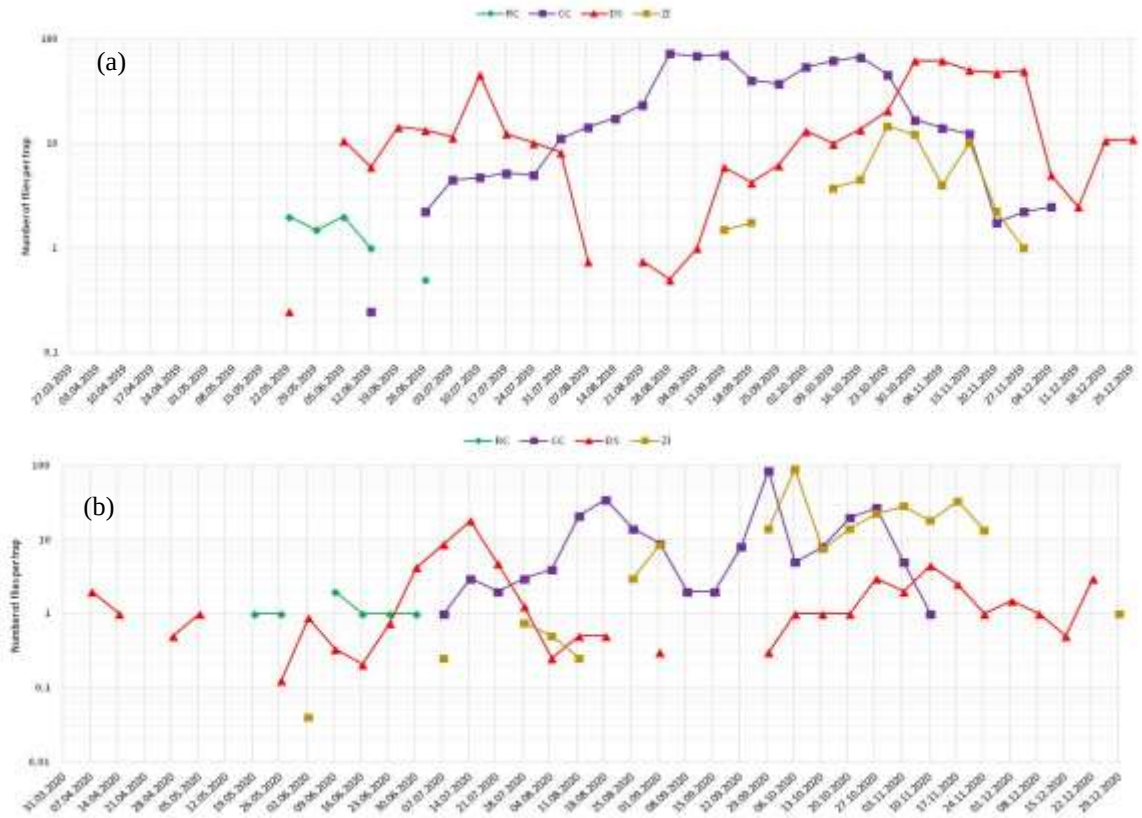


Figure 2. Flight activity of *Rhagoletis cerasi* (RC), *Ceratitis capitata* (CC), *Drosophila suzukii* (DS) and *Zaprionus indianus* (Zİ) in Belemedik in 2019 (a) and 2020 (b).

Şekil 2. Rhagoletis cerasi (RC), Ceratitis capitata (CC), Drosophila suzukii (DS) ve Zaprionus indianus' un (Zİ) 2019 (a) ve 2020 (b) yıllarında Belemedik'deki uçuş aktivitesi.

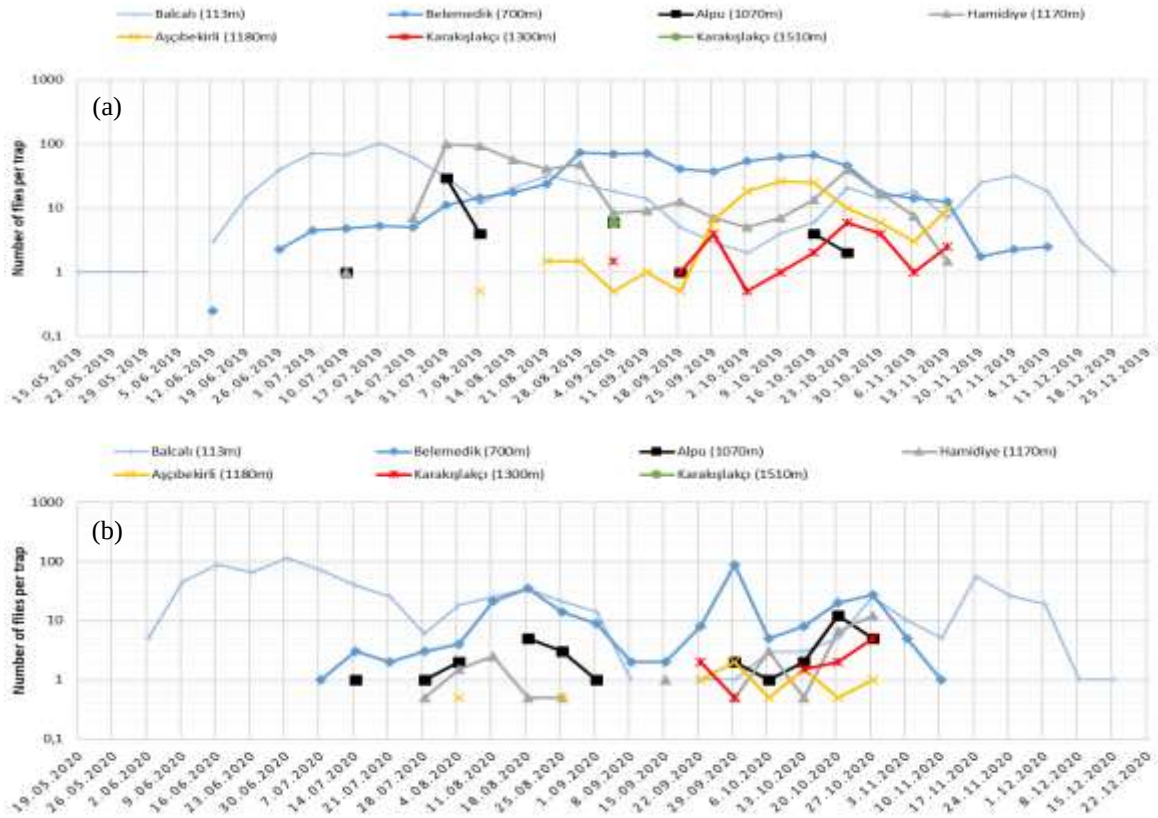


Figure 3. Flight activity of *Ceratitis capitata* in 2019 (a) and 2020 (b) at different altitudes.
Şekil 3. Ceratitis capitata 'nın 2019 (a) ve 2020 (b) yıllarında farklı yüksekliklerdeki uçuş aktivitesi.

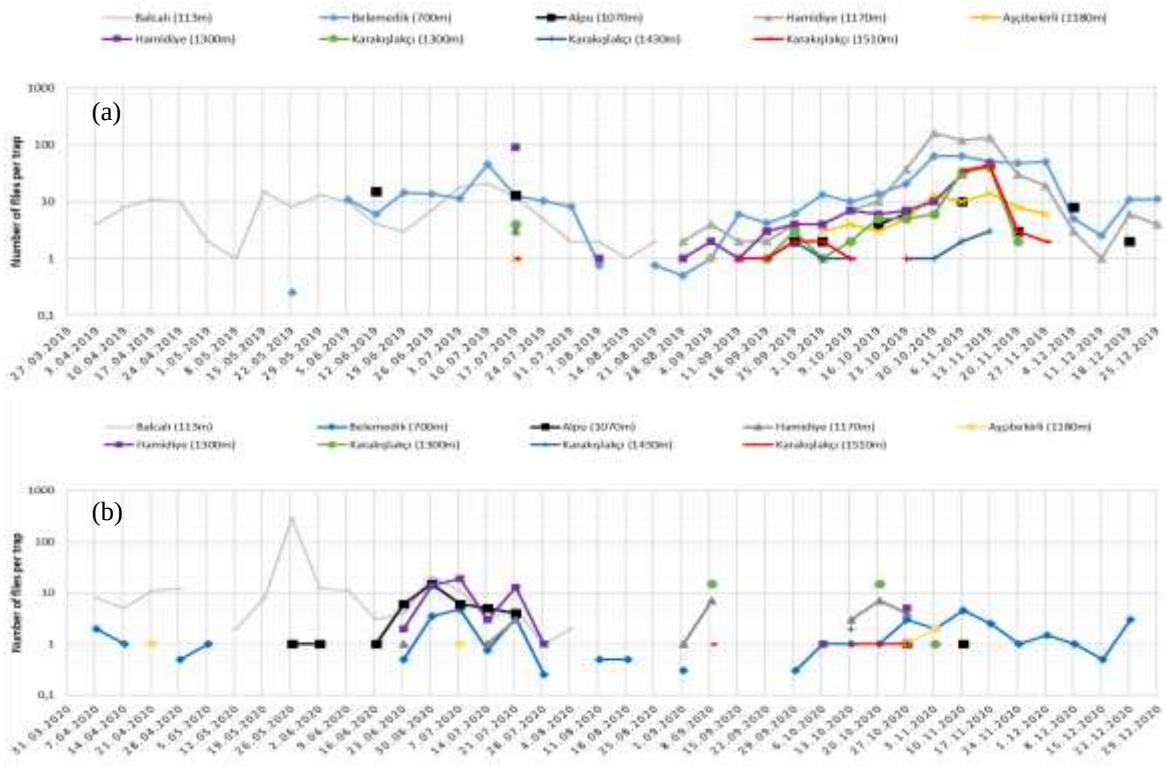


Figure 4. Flight activity of *Drosophila suzukii* in 2019 (a) and 2020 (b) at different altitudes.
Şekil 4. Drosophila suzukii 'nin 2019 (a) ve 2020 (b) yıllarında farklı yüksekliklerdeki uçuş aktivitesi.

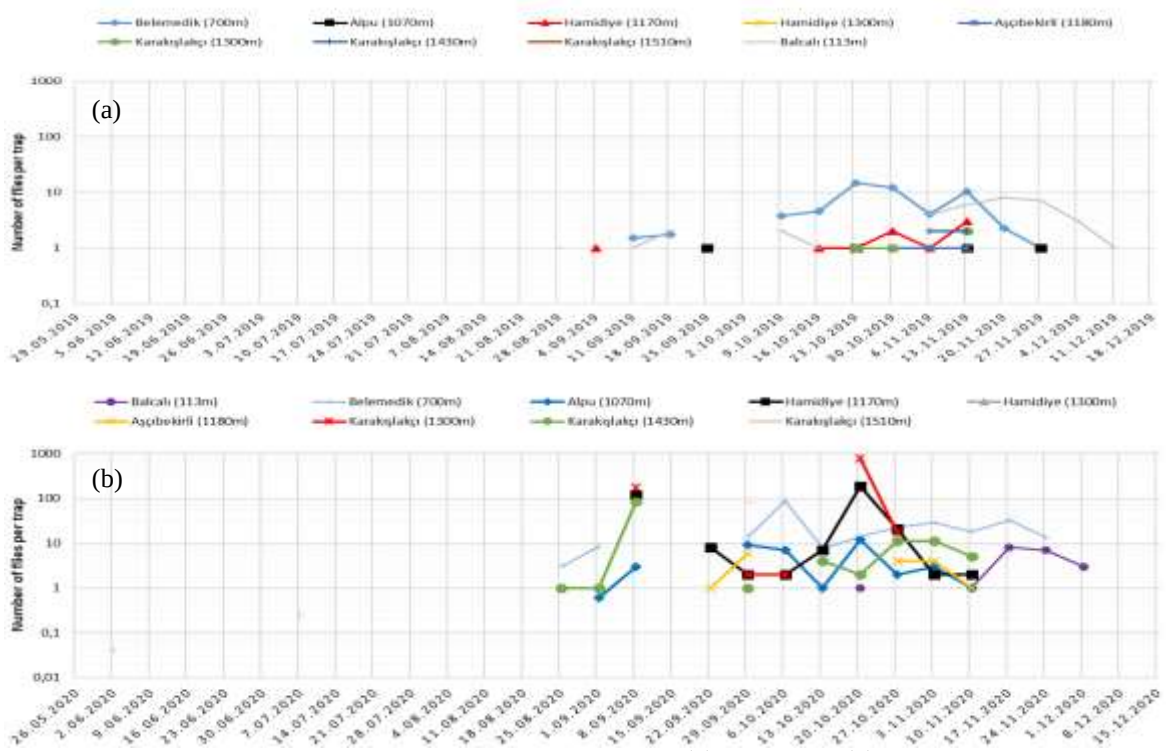


Figure 5. Flight activity of *Zaprionus indianus* in 2019 (a) and 2020 (b) at different altitudes.
Şekil 5. Zaprionus indianus ' un 2019 (a) ve 2020 (b) yıllarında farklı yüksekliklerdeki uçuş aktivitesi.

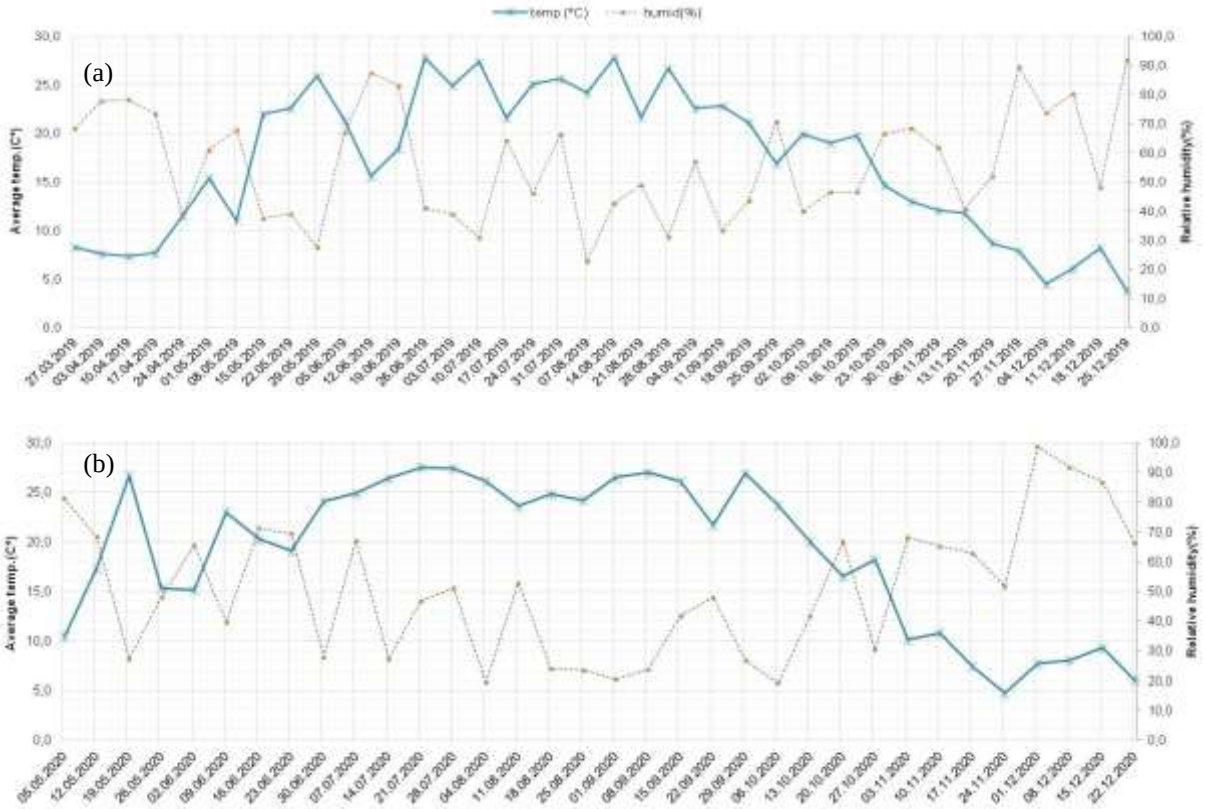


Figure 6. Adana-Pozantı district average temperature (°C) and relative humidity (%) values for March - December 2019 (a) – 2020 (b).
Şekil 6. 2019 (a) – 2020 (b) yıllarında Adana-Pozantı ilçesi Mart – Aralık ayları ortalama sıcaklık (°C) ve bağıl nem (%) değerleri.

Although there was an elevation difference of 300 m between the two regions, there was no difference in the first emergence of adults and the duration of their appearance in nature. The reason for this was the topographic structure of the region. Namely, Belemedik is located in a valley between two mountains overshadowed by the mountains, while Alpu, although it is higher, is open on all sides and has more than 12 hours of sunshine in the summer months. We believe that these topographical features directly impact the time of emergence of the cherry fruit fly from its wintering grounds, the flight, and the duration of its stay in the wild, in light of similar results obtained despite the difference in altitude of 300 m.

Drosophila suzukii flies began hatching in April at 703 m elevation (Belemedik); at 1077 m elevation (Alpu), the timing of hatching was slightly later due to increasing height, and they were found to survive in the wild until late December. The highest population concentrations were observed in late June to mid-July and late October to November.

Zaprionus indianus appeared in late August and early September, was observed in the wild until late November, and was detected once in traps in late December. The highest population was observed in mid-October. Only in Belemedik were adults seen once in June in the second year, detected again a month later, and appearances lasted until December. *Zaprionus indianus* adults were observed in nature following cherry fruit harvest in both years.

Vinegar flies feed mainly on spilled, damaged, decaying fruits and vegetables (Kaneshiro, 2006; O'Grady, 2009). They also survive on other thin-skinned fruits (plums, apples, pears) and fruit-bearing plants such as hawthorn, blackthorn, rose, nightshade, etc., in and around gardens. In gardens with all types of fruits, it is possible to come across adults all year round if climatic conditions allow. Of these species, *D. suzukii* also damages ripening, intact fruit using its sawtooth-like ovipositor. *Zaprionus indianus*, on the other hand, is known as a fig pest and lays its eggs in figs primarily in natural openings where the blowfly makes an entrance and on the ruptured fruit skin of fully ripe figs. These pests are spotted in gardens during harvest time of ripening fruit. Aromatic odors emanating from fruits attract these pests. While there is more damage in orchards having a delayed harvest, their population decreases significantly in gardens harvested on time. For this reason, they are known as sap-sucking insects feeding on fruit that falls to the ground or is left on the tree. We concluded that the irregularity of the populations of both pests was because the orchards were mixed and the harvesting seasons were different. The abiotic factors affecting this situation include the daily variations in daytime and nighttime temperatures between April and June and between September and December, sudden rainfall, and the rapidly decreasing ambient temperature.

Ceratitis capitata adults were observed in nature following cherry fruit harvest in both years. The adults of this species were observed in Alpu and Belemedik from July to December. There was also an increase in their population between the end of July and October. In both years, they were observed to appear in direct proportion to increasing altitude (from 703 m to 1077 m), appeared earliest at lower altitudes, and the duration of sightings in the wild was longer. Most adult *C. capitata* were found in July-August and October-November.

The adults of the Mediterranean fruit fly are brought to the cool climate regions by the commercial transport of fruits such as oranges, tangerines, peaches, nectarines, and apricots grown in the subtropical region. Study region, the pest emerged after the cherry harvest, causing no damage to cherries. However, in the first week of July, the pest was detected in fruits such as sour cherry, apple, pear, and quince grown in cool climate regions. It is assumed that the ambient temperature between May and November is suitable for the larvae of the Mediterranean fruit fly transported to the area with contaminated fruit to enter the pupal stage and for the adults hatching from the pupae to perform their biological activities. That altitude does not play an important role. Kansu, (2000) indicated in one of the sketches for the Mediterranean fruit fly that it could theoretically produce three offspring in Ankara during the summer months.

In conclusion, the results of this present study, intended to monitor the population of *R. cerasi*, *C. capitata*, *D. suzukii* and *Z. indianus* in different orchards at different altitudes, will serve as primary data for future studies and researchers and help with pest management in cherry-cultivation areas.

ACKNOWLEDGMENTS

Çukurova University supported this research as a scientific research project number FBA-2019-11905. This research was presented orally at the 3rd International Agriculture, Environment and Health Congress held in Aydın, Turkey, on November 12-14, 2020. The abstract of the study was also published in the meeting book.

Author's Contributions

Each author's contribution is equal.

Statement of Conflict of Interest

The authors have stated that there is no conflict of interest.

REFERENCES

Anonymous, (2011). *Kiraz Bahçelerinde Entegre Mücadele Teknik Talimatı*. Ankara: Tarım ve Köy İşleri Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı, 1-84 pp.

Asplen, M.K., Anfora, G., Biondi, A., Choi, D-S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z-L., Kárpáti, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vétek, G., Vogt, H., Walton, V.M., Yu, Y., Zappalá, L. & Desneux, N. (2015). Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science, 88*, 469–494.

Araripe, L.O., Klaczko, L.B., Moreteau, B., & David, J.R. (2004). Male sterility thresholds in a tropical cosmopolitan drosophilid, *Zaprionus indianus*. *Journal of Thermal Biology, 29*(2), 73-80.

Boller, E. & Prokopy, R.J. (1976). Bionomics and Management of Rhagoletis. *Annual Review of Entomology, 21*, 223-246.

Buğday, M. & Keçeci, M. (2020). Elazığ ve Malatya illerinde, Akdeniz Meyve sineği'nin bazı biyo-ekolojik özellikleri ve zarar durumu üzerine çalışmalar. *Derim, 37*(1), 64-75.

Briem, F., Dominic, A.R., Golla, B., Hoffmann, C., Englert, C., Herz, A., & Vogt, H. (2018). Explorative data analysis of *Drosophila suzukii* trap catches from a seven-year monitoring program in Southwest Germany. *Insects, 9*(4), 125.

Chassagnard, M.T. & Kraaijveld, A.R. (1996). The occurrence of Zaprionus sensu stricto in the Palearctic region (Diptera: Drosophilidae). *Annales de la Société entomologique de France (N.S.), 27*, 495–496.

Chassagnard, M.T. & Tsacas, L. (1993). Le sous-genre Zaprionus S. Str.: définition de groupes d'espèces et révision du sous-groupe vittiger (Diptera: Drosophilidae). *Annales de la Société entomologique de France, 29*(2), 173-194.

Cini, A., Ioriatti, C., & Anfora, G. (2012). A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. *Bulletin of Insectology, 65*, 149–160.

Commar, L.S., Galego, L.G.D., Ceron, C.R., & Carareto, C.M.A. (2012). Taxonomic and evolutionary analysis of *Zaprionus indianus* and its colonization of Palearctic and Neotropical regions. *Genetics and Molecular Biology, 35*, 395–406.

Çatal, B.Ö., Çalışkan Keçe, A.F., & Ulusoy, M.R. (2018). Adana İli Kiraz Yetiştiriciliği Yapılan Alanlarda Zararlı Böcek Türleri. Uluslararası Erdemli Sempozyumu, Mersin, Türkiye, 19-21 Nisan 2018, ss.109.

Çatal, B.O., Kece, A.F.C., & Ulusoy, M.R. (2019). New invasive species in Turkey: *Zaprionus indianus* (Gupta) (Diptera: Drosophilidae). *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22*, 110-113.

Dreves, A.J., Walton, V., & Fisher, G. (2009). A New Pest Attacking Healthy Ripening Fruit in Oregon. Spotted wing Drosophila: *Drosophila suzukii* (Matsumura). Retrieved from https://catalog.extension.oregonstate.edu/em8991

Duyck, P.F. & Quilici, S. (2002). Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bulletin of Entomological Research, 92*(6), 461-469.

Escudero-Colomar, L.A., Vilajeliu, M., & Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in the north-east of Spain. *Journal of Applied Entomology, 132*(9-10), 714-721.

Gupta, J.P. (1970). Description of a new species of *Phorticella zaprionus* (Drosophilidae) from India. *Proceedings of the Indian National Science Academy, 36*(1), 62-70.

Kaneshiro, K.Y. (2006). Dynamics of sexual selection in the Hawaiian Drosophilidae: a paradigm for evolutionary change. *Proceedings of the Hawaiian Entomological Society, 38*, 1–19.

Kansu, İ.A. (2000). *Genel entomoloji*. Ankara: Kıvanç Basımevi.

Kanzawa, T. (1939). Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi Agricultural Experiment Station 49 (abstr.). *Review of Applied Entomology, 29*, 622.

Kasap, I. & Özdamar, E. (2019). Population development of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in vineyards of Çanakkale province. *Turkish Journal of Entomology, 43*(1), 57-62.

Katsoyannos, B.I., Boller, E.F., & Benz, G. (1986). The behavior of the cherry fruit fly, *Rhagoletis cerasi* L., in its hostplant selection and its dispersal. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft, 59*, 315-335.

Mansour, M. & Mohamad, F. (2016). Seasonal occurrence of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) in southern Syria. *Polish Journal of Entomology, 85*(3), 311.

Mazzetto, F., Pansa, M.G., Ingegno, B.L., Tavella, L., & Alma, A. (2015). Monitoring of the exotic fly *Drosophila suzukii* in stone, pome, and soft fruit orchards in NW Italy. *Journal of Asia-Pacific Entomology, 18*(2), 321-329.

O'Grady P.M. (2009). *Drosophila melanogaster*. In: V. H. Resh & R. T. Cardé, Encyclopedia of insects (Second Edition) (pp. 301-303). NewYork: Academic Press.

Orhan, A., Aslantaş, R., Önder, B.Ş., & Tozlu, G. (2016). First record of the invasive vinegar fly

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) from eastern Turkey. *Turkish Journal of Zoology, 40*(2), 290-293.

Ögür, E., Ünlü, L., & Canbulat, F. (2018). New record for spotted wing Drosophila, *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in Karaman, Central Anatolia. *Agricultural & Forestry, 64* (1), 189-197.

Parkash, R. & Yadav, J.P. (1993). Geographical clinal variation at 7 esterase-coding loci in Indian populations of *Zaprionus indianus*. *Hereditas, 119*, 161–170.

Santos, R.R.S. (2014). *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) atacando frutos de morangueiro no Brasil. *Enciclopédia Biosfera, 10* (18), 4005–4011.

Tochen, S., Dalton, D.T., Wiman, N., Hamm, C., Shearer, P.W., & Walton, V.M. (2014). Temperature-related development and population parameters for *Drosophila suzukii* (Diptera: Drosophilidae) on cherry and blueberry. *Environmental Entomology, 43*, 501–510.

Ulusoy, M.R., Kazak, C., Karut, K., & Çalışkan Keçe, A.F. (2022). *Genel Entomoloji (Tarımsal)*. Adana: Karahan Kitabevi Yayınları.

Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J., Bruck, D.J., Walton, V.M., O'Neal, S.D., & Zalom F.G. (2011). *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. *Journal of Integrated Pest Management, 2*, 1–7.

Wiesmann, R. (1933). Untersuchungen u¨ber die Labensgeschichte und Beka¨mpfung der Kirschßiege *Rhagoletis cerasi* L. *I.Mitt. Landwirrtsch. Jahrb. Schweiz, 47*, 711-760.

Yassin, A. & David, J. R. (2010). Revision of the Afrotropical species of *Zaprionus* (Diptera, Drosophilidae), with descriptions of two new species and notes on internal reproductive structures and immature stages. *Zookeys, 51*, 33– 72.

Zengin, E. & Karaca, İ. (2019). Dynamics of trapped adult populations of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) and its parasitoids in Uşak Province, Turkey. *Egyptian Journal of Biological Pest Control*, *29*(1), 1-6.

KSÜ Tarım ve Doğa Derg 27 (4), 881-891, 2024
KSU J. Agric Nat 27 (4), 881-891, 2024
https://doi.org/10.18016/ksutarimdoga.1388051

Aelia rostrata (Heteroptera: Pentatomidae)'un Sindirim Kanalının Ultrastrüktürü

Şermin GENÇ[1✉], Selami CANDAN[2]
[1,2]Science Faculty, Department of Biology Ankara, Gazi University, Ankara,
[1]https://orcid.org/0000-0003-1921-110X, [2]https://orcid.org/0000-0002-7402-1360
✉: sermingenc.00@gmail.com

ÖZET

Aelia rostrata (Fabricius, 1803) (Heteroptera: Pentatomidae) delici emici ağız tipine sahip olduğu için bitki özsuyu ile beslenmektedir. *Aelia rostrata,* başta buğday olmak üzere yabani Gramineae türleriyle de beslenerek zarar yapmaktadır. Buğday pis böceği (Kımıl) olarak da bilinen zararlı önemli hale gelmiştir. Bu çalışmada, 2014 yılının Ağustos-Ekim aylarında Ankara ili, Bala ilçesi ve civarındaki çeşitli tarımsal ve yabani bitki alanlarından *A. rostrata* örnekleri toplanmıştır. Ardından ışık mikroskobu ve taramalı elektron mikroskobu (SEM) kullanılarak böceğin sindirim kanalının yapısı incelenmiştir. *A. rostrata*'nın sindirim kanalının üç farklı bölgeden oluştuğunu göstermiştir: ön bağırsak, orta bağırsak ve arka bağırsak. Ön bağırsak tükürük bezleri, yutak, yemek borusu ve ön bağırsaktan oluşmaktadır. Orta bağırsakta ön, medyan (orta bağırsağın kanal yapısı), arka orta bağırsak (orta bağırsağın ampul yapısı) bulunmaktadır. Arka bağırsak, ileum ve rektumdan oluşmak tadır. İleum'a bağlı olarak Malpighi tüpleri ve gastrik çekumlar vardır. Ön bağırsakta tükürük bezleri ve mide silindirik epitelden, yardımcı tükürük bezi ve orta bağırsak kanalı kübik epitelden, proventrikulus ise yalancı çok tabakalı epitelden meydana gelmektedir. Orta bağırsakta; orta bağırsak kanalı kübik epitelden, bulb yassı epitelden oluşmaktadır. Arka bağırsakta ileum silindirik epitelden, rektum kübik silindirik epitelden oluşmaktadır. Araştırma böceklerin sindirim kanalı yapısı ile ilgili çalışmalarda bilim dünyasına katkıda bulunacaktır.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 08.11.2023
Kabul Tarihi : 21.01.2024

Anahtar Kelimeler
Aelia rostrata
Bağırsak
Malpighi tüpleri
Işık mikroskobu
Taramalı elektron mikroskobu

Ultrastructure of Digestive Canal of *Aelia rostrata* (Heteroptera: Pentatomidae)

ABSTRACT

Aelia rostrata (Fabricius, 1803) (Heteroptera: Pentatomidae) has a piercing mouthpiece type that is supplied with a plant sap-absorbing nose type. *Aelia rostrata* mainly consumes wheat but also nourishes wild Gramineae species, making it an important pest known as the wheat stink bug. In this study, A. rostrata samples were collected in August-October 2014 from various fields of agricultural and wild plants in and around the Bala district of Ankara province. Then the structure of the insect digestive canal was investigated using a light microscope and scanning electron microscope (SEM). The results showed that the digestive canal of *A. rostrata* consists of three distinct regions: foregut, midgut, and hindgut. The foregut consists of the salivary glands, pharynx, esophagus, and proventriculus. The midgut has an anterior, median (canal structure of the midgut), and posterior midgut (bulb structure of the midgut). Hindgut has the ileum and rectum. There are Malpighian and gastric caeca depending on the ileum. In the foregut, salivary glands and stomach are composed of cylindrical epithelium, have cylindrical epithelium while the accessory salivary gland and the midgut canal are formed from cuboidal epithelium and also the proventriculus is made from pseudo-stratified epithelium. In the midgut, the cylindrical channel of the midgut causes the cubic epithelium, while the "bulb" causes squamous epithelium. In the hindgut, the ileum occurs as cylindrical epitheliums, and the rectum consists of cubic-cylindrical epithelium. This study will contribute greatly to the scientific world of studies on the digestive tract structure of insects.-

Entomology

Research Article

Article History
Received : 08.11.2023
Accepted : 21.01.2024

Keywords
Aelia rostrata
Midgut
Malpighian tubules
Light microscope
Scanning electron microscope

Atıf Şekli: Genç, Ş., & Candan, S., (2024). *Aelia rostrata* (Heteroptera: Pentatomidae)'un Sindirim Kanalının Ultrastrüktürü. *KSÜ Tarım ve Doğa Derg 27*(4), 881-891. https://doi.org/10.18016/ksutarimdoga.1388051
To Cite : Genç, Ş., & Candan, S., (2024). Ultrastructure of Digestive Canal of *Aelia rostrata* (Heteroptera: Pentatomidae). *KSU J. Agric Nat 27*(4), 881-891. https://doi.org/10.18016/ksutarimdoga.1388051

INTRODUCTION

Pentatomidae is one of the largest families of the Heteroptera suborder, while Heteroptera itself is the largest group of the Exopterygota division of the Pterygota subclass. About 4700 species of the Pentatomidae family are known around the world, grouped into about 900 genera (Rider, 2006). Approximately 170 species belonging to 57 genera from this family are known in Turkey up to date (Rider, 2006; Önder et al., 2006; Fent and Aktaç, 2007; Fent et al., 2010 a,b). Most of the species belonging to this family are phytophagous and have a wide host distribution (Rider, 2006).

Aelia rostrata (Fabricius, 1803), popularly known as Kımıl in Turkish and wheat stink bug in English, is a species belonging to the Pentatomidae family of the Heteroptera order and causes considerable yield loss. The stink bug is known as the most harmful insect species in wheat fields in Turkey (Lodos, 1982). As a phytophagous species that acquires nutrients by absorbing plant sap, it fed primarily on wheat, and other cultivated and wild Gramineae species: oak, even, hedgehog, pine, bear ears, etc. They feed, mate, and lay eggs while living mostly under the leaves. The offspring that emerge from the egg become a new generation of adult insects, and they retreat to the surrounding mountains and forest after the wheat harvest in Turkey (Lodos and Önder, 1986).

The insect digestive system usually has a tube that continues from the mouth to the anus. It is mainly divided into three regions: foregut, midgut, and hindgut (Hood, 1937; Wigglesworth, 1977; Chapman, 1985; Dow, 1986; Gullan and Cranston, 2005; Borges et al.,2015). The foregut (stomodeum), which is of ectodermal origin, usually; consists of the pharynx, esophagus, crop, and proventriculus. Foregut cells are usually flat. These cells are undifferentiated because they do not secrete or absorb. However, the cuticle layer is located in different regions. It usually consists of only the endocuticle and epicuticle. This structure varies from species to species and in different regions of the foregut (Chapman, 1988). The pharynx provides the intake and backward passage of food. The esophagus is usually tubular and provides a connection between the pharynx and the crop. This structure has been described mostly in hemimetabolous insects. Nutrients are stored in the crop. Annular and longitudinal muscles are very well developed in the proventriculus. On the inner side of the proventriculus, the spines, teeth, and many projections of various shapes of the intima are rubbing against each other under the action of the muscles. With this friction, food particles are ground (Chapman, 1998). The midgut is of endodermal origin and is generally not compartmentalized. Digestion usually occurs here. Cells in the midgut are actively involved in the absorption of nutrients as well as enzyme production and secretion (Chapman, 1988). The hindgut (proctodeum), like the foregut, is of ectodermal origin. Removal of waste materials, water, and salt absorption occurs here. It consists of 3 parts: the ileum, colon, and rectum. The anterior part of the hindgut consists of the ileum, the narrow middle part of the colon, and the wider posterior part of the rectum. In many terrestrial insects, the rectum is the only intestinal site where water and solutions in feces are absorbed, but in some insects, the ileum also provides osmoregulation. Malpighi tubes, responsible for excretion, are located at the junction of the midgut and hindgut. It removes nitrogenous waste (especially ammonium ions) from the hemolymph (Chapman, 1988).

To date, the structure of the digestive system of some species belonging to the Heteroptera order has been examined and the structures of the digestive system have been revealed (Çetin, 2014; Metin, 2014; Amutkan et al., 2015; Demirkol, 2016; Candan et al., 2020). Similarly, knowing the structure of the digestive system in detail in *Aelia rostrata*, whose digestive system has not been studied before, and which is an economically important agricultural pest in our country, will shed light on the development of control methods, as well as will be beneficial for systematic and taxonomic studies. In this study, the digestive system of *Aelia rostrata* was examined in detail using light microscopy and scanning electron microscopy.

MATERIALS and METHODS

Adult females and males of *Aelia rostrata* (Heteroptera: Pentatomidae) were collected in the Bala district of Ankara province in August-October 2014. The samples brought to the Gazi University laboratory were preserved in jars containing food plants and moistened cotton.

Preparation of Samples for a Light Microscope (LM)

Live adult *Aelia rostrata* specimens were kept in ethyl acetate vapor in glass containers. The digestive tract of *Aelia rostrata* was dissected under a stereomicroscope in a 70% alcohol medium. The general structure of the removed digestive system was detected in Bouin fixative fluid after being photographed with a stereo-microscope. The detected samples were washed with 70% ethyl alcohol and the

digestive system was transferred into paraffin blocks, which were the embedding medium, after dehydration with rising ethyl alcohol series. Sections of approximately 5–7 mm thick were taken from paraffin blocks using a microtome. Sections were stained with Hemotoxylene-Eosin and Mallory 3 staining, closed with Stellan and turned into a permanent prepare. Sections of the parts of the digestive system were examined under an Olympus BX51 light microscope at 4X, 10X, 20X, 40X, and 100X (with immersion oil) magnifications. Photographs were taken after the examination.

Preparation of Samples for Scanning Electron Microscope (SEM)

Samples for scanning electron microscopy were prepared with (0.1-M; pH: 7.2) phosphate buffer with pH: 7.2, fixed in 2.5% Glutaraldehyde for at least 1 day; and washed with phosphate buffer (pH: 7.2) by making two 15-minute changes. Then, it was passed through 70%, 80%, 96%, 100%, and 100% alcohol series for 15 min each. The samples, which were kept in amyl acetate 2 times for 15 min, were dried at the critical point, then they were broken in their entirety or from various parts and attached to the staples with double-sided adhesive tapes. The digestive tracts covered with gold in the Polaron SC 502 coating device were examined in the JEOL JSM 6060 brand scanning electron microscope (SEM) at 5–10 kV and their photographs were taken.

RESEARCH FINDINGS

Gross Morphology of the Alimentary Canal

The digestive system of *Aelia rostrata* is divided into three parts: foregut, midgut, and hindgut (Figure 1). The alimentary canal is long, muscular, and tubular in structure and extends from the mouth to the anus. The foregut consists of the salivary gland, accessory salivary gland, pharynx, esophagus, and proventriculus. Since the pharynx and esophagus parts are not separated from the hard chitin part of the head, they are not examined in this study. The midgut has an anterior, median (canal structure of the midgut), and posterior midgut (bulb structure of the midgut). Hindgut has the ileum and rectum (Figure1).

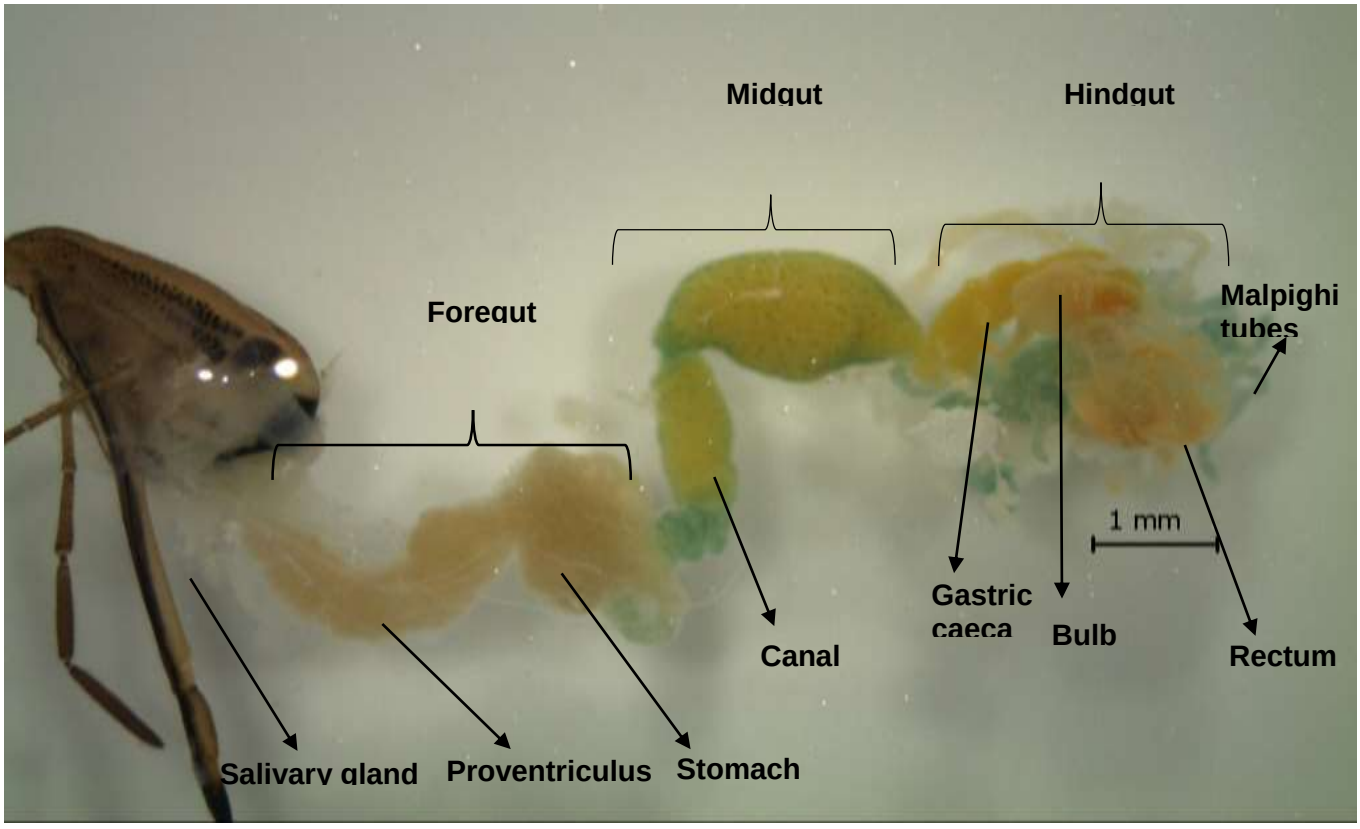


Figure 1. The general view of the alimentary canal of *Aelia rostrata* – Stereomicroscope
Şekil 1. Aelia rostrata sindirim kanalının genel görünüşü-Stereomikroskop

Foregut

The foregut is of ectoderm origin. It begins with the salivary glands opening into the oral cavity. *Aelia rostrata* contains a pair of salivary glands attached to the foregut. Each salivary gland also consists of two parts, posterior and anterior regions (Figure 2a – 2c). Thinning was observed in the region where the posterior part connects with the anterior part (Figure 2a). In SEM images, the folds on the surface of the posterior part connecting to the foregut are less than those on the anterior part (Figure 2b). The outer surface of the anterior part of the salivary glands has

the appearance of corn grains (Figure 2c, 2d). The nuclei of the cells are close to the lumen of the cell (Figure 2d). Light and SEM images of the salivary gland showed that the lumen is filled with saliva (Figure 2d, 2e). Each salivary gland also consists of two parts, posterior and anterior (Figure 2a - 2d). The salivary gland is surrounded by a thin monolayer epithelium from the outside in both regions and consists of cubic cells.

Aelia rostrata has an accessory salivary gland attached to each of the two salivary glands attached to the foregut. In the SEM images, it is seen that the accessory salivary gland makes "S"-shaped folds. In the light microscope images, it was observed that the accessory salivary gland consists of a single-layered cuboidal epithelium, and the part facing the lumen is lined with the cuticle layer (Figure 2g, 2h).

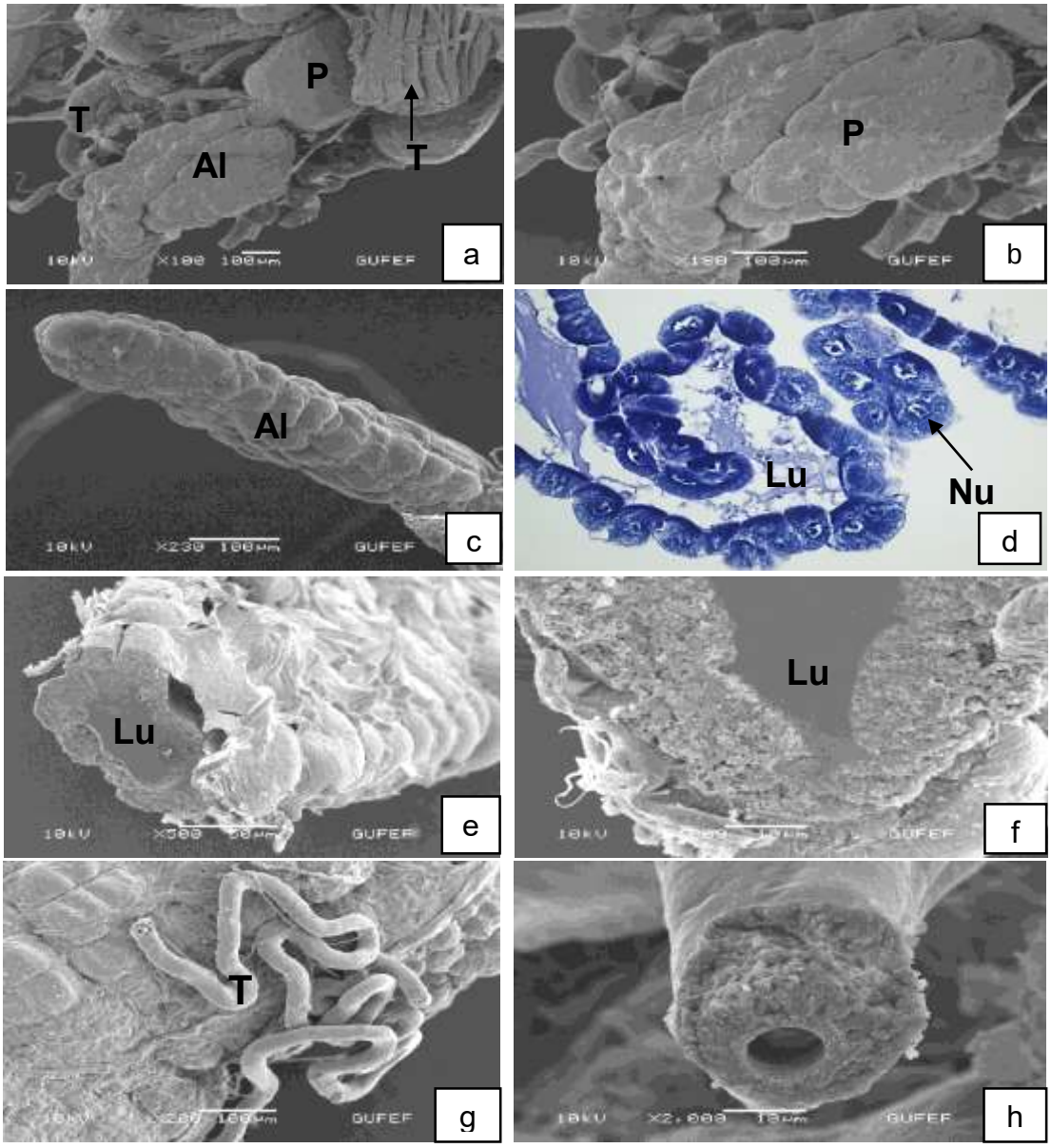


Figure 2. (a) Connecting part of the anterior and posterior region of the salivary gland and trachea (SEM). (b) Posterior region of salivary gland (SEM). (c) Anterior region of the salivary gland (SEM). (d) Longitudinal section view of the salivary gland (LM) (X400). (e,f) Cross-section of the inner surface of the salivary gland (SEM). (g) General view of accessory salivary gland (SEM). (h) Sectional view of the duct of the accessory salivary gland (SEM). Al, anterior lobe; Lu, lumen; Nu, nucleus; Pl, posterior lobe; T, trachea.

Şekil 2. a) Tükürük bezinin ve trakenin ön ve arka bölgesinin birleşen kısmı (SEM). (b) Tükürük bezinin arka bölgesi (SEM). (c) Tükürük bezinin ön bölgesi (SEM). (d) Tükürük bezinin (LM) boyuna kesit görünümü (X400). (e,f) Tükürük bezinin iç yüzeyinin kesiti (SEM). (g) Aksesuar tükürük bezinin (SEM) genel görünümü. (h) Aksesuar tükürük bezi kanalının kesit görünümü (SEM). Al, ön lob; Lu, lümen; Nu, çekirdek; Pl, arka lob; T, soluk borusu.

Proventriculus and Stomach (Ventriculus)

The proventriculus and the stomach, which is the anterior part of the midgut, are parts of the digestive system that help break down food (Figure 3a). Both these regions are externally equipped with a dense tracheal network and longitudinal muscles (Figure 3b). This image shows that the junctions of the monolayer cells are in the pit and the central parts are elevated apically (Figure 3c). The apical membranes of the cells of the single-layered cylindrical epithelial layer are abundant with microvilli, and the basal parts are indented inward. The apical parts of the cells protruded into the lumen. SEM images show that the cells are filled with secretory granules (Figure 3d). The proventriculus and stomach are not completely separated from each other, and the stomach forms the larger part of this structure (Figure 3a). The indentation of the inner surface of the stomach can be seen in SEM images and cross-sections (Figure 3e).

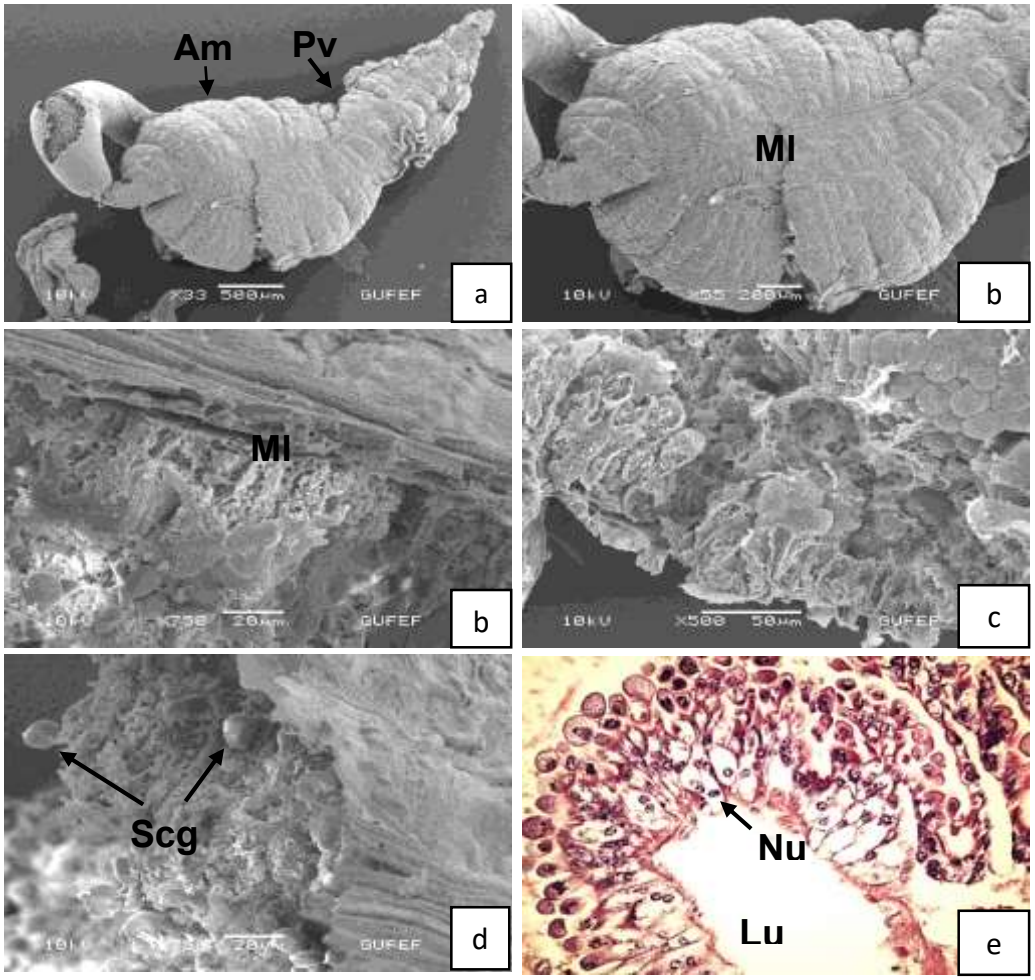


Figure 3. (a) General view of the proventriculus and anterior midgut (SEM). (b) muscle layer on the outer surface of the proventriculus and stomach (SEM). (c) Proventriculus and protrusions on the apical surface of the stomach (SEM). (d) proventriculus and secretory granules in the stomach (SEM). (e) Proventriculus and cells in the stomach and lumen. Am, anterior midgut; L, lumen; Ml, muscle layer; Nu, nucleus; Pv, proventriculus, S, stomach; Scg, secretory granules.

Şekil 3. (a) Proventrikulus ve ön orta bağırsağın genel görünümü (SEM). (b) Proventrikulus ve midenin dış yüzeyindeki kas tabakası SEM). (c) Midenin apikal yüzeyindeki Proventrikül ve çıkıntılar (SEM). (d) Midedeki proventrikulus ve salgı granülleri (SEM). (e) Proventrikül, mide ve lümendeki hücreler. Am, ön orta bağırsak; L, lümen; Ml, kas tabakası; Nu, çekirdek; Pv, proventrikulus, S, mide; Scg, salgı granülleri.

Midgut

The midgut canal (median midgut) is a long tube with one end connected to the stomach and the other to the bulb (Figure 4a-4c). Digestion occurs in this channel. The surface of the midgut canal is surrounded by an abundant tracheal network and muscles (Figure 4d). The cells are composed of a monolayer cubic epithelium. There are microvilli in the lumen-facing part of the epithelial cells (Figure 4e). It consists of a monolayer of cylindrical intestinal epithelial cells of varying heights with abundant trachea and a thin

basal lamina (Figure 4f). Cells have microvilli directed toward the lumen side. Epithelial cells contain numerous rough endoplasmic reticulum and secretory granules (Figure 4g).

The short enlarged posterior region of the midgut canal is called the "bulb "posterior midgut. It is attached to the midgut wedge with one end and the ileum with the other end (Figure 4h). This part helps absorb the excess water in the food before it passes to the hindgut. In the SEM images, the outer surface was smooth and surrounded by muscles. Its interior is in the form of a large cavity containing digestive products (Figure 4i). The wall structure is thin, and the epithelial cells are shortened in size, so there are differences in their heights, and there are sometimes cubic or even flat cells. There are microvilli on the apical surface of the cells.

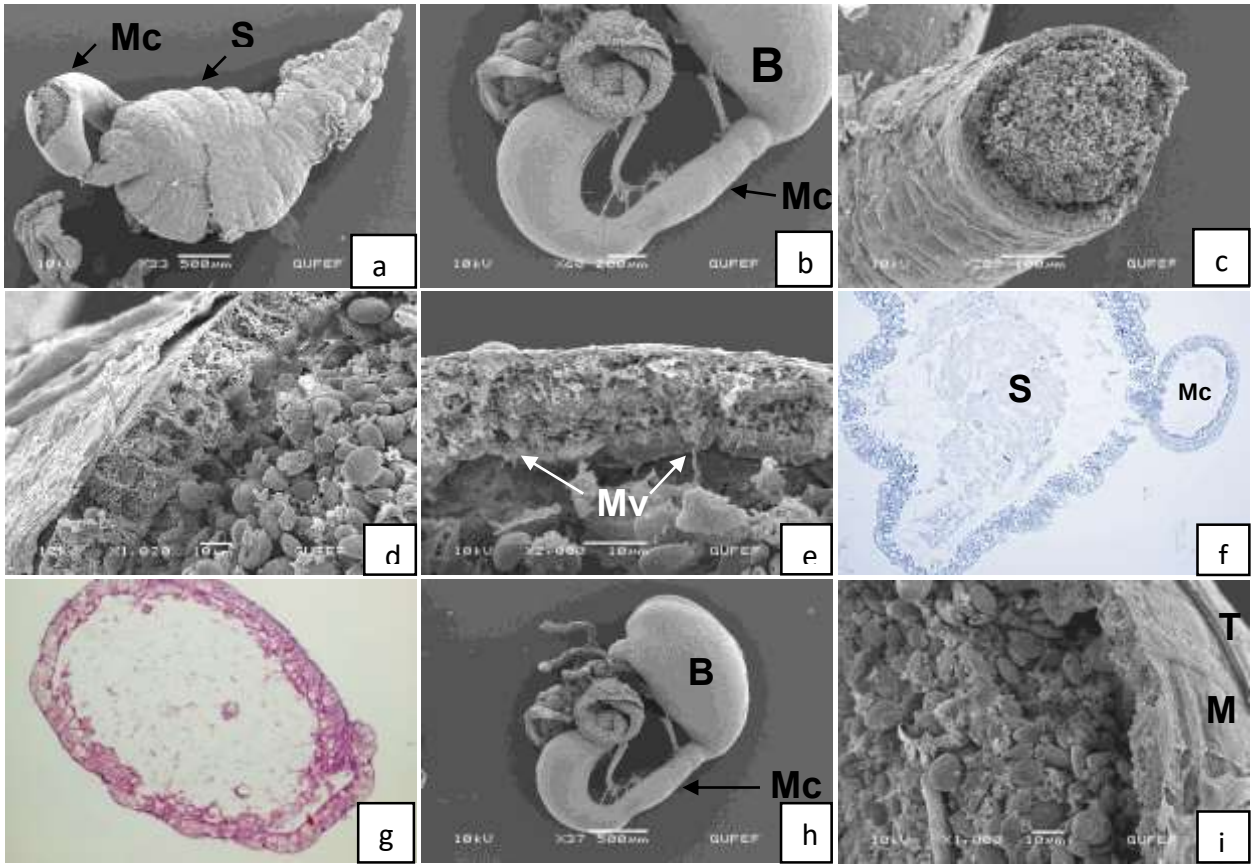


Figure 4. (a) The part where the midgut canal connects to the stomach-SEM. (b) The part of the midgut duct where it attaches to the bulb-SEM. (c) The inner surface structure of midgut cross-sectional canal-SEM. (d) The inner surface structure of the midgut tract-SEM. (e) View of microvilli in the midgut tract-SEM. (f) General view of the stomach and mid-intestinal tract (LM) (X40). (g) Light microscope view of the midgut tract (X40). (h) The part where the bulb structure attaches to the midgut-SEM. (i) Thin cell layer in the bulb and trachea and muscles on the outer surface-SEM. B, bulb; M, muscle; Mc, midgut canal; Mv, microvillus; S, stomach; T, trachea.

Şekil 4. (a) Midgut kanalının mide-SEM'e bağlandığı kısım. (b) Orta bağırsak kanalının ampul-SEM'e bağlandığı kısmı. (c) Orta bağırsak kesit kanalının iç yüzey yapısı-SEM. (d) Orta bağırsak kanalının iç yüzey yapısı-SEM. (e) Orta bağırsak kanalındaki mikrovillusların görünümü-SEM. (f) Mide ve orta bağırsak kanalının genel görünümü (LM) (X40). (g) Orta bağırsak kanalının ışık mikroskobu görünümü (X40). (h) Ampul yapısının orta bağırsağa bağlandığı kısım-SEM. (i) Ampul ve trakedeki ince hücre tabakası ve dış yüzeydeki kaslar-SEM. B, ampul; M, kas; Mc, orta bağırsak kanalı; Mv, mikrovillus; S, mide; T, soluk borusu.

Hindgut

The gastric caeca includes a structure consisting of four channels, one end of which is connected to the rectum and the other end to the midgut. The ileum, which consists of a monolayer cubic-cylindrical epithelium, passes through the middle of the channels in the gastric caeca (Figure 5a, 5c- 5e). In SEM images, it is seen that the caeca has a transverse nodular structure and its outer surface is surrounded by the trachea (Figure 5b, 5e, 5f).

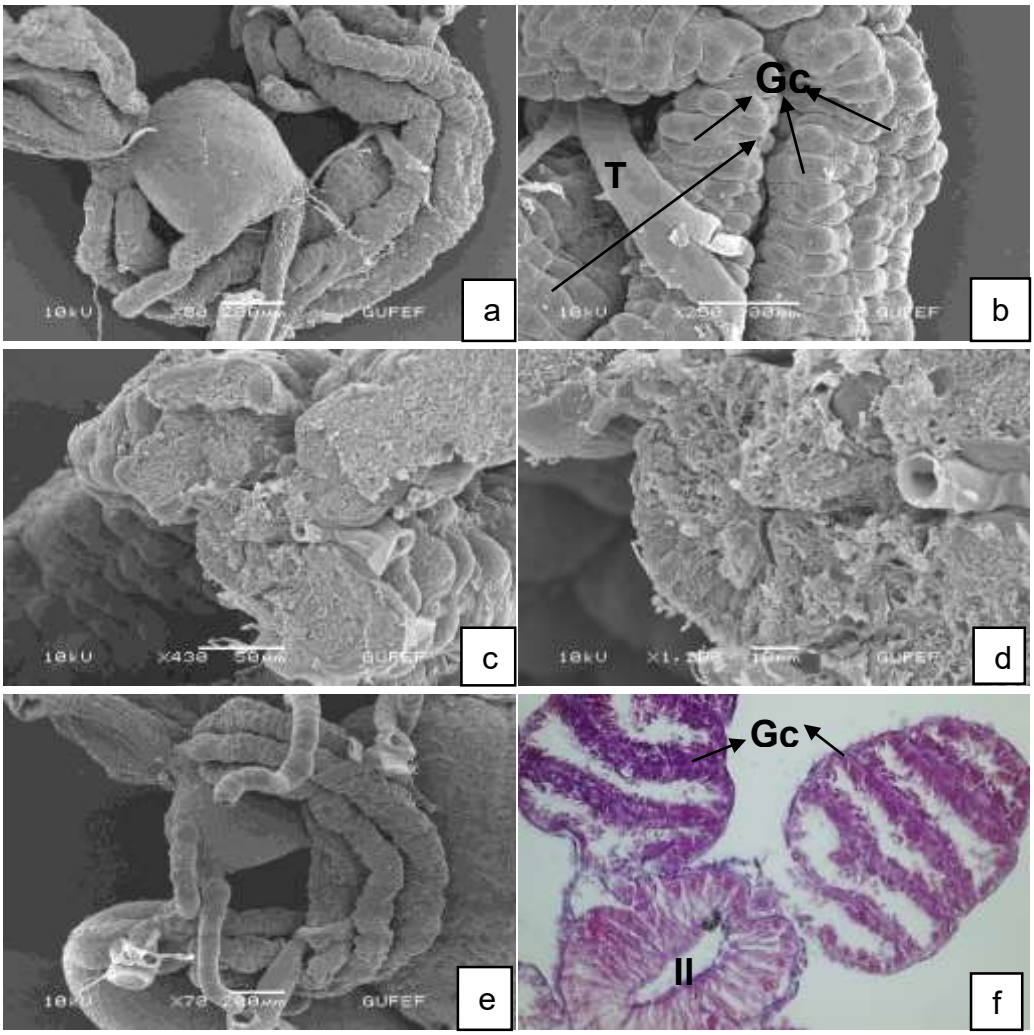


Figure 5. (a) General view of gastric caeca-SEM. (b) Trachea-SEM on the outer surface of the gastric caeca. (c-d) General view of the ducts and ileum in the gastric caeca-SEM. (e) General view of ileum-SEM. (f) View of the gastric cecum and ileum with a light microscope (X40). Gc, gastric caeaca; Il, ileum; T, trachea.

Şekil 5. (a) Gastrik çekumun genel görünümü-SEM. (b) Mide boşluğunun dış yüzeyindeki Trake-SEM. (c-d) Gastrik çekum kanalları ve ileumun genel görünümü-SEM. (e) İleum'un genel görünümü-SEM. (f) Gastrik çekum ve ileumun ışık mikroskobuyla görünümü (X40). Gc, gastrik çekum; Il, ileum; T, soluk borusu.

Malpighian tubules are connected to the digestive canal at the junction of the midgut and hindgut (Figure 5g). One end of the Malpighi tubes, which are found as a pair, is connected to the digestive tract, and the other end is free in the body cavity. While the part connecting to the digestive tract is flatter, the ends are seen as beads (Figure 5h). In the SEM images, the outer surface of the Malpighi tubes is surrounded by the trachea (Figure 5h). Its cells consist of a monolayer cubic epithelium (Figure 5j). The parts of the cells facing the lumen are surrounded by microvilli (Figure 5i, 5j).

The rectum forms the last region of the hindgut (Figure 5k). The surface of the rectum was surrounded by the trachea and muscles (Figure 5l). Intense folds are seen in the parts of the cells facing the lumen (Figure 5m). The cell wall consists of a monolayer cubic epithelium (Figure 5n).

DISCUSSION

Intestinal physiology and morphology differ in insects depending on their nutritional diversity. Insects that feed on solid foods have large, flat, short intestines equipped with strong muscles that protect against abrasion. These structures are very prominent in plant-feeding caterpillars, which consume their food fast and eat solid foods (Harris, 1938; Edmonds 1974, Fontanetti et al., 2002; Silva et al., 2004; Nardi et al., 2009; Metin, 2014; Candan et al. 2019). In contrast, in insects that feed on liquid nutrients such as blood, plant sap, or nectar, the intestine is usually long, narrow, and curved to ensure maximum contact with the liquid food. In this type of nutrition, there is no need to protect the intestine from erosion (Demirsoy, 2003).

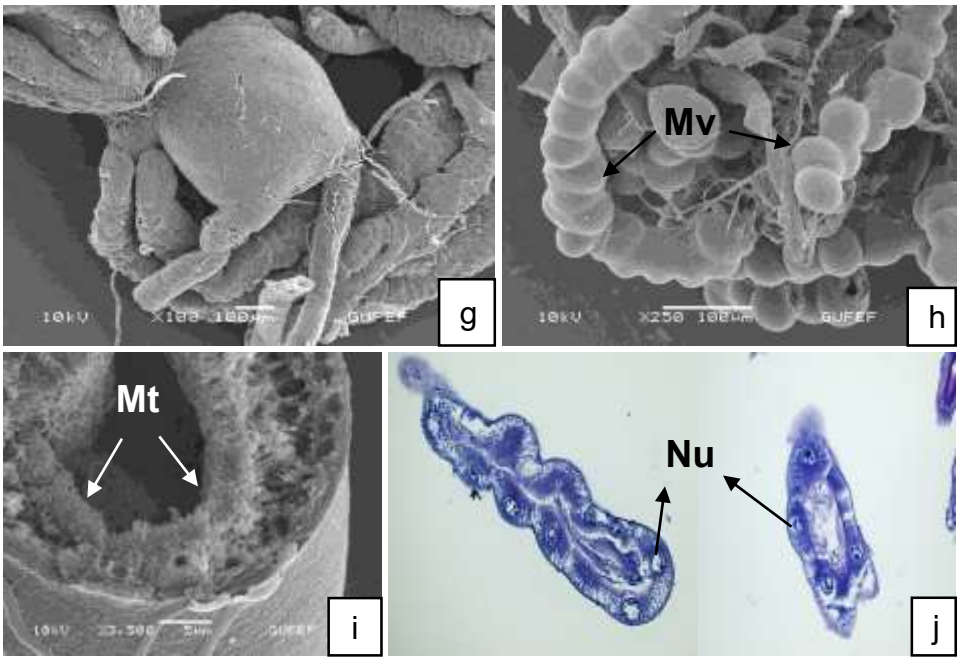


Figure 5. (g) The part where Malpighi tubes connect to the digestive tract-SEM. (h) Bead-shaped structure of Malpighi tubes-SEM. (i) Microvilli in Malpighi tubes-SEM. (j) Longitudinal and cross-section of Malpighi tube-LM (X40) (Mallory). Mt, malpighian tubules; Mv, microvillus; Nu, nucleus; T, trachea.

Şekil 5. (g) Malpighi tüplerinin sindirim sistemine bağlandığı kısım-SEM. (h) Malpighi tüplerinin boncuk şeklindeki yapısı-SEM. (i) Malpighi tüplerindeki mikrovilluslar-SEM. (j) Malpighi tüpü-LM (X40) (Mallory) boyuna ve enine kesiti. Mt, malpighian tübüller; Mv, mikrovillus; Nu, çekirdek; T, soluk borusu.

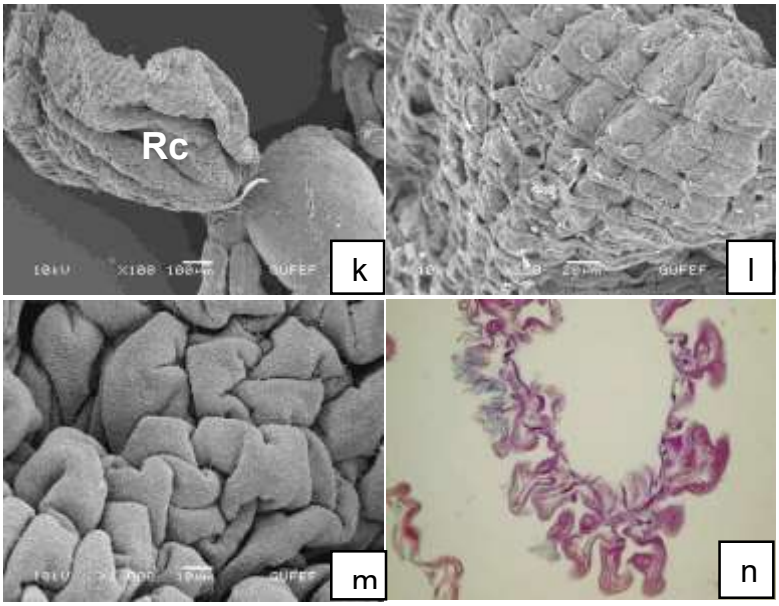


Figure 5. (k) General view of the rectum-SEM. (l) External surface view of the rectum-SEM. (m) Folds on the inner surface of the rectum-SEM. (n) Cubic-cylindrical cell structures of the rectum-LM (X40, HE). Rc, rectum.

Şekil 5. (k) Rektumun genel görünümü-SEM. (l) Rektumun dış yüzey görünümü-SEM. (m) Rektumun iç yüzeyindeki kıvrımlar-SEM. (n) Rektum-LM'nin kübik-silindirik hücre yapıları (X40, HE). Rc, rektum.

The digestive system of *Aelia rostrata*, which consists of the foregut, midgut, and hindgut is similar to the digestive systems of other Heteroptera species (Hamner, 1936; Harris, 1938; Barber, 1980; Pastle and Woodward, 1988; Habibi et al., 2008; Bandani et al., 2010; Çetin, 2014; Metin, 2014; Amutkan et al., 2015; Demirkol, 2016; Candan et al., 2020; Özyurt Koçakoğlu, 2021). The anterior and posterior intestines are of ectodermal origin, and the midgut is of endodermal origin (Candan et al., 2020; Özyurt Koçakoğlu, 2021). The foregut and hindgut are short and the midgut is long. The foregut consists of the

salivary glands, pharynx, esophagus, and proventriculus.

There is a pair of salivary glands attached to the foregut. Each salivary gland consists of two parts, posterior and anterior. The salivary glands are surrounded by a thin muscle layer from the outside in both regions and consist of a monolayer of cubic cells. The folds on the surface of the posterior part of the salivary glands are smaller than those of the anterior part. The outer surface of the anterior part of the salivary glands has the appearance of corn grains. Tracheal structures were also observed on the surface and sections of the salivary gland. In terms of possessing a pair of salivary glands, *Dolycoris baccara* (Linneaus, 1758) (Heteroptera: Pentatomidae) (Çetin, 2014), *Carpocoris predicts* (Heteroptera: Pentatomidae) (Metin, 2014), *Lygaeus equestris* (Linneaus, 1758) (Heteroptera: Lygaeidae) (Demirkol, 2016), and *Rhaphigaster nebulosa* (Heteroptera: Pentatomidae) (Bayramova, 2015) show similarity with *Aelia rostrata*. However, Amutkan (2012) showed in his study that there are two pairs of salivary glands attached to the foregut in *Graphosoma lineatum* (Linnaeus, 1758) (Heteroptera: Pentatomidae). In *Aelia rostrata*, the anterior region of the salivary gland has the appearance of corn grains, while it is finger-like protruding in *Graphosoma lineatum* (Amutkan, 2012). *Aelia rostrata* has an accessory salivary gland attached to each of the two salivary glands, which in turn attaches to the foregut. In the SEM images, it is seen that the accessory salivary gland makes "S"-shaped folds. The accessory salivary gland consists of a monolayer cubical epithelium according to light microscope images.

The proventriculus and the stomach, which is the anterior part of the midgut, are parts of the digestive system that help break down food. *Aelia rostrata* does not have tooth-like chitin protrusions as it is fed with plant sap as seen in *Graphosoma lineatum* (Amutkan, 2012), *Carpocoris pudicus* (Metin, 2014), *Dolycoris baccarrum* (Çetin, 2014) and *Rhaphigaster nebulosa* (Bayramova, 2015). It protruded toward the lumen. In Coleoptera, Hymenoptera, and Orthoptera species that feed on solid foods, the structure consists of tooth-like chitin protrusions because the proventriculus is the place where the food is broken down. Because of the studies conducted with the proventriculus, it was observed that this structure changes depending on liquid and solid nutrition. The stomach is in the form of a thin tube, as in *Solube pugnca* Fab. (Heteroptera: Pentatomidae) (Hamner, 1936), *Graphosoma lineatum* (Amutkan, 2012), and *Carpocoris pudicus* (Metin, 2014). Since the is fed with liquid food, no spiny protrusions are observed in the stomach. In *Aelia rostrata*, the inner surface of the stomach is less protruding than the proventriculus.

The midgut canal is in the form of a long tube and consists of a monolayer of cuboidal cells. In *A. rostrata*, the midgut canal is in the form of a long tube, as in *Graphosoma lineatum* (Amutkan, 2012), *Carpocoris pudicus* (Metin, 2014), *Rhaphigaster nebulosa* (Bayramova, 2015), and *Lygaeus equestris* (Demirkol, 2016). Although the midgut canal is composed of monolayered cubic cells in *A. rostrata*, it consists of cylindrical cells in *Carpocoris pudicus* (Metin, 2014). In *A. rostrata* and *Rhaphigaster nebulosa*, the midgut canal is similar in that one end is connected to the stomach and the other to the bulb (Bayramova, 2015).

The short enlarged posterior region of the midgut canal is called the "bulb." It is connected to the midgut wedge with one end and the ileum with the other end. The bulb structure found in *A. rostrata* is similar to that of *Graphosoma lineatum* (Amutkan, 2012) and *Carpocoris pudicus* (Metin, 2014).

In *A. rostrata*, the hindgut consists of the ileum and rectum, whereas *Melanogryllus desertus* (Orthoptera: Gryllidae) consists of the ileum, colon, and rectum (Çakıcı, 2008). Four gastric caeca resembling corn kernels are attached to the ileum. *A. rostrata* is similar to *Carpocoris pudicus* (Metin, 2014) and *Rhaphigaster nebulosa* (Bayramova, 2015) in terms of attachment of gastric caeca and formation of four channels. In *A. rostrata*, the gastric caeca attaches to the hindgut, whereas in *Melanogryllus desertus* it attaches to the midgut (Çakıcı, 2008). Although there are four gastric caeca in A. rostrata, there are two in *Melanogryllus desertus* (Çakıcı, 2008). The gastric caeca is absent in *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae) (Koçakoğlu, 2021) and *Lygus hesperus* (Heteroptera: Miridae) (Habibi, 2008).

In *A. rostrata*, the Malpighi tube is connected to the area where the ileum and rectum are connected. Two pairs of Malpighi tubes connected to the ampulla structure branch show a knotty structure and end with closed ends. The presence of constricted and two pairs of malpighi tubes of *A. rostrata* is similar to that of *Carpocoris pudicus* (Metin, 2014) and *Rhaphigaster nebulosa* (Bayramova, 2015) in that the malpighi cells consist of monolayer cubic cells. Although there are 2 pairs of Malpighi tubes in *A. rostrata* and 1 pair in *Pyrrhocoris apterus* (Koçakoğlu, 2021), the Malpighi cells are similar in that they consist of monolayer cubic cells. There are two pairs of Malpighi tubes in *A. rostrata*, *Lygaeus equestris* (Demirkol, 2016), *Oncopeltus fasciatus* (Heteroptera: Lygaeidae) (Hamner, 1936), *Graphosoma lineatum* (Amutkan,2012), *Dolycoris baccarum* (Çetin,2014), and *Psammotettix striatus* (L.)(Hemiptera: Cicadellidae) (Zhang et al., 2012). There are two pairs of Malpighi tubes, as in Zhang et al., 2012). Malpighi tubes *Solubea pugncvc* (Hamner, 1936) 1 pair, *Pezodrymedusa lata* Karadağ (Orthoptera: Tettigoniidae) (Bursalı, 1996) 3 pairs. Ghanim et al. (2001) found that *Besimia tabaci* Gennadius

(Hemiptera: Aleyrodidae) lacks Malpighi tubes, but instead has a special Malpighi-like cell in the inside of the ileum and the filter chamber. In *A.rostrata*, Malpighi cells are nodular, while in *Sarcophaga ruficornis* it consists of principal and star-shaped cells.

The surface of the rectum is surrounded by the trachea and muscles. The cell wall consists of a single layer of cubic cylindrical epithelium. Instead of microvilli, dense folds are seen in the parts of the cells facing the lumen. These features are similar to *Carpocoris pudicus* (Metin, 2014), *Rhaphigaster nebulosa* (Bayramova, 2015), and *Lygaeus equestris* (Demirkol, 2016). Rectal papillae were not observed in *A. rostrata*. Rectal papillae are generally seen in groups with chewing mouth structures such as Orthoptera and Coleoptera. Therefore, the rectal papillae cause the solidification of the waste product with final absorption.

Because of this study on the examined *Aelia rostrata*, it was found that the digestive tract was generally similar to Heteroptera species. However, there are not enough studies on the fine structure of the digestive tract. Due to the structural and morphological similarities of the digestive system of insects, this study will have an important place in future studies and in illuminating insect control studies in terms of agricultural control. It aims to develop mechanisms that will affect the digestive system in the fight against this insect by investigating the structure of the digestive system of *A. rostrata*.

Acknowledgments

This article is prepared according to the master thesis of Şermin GENÇ. The thesis was conducted under the supervision of Professor Doctor Selami CANDAN. This study was presented as a poster at the 4th National Biologists Congress (International Participation), on May 06, 2017, in Ankara. The Authors thank Adyatma Irawan Santosa (Universitas Gadjah Mada, Indonesia) for the improvement of the English of the manuscript.

Author's Contributions

Selami CANDAN planned the study and Şermin GENÇ conducted the study. The findings obtained in the study were analyzed by Selami CANDAN and the article was written by Şermin GENÇ.

Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

Amutkan, D., Suludere Z., & Candan, S. (2012). Ultrastructure of Digestive Canal of Graphosoma lineatum (Linnaeus, 1758) (Heteroptera: Pentatomidae). *Journal of the Entomological Research Society, 17*(3), 75- 96.

Amutkan, D., Suludere, Z., & Candan, S. (2015). Ultrastructure of the digestive canal of Graphosoma lineatum (Linnaeus, 1758) (Heteroptera: Pentatomidae). *Journal of Entomological Research Society,17*, 75–96.

Barber, D.T., Cooksey, L.M., & Abell, D.W. (1980). A study of the anatomy of the alimentary canal of Brochymena quadripustulata (Hemiptera: Pentatomidae). *Arkansas Academy of Science Proceedings, 34*, 16-18.

Bayramova, G. (2015). Ultrastructure of the digestive tract of Rhaphigaster nebulosa (Poda, 1761) (Heteroptera: Pentatomidae) (Master's thesis, Gazi University Institute of Science and Technology, Ankara).

Borges I, Nóia M, Camarinho R, Rodrigues AS, & Soares AO. (2015). Characterization of the alimentary canal of the aphidophagous ladybird, Adalia bipunctata (Coleoptera: Coccinellidae): anatomical and histological approaches. *Entomological Science, 18*(1), 66-73.

Bursalı, A. (1996). Investigation of the histological and histochemical structure of the digestive tract of Pezodrymedusa lata Karabağ (Orthoptera: Tettigoniidae) (Master's thesis, Gaziosmanpaşa University Institute of Science and Technology, Tokat).

Candan S, Özyurt Koçakoğlu N, & Erbey M. (2019). Morphology and histology of the alimentary canal of Epiphaneus malachiticus Boheman, 1842 (Coleoptera, Curculionidae). *Entomological Review, 99*(3), 326–336.

Candan, S., Özyurt Koçakoğlu, N., Güllü, M., & Çağlar, Ü. (2020). Anatomical and histological studies of the alimentary canal of adult maize leaf weevil, Tanymecus dilaticollis Gyllenhal, 1834 (Coleoptera: Curculionidae). *Microscopy Research and Technique, 83*(9), 1153-1162.

Chapman RF. (1985). Comprehensive and insect physiology. In Kerkut GA & Gilbert LI (Eds.), *Biochemistry and Pharmacology* (pp. xx-xx). Oxford: Pergamon.

Chapman, M. (1988). *Constructive evolution: Origins and development of Piaget's thought*. Cambridge University Press.

Chapman, R. F. (1998). Alimentary canal, digestion, and absorption. In Simpson, S. J., & Douglas, A. E. (Eds.), *The Insect: Structure and Function* (pp. 38-68). Cambridge University Press.

Çakıcı, Ö. (2008). Histological and ultrastructural studies in the digestive system of Melanogryllus desertus pallas (Orthoptera: Gryllidae) (Doctoral dissertation, Ege University Institute of Science and Technology, İzmir).

Çetin, T. K. (2014). Ultrastructure of Digestive Canal of Dolycoris baccara (Linnaeus, 1758) (Heteroptera:

Pentatomidae) (Master's thesis, Gazi University Institute of Science and Technology, Ankara).

Demirkol, E. (2016). Ultrastructure of the digestive tract of Lygaeus equestrians (Linnaeus, 1758) (Heteroptera: Lygaeidae) (Master's thesis, Gazi University Institute of Science and Technology, Ankara).

Demirsoy, A. (2003). *Basic Rules of Life*. (Eighth Edition). Turkey: Meteksan.

Dow JA. (1986). Insect midgut function. *Advances In Insect Physiology, 19*, 187-328.

Edmonds WD. (1974). Internal anatomy of Coprophanaeus lancifer (L.) (Coleoptera: Scarabaeidae). *International Journal of Insect Morphology and Embryology, 3*, 257–272.

Fontanetti, C.S., Zefa, E., Pasetti, F., & Mesa, A. (2002). Morphological characterization and comparative analysis of the proventriculus from three species of Endecous Saussure, 1878 (Orthoptera: Gryllidae: Phalangopsinae). *Entomotropica, 17*(1), 15-23.

Ghanim, M., Rosell, R.C., Campbell, L.R., Czosnek, H., Brown, J.K., & Ullman, D.E. (2001). Digestive, Salivary, and Reproductive Organs of Bemisia tabaci (Gennadius)(Hemiptera:Aleyrodidae) B Type. *Journal of Morphology, 248*(1), 22–40.

Gullan DJ, & Cranston PS. (2005). *The Insects: An outline of entomology* (3rd ed.). Blackwell Publishing Ltd.

Habibi, J., Coudron, T.A., Backus, E. A., Brandt, S. L., Wagner, R. M., Wright, M. K., & Huesing, J.E. (2008). Morphology and histology of the alimentary canal of Lygus hesperus (Heteroptera: Cimicomoropha: Miridae). *Annals of the Entomological Society of America, 100*(1), 159-171.

Hamner, A. L. (1936). The gross anatomy of the alimentary canal of the Solubea pugnca (Fab.) (Heteroptera, Pentatomidae). *The Ohio Journal of Science, 36*(3), 157-160.

Harris, C. S. (1938). The anatomy and histology of the alimentary system of the harlequin cabbage bug, Murgantia histrionica Hahn. (Hemiptera, Pentatomidae). *The Ohio Journal of Science, 38*(6), 316-331.

Hood, Charles W. (1937). The Anatomy of the Digestive System of Oncopeltus Fasciatus Dall. (Heteroptera: Lygaeidae). *The Ohio Journal of Science, 37*(3), 151-160.

Lodos, N. (1982). Turkey Entomology II (General Applied and Faunistic). Izmir: Ege University Faculty of Agriculture Publications. (No. 429).

Lodos, N. (1986). Turkish Entomology General, Applied and Faunistic (2nd ed.). Izmir-Bornova: EÜZF.

Metin, H. (2014). Ultrastructure of the Digestive Canal of Carpocoris pudicus (Poda, 1761) (Heteroptera: Pentatomidae) [Master's thesis, Gazi University Institute of Science and Technology].

Nardi, J. B., Bee, C. M., Miller, L. A., & Taylor, S. J. (2009). Distinctive features of the alimentary canal of a fungus-feeding hemipteran, Mezira granulata (Heteroptera: Aradidae). *Arthropod Structure & Development, 38*(3), 206-215.

Özyurt Koçakoğlu, N. (2021). Morphology and histology of the alimentary canal, salivary glands and Malpighian tubules in Pyrrhocoris apterus (Linnaeus, 1758) (Hemiptera: Pyrrhocoridae): A scanning electron and light microscopies study. International *Journal of Tropical Insect Science, 41*(2), 1845-1862.

Silva, C. P., Silva, J. R., Vasconcelos, F. F., Petreski, M. D., Damatta, R. A., Ribeiro, A. F., & Terra, W. R. (2004). Occurrence of midgut peri microvillar membranes in paraneopteran insect orders with comments on their function and evolutionary significance. *Arthropod Structure & Development, 33*(2), 139-148.

Wigglesworth, V. B. (1977). The principles of insect physiology. London: Chapman and Hall Ltd.

KSÜ Tarım ve Doğa Derg 27 (4), 892-900, 2024
KSU J. Agric Nat 27 (4), 892-900, 2043
https://doi.org/10.18016/ksutarimdoga.vi.1329294

The Effects of Priming With NaCl Solutions on Salt Stress During Germination and Seedling Stages in Maize

Sema NAZLI[1], Uğur BAŞARAN[2] ✉
[1]Faculty of Agriculture, Department of Field Crops, Yozgat Bozok University, Yozgat-Türkiye. [2]Faculty of Agriculture, Department of Field Crops, Yozgat Bozok University, Yozgat-Türkiye.
[1]https://orcid.org/0000-0002-3551-3194, [2]https://orcid.org/0000-0002-6644-5892
✉: ugur.basaran@bozok.edu.tr

ABSTRACT
In this study, germination and seedling growth under salt stress (175 mM) of maize pretreated (priming) with different salt (NaCl) solutions (0, 150, 175, and 200 mM) were investigated. Unprimed seeds were used as control. The study was carried out with two maize cultivars (ADA-9510 and Simpatico) in a petri dish and pot media. The effects of cultivar and priming treatments on germination and seedling characteristics of maize under salt stress were significant (P<0.01) in both environments. In the ADA-9510 variety, the average values of the examined traits were found to be higher. The germination rate of the Simpatico variety was very low in salt stress, but after priming, it showed an increase of up to 224% in Petri dishes and up to 44% in pots. In pot conditions, priming improved crude protein content, root dry matter ratio, and emergence speed in ADA-9510, while root dry matter ratio, emergence rate, and speed of the Simpatico variety improved when compared to control. Accordingly, as a result of the study, it was determined that the application of priming with 150 and 175 mM NaCl solutions, even with normal water, reduced the negative effects of salt stress on germination and seedling growth in maize.

Field Crops

Research Article

Article History
Received : 18.07.2023
Accepted : 18.01.2024

Keywords
Priming
Salt stress
Maize
Germination
Seedling growth

Tohumlara NaCl Çözeltileri ile Priming Uygulanmasının Mısırda Çimlenme ve Fide Aşamalarında Tuz Stresine Etkileri

ABSTRACT
Bu çalışmada farklı tuz (NaCl) çözeltileriyle (0, 150, 175 ve 200 mM) ön işlem uygulanan (priming) mısırın tuz stresi (175 mM) altında çimlenme ve fide gelişimi incelenmiştir. Kontrol olarak priming uygulanmamış tohumlar kullanılmıştır. Çalışma iki adet mısır çeşidi (ADA-9510 ve Simpatico) ile petri ve saksı koşullarında yürütülmüştür. Mısırın tuz stresi altında çimlenme ve fide özellikleri üzerinde çeşit ve priming uygulamalarının etkisi hem petri hem de saksı ortamında önemli (P<0.01) olmuştur. ADA-9510 çeşidinde, incelen özelliklere ait ortama değerler daha yüksek bulunmuştur. Simpatico çeşidinin çimlenme oranı tuz stresinde çok düşük olmuş, ancak priming sonrası petri kaplarında %224'e, saksılarda %44'e varan bir artış göstermiştir. Saksı koşullarında, kontrolle kıyaslandığında priming uygulamaları ADA-9510 çeşidinde ham protein içeriğini, kök kuru madde oranı ve sürme hızını, Simpatico çeşidinde ise kök kuru madde oranı, sürme oranı ve hızını geliştirmiştir. Buna göre çalışma sonucunda 150 ve 175 mM NaCl çözeltileri ile hatta su ile yapılan priming uygulamasının da mısırda tuz stresinin çimlenme ve fide gelişimi üzerindeki olumsuz etkilerini azalttığı belirlenmiştir.

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 18.07.2023
Kabul Tarihi : 18.01.2024

Anahtar Kelimeler
Priming
Tuz stresi
Mısır
Çimlenme
Fide gelişimi

To Cite : Nazlı, S., & Başaran, U., (2024). Priming The Seeds With NaCl Solutions Alleviates Salt Stress During Germination And Seedling Stages İn Maize. *KSU J. Agric Nat 27* (4), 892-900. https://doi.org/10.18016/ksutarimdoga.vi.1329294

Atıf Şekli: Nazlı, S., & Başaran, U., (2024). Tohumlara NaCl Çözeltileri İle Priming Uygulanmasının Mısırda Çimlenme ve Fide Aşamalarında Tuz Stresine Etkileri. *KSÜ Tarım ve Doğa Derg 27* (4), 892-900. https://doi.org/10.18016/ksutarimdoga.vi.1329294

INTRODUCTION

Salt stress is one of the most important and common abiotic stress factors that negatively affect agricultural production (Vinocur & Altman, 2005). Salinity makes water uptake difficult due to osmotic degradation and causes disruption of physiological processes in plants by ion stress. In addition, it limits photosynthesis by reducing the amount of green tissue in plants and reduces the nutrient content by limiting the activity of various enzymes (Munns et al., 2006). Soil salinity can be caused by many compounds such as chlorides (NaCl, $CaCl_2$, $MgCl_2$), Sulfates (Na_2SO_4, $MgSO_4$), nitrates (Na_2NO_3, KNO_3), Carbonates, bicarbonates ($CaCO_3$, Na_2CO3, $NaHCO_3$) and borates (Yetissin & Karakaya, 2022). The most important and most common of these compounds is sodium chloride (NaCl) and, high NaCl content in the environment leads to ion competition and reduces Ca^{+2}, $K^{+1,}$ and Mg^{+2} uptake (Parida & Das, 2005).

Although there is a general perception that salinization occurs only in arid and semi-arid regions, no climatic region is exempt from this problem (Rengasamy, 2006). Current information from 118 countries covering 85% of global land area, express that more than 424 million hectares of topsoil (0-30 cm) and 833 million hectares of subsoil (30-100 cm) are salt-affected (FAO, 2023). Salinity is encountered in an area of approximately 1.5 million hectares in Turkey, which constitutes 5.48% of the country's total arable land (Sönmez, 2003). The salinity seen in agricultural areas may be due to soil, as well as incorrect agricultural practices, excessive or insufficient irrigation, irrigation water, and temperature increase may be effective in the formation of salinity or its reaching further dimensions. The continuation of wrong agricultural practices and especially the changes in the climate in recent years may cause an increase in the salinity problem. It is predicted that global climate change will cause an increase in temperatures in large areas, including Turkey, and a decrease in precipitation and water resources. The expansion of salt-affected areas poses a major threat to food supply security around the world. It is predicted that 10% of agricultural lands will become salinized every year due to irrigation with salt water, low precipitation, and high evapotranspiration, and salinity problems may occur in more than 50% of arable land rapidly by 2050 (Jamil et al., 2011). Therefore, cultural, physiological, and genetic studies that increase salt tolerance of basic crops are of great importance for sustainable agricultural production.

Maize (*Zea mays* L.) is grown in many countries and is one of the most basic products for human and animal nutrition worldwide (Agami, 2013). Maize is the third cereal after wheat and barley in terms of production amount in Turkey and it is of great importance, especially for animal nutrition. It is a highly crucial crop for both plant and animal production in the Turkish agricultural system. Moreover, its importance is gradually increasing due to the increase in demand, especially in the livestock sector. Maize is a moderately sensitive plant to salt (Carpıcı et al., 2009) and soil salinity is a serious limiting factor for maize production all over the world (Farooq et al., 2015). Pitann et al. (2009) reported differential responses of maize cultivars to salt stress based on biochemical data at the cellular level. Salinity causes stress for plants at all stages of development. However, it poses a greater risk, especially during the germination and seedling stages (Goldsworthy, 1994). Like all plants, Mize is much more sensitive to salinity during germination and seedling periods. Therefore, improving the salinity tolerance of maize during the germination and early development period is critical for the yield and quality of maize and, of course, its sustainable production. The susceptibility of maize to high salt stress was revealed by the decrease in growth and development of underground and aboveground parts (Neubert et al., 2005; Sazalai & Janda, 2009). Therefore, intensive efforts are made to increase the resistance of maize to salt stress, including seed priming (Bakht et al., 2011; Gebreegziabher & Qufa, 2017).

One of the most common priming is halo-priming in which water uptake is ensured in 50 - 200 mM salty (KCl and NaCl) solutions (Kumar et al., 2016). Priming is based on the principle of stimulating seed germination by controlling moisture, ensuring certain metabolic processes, and stopping the process before root emergence occurs (Singh & Kumar, 2021). With priming, homogeneous and strong seedling development is aimed at increasing the germination and seedling emergence rate. In addition to germination performance, protection systems, and stress tolerance increase in seeds stimulated by priming (Khan et al., 2022). In many studies, it has been revealed that priming positively affects the germination and development of salt-stressed plants and has an effect on tolerance to different stress factors (Agami, 2013; Munns & Gilliham, 2015; Baghel et al., 2019; Farooq et al., 2019; Yetissin & Karakaya, 2022). Ashraf & Rauf (2001) reported that priming with chlorinated salts can reduce the negative effect of salt stress on germination (Mahara et al., 2022), seedling development (Kaya & Gözübenli 2020), and growth (Bakht et al., 2011) in maize.

As it is known, the pretreatment of seeds with biological, chemical, or physical agents has changed germination characteristics and seedling growth in many plants. These findings inspired new studies by raising the question of whether the plant's response to stress conditions can be ameliorated by priming

(Faroq et al., 2015). Priming is not simply a process of biochemical changes associated with early germination in seeds. In the priming stage, metabolic activities occur in RNA and protein production, structural and genetic repair, and antioxidant mechanisms, which are decisive for good germination and seedling development (Akshay et al., 2022; Li et al., 2022). At this stage, by applying sufficient stress to the seeds, the systems that respond to stress are activated, and thanks to this information, the seeds are better prepared for the stress they will encounter (Bhanuprakash & Yogeesha, 2016). Therefore, the present study was planned to determine the response of maize seeds primed with solutions with different salt concentrations to salt stress during germination and seedling stages.

MATERIAL and METOD

This study was carried out as a petri dish and pot experiment in the laboratory of the Department of Field Crops, Faculty of Agriculture, Yozgat Bozok University. Plant material Simpatico (FAO 300) and Ada-9510 (FAO 650) maize varieties were used in the study. Maize seeds were primed with different salt solutions and then germination and seedling growth under salt stress were investigated. Unprimed seeds were used as a control

Priming

For priming, maize seeds were kept in solutions containing 0, 150, 175, and 200 mM salt (NaCl) for 18 hours at 24 °C. Then they were washed thoroughly with distilled water and dried in an oven at 40 °C for 5 hours. Finally, the seeds were packaged and made ready for use in the experiment.

Salt Stress

Primed and Control seeds were tested in petri dishes and pots. To create salt stress, saline solution (175 mM, NaCl) was used in the initial irrigation in both media, and subsequent irrigations (for the pot media) were made with distilled water.

Petri Trial

The effect of treatments on the germination rate and speed of maize in the Petri dish was investigated. 10 seeds of all treatments were placed in petri dishes with a layer of filter paper in triplicate and then saline solution (175 mM) was added to the petri dishes. The prepared petri dishes were wrapped with parafilm and left to germinate. They were kept in dark conditions for the first two days, then in 16 hours light-8 hours dark conditions at a constant temperature of 24 °C. Germination results were started on the second day after planting and were completed at the end of the 7th day. In the experiment, the seeds whose root length reached 0.5 cm were accepted as germinated and the following characteristics were examined.

Germination rate: GR (%) = (Number of germinated seeds/total number of seeds) ×100 (Kayaçetin et al., 2018).

Germination speed: GS= $\sum[(G_1/D_1) + (G_2/D_2) + \ldots + (Gn/Dn)]$. G: number of germinated seeds, D: number of days (Czabator, 1962).

Pot Trial

Primed and control seeds were tested in 0.5 L pots to determine emergence and early seedling growth characteristics. 300 grams of peat was placed in each pot and 10 seeds were planted at a depth of 3 cm. After planting, the pots were irrigated with salt solution (175 mM) to field capacity and placed in conditions with 16 hours of light - 8 hours of darkness, and a constant temperature of 24 °C. This process was repeated 3 times for each application. Follow-up irrigations were made with pure water at 100% when each pot reached half of the field capacity. The pot experiment was continued for 21 days. Emerging speed and rate were determined in the first 10 days, and seedling characteristics were determined at the end of the 21st day.

Emergence traits

Emergence rate*: ER (%) = (Number of seeds to emergence/total number of seeds)×100

Emergence speed*: ES (day^{-1}) = $\sum[(E_1/D_1) + (E_2/D_2) + \ldots + (En/Dn)]$

E: number of emergence in the counting day, D: days.
* those with a coleoptile length of 1 cm were counted for emergence.

Seedling characteristics

At the end of the 21st day, shoot and root lengths were determined by measuring the actual lengths of the above-ground and underground parts of 5 seedlings in each pot. For the shoot and root dry matter ratio, the roots and shoots of all seedlings in each pot were weighed freshly and then dried in an oven at 65°C until constant weight. Finally, dry matter ratios were determined by the formula (Dry weight/fresh weight x 100).

Dried samples were ground in a mill to pass through a sieve with a diameter of 1 mm. Then crude protein, ADF (Fiber insoluble in acid detergent), NDF (Fiber Insoluble in Neutral Detergent), Potassium (K), Phosphorus (P), Calcium (Ca), and Magnesium (Mg) (%) contents were determined using Near Infrared Reflectance Spectroscopy (NIRS) (Foss 6500 and IC-0904-FE package program).

Statistic Analysis

The experiment was arranged in a split-plot design with three replications. Varieties were analyzed separately for a priming effect. The analysis of the data was made using the MSTAT_C statistical package program and differences between treatments were compared with Duncan's multiple range test (Bricker, 1991).

RESULTS and DISCUSSION

The effect of variety and priming on the germination rate and speed of maize in Petri media is given in Table 1. The difference between varieties was found to be significant (P<0.01) and, ADA-9510 had higher values in terms of both germination rate (75.33%) and germination speed (3.79 day^{-1}).

The effect of priming treatments on germination rate and speed was significant (P<0.01) in both cultivars. The highest germination rate of ADA-9510 was determined in control (93.33%), P0 (86.67%), and P3 (76.67%) applications, and the lowest in P2 (56.67%) and P1 (56.67%) applications. Accordingly, a positive effect of priming processes on the germination rate of the ADA-9510 variety was not observed in the petri dish. The germination rate of the Simpatico cultivar was significantly higher than the control (26.67%) in all priming treatments and was the highest in P0 (86.67%), P2 (76.67%) and P3 (63.33%) treatments (Table 1). These results show that Simpatico has a germination problem in saline conditions (175 mM), and this problem can be largely overcome by priming.

Table 1. Effect of priming on germination characteristics of maize varieties under saline (175 mM) conditions (Petri media)

Çizelge 1. Priming işleminin mısır çeşitlerinin tuzlu koşullarda (175 mM) çimlenme özelliklerine etkisi (petri ortamı)

Variety**	Priming **	Characteristic			
		GR (%)	±*Se*	GS (day^{-1})	±*Se*
ADA-9510	Control	93.33 a	*2.72*	3.73 ab	*0.12*
	P0 (0 mM)	86.67 a	*3.45*	4.53 a	*0.32*
	P1 (150 mM)	63.33 b	*4.21*	3.57 ab	*0.31*
	P2 (175 mM	56.67 b	*3.45*	2.63 b	*0.31*
	P3 (200 mM)	76.67 ab	*2.72*	4.50 a	*0.43*
	Mean	*75.33 A*		*3.79 A*	
Simpatico	Control	26.67 c	*3.82*	0.97 c	*0.27*
	P0 (0 mM)	86.67 a	*2.72*	5.30 a	*0.37*
	P1 (150 mM)	46.67 bc	*3.45*	1.53 c	*0.15*
	P2 (175 mM	76.67 ab	*3.90*	2.53 bc	*0.14*
	P3 (200 mM)	63.33 ab	*3.82*	3.43 b	*0.39*
	Mean	*60.00 B*		*2.75 B*	

**:P<0.01. *Se*: Standard error. There is no difference between the averages in the same column and with the same letter for each variety. GR: germination rate, GS: germination speed.

Similarly, germination speed was affected by priming in both cultivars and showed a parallel change with germination rate. The lowest germination speed in ADA-9510 was determined in the P2 (2.63 day^{-1}) treatment while the other treatments were in the highest group with values ranging from 3.73 to 4.53 days^{-1}. The germination rate of the Simpatico cultivar was highest in the P0 (5.30 day^{-1}) treatment while it was lowest in control (0.97 day^{-1}) and P1 (1.53 day^{-1}) treatments (Table 1). Accordingly, in terms of germination properties, priming application did not show any relieving effect on salt stress in ADA-9510 in petri conditions. On the other hand, priming affected the germination rate and speed positively in the Simpatico variety, but this effect was higher in water priming than in salty priming and decreased in salty priming depending on salt concentration.

In the pot conditions, slightly different results were found, which indicates that the growing conditions are also determinant in the response of plants to salt stress. The effect of variety and priming treatments on the emergence and seedling growth characteristics of maize was also significant (P<0.01) in the pot conditions (Table 2). On average, the highest emergence rate (66.31%), emergence speed (3.23 day^{-1}), root length (17.3 cm), and shoot length (31.63 cm) were determined in ADA-9510, and root dry weight (13.09 g) in Simpatico variety (Table 2). The effect of priming on emergence rate and speed was significant (P<0.01) in both cultivars. In terms of emergence rate, control (84.33%) and P0 (80.55%) treatments were in the highest group together in the ADA-9510 variety, while all other treatments formed the lowest group. In the Simpatico, the priming, except for P3, was found to be above the control. Priming treatments had a positive effect on the emergence speed in both varieties. P0 and P1 in the ADA-9510 variety and all priming treatments in Simpatico were above the

control regarding emergence speed. In this context, apart from the emergence rate of ADA-9510, priming with saline agents significantly improved the germination properties at low concentrations (150 and 175 mM). When seeds were primed with 200 mM solution, the positive effect was reduced and germination values were similar to the control. This indicates that the high salt content in the priming agent may have a toxic effect on maize. Many previous studies seem to be in great agreement with these results. Kaya & Gözübenli (2020) investigated the effect of priming with NaCl solutions on seedling development in two maize varieties (Pasha and P-31A34) in saline soils and reported that priming seeds with 5 g L^{-1} NaCl positively affects seedling growth. They also emphasized the importance of variety selection in saline soils. Similarly, Mahara et al. (2022) reported that seed priming with 5 g L^{-1} NaCl solution alleviated the inhibitory effect of salt stress on germination and seedling growth of maize.

Table 2. The effect of priming on emergence and seedling characteristics of maize cultivars under saline (175 mM) conditions (Pot media)

Çizelge 2. Priming işleminin mısır çeşitlerinin tuzlu koşullarda (175 mM) çıkış ve fide özelliklerine etkisi (saksı ortamı)

Variety**	Priming **	Characteristic											
		ER (%)	±*Se*	ES (gün^{-1})	±*Se*	RL (cm)	±*Se*	SL (cm)	±*Se*	RDMR (%)	±*Se*	SDMR (%)	±*Se*
ADA-9510	Control	84.33a	2.84	3.44 b	0.35	17.8	1.61	33.27 a	2.61	9.37 bc	1.13	9.52	1.71
	P0 (0 mM)	80.55 a	3.06	4.89 a	0.26	15.1	2.21	34.13 a	1.04	6.92 c	1.28	8.84	0.53
	P1 (150 mM)	61.11 b	3.84	3.31 ab	0.43	18.0	1.53	31.60 ab	1.89	15.44 a	3.92	9.00	0.25
	P2 (175 mM	55.56 b	3.54	2.36 b	0.13	17.7	0.52	32.20 ab	0.57	11.42 abc	1.07	10.28	0.94
	P3 (200 mM)	50.00 b	4.87	2.17 b	0.22	17.9	0.88	26.95 b	2.32	11.92 ab	3.21	9.80	0.73
	Mean	*66.31 A*		*3.23 A*		*17.3 A*		*31.63 A*		*11.02 B*		*9.49*	
Simpatico	Control	55.56 b	3.35	2.30 b	0.23	12.3	1.10	30.67 a	2.79	10.34 b	0.92	8.75	*0.42*
	P0 (0 mM)	80.56 a	1.36	3.81 a	0.38	12.5	1.00	24.47 b	1.91	11.99 b	0.37	9.15	0.60
	P1 (150 mM)	63.89 ab	5.90	3.33 a	0.30	13.1	0.48	24.20 b	1.23	17.15 a	1.71	9.20	0.28
	P2 (175 mM	75.00 a	5.19	3.64 a	0.28	13.2	1.37	27.88 ab	2.27	12.47 b	1.16	7.83	1.09
	P3 (200 mM)	55.56 b	3.01	2.53 ab	0.36	12.9	1.46	27.13 ab	1.98	13.52 b	1.44	8.06	0.58
	Mean	*66.11 B*		*3.12 B*		*12.8 B*		*26.87 B*		*13.09 A*		*8.60*	

**: P<0.01. *Se*: Standard error. There is no difference between the averages in the same column and with the same letter for each variety. ER: Emergence rate, ES: Emergence speed, RL: Root length, SL: Shoot length, RDMR: Root dry matter ratio, SDMR: Shoot dry matter ratio.

While shoot length and root dry matter ratio were significantly (P<0.01) different, root length and shoot dry matter ratio were found to be similar among the priming treatments in maize varieties (Table 2). The shoot length of ADA-9510 showed a significant decrease in P3 (26.95 g) but was similar in other treatments. In Simpatico, P2, P3, and control were in the highest group in terms of shoot length, but a significant decrease was recorded in P0 and P1 treatments. Interestingly, although the roots of the plants are first exposed to salt stress, the shoots may be more affected (Muns & Sharp, 1993). In both cultivars, an increase was recorded in the root dry matter ratio when priming with saline solution. The root dry matter ratio of the ADA-9510 variety was determined to be significantly higher, especially in saline priming (P1, P2, and P3). The highest root dry matter ratio of Simpatico was determined in P1 (17.15 g), and all other treatments were in the lowest group together (Table 2). These results show that priming in saline solutions has a positive effect on root dry matter ratio in maize, but this effect may vary depending on the variety and salt dose in the priming solution.

The effect of variety and priming on crude protein and mineral content of maize is given in Table 3. Crude protein content was affected by cultivars and was found to be higher in Simpatico (25.86%) on average. The effect of priming treatments on crude protein content was significant only in the ADA-9510 variety. The crude protein content of the ADA-9510 variety was above the control (23.04%) in priming, and the highest was detected in P1 (25.20%) and P2 (25.44%) treatments. The crude protein content of Simpatico was similar in all treatments and was between 25.18% (P3) and 26.86% (control). Growth and development in plants is a process dependent on physiological and biochemical mechanisms. In this

respect, salt stress affects the chemical content of plants as well as their morphological features, and this effect is due to the combination of dry matter accumulation, ion uptake and relationships, water status, biochemical reactions, and/or many physiological reactions (Sohan et al., 1999). Therefore, the response of plants against salt stress depends on the effectiveness of these mechanisms. The protein mechanism and accumulation of mazie undergo significant changes under salt stress, and the level of salt stress is also important in this change. Indeed, the change in protein regulation of protein roots and shoots in maize under 25 mM NaCl stress was 45% and 31%, respectively, while this level was 80% for total separated proteins under 100 mM NaCl salt stress (Zörb et al., 2004). Arora et al. (2008) found that the pre-sowing treatment of maize seeds with 28-homobrassinolide promoted antioxidative enzyme activity, resulting in a decrease in lipid peroxidation and an increase in protein content.

Table 3. Effect of priming on crude protein and mineral matter (P-K-Ca-Mg) content of maize seedlings grown in salty conditions (175 mM) (Pot media)

Çizelge 3. Priming işleminin tuzlu koşullarda (175 mM) yetişen mısır fidelerinin ham protein ve mineral madde (P-K-Ca-Mg) içeriğine etkisi (saksı ortamı)

Variety**	Priming **	Characteristic					
		Crude protein (%)	±*Se*	P (%)†	K (%)†	Ca (%)†	Mg (%)†
ADA-9510	Control	23.04 c	*0.08*	0.57 bc	4.40 d	0.40 a	0.17 b
	P0 (0 mM)	24.76 b	*0.05*	0.58 b	4.65 b	0.41 a	0.18 b
	P1 (150 mM)	25.20 a	*0.10*	0.59 a	4.62 bc	0.41 a	0.19 a
	P2 (175 mM	25.44 a	*0.08*	0.59 ab	4.71 a	0.41 a	0.17 b
	P3 (200 mM)	21.77 d	*0.08*	0.56 c	4.59 c	0.34 b	0.17 b
	Mean	*24.04 B*		*0.58 B*	*4.59 A*	*0.39*	*0.18 A*
Simpatico	Control	26.86	*0.33*	0.62	4.76	0.32	0.14 b
	P0 (0 mM)	25.31	*0.68*	0.62	4.32	0.42	0.21 a
	P1 (150 mM)	26.39	*0.65*	0.58	3.79	0.37	0.22 a
	P2 (175 mM	25.58	*0.27*	0.60	4.28	0.43	0.18 ab
	P3 (200 mM)	25.18	*0.19*	0.60	4.15	0.35	0.19 ab
	Mean	*25.86 A*		*0.60 A*	*4.26 B*	*0.38*	*0.19 B*

*:P<0.05, **: P<0.01. *Se*: Standard error. †: *Se*<0.01. There is no difference between the averages in the same column and with the same letter for each variety.

The effect of variety on the P, K, and Mg content of maize was significant (P<0.01). The average P content was found to be higher in Simpatico (0.60%) and, the K and Mg content was higher in ADA-9510 (4.59% and 0.18%) (Table 3).

Priming treatments affected the mineral content significantly (P<0.01), especially in ADA-9510. In general, the mineral content of ADA-9510 was positively affected by priming except Ca. The highest P, K, and Mg contents in ADA-9510 were detected in priming with saline solution. This positive effect is particularly evident in priming with low-salt solutions (P1 and P2). The Ca content of ADA-9510 was adversely affected by high-salt solution priming (P3) but was similar in other treatments. In Simpatico, treatments were only effective on Mg content and all priming treatments were in the same group and above control in terms of Mg. Under salt stress, the uptake of nitrogen, calcium, potassium, magnesium, and iron by plants decreases (Karimi et al., 2005; Kaya et al., 2010) and excess sodium and chlorine in salty soils limit the uptake of other elements, leading to imbalance in the chemical content of maize (Turan et al., 2010). Present results showed that priming affected the chemical response of maize to salt stress related to protein and mineral content. In previous studies, the stress-reducing effect of different priming agents has been reported. Seed priming with NaCl stimulates various metabolic and physiological processes in plants during germination and early growth stages (Abraha & Yohannes, 2013; Kaya & Gozubenli, 2020). It was reported that 60 and 120 mM NaCl significantly reduced growth, development, photosynthesis, and enzyme (catalase and peroxidase) activity and leaf anatomy in maize, but the toxic effect of salt stress was alleviated by presoaking the seed with salicylic acid and 24-epibrassinolide (Agami, 2013). Gunes et al. (2005) reported that exogenously applied salicylic acid significantly enhances plant growth in both saline and unsalted conditions by acting as an endogenous signaling molecule responsible for inducing abiotic

stress tolerance. The authors also reported that salicylic acid strongly inhibited the accumulation of Na and Cl, but stimulated N, Mg, Fe, Mn, and Cu concentrations of salt-stressed maize plants, so it could be used as a potential growth regulator to improve salinity stress resistance of the plant. Similarly, the application of Brassinosteroids, a phytohormone group of steroids to rice seeds alleviated the adverse effects of salt stress by restoring the pigment level, increasing nitrate reductase, nucleic acid, and proteins (Anuradha & Rao 2001).

CONCLUSION

Seed priming with water and salty (NaCl) solutions improved germination characteristics, seedling growth, and the chemical content of maize under salinity stress. However, this effect was significantly dependent on the variety and the salt concentration of the priming agent. Priming with water was also effective in reducing the negative consequences of salt stress. However, it was observed that saline agents at certain concentrations could be more effective. In this study, 150 and 175 mM NaCl solutions were found to be effective for maize. On the other hand, the results showed that the cultivars differed very significantly in both salt resistance and priming response. The germination and emergence results in the control treatment showed that Simpatico variety is more sensitive to salinity (175 mM), but this can be alleviated highly by priming. According to general results, the ADA-9510 variety is more durable under salt stress and also responded positively to the applied priming treatments. This shows that priming with low-salty solutions and water may alleviate the negative effects of salt stress in both salt-tolerant and sensitive varieties.

ACKNOWLEDGEMENTS

Produced from Sema Nazlı's master's thesis

Statement of Conflict of Interest

The authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

REFERENCES

Abraha, B., & Yohannes, G. (2013). The role of seed priming in improving seedling growth of maize (Zea mays L.) under salt stress at field conditions. *Agricultural Sciences, 4*(12), 666-672.

Agami, R.A. (2013). Alleviating the adverse effects of NaCl stress in maize seedlings by pretreating seeds with salicylic acid and 24-epibrassinolide. *South African Journal of Botany, 88*, 171-177.

Ajouri, A., Asgedom, H., & Becker, M. (2004). Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. *Journal of Soil Science and Plant Nutrition, 167*(5), 630-636.

Akshay, U. N, Durga, P. N. B., Ramanjulu, S., Sreenivas, C., & Annapurna, D. A. (2022). Molecular basis of priming-induced acquired tolerance to multiple abiotic stresses in plants. *Journal of Experimental Botany, 73*(11), 3355–3371.

Anuradha, S., & Seeta, R. R. S. (2001). Effect of brassinosteroids on salinity stress-induced inhibition of seed germination and seedling growth of rice (Oryza sativa L.). *Plant Growth Regulation, 33*, 151–153. https://doi.org/10.1023/A:1017590108484

Arora, N., Bhardwaj, R., Sharma, P., & Arora H. K. (2008). 28-Homobrassinolide alleviates oxidative stress in salt-treated maize (Zea mays L.) plants. *Brazilian Journal of Plant Physiology, 20*, 153-157.

Ashraf, M., & Rauf, H. (2001). Inducing salt tolerance in maize (Zea mays L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. *Acta Physiologiae Plantarum, 23*, 407-417.

Baghel, L., Kataria, S., & Jain, M. (2019). Mitigation of adverse effects of salt stress on germination, growth, photosynthetic efficiency, and yield in maize (Zea mays L.) through magneto priming. *Acta Agrobot., 72*(1), 1757. https://doi.org/10.5586/aa.1757

Bakht, J., Shafi, M., Jamal, Y., & Sher, H. (2011). Response of maize (Zea mays L.) to seed priming with NaCl and salinity stress. *Spanish Journal of Agricultural Research 9*(1), 252-261.

Bhanuprakash, K., & Yogeesha, H.S. (2016). Seed priming for abiotic stress tolerance: an overview. N.K. Srinivasa Rao, K.S. Shivashankara, R.H. Laxman (Eds.), Abiotic Stress Physiology of Horticultural Crops. Springer, pp. 103-117.

Bricker, B. (1991). MSTATC: A Micro-Computer Program for the Design, Management, and Analysis of Agronomic Research Experimentation. Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan.

Carpıcı, E. B., Celik, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (Zea mays L.) cultivars. *African Journal of Biotechnology 8*(19), 4918- 4922.

Czabator, F. J. 1962. Germination value: An index combining speed and completeness of pine seed germination. *Forest Science, 8*, 386-395.

FAO Soil Portal, (2023) https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/global-map-of-salt-affected-soils/en/ (Accessed: May 8, 2023).

Farooq, M., Hussain, M., Wakel, A., & Siddique, K. H. M. (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development, 35*, 461–481.

Gebreegziabher, B. G., & Qufa, C. A. 2017. Plant Physiological Stimulation by Seeds Salt Priming in Maize (Zea mays): Prospect for Salt Tolerance. *African Journal of Biotechnology, 16*, 209-223.

Goldsworthy, (1994). Calcium and salinity. *Appl Biol.*, *4*, 1-6.

Gunes, A., Inal, M., Alpaslan, F., Eraslan, E. G., & Bagci, N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity, *Journal of Plant Physiology, 164* (6), 728-736.

Jamil, A., Riaz, S., Ashraf, M., & Foolad, M.R. (2011). Gene expression profiling of plants under salt stress. *CRC Crit. Rev. Plant Sci.*, *30*, 435–458. doi: 10.1080/07352689.2011.605739.

Karimi, G., Ghorbanli, M., Heidari, H., Khavari Nejad, R.A., & Assareh, M.H. (2005). The effects of NaCl on growth, water relations, osmolytes, and ion content in Kochia prostrata. *Biologia plantarum, 49*(2), 301-304. doi: 10.1007/s10535-005-1304-y

Kaya, A., & Gözübenli, H. (2020). Tohumları farklı NaCl dozları ile muamele edilen mısırın tuzlu topraklarda fide gelişiminin belirlenmesi. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 25* (3), 394-405 . DOI: 10.37908/mkutbd.755170

Kaya, C., Tuna., A. L., & Okant, A. M. (2010). Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. *Turk J Agric For., 34*, 529–538. doi:10.3906/tar-0906-173

Kayacetin, F., Efeoglu, B., & Alizadeh, B. (2018). Effect of NaCl and PEG-Induced Osmotic Stress on Germination and Seedling Growth Properties in Wild Mustard (Sinapis arvensis L.). *ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 28*(1), 62-68.

Khan, I., Muhammad, A., Chattha, M. U., Skalicky, M., Bilal Chattha, M., Ahsin Ayub, M., Rizwan Anwar, M., Soufan, W., Hassan, M.U., Rahman, M.A., Brestic, M., Zivcak, M., & El Sabagh, A. (2022). Mitigation of Salinity-Induced Oxidative Damage, Growth, and Yield Reduction in Fine Rice by Sugarcane Press Mud Application. *Front. Plant Sci., 13*, 840900. doi: 10.3389/fpls.2022.840900

Kumar, M., Pant, B., Mondal, S., & Bose, B. (2016). Hydro and halo priming: influenced germination responses in wheat var-HUW-468 under heavy metal stress. *Acta Physiol. Plant., 38*(9), 217. doi:10.1007/s11738-016-2226-3

Li, P., Yang, H., Wang, L., Liu, H., Huo, H., Zhang, C., Liu, A., Zhu, A., Hu, J., Lin, Y., & Liu, L. (2019). Physiological and transcriptome analyses reveal short-term responses and the formation of memory under drought stress in rice. *Front. Genet., 10*, 55. doi: 10.3389/fgene.2019.00055

Mahara, G., Bam, R., Kandel, M., Timilsina, S., Chaudhary, D., Lamichhane, J., Bajgai, T. R., Pant, B. R., Bhattarai, U., & Upadhyaya, J. (2022). Seed priming with NaCl improves germination in maize under saline soil conditions. *Eurasian Journal of Soil Science, 11*(2), 151-156. doi: 10.18393/ejss.1027558

Munns, R., & Gilliham, M. 2015. Salinity tolerance of crops – what is the cost? New Phytol., 208, 668-673.

Munns, R., & Sharp, R. E. (1993). Involvement of abscisic acid in controlling plant growth in soils of low water potential. *Aust J Plant Physiol., 20,* 425–437. doi:10.1071/PP9930425

Munns, R., James, R. A., & Lauchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany, 57*, 1025-1043.

Neubert, A. B., Zörb, C., & Schubert, S. (2005). Expression of vacuolar Na+/H+antiporters (ZmNHX) and Na+ exclusion in roots of maize (Zea mays L.) genotypes with improved salt resistance In Li CJ et al. (eds)Plant nutrition for food security, human health, and environmental protection, Tsinghua University Press, Bejing, China, pp 544–54.

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety, 60,* 324-349.

Pitann, B., Zörb, C., & Mühling, K. H. (2009). Comparative proteome analysis of maize (Zea mays L.) expansins under salinity. *J Plant Nutr Soil Sci., 172*, 75–77. doi:10.1002/jpln.200800265

Rengasamy, P. (2006). World salinization with emphasis on Australia. *J Exp Bot., 57*(5), 1017-23. doi: 10.1093/jxb/erj108.

Singh, D., & Kumar, A. (2021). A multivariate screening approach indicated adaptive tolerance to salt stress in the seedlings of an agroforestry tree, Eucalyptus tereticornis Sm. *Plant Cell Tissue Organ Cult., 145*(3), 545–560. doi:10.1007/s11240-021-02025-2

Sohan, D., Jasoni., & R, Zajicek, J. (1999). Plant-water relation of NaCl and calcium treated sunflowers plants. *Envi. Experi. Bot., 42*, 105-111.

Sonmez, B. (2003). Guide for Salinity Control in Turkey. Ankara, Turkey; 2003.

Szalai, G., & Janda, T. (2009). Effect of salt stress on the salicylic acid synthesis in young maize (Zea mays L.) plants. *J Agron Crop Sci., 195*, 165–171. doi:10.1111/j.1439-037x.2008.00352.x

Turan, M. A., Elkarim, A. H. A., Taban, N., & Taban, S. (2010). Effect of salt stress on growth and ion distribution and accumulation in shoot and root of maize plant. *Afr J Agric Res., 5*, 584-588.

Vinocur, B., & Altman, A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology, 16,* 123-132.

Yetissin F., & Karakaya A. (2022). Investigation of the effects of acetone o-(4 chlorophenylsulfonyl) oxime pre-application on biochemical parameters of maize seedlings under salt stress. *ACUJFF, 23*(1), 74-83.

Zörb, C., Schmitt, S., Neeb, A., Karl, S., Linder, M., & Schubert, S. (2004). The biochemical reaction of maize (Zea mays L.) to salt stress is characterized by mitigation of symptoms and not by a specific adaptation. *Plant Sci., 167*, 91-100. doi:10.1016/j.plantsci.2004.03.004.

KSÜ Tarım ve Doğa Derg 27 (4), 901-909, 2024
KSU J. Agric Nat 27 (4), 901-909, 2024
DOI:10.18016/ksutarimdoga.vi.1285098

The Performance of Multi-Parental Cotton (*Gossypium hirsutum* L.) Hybrid Genotypes

Ferhat ÇAKMAK[1], Volkan Mehmet ÇINAR[2], Şerife BALCI[3], Aydın ÜNAY[4]✉
[1]Aydın Adnan Menderes University, Graduate School of Natural and Applied Sciences, Aydın/TÜRKİYE, [2]Postdoctoral Researcher, Aydın Adnan Menderes University, Faculty of Agriculture, Department of Field Crops, Aydın/TÜRKİYE, [3]Cotton Research Institute, Nazilli-Aydın/TÜRKİYE, [4]Aydın Adnan Menderes University, Faculty of Agriculture, Department of Field Crops, Aydın/TÜRKİYE
[1]https://orcid.org/0000-0003-2211-2145, [2]https://orcid.org/0000-0001-5822-5649, [3]https://orcid.org0000-0001-8348-3674
[4]https://orcid.org/0000-0002-7278-4428
✉: aunay@adu.edu.tr

ABSTRACT

We aimed to evaluate the possibilities of increasing the ginning out-turn in multi-parent hybrid populations of cotton. Two genotypes with high ginning out-turn were crossed with seven F_3 populations obtained from double crosses, and then fourteen F_1 populations were created in 2020. The F_1 populations, their grandparents, and two parents, a total of 23 genotypes, were compared by Randomized Complete Block Design with three replications in 2021. Significant differences were detected among genotypes, including crosses and parents for seed cotton yield per plant, ginning out-turn, fiber fineness, and fiber strength. The orthogonal contrasts indicated that the average performance of hybrids was significantly higher than that of parents for ginning out-turn, seed cotton yield per plant, and fiber fineness. Standard heterosis was between -11.19% and 20.54% for seed cotton yield per plant; 4.71% and 16.03% for ginning out-turn. [(ST-468 × Claudia) × (Gloria × Carisma)] × Esperia should be transferred to further generations. Multi-parent hybrids could be used to create the required variance and maintain dominance for the improvement of yield and ginning out-turn.

Field Crops

Research Article

Article History
Received : 18.04.2023
Accepted : 04.01.2024

Keywords
Fiber Quality
F_1 Populations
Ginning Out-Turn
Orthogonal Contrasts
Standard Heterosis

Çok Ebeveynli Pamuk (*Gossypium hirsutum* L.) Melez Genotiplerinin Performansı

ÖZET

Çok ebeveynli melez pamuk popülasyonlarında çırçır randımanını artırma olanakları değerlendirilmiştir. Sekiz adet çift melez F_3 melez popülasyonu ile yüksek çırçır randımanına sahip iki genotip melezlenmiş ve 14 farklı F_1 melez popülasyonu elde edilmiştir. Melezlerden ve ebeveynlerden oluşan genotipik farklılık tek bitki kütlü pamuk verimi, çırçır randımanı, lif inceliği ve lif dayanıklılığı yönünden önemli bulunmuştur. Ortogonal karşılaştırmalar melezlerin ortalama performansının çırçır randımanı, tek bitki kütlü pamuk verimi ve lif inceliği yönünden ebeveyn ortalamasından önemli düzeyde farklı olduğunu göstermiştir. Standart heterosis tek bitki kütlü pamuk için %-11.19 ile %20.54; çırçır randımanı için %4.71 ile %16.03 arasında değişmiştir. [(ST-468 × Claudia) × (Gloria × Carisma)] × Esperya melez kombinasyonunun ileri generasyonlara aktarılması gerektiği saptanmıştır. Çırçır randımanı ve kütlü pamuk verimini artırmayı amaçlayan ıslah çalışmalarında dominantlığı sürdürmek ve varyasyonu oluşturmak için çok ebeveynli melezlerin kullanılabileceği sonucuna varılmıştır.

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 18.04.2023
Kabul Tarihi : 04.01.2024

Anahtar Kelimeler
Çırçır Randımanı
F_1 Popülasyonları
Lif Kalitesi
Ortogonal Karşılaştırma
Standart Heterosis

Atıf İçin: Çakmak, F., Çınar, V.M., Balcı, Ş., & Ünay, A. (2024). Çok Ebeveynli Pamuk (*Gossypium hirsutum* L.) Melez Genotiplerinin Performansı. *KSÜ Tarım ve Doğa Derg 27* (4), 901-909. DOI: 10.18016/ksutarimdoga.vi.1285098

To Cite: Çakmak, F., Çınar, V.M., Balcı, Ş., & Ünay, A. (2024). The Performance of Multi-Parental Cotton (*Gossypium hirsutum* L.) Hybrid Genotypes. *KSU J. Agric Nat 27* (4), 901-909. DOI: 10.18016/ksutarimdoga.vi.1285098

INTRODUCTION

Cotton, grown in tropical, semi-tropical, and semi-arid areas of the world, is included in the genus Gossypium. Cotton fiber and seed provide raw materials to the textile and edible oil industries,

respectively (Shahzad et al., 2019; Tarazi et al., 2020). In Türkiye, the pricing of cotton by cooperatives and private buyers is primarily based on the ginning out-turn (GOT). In addition, GOT is one of the most important yield components, and genotypic performance for high GOT has been extensively used in cotton breeding (Desalegn, 2016). The quantitative characteristics such as seed cotton yield and ginning out-turn exhibited polygenic heritage and broad variations in segregating generation of cotton (Memon et al., 2017; Monicashree et al., 2017; Premalatha et al., 2020; Balci et al., 2021b). Therefore, cotton breeders concentrate on optimizing the high ginning out-turn and seed cotton yield with fiber characteristics within commercial limits (Akbar et al., 2009; Ahuja et al., 2018). The results of simple correlation and path analysis demonstrated that ginning out-turn is one of the attributes most to seed cotton yield (Zhou, 1986; Choudhari et al., 1988; Ahmad ve Azhar, 2000; Salahuddin et al., 2010; Erande et al., 2014).

Ibragimov (1989) announced that higher seed cotton yield and ginning out-turn brought with coarse fiber in cotton genotypes. The significant and negative association between seed cotton yield and ginning out-turn (Dinakaran et al., 2012; Parmar et al., 2015) and ginning out-turn with seed weight, boll weight, fiber length, and fiber fineness (Karademir et al., 2010) were underlined. The presence of a negative genetic correlation among ginning out-turn, seed cotton yield, and fiber quality characters can often limit the success of breeding (Yu et al., 2013), and this negative association needs to be broken by the different methods (Islam et al., 2016). A multiparent advanced generation inter-cross (MAGIC) population can be a good method to eliminate the mentioned negative linkage compared with bi-parental populations having low allelic diversity (Jenkins et al., 2008). MAGIC populations can result from cycles of recurrent selection aimed at combining favorable alleles.

In another study, F_4 and F_5 cotton plants were crossed with opposite and diverse testers to determine the performances of heterotic groups and the combining abilities of testers (Girish et al., 2019). To compare the heterotic populations and to predict the double cross performance, the robust and compact cotton types were crossed in Line × Tester mating design, and it was concluded that crosses involving between group genotypes (interplant type) are highly heterotic (Ranganatha et al., 2013).

Heterobeltiosis and standard heterosis are the performance of F_1 over the better parent and over the standard check to identify the best cross combination (Shashibhushan & Patel, 2019; Kumbhalkar et al., 2021; Chapara & Madugula, 2021).

The previous studies on double cross population assessed the hybrid performance in terms of yield, yield components, and fiber quality characteristics (Yehia et al., 2009; El-Hashash, 2013; Ekinci et al., 2016). In this study, seven F_3 populations of double crosses obtained from recurrent selection were crossed with two parents with high ginning out-turn, and then 14 F_1 populations having five parents were obtained. We aimed (1) to evaluate the cross combinations, (2) to compare the performance of cross combinations over different check varieties via contrast parameters, and (3) to determine the cross combinations to be transferred to the further generations.

MATERIALS and METHODS

The 7 F_3 populations derived from 4 × 3 reciprocal line × tester crosses and recurrent selection (cycle 1) were crossed with Esperia (ES) and advanced line (Genotype-I; G1) having high ginning out-turn in 2020. The details of the F_3 population development were described by Balci et al., 2021a; Balci et al., 2021b) and summarized in Figure 1.

Since the theoretical segregation in the F_3 generation was 75% homozygous and 25% heterozygous, at least 1 cross was made from each plant of all F_3 populations. Fourteen F_1 populations and 9 of their grandparents and parents, 23 genotypes, were planted in Randomized Complete Block Design with three replications in 2021. The weather of the experimental location (Nazilli-Aydin/Turkey; 37˚86' N, 28˚37' E) was defined as mild, generally warm, and temperate within the class of Csa by Köppen and Geiger. Experimental soil characteristics were slightly alkali, high lime content, adequate potassium, low organic matter, nitrogen, and phosphorus.

The seeds of F_1 and their parents were planted 0.12 m apart with 0.70 m of row spacings. Each plot consisted of one row of 6 m long. Field practices such as fertilization, irrigation, pest, and weed control were managed according to the national recommendation for the cotton growing of the Aegean Region in Türkiye. Ten plants per experimental unit were randomly exampled for data collection, as suggested by Sahito et al. (2016). Seed cotton yield per plant (g), ginning out-turn (%), fiber fineness (mic.), fiber length (mm), and fiber strength (g tex^{-1}) were recorded. The laboratory roller gin was used for ginning out-turn, and the USTER® HVI-1000 instrument was used to determine fiber quality properties.

Data were subjected to analysis of variance using the JMP® 14 statistical program (SAS Institute Inc. 2018), and genotypic differences were tested by using the LSD (0.05) test (Steel & Torrie, 1980). The LSD means contrast function, as implemented in JMP® 14, has been used to test orthogonal contrasts between

treatments using F statistics for different means. Orthogonal contrasts for ginning out-turn; C_1; Esperia crosses vs. Genotype-I crosses, C_2; All crosses vs. mean of Esperia and Genotype-I, C_3; All crosses vs. all parents, C_4; Esperia crosses vs. best variety, C_5; Genotype-I crosses vs. best variety and C_6; all crosses vs. best variety. Orthogonal contrasts for other characters; C_7; all crosses vs. check variety (Gloria), and C_8; all crosses vs. best variety.

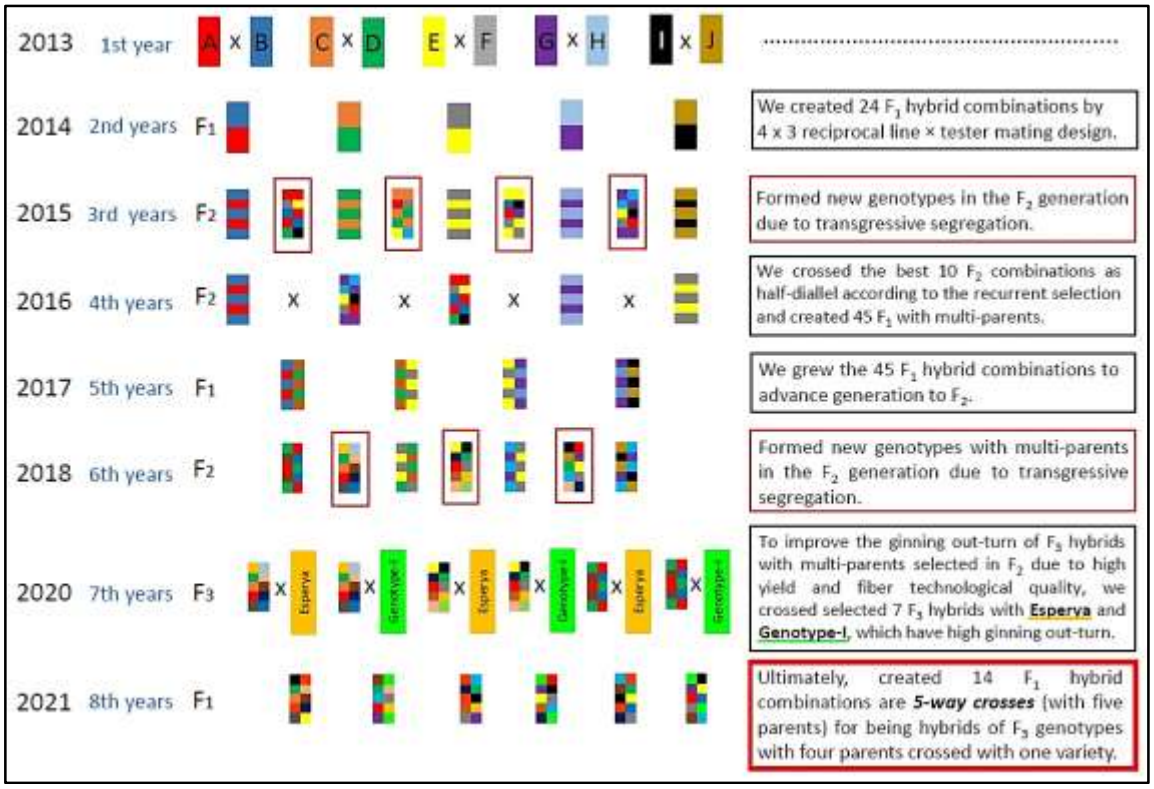


Figure 1. Breeding actions between 2013 and 2021
Şekil 1. 2013 ve 2021 yılları arasında sürdürülen ıslah çalışmaları

The F_1 performance over standard over-check variety was defined as standard heterosis, and the formula;

Standard heterosis (%) = $\frac{F1-check\ variety}{check\ variety} \times 100$ (Fonseca and Patterson, 1968). (1)

The standard error for the significance of standard heterosis was $\sqrt[2]{\frac{2 \times mean\ sum\ of\ square\ due\ to\ error}{number\ of\ replications}}$, according to Cohran & Cox (1957). (2)

RESULTS and DISCUSSION

Genotypic differences were found to be significant for seed cotton yield per plant, ginning out-turn, fiber fineness, and fiber strength (Table 1). The results are in concordance with the findings of Arain (2015), Baloch et al. (2015), Memon et al. (2017), Monicashree et al. (2017) and Premalatha et al. (2020).

Seed cotton yield

[(CA × ST) × (ST × CL)] × G1 (53.66 g), [(ST × CL) × (GL × CR)] × G1 (54.04 g) and [(CR × CA) × (GL × FL)] × ES (54.44 g) were exhibited the lowest seed cotton yield per plant in crosses, whereas the highest seed cotton yield per plant recorded in [(ST × CL) × (GL × CR)] × ES (73.89 g). The comparison of genotypic means indicated that Esperia had the highest seed cotton yield per plant (71.82 g) among parents (Table 2). In addition, mean data showed that seed cotton yield per plant of 6 out of 14 combinations was over the grand mean of crosses (60.57 g). Although orthogonal contrast (C_3) between parents (55.76 g) and crosses means (60.57 g) was favorable significant, crosses vs. check variety (61.30 g) and crosses vs. best variety (71.82 g) were unfavorable significant (C_7 and C_8) (Table 3). In this case, [(ST × CL) × (GL × CR)] × ES was superior for seed cotton yield per plant and was followed by combinations [(GL × FL) × (GL × CR)] × G1, [(CR × CA) × (GL × FL)] × G1 and [(JU × ST) × (CR × CA)] × ES. The positive and significant standard heterosis value of all four combinations corrected the performance of cross combinations. In previous studies, it was revealed that standard heterosis ranged from -12.63% to 30.90%, with 25 of 36 crosses being positive (Bilwal et al., 2018) and from -28.29 to 47.03% with significant and positive in 9 out of 32

crosses (Rathava et al., 2018). We also estimated that standard heterosis was between -12.47% and 20.53%, and 4 out of 14 crosses had significant and positive values (Table 4). In this study, the grandparents and parents in pedigrees of hybrids were the important cultivars grown in recent years. Therefore, the maximum level of standard heterosis in our crosses was lower than that of Bilwal et al. (2018) and Rathawa et al. (2018).

Table 1. Means used for orthogonal contrasts
Çizelge 1. Ortogonal karşılaştırmalar için kullanılan ortalama değerler

Means	SCY	GOT	FL	FF	FS
The grand mean of Crosses	60.57±0.91	45.22±0.24	29.58±0.17	5.34±0.04	31.22±0.19
The grand mean of Parents	55.76±1.44	42.64±0.30	29.66±0.18	4.91±0.05	31.42±0.33
Grand Mean of Genotypes	58.69±0.84	44.21±0.24	29.61±0.13	5.17±0.04	31.30±0.18
Mean of Esperia Crosses		43.99±0.17			
Mean of Genotype-I Crosses		46.45±0.23			
Mean of Esperia and Genotype-I		44.89±0.51			

SCY: Seed cotton yield (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (micronaire); FS: Fiber strength (g tex^{-1}).

Ginning Out-Turn

The main purpose of our study is to increase the ginning out-turn in cross combinations. The range of ginning out-turn was 43.32 - 48.00% in cross combinations and 40.73 - 46.11% in parents and grandparents. The ginning out-turn of all cross combinations was higher than all parents except Genotype-I, while four cross combinations, [(JU × ST) × (CR × CA)] × G1, [(GL × FL) × (GL × CR)] × G1, [(CA × ST) × (GL × FL)] × G1 and [(JU × ST) × (GL × CR)] × G1, were higher than Genotype-I (Table 2). In addition, the mean of all crosses and Genotype I crosses were 45.22% and 46.45%, respectively (Table 1). The significant orthogonal contrast values, such as C_2 and C_3, indicated that the performance of all crosses was superior to the mean of Esperia and Genotype-I and all parents (Table 3). Although no significant C_5 confirmed the equality of the mean of the Genotype-I crosses and the best variety (Genotype-I), the mean of all crosses was significantly lower than the best variety Genotype-I (C_6). Moreover, standard heterosis (compared to Gloria) was between 4.71% and 16.03%, with 9 out of 14 crosses being significant. Murthy et al. (2017) found that the mean performance of 4 F_1 populations ranged from 35.74% to 40.98%, and heterosis over two check varieties was completely favorable. Mudhalvan et al. (2021) determined that the ginning out-turn was between 25.47% and 39.10% in 30 F_1 combinations (*Gossypium hirsutum* L), and standard heterosis was mostly negative and significant (Table 4). In another study, the values of the advanced lines selected for ginning out-turn varied between 36.50% and 45.50%, and the average of the control varieties was 40.9% (Çoban and Çiçek, 2017). We recorded higher performance for all cross combinations than the mentioned studies regarding ginning out-turn.

Fiber Length

The most important fiber quality characteristics are fiber length, fineness, and strength in terms of the fiber value for spinning into yarn and marketing. Developing new cultivars with improved fiber properties is the main target of cotton breeders (Constable et al., 2015). The range of fiber length was 28.71 - 30.87 mm for cross combination and 28.63 – 30.87 mm for all parents (Table 2). Although genotypic differences were nonsignificant, the mean of all crosses (29.58) was significantly shorter than that of the best variety, Carmen (30.87 mm). These findings were incompatible with the results obtained by Monicashree et al. (2017) and Premalatha et al. (2020). Despite all this, the maximum fiber length value in our study was higher than that of Arain et al. (2015) and Baloch et al. (2015). [(ST × CL) × (GL × CR)] × G1, [(CA × ST) × (ST × CL)] × G1, [(CA × ST) × (GL × FL)] × G1 and [(CA × ST) × (GL × FL)] × ES were highly performed for fiber length over 30.0 mm, while standard heterosis was favorable in the first three of these hybrids (Table 4). Earlier studies reported that the means of fiber length were 30.0 mm for crosses and 28.1 mm for parents, and standard heterosis was between 2.75% and 14.43% (Ashokkumar et al., 2013). It was seen that genetic variability and standard heterosis were not created for fiber length in the population where nine commercial varieties and advanced lines were crossed.

Fiber Fineness

The stronger and faster yarn process can only be realized thanks to finer mature fibers (Steadman, 1997). The earlier opposite association has been reported among ginning out-turn and fiber length and micronaire, while the direct association between ginning out-turn and strength and micronaire (Percy et al., 2006). Fiber fineness of cross combinations and

parents were 5.06 - 5.58 (mic.) and 4.61 - 5.11 (mic.), respectively (Table 2). Although, [(ST × CL) × (GL × CR)] × ES, [(ST × CL) × (GL × CR)] × G1 and [(JU × ST) × (GL × CR)] × ES exhibited the fiber fineness between 5.06 and 5.13 (mic.), other 11 cross combinations had coarse fibers. The fiber fineness of Carisma was considerably finer, and it was seen that three combinations with acceptable fibers have Carmen in their pedigrees. All defined orthogonal contrasts for fiber fineness were significant, and the mean of crosses (5.34 min.) was coarser than the mean of all parents (4.91 min.), standard variety (Gloria; 4.88 mic.), the finest variety (Carisma; 4.61 mic.). These findings indicated that our hybrid population evolved in the direction of coarse fibers. The significant association between ginning out-turn and fiber fineness (Saeed et al., 2014) indicated that coarse fibers could arise from high ginning out-turn in cotton breeding. As Çakmak et al. (2023) emphasized, ginning out-turns should be kept at a certain level to obtain fiber fineness values between certain limits.

Table 2. Mean values of the grandparents, parents, and cross combinations.
Çizelge 2. Ebeveynler ve melez kombinasyonlara ilişkin ortalama değerler

	SCY	GOT	FL	FF	FS
Combinations					
[(CA × ST) × (ST × CL)] × ES	56.60 de	43.78 e-g	28.77	5.48 a-c	31.13 b-g
[(CA × ST) × (ST × CL)] × G1	53.66 fg	45.62 c	30.74	5.32 a-f	29.60 g
[(JU × ST) × (GL × CR)] × ES	60.58 c	43.32 g	29.87	5.13 b-h	31.10 b-g
[(JU × ST) × (GL × CR)] × G1	60.50 c	46.16 bc	29.45	5.38 a-e	30.90 d-g
[(CR × CA) × (GL × FL)] × ES	54.44 e-g	45.63 c	28.76	5.58 a	31.23 b-g
[(CR × CA) × (GL × FL)] × G1	66.90 b	45.01 d	29.22	5.45 a-d	30.17 e-g
[(JU × ST) × (CR × CA)] × ES	65.59 b	43.47 fg	28.71	5.15 b-h	31.37 b-g
[(JU × ST) × (CR × CA)] × G1	57.76 d	48.00 a	29.36	5.41 a-e	31.00 c-g
[(ST × CL) × (GL × CR)] × ES	73.89 a	43.95 of	29.08	5.06 e-h	32.50 a-d
[(ST × CL) × (GL × CR)] × G1	54.04 fg	45.84 c	30.87	5.12 b-h	31.63 b-g
[(GL × FL) × (GL × CR)] × ES	62.78 c	44.10 e	29.50	5.49 ab	32.13 a-e
[(GL × FL) × (GL × CR)] × G1	66.97 b	47.84 a	29.47	5.58 a	30.93 d-g
[(CA × ST) × (GL × FL)] × ES	57.74 d	43.67 e-g	30.06	5.40 a-e	31.63 b-g
[(CA × ST) × (GL × FL)] × G1	56.59 de	46.65 b	30.33	5.22 a-g	31.70 b-f
Grandparents					
ST-468 (ST)	61.53 cd	42.71 hı	28.63	5.10 d-h	30.37 e-g
GLORIA (GL)	61.30 c	41.37 j	30.15	4.88 g-ı	30.70 d-g
FLASH (FL)	53.28 g	40.73 k	29.94	4.89 g-ı	29.83 fg
CARISMA (CR)	53.21 g	42.45 ı	28.88	4.64 ı	30.40 e-g
JULIA (JU)	48.60 h	41.64 j	29.86	5.11 c-h	33.13 ab
CARMEN (CA)	47.11 h	41.83 j	30.87	4.83 h-ı	33.03 a-c
CLAUDİA (CL)	49.32 h	43.31 gh	30.12	4.96 f-ı	33.90 a
Parents					
Genotype-I (G1)	55.70 d-f	46.11 bc	29.64	4.84 h-ı	30.57 d-g
ESPERIA (ES)	71.82 a	43.66 e-g	28.87	4.97 f-ı	30.87 d-g
LSD $_{(0.05)}$	2.33	0.61	-	0.38	2.05

SCY: Seed cotton yield (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (micronaire); FS: Fiber strength (g tex^{-1}).

Table 3. The significance of orthogonal contrasts
Çizelge 3. Ortogonal karşılaştırmaların önemliliği

	Orthogonal contrasts	SCY	GOT	FL	FF	FS
C_1	Esperia crosses vs. Genotype-I crosses		**			
C_2	All crosses vs. mean of Esperia and Genotype-I		**			
C_3	All crosses vs. all parents	**	**	ns	**	ns
C_4	Esperia crosses vs. best variety		**			
C_5	Genotype-I crosses vs. best variety		ns			
C_6	All crosses vs. best variety		**			
C_7	All crosses vs. check variety (Gloria)	**	ns	ns	**	ns
C_8	All crosses vs. best variety	**	ns	*	**	**

*: Significant at P < 0.05 and **: Significant at P < 0.01. SCY: Seed cotton yield (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (micronaire); FS: Fiber strength (g tex^{-1}).

Table 4. Standard heterosis (%)
Çizelge 4. Standart heterosis değerleri (%)

Combinations	SCY	GOT	FL	FF	FS
[(CA × ST) × (ST × CL)] × ES	-7.66**	5.83	-4.59	12.36**	1.41
[(CA × ST) × (ST × CL)] × G1	-12.47**	10.27**	1.97	8.95*	-3.58
[(JU × ST) × (GL × CR)] × ES	-1.17	4.71	-0.94	5.05	1.30
[(JU × ST) × (GL × CR)] × G1	-1.31	11.58**	-2.33	10.25**	0.65
[(CR × CA) × (GL × FL)] × ES	-11.19**	10.30**	-4.62	14.41**	1.74
[(CR × CA) × (GL × FL)] × G1	9.14**	8.80**	-3.10	11.61**	-1.74
[(JU × ST) × (CR × CA)] × ES	7.00**	5.08	-4.79	5.60	2.17
[(JU × ST) × (CR × CA)] × G1	-5.78**	16.03**	-2.62	10.79**	0.98
[(ST × CL) × (GL × CR)] × ES	20.54**	6.24*	-3.56	3.62	5.86
[(ST × CL) × (GL × CR)] × G1	-11.84**	10.80**	2.39	4.85	3.04
[(GL × FL) × (GL × CR)] × ES	2.41	6.60*	-2.14	12.50**	4.67
[(GL × FL) × (GL × CR)] × G1	9.25**	15.64**	-2.27	14.28**	0.76
[(CA × ST) × (GL × FL)] × ES	-5.81**	5.56	-0.30	10.66**	3.04
[(CA × ST) × (GL × FL)] × G1	-7.68**	12.76**	0.61	7.04	3.26

*: Significant at P < 0.05 and **: Significant at P < 0.01. SCY: Seed cotton yield (g); GOT: Ginning out-turn (%); FL: Fiber length (mm); FF: Fiber fineness (micronaire); FS: Fiber strength (g tex^{-1}).

Fiber Strength

The use of favorable fiber strength improved both ring- and open-end spinning and yarn strength (Simpson & Murray, 1978), and modern textile industries demand stronger, longer, finer, and more uniform cotton fibers (Chapara & Madugula, 2021). In our study, the highest fiber strength recorded in 32.50 g tex^{-1} [(ST × CL) × (GL × CR)] × ES and 32.13 g tex^{-1} [(GL × FL) × (GL × CR)] × ES cross combinations, while Claudia (33.90 g tex^{-1}), Julia (33.13 g tex^{-1}) and Carmen (33.03 g tex^{-1}) were the grandparents with the highest fiber strength (Table 2). When the mean of all crosses (31.22 g tex^{-1}) was compared with the grand mean of parents (31.42 g tex^{-1}) and check variety, Gloria (30.70 g tex^{-1}), the differences were nonsignificant as confirmed by orthogonal comparisons (C_3 and C_7), whereas all cross combinations performed poorly against the best variety, Claudia (C_8) (Table 3). The standard heterosis of all cross combinations except [(CA × ST) × (ST × CL)] × G1 and [(CR × CA) × (GL × FL)] × G1 indicated that a certain genetic improvement over 31.0 g tex^{-1} was achieved in the cross population (Table 4). Sirisha et al. (2019) mostly detected positive standard heterosis between -6.52% and 13.94%. In a study evaluating the mean performances and standard heterosis for fiber strength, Chapara & Madugula (2021) reported performance between 25.20 - 31.15 g tex^{-1} and significant and positive standard heterosis in only 2 of the 20 crosses. When the findings of our study and related literature are evaluated together, standard heterosis varies widely, depending on the performance of the parents used and the standard variety used.

CONCLUSIONS

The F_1 cross combinations of double cross F_3 populations with variety (Esperia) or advanced line (Genotype-I) were successful in terms of seed cotton yield and ginning out-turn, whereas cross combinations have evolved in the direction of coarse fiber, and improvement in fiber length and strength was limited. It was concluded that optimizing yield, ginning out-turn and fiber quality is a challenge for cotton breeding. Based on this information, [(ST-468 × Claudia) × (Gloria × Carisma)] × Esperia with high yielding, medium-high ginning out-turn, fiber fineness of 5.06 mic, fiber length of 29.08 mm and fiber strength of 32.50 g tex^{-1} should be evaluated in advanced generations. Genotype-I could be used as a parent in breeding programs, aiming to increase ginning out-turn. However, [(Gloria × Flash) × (Gloria × Carisma)] × Genotype-I and [(Carisma × Carmen) × (Gloria × Flash)] × Genotype-I hybrids have high ginning out-turn, medium-high seed cotton yield but coarse fibers. It would be beneficial to cross these genotypes with *Gossypium hirsutum* L., *Gossypium barbadense* L., or a hybrid of two species, which can adapt in terms of fine fiber.

ACKNOWLEDGEMENTS

This manuscript is based on an ongoing recurrent selection breeding study and is part of a master thesis carried out by Ferhat ÇAKMAK. The authors gratefully thank the editors and anonymous reviewers for their constructive comments and suggestions to improve the quality of this manuscript.

Contribution of the Authors as Summary

The authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

KAYNAKLAR

Ahmad, M., & Azhar, F. M. (2000). Genetic correlation and path coefficient analysis of oil and protein contents and other quantitative characters in F_2 generation of *G. hirsutum* L. *Pakistan Journal of Biological Sciences 3*, 1049-1051.

Ahuja, S. L., Ahmad, S., Saim, S. K., & Meena, R. A. (2018) Heterosis studies for high ginning out-turn percent and related traits in *G. hirsutum* cotton. *International Journal of Agriculture Sciences, 10*(12), 6465-6468.

Akbar, M., Anwar, J., Hussain, M., Qureshi, M. H., & Khan, S. (2009). Line tester analysis in bread wheat (*Triticum aestivum* L.). *Journal of Agricultural Research 47*(1), 411-420.

Arain, B. T., Baloch, M. J., Sial, P., Arain, M. A., & Baloch, A. (2015). Estimation of heterosis and combining ability in F_1 hybrids of upland cotton for yield and fiber traits: upland cotton yield and fiber traits. *Biological Sciences-PJSIR 58*(3), 132-139.

Ashokkumar, K., & Ravikesavan, R. (2013). Genetic variation and heterotic effects for seed oil, seed protein, and yield attributing traits in upland cotton (*Gossypium hirsutum* L.). *African Journal of Biotechnology 12*(33), 5183-5191. DOI: https://doi.org/10.5897/AJB2013.13016

Balci, S., Cinar, V.M., & Unay, A. (2021a). The effects of modified recurrent selection on fiber characteristics and neps in cotton (*Gossypium hirsutum* L.). *ANADOLU Journal of Aegean Agricultural Research Institute 31*(2), 137-142. DOI: https://doi.org/10.18615/anadolu.1029812

Balci, S., Cinar, V. M., & Unay, A. (2021b). Genetic analysis for yield and ginning out turn in F_1 and F_2 populations of upland cotton. *Indian Journal of Genetics and Plant Breeding 81*(4), 104-106. DOI: https://doi.org/10.31742/ISGPB.81.4.15

Baloch, M. J., Sial, P., Arain, B. T., & Arain, M. A. (2015). Assessment of heterotic effects in F_1 hybrids of cotton (*Gossypium hirsutum* L.). *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences 31*(2), 193-202.

Bilwal, B. B., Vadodariya, K. V., Lahane, G. R., & Rajkumar, B. K. (2018). Heterosis study for seed cotton yield and its yield attributing traits in upland cotton (*Gossypium hirsutum* L.). *Journal of Pharmacognosy and Phytochemistry 7*(1), 1963-1967.

Çakmak, F., Çınar, V. M., Balcı, Ş., & Ünay, A. (2023). Yield and fiber quality balance in upland cotton (*Gossypium hirsutum* L.) breeding. *Bangladesh Journal of Botany, 52*(2), 283–290. https://doi.org/10.3329/bjb.v52i2.67025

Chapara, R., & Madugula, S. (2021). Heterosis for seed fiber quality traits cotton (*Gossypium hirsutum* L.). *Journal of Forest Research 10,* 253-258.

Choudhari, P. N., Borole, D. N., Patil, S. D., & Narkhede, B. N. (1988). Path analysis in deshi cotton. *Journal of Maharashtra Agricultural Universities 13*, 54-55.

Çoban, M., & Çiçek, S. (2017). Identification of yield and fiber quality properties of some cotton lines for Nazilli conditions. *KSU Journal of Agriculture and Nature 20*, 222-226. https://doi.org/10.18016/ksudobil.349210

Cohran, W. G., & Cox, G. M. (1957). *Experimental designs.* John Willey Sons Inc, New York.

Constable, G., Llewellyn, D., Walford, S. A., & Clement, J. D. (2015). Cotton breeding for fiber quality improvement. In: Cruz VMV, Dierig DA (eds) Industrial Crops. Handbook of Plant Breeding, vol 9. Springer, New York, NY, pp. 191-232. DOI: https://doi.org/10.1007/978-1-4939-1447-0_10

Desalegn, Z. (2016). High ginning out turn and the improvement of Ethiopian cotton production. World Cotton Research Conference - 6Goiânia - Goiás, Brazil, 2 - 6 May.

Dinakaran, E., Thirumeni, S., & Paramasivam, K. (2012). Yield and fiber quality components analysis in upland cotton (*Gossypium hirsutum* L.) under salinity. *Annals of Biological Research 3*(8), 3910-3915.

Ekinci, R., Basbag, S., & Gencer, O. (2016). Heterotic effects for lint yield in double cross hybrids on cotton. *Ekin Journal of Crop Breeding and Genetics 2*(1), 40-44.

El-Hashash, E. F. (2013). Heterosis and gene action among single and double-cross hybrids performances in cotton. *American-Eurasian Journal of Sustainable Agriculture 13*(4), 505-516. DOI: https://doi.org/10.5829/idosi.aejaes.2013.13.04.1955

Erande, C. S., Kalpande, H. V., Deosarkar, D. B., Chavan, S. K., Patil, V. S., Deshmukh, J. D., ... & Puttawar, M. R. (2014). Genetic variability, correlation, and path analysis among different traits in desi cotton (*Gossypium hirsutum* L.). *African Journal of Agricultural Research 9*(29), 2278-2286. DOI: https://doi.org/10.5897/AJAR2013.7580

Fonseca, S., & Patterson, F. L. (1968). Hybrid vigor in seven parent diallel cross in common wheat (*Triticum aestivum* L.). *Crop Science, 8,* 85–88. DOI: https://doi.org/10.2135/cropsci1968.0011183X000800010025x

Girish, T., Patil, S. S., & Kencharaddi, H. G. (2019). Consistency of combining ability in segregating generations of a heterotic box subjected to reciprocal selection in cotton. *Journal of Cotton Research and Development 33*(1), 26-35.

Ibragimov, P. (1989). *Genetic Correlations Between Characters.* Khlopok, No.4, 45.

Islam, M. S., Thyssen, G. N., Jenkins, J. N., Zeng, L., Delhom, C. D., McCarty, J. C., ... & Fang, D. D. (2016). A MAGIC population-based genome-wide association study reveals a functional association of the GhRBB1_A07 gene with superior fiber quality in cotton. *BMC Genomics 17*(1), 903-920. DOI: https://doi.org/10.1186/s12864-016-3249-2

Jenkins, J. N., McCarty Jr, J. C., Gutierrez, O. A., Hayes, R. W., Bowman, D. T., Watson, C. E., & Jones, D. C. (2008). Registration of RMUP-C5, a random mated population of Upland cotton germplasm. *Journal of Plant Registrations 2*(3), 239–242. DOI: https://doi.org/10.3198/jpr2008.02.0080crg

JMP. (2018). SAS Institute Inc. 2018. JMP Statistical Software, Version 14. Cary, USA.

Karademir, E., Karademir, Ç., Ekinci, R., & Gençer, O. (2010). Relationship between yield, fiber length and other fiber-related traits in advanced cotton strains. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38*(3), 111-116. https://doi.org/10.15835/nbha3834889

Kumbhalkar, H. B., Gawande, V. L., Desmukh, S. B., Gotmare, V. P., & Waghmare, V. N. (2021). Stabilized heterosis studies for seed cotton yield and component traits in upland cotton (*Gossypium hirsutum* L.). *International Journal of Current Microbiology and Applied Sciences 10*(7), 99-113.

Memon, S., Jatoi, W. A., Khanzada, S., Kamboh, N., & Rajput, L. (2017). Line × tester analysis for earliness yield and yield contributing traits in *Gossypium hirsutum* L. *Journal of Basic & Applied Sciences 13,* 287-292. DOI: https://doi.org/10.6000/1927-5129.2017.13.47

Monicashree, C., Balu, P. A., & Gunasekaran, M. (2017). Combining ability and heterosis studies on yield and fiber quality traits in upland cotton (*Gossypium hirsutum* L.). *International Journal of Current Microbiology and Applied Sciences 6*(8), 912-927.

Mudhalvan, S., Rajeswari, S., Mahalingam, L., Jeyakumar, P., Muthuswami, M., & Premalatha, N. (2021). Combining ability estimates and heterosis analysis on major yield attributing traits and lint quality in American cotton (*Gossypium hirsutum* L.). *Electronic Journal of Plant Breeding 12*(4), 1111-1119. DOI: https://doi.org/10.37992/2021.1204.153

Murthy, K. G. K., Pradeep, T., Reddy, S. S., & Krishna, K. R. (2017) Estimation of standard heterosis in multiple cross derivatives of upland cotton (*Gossypium hirsutum* L.) for yield, plant type, and fiber quality. *International Journal of Pure & Applied Bioscience 5*(6), 691-697. DOI: http://dx.doi.org/10.18782/2320-7051.4062

Parmar, M. B., Joshi, N. R., Patel, S. M., & Kapadia, V. N. (2015). Genetic variability studies in bt cotton hybrids (H x H). *AGRES – An International e-Journal 4*(2), 145-150.

Percy, R. G., Cantrell, R. G., & Zhang, J. (2006). Genetic variation for agronomic and fiber properties in an introgressed recombinant inbred population of cotton. *Crop Science, 46*(3), 1311-1317. https://doi.org/10.2135/cropsci2005.08-0284

Premalatha, N., Kumar, M., & Mahalingam, L. (2020). Combining ability analysis for yield and fiber quality traits in intraspecific hybrids of *Gossypium hirsutum* L. *Electronic Journal of Plant Breeding 11*(4), 1085-1092. DOI: https://doi.org/10.37992/2020.1104.176

Ranganatha, H. M., Patil, S. S., Swathi, P., Rajeev, S., Srivalli, P., & Kanti, V. M. (2013). Development of heterotic pairs or groups of cotton genotypes based on predicted double cross performance. *International Journal of Agriculture and Crop Sciences 6*(5), 231-235.

Rathava, P., Patel, S. R., Patel, D. M., Patel, H. N., Dinisha, A., & Patil, S. S. (2018). Heterosis studies for seed cotton yield and other traits in tetraploid cotton (*Gossypium hirsutum* L.). *Journal of Pharmacognosy and Phytochemistry 7*(4), 1642-1648.

Saeed, F., Farooq, J., Mahmood, A., Riaz, M., Hussain, T., & Majeed, A. (2014). Assessment of genetic diversity for Cotton leaf curl virus (CLCuD), fiber quality and some morphological traits using different statistical procedures in *Gossypium hirsutum* L. *Australian Journal of Crop Science 8*(3), 442-447.

Salahuddin, S., Abro, S., Kandhro, M. M., Salahuddin, L., & Laghari, S. (2010). Correlation and path coefficient analysis of yield components of upland cotton (*Gossypium hirsutum* L.) sympodial. *World Applied Sciences Journal 8*(8), 71-75.

Shahzad, K., Li, X., Qi, T., Guo, L., Tang, H., Zhang, X., Wang, H., Zhang, M., Zhang, B., Qiao, X., & Xing, C. (2019). Genetic analysis of yield and fiber quality traits in upland cotton (*Gossypium hirsutum* L.) cultivated in different ecological regions of China. *Journal of Cotton Research 2*(1), 14-25. DOI: https://doi.org/10.1186/s42397-019-0031-4

Shashibhushan, D., & Patel, U. G. (2019). Heterosis for seed cotton yield and other agro morphological traits in GMS-based hybrids of upland cotton (*Gossypium hirsutum* L.). *Journal of Pharmacognosy and Phytochemistry 8*(5), 572-576.

Simpson, J., & Murray, M. F. (1978). Effect of cotton fiber fineness and strength on mechanical processing, open-end spinning, and yarn properties. *Textile Research Journal 48*(5), 270-276. DOI: https://doi.org/10.1177/004051757804800505

Sirisha, A. B. M., Ahamed, M. L., Kumar, P. R., Kumari, S. R., & Rao, V. S. (2019). Heterosis

studies for fiber quality traits in upland cotton (*Gossypium hirsutum* L.). *The Andhra Agricultural Journal, 66*(2), 293-295.

Steadman, R. G. (1997). Cotton testing. *Textile Progress 27*(1), 1-63. DOI: https://doi.org/10.1080/00405169708688868

Steel, R. G. D., & Torrie, J. H. (1980). *Principles and Procedures of Statistics.* 2nd ed., McGraw Hill Book Co, New York, U.S.A.

Tarazi, R., Jimenez, J. L. S., & Vaslin, M. F. (2020). Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. *Biotechnology Research and Innovation 3*, 19–26. DOI: https://doi.org/10.1016/j.biori.2020.01.001

Yehia, W. M. B., Hamoud, H. M. E., & Abo, E. Y. (2009). Double crosses analysis for yield component and fiber traits in Egyptian cotton (*Gossypium barbadense* L.). *Journal of Agricultural Chemistry and Biotechnology 34*(3), 1581-1598. DOI: https://doi.org/10.21608/jacb.2009.90290

Yu, J., Zhang, K., Li, S., Yu, S., Zhai, H., Wu, M., ... & Zhang, J. (2013). Mapping quantitative trait loci for lint yield and fiber quality across environments in a *Gossypium hirsutum* x *Gossypium barbadense* backcross inbred line population. *Theoretical and Applied Genetics 126*, 275–287. DOI: https://doi.org/10.1007/s00122-012-1980-x

Zhou, Y. Y. (1986). Yield components in upland cotton. *Acta Agricultura Universitatis Pekinensis 12*(3), 269-274.

KSÜ Tarım ve Doğa Derg 27 (4), 910-919, 2024
KSU J. Agric Nat 27 (4), 910-919, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1395888

Çukurova Bölgesi İkinci Ürün Koşullarında Bazı İnci Darı (*Pennisetum glaucum* (L.) R. Br.) Çeşitlerinin Ot Verimleri ve Agromorfolojik Özellikleri Üzerinde Bir Araştırma

Hasan Beytullah DÖNMEZ[1]✉, Rüştü HATİPOĞLU[2]
[1]Çukurova Üniversitesi, Tufanbeyli Meslek Yüksekokulu, Bitkisel ve Hayvansal Üretim Bölümü, 01640, Adana/Türkiye, [2]Kırşehir Ahi Evran Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, 40100, Kırşehir/Türkiye
[1]https://orcid.org/0000-0003-1495-4553, [2]https://orcid.org/0000-0002-7977-0782
✉: bdonmez@cu.edu.tr

ÖZET
Bu araştırma, Çukurova Bölgesi ikinci ürün koşullarında yetiştirilebilecek bazı inci darı (*Pennisetum glaucum* (L.) R. Br.) çeşitlerinin ot verimleri ve bazı agromorfolojik özelliklerinin belirlenmesi amacıyla yürütülmüştür. Deneme, Çukurova Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü Araştırma ve Uygulama arazisinde, tesadüf blokları deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Araştırmada 5 adet inci darı çeşidi (Ashana, Heveahri, White, Yellow, Tifleaf 3) test edilmiştir. Araştırmada, bitki boyu, yeşil ot verimi, kuru ot verimi, yaprak oranı, sap çapı ve kardeş sayısı bakımından inci darı çeşitleri arasında önemli farklılıklar tespit edilmiştir. Ayrıca, bitki boyu ile yeşil ot verimi (0.9773**) arasında çok önemli ve olumlu, kuru ot verimi (0.9562*) arasında önemli ve olumlu, yaprak oranı (-0.9417*) arasında önemli ve olumsuz; yeşil ot verimi ile kuru ot verimi (0.9932**) arasında çok önemli ve olumlu, yaprak oranı (-0.9889**) arasında çok önemli ve olumsuz; kuru ot verimi ile yaprak oranı (-0.9916**) arasında çok önemli ve negatif ilişki olduğu saptanmıştır. Mevcut bulgular doğrultusunda, inci darı çeşitlerinin ikinci ürün koşullarında mısır ve sorguma alternatif olabileceği, ot verimi bakımından White ve Heveahri çeşitlerinin diğer çeşitlere göre daha üstün olduğu, incelenen çeşitlerin ot kaliteleri ve silaj verimlerinin belirlenerek yeni araştırmaların yapılması gerektiği sonucuna varılmıştır.

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 25.11.2023
Kabul Tarihi : 04.01.2024

Anahtar Kelimeler
İnci darı
İkinci ürün
Ot verimi
Agromorfolojik özellikler

A Research on the Forage Yield and Agromorphological Characteristics of Some Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) Cultivars under Second Crop Conditions in the Cukurova Region

ABSTRACT [Century 10]
This study was conducted to determine the herbage yield and some geomorphological characteristics of some pearl millet (*Pennisetum glaucum* (L.) R. Br.) cultivars that can be grown under second-crop conditions in the Cukurova Region. The experiment was established according to a randomized block design with 3 replications in the research area of the Field Crops Department of the Agricultural Faculty, Cukurova University, Adana, Turkey. Five pearl millet cultivars (Ashana, Heveahri, White, Yellow, and Tifleaf 3) were tested in the study. In the study, significant differences were found among pearl millet cultivars in terms of plant height, green forage yield, hay yield, leaf ratio, stem diameter, and number of tillers. In addition, there were significant and positive correlations between plant height and green herb yield (0.9773**), significant and positive correlations between green forage yield (0.9562*), significant and negative correlations between leaf ratio (-0.9417*), significant and positive correlations between green forage yield and hay yield (0.9932**), significant and negative correlations between leaf ratio (-0.9889**), and significant and negative correlations between hay yield and leaf ratio (-0.9916**). In light of the present findings, it was concluded that pearl millet can be an alternative to corn and sorghum in second-crop conditions. White and Heveahri cultivars were superior to other cultivars in terms of hay yield, and new research should be carried

Field Crops

Research Article

Article History
Received : 25.11.2023
Accepted : 04.01.2024

Keywords
Pearl millet
Second crop
Hay yield
Agromorphological characteristics

out to determine the hay quality and silage yield of the cultivars examined.

Atıf Şekli: Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Çukurova Bölgesi İkinci Ürün Koşullarında Bazı İnci Darı (Pennisetum glaucum (L.) R. Br.) Çeşitlerinin Ot Verimleri ve Agromorfolojik Özellikleri Üzerinde Bir Araştırma. *KSÜ Tarım ve Doğa Derg*, *27*(4), 910-919. https://doi.org/10.18016/ksutarimdoga.vi.1395888

To Cite : Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Çukurova Bölgesi İkinci Ürün Koşullarında Bazı İnci Darı (Pennisetum glaucum (L.) R. Br.) Çeşitlerinin Ot Verimleri ve Agromorfolojik Özellikleri Üzerinde Bir Araştırma. KSU J. Agric Nat. *27*(4), 910-919. https://doi.org/10.18016/ksutarimdoga.vi.1395888

GİRİŞ

Türkiye'de, gelişmekte olan ülkelerde olduğu gibi, artan nüfusa bağlı olarak hayvansal ürünlere yönelik talep her geçen gün artmaktadır. Hayvansal ürünlere artan talebin karşılanması için hayvan sayısının yeterli düzeye getirilmesi ve hayvan başına verimin artırılması gerekmektedir. Hayvan başına verim; hayvanın genetik potansiyeli, beslenme, barınma ve hastalık ve zararlıları gibi faktörlerle ilişkilidir. Türkiye hayvancılığında hayvanların beslenmesi ile ilgili önemli problemler bulunmaktadır. Nitekim, 19.4 milyon BBHB'ine eşdeğer hayvan varlığının yaşama payı için gerekli olan yıllık kaba yem miktarının (12 kg/gün mısır silajı+ 2 kg/gün fiğ/yulaf + 3 kg/gün saman) 120.8 milyon ton olduğu (Alçiçek, 2021) ve söz konusu kaliteli kaba yemin yeterli düzeyde üretilemediği bilinmektedir (Hatipoğlu ve ark., 2020; Açıkgöz, 2021). Çiftlik hayvanlarının yaşama payı ihtiyaçlarının yanında verim paylarının bir bölümünün de karşılanması ve kaba yemdeki arz-talep dengesindeki açığı kapatmak için birçok seçenek bulunmaktadır. Bunlardan birisi de birim alanda yüksek biyomas verimine ve biyomas kalitesine sahip yüksek verimli yembitkilerinin üretilmesidir (Hassan ve ark., 2014). Özellikle iklim değişikliğine karşı adaptasyonu iyi olan, kabul edilebilir verimleri ve kaliteleri nedeniyle darılar popülerlik kazanmaktadır (Jukanti ve ark., 2016).

İnci darı (*Pennisetum glaucum* (L.) R. Br.), *Poaceae* familyasına ait yabancı döllenen, yüksek heterosise sahip tek yıllık sıcak mevsim C4 bitkisidir (Dumanoğlu ve ark., 2022). Türkiye'de henüz yetiştiriciliği yapılmayan bu bitki, kuraklığa ve sıcağa toleransı yüksek, fakir, kumlu ve tuzlu topraklarda rahatlıkla yetiştirilebilmektedir (Benek & Geren, 2023). İnci darının, özellikle güney bölgelerde ikinci ürün koşullarında yetiştirilen ve kaliteli kaba yem kaynağı olan silajlık mısırın su tüketiminin fazla olması, sap ve koçan kurdu gibi zararlı popülasyonunun yoğun olması ve bu nedenle verim ve yem kalitesinin düşmesinden dolayı, söz konusu koşullarda mısırın yerini alarak avantaj sağlayacağı bildirilmiştir (Hatipoğlu & Tükel, 2009; Yücel & Yücel, 2022). Bunların yanında, inci darının hidrosiyanik ve prusik asit içermemesi sorgum ve sudan otuna besinsel üstünlük sağlamaktadır (Silungwe, 2011; Lemus, 2015).

Akdeniz ikliminin hüküm sürdüğü güney kesimlerde yaz dönemi ortalama sıcaklıklar Haziran ayından itibaren 25 °C'nin üzerine çıkmakta ve uzun süreli devam eden bu sıcaklıklardan dolayı serin mevsim yembitkileri dormant hale geçmektedir (Dönmez, 2022). Söz konusu alanlarda bugüne kadar sürdürülen araştırmalarda; serin dönemlerde yetiştirilebilecek yembitkileri türlerinin saptanması amacıyla birçok araştırma yürütülmüş olmasına karşılık, mısır ve sorgum dışında yaz döneminde yetiştirilebilecek yembitkisi türleri ile ilgili araştırmalar sınırlı kalmıştır.

Bu araştırmada, Akdeniz ikliminin hüküm sürdüğü bölgelerde ikinci ürün koşullarında yetiştirilebilecek biryıllık yembitkisi türlerinden olan inci darısı (*Pennisetum glaucum* (L.) R. Br.) türüne ait bazı çeşitlerin performansları değerlendirilmiştir.

MATERYAL ve METOD

Deneme, Çukurova Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü Araştırma ve Uygulama arazisinde (37° 01' 00" N, 35° 21' 42" E, Deniz seviyesinden yükseklik 35 m), 2018 ve 2019 yetiştirme mevsimi ikinci ürün koşullarında 2 yıl süreyle yürütülmüştür. Deneme alanından alınan toprak örnekleri Çukurova Üniversitesi Ziraat Fakültesi Toprak Bölümü laboratuvarında incelenmiş ve deneme alanı topraklarının kili yapıda ve ağır bünyeli olduğu, kireç oranının (%27.9) oldukça yüksek, pH'ının 7.40, organik madde (%1.9) ve fosfor (29 kg ha^{-1}) bakımından fakir, potasyum (713 kg ha^{-1}) bakımından ise oldukça zengin olduğu ortaya çıkmıştır (Kızılkaya, 2020).

Araştırmanın yürütüldüğü Adana iline ait 2018 ve 2019 yılları ile uzun yıllar ortalaması Haziran-Ekim dönemi iklim değerleri Çizelge 1'de verilmiştir (Anonim, 2019).

Çizelge 1 izlendiğinde, 2018 yılında gerçekleşen toplam yağış (100.6 mm), uzun yıllar ortalaması gerçekleşen yağış toplamına (92.0 mm) göre daha yüksek olurken, 2019 yılında gerçekleşen toplam yağışın (77.3 mm) uzun yıllar ortalaması gerçekleşen yağış toplamından daha düşük olduğu anlaşılmaktadır. Bunun yanında, 2018 ve 2019 yıllarında ortalama sıcaklık ve ortalama nispi nem değerlerinin sırasıyla 27.2 °C-27.3 °C ve %66.2-%65.8

olarak gerçekleştiği ve uzun yıllar ortalama sıcaklık ve nispi nem değerlerine göre her iki yetiştirme mevsiminin de daha sıcak ve daha nemli olduğu ortaya çıkmıştır.

Çizelge 1. Araştırmanın yürütüldüğü Adana iline ait iklim verileri
Table 1 Climatic data of Adana province where the research was conducted

Aylar *Months*	**Toplam Yağış (mm)** *Total precipitation (mm)*			**Ortalama Sıcaklık (°C)** *Average temperature (°C)*			**Ortalama Nispi Nem (%)** *Relative humidity (%)*		
	UYO* LYA*	**2018**	**2019**	**UYO LYA***	**2018**	**2019**	**UYO LYA***	**2018**	**2019**
Haziran *June*	20.5	25.6	21.3	25.6	26.4	27.1	66.5	70.2	68.7
Temmuz *July*	6.5	0.0	30.9	28.2	29.1	28.4	69.0	69.8	68.8
Ağustos *August*	5.4	0.0	0.0	28.7	29.6	29.6	68.5	68.8	68.0
Eylül *September*	17.4	1.3	4.0	26.1	27.9	27.3	63.5	63.6	62.1
Ekim *October*	42.2	73.7	21.1	21.7	22.9	24.2	60.7	58.6	61.6
Top./Ort. *Totl/Avg.*	92.0	100.6	77.3	26.1	27.2	27.3	65.7	66.2	65.8

*Uzun yıllar ortalaması *(Long year average)*

Araştırmada, Sudan orjinli dört adet (Ashana, Heveahri, White, Yellow) ve ABD orijinli bir adet (Tifleaf 3) olmak üzere toplam beş adet inci darı (*Pennisetum glaucum* (L.) R. Br.) çeşidi kullanılmıştır. Deneme, tesadüf blokları deneme desenine göre, 3 tekerrürlü olarak tesis edilmiştir. Denemede, sıra aralığı 70 cm ve sıra üzeri mesafe 10 cm olarak belirlenmiş ve her parsel 5 m uzunluğunda 4 sıradan oluşmuştur. Ekim işlemi, her iki yetiştirme mevsiminde de ikinci ürün koşulları dikkate alınarak, 2018 yılında 24 Haziran ve 2019 yılında 26 Haziran tarihinde gerçekleştirilmiştir. Ekim işleminden 15 gün sonra sıra üzeri mesafenin 10 cm olması için seyreltme ve aşılama yapılmıştır. Her iki yetiştirme mevsiminde de 85 kg ha^{-1} saf N ve 85 kg ha^{-1} saf P_2O_5 olacak şekilde deneme alanında gübreleme yapılmıştır. Azotun yarısı ve fosforun tamamı ekimle birlikte, azotun diğer yarısı ise bitkilerin 20-25 cm olduğu dönemde üst gübre olarak sıra aralarına uygulanmıştır. Araştırmanın yürütüldüğü yıllarda gerektikçe elle yabancı ot mücadelesi yapılmış olup, ekim sonrası çıkış sağlamak için ve bitkinin ihtiyacına göre belirli aralıklara sulama (salma sulama) yapılmıştır. Araştırmada hasat çeşitlerin çiçeklenme döneminde gerçekleştirilmiştir. Test edilen çeşitlerin çiçeklenme tarihleri farklılık göstermiş olup, çeşitlerin hasat tarihleri Çizelge 2'de verilmiştir.

Çizelge 2. Araştırmada incelenen inci darı çeşitlerinin hasat tarihleri
Table 2 Harvest dates of pearl millet varieties examined in the study

Yetiştirme Mevsimi *Growing season*	**İnci Darı Çeşitleri ve Hasat Tarihleri** *Pearl millet cultivars and harvest dates*				
	Ashana	**Heveahri**	**White**	**Yellow**	**Tifleaf 3**
2018	22.08.2018	10.10.2018	10.10.2018	10.10.2018	22.08.2018
2019	24.08.2019	25.09.2019	01.10.2019	25.09.2019	30.08.2019

Denemede, parsel başlarından 50 cm ve parselin her iki kenarından birer sıra kenar tesiri olarak atıldıktan sonra, ortada bulunan 2 sıradan tesadüfi olarak seçilen 5 bitkide bitki boyu ölçülerek kaydedilmiş ve bu bitkiler 10 cm yükseklikten biçilerek tartılmıştır. Her parselde kenar tesiri atıldıktan sonra geriye kalan ortadaki 2 sırada tüm bitkiler biçilip tartılmış ve 5 bitkinin ağırlıkları da eklenerek parselin yeşil ot ağırlığı belirlenmiştir. Gerekli dönüşümler yapılarak çeşitlerin hektara yeşil ot verimleri hesaplanmıştır. Hasat edilen yeşil bitkilerden ortalama 500 g örnek alınmış ve önce 10 gün süreyle açık havada, ardından 70 °C'de 24 saat etüvde kurutularak çeşitlerin kuru ağırlıkları ve kuru ot oranları belirlenmiştir. Gerekli dönüşümler yapılarak her çeşidin hektara kuru ot verimleri hesaplanmıştır. Her parselden tesadüfi olarak seçilen ve bitki boyu ölçülen 5 bitkinin her birinde kardeş sayıları belirlenmiş ve kardeş sayısı olarak kaydedilmiştir. Ayrıca, söz konusu bitkilerde ana sapın 2. ve 3. boğum arası kumpas yardımıyla ölçülmüş ve sap çapı olarak kaydedilmiştir. Sap çapı ölçülen 5 bitkinin yaprakları, yaprak kınından koparılmış ve aynı bitkilerin yaprakları ve sapları ayrı ayrı 70 °C'de etüvde kurutulmuştur. Kurutulan

yaprak ve sap örnekleri tartılmış ve aşağıda açıklanan Eşitlik 1 yardımıyla yaprak oranı hesaplanmıştır.

Yaprak Oranı (%): Kuru yaprak ağırlığı/Kuru ağırlık toplamı (1)

Araştırmadan elde edilen verilere, MSTAT-C (Michigan State University V.2.10) istatistik paket programında varyans analizi uygulanmıştır. İstatistiksel olarak önemli çıkan özellik ortalamaları P≤0.05 önem düzeyinde Duncan testi ile karşılaştırılmıştır. İncelenen özelliklerin arasındaki ikili ilişkiler, korelasyon katsayılarıyla tanımlanmıştır (Yurtsever, 2011).

BULGULAR ve TARTIŞMA

Bu Araştırmada test edilen inci darı çeşitlerinin ot verimi ve bazı agromorfolojik özelliklerine uygulanan varyans analiz sonuçları, Çizelge 3'te verilmiştir.

Çizelge 3. İnci darı çeşitlerinde saptanan ot verimi ve bazı agromorfolojik özelliklerine uygulanan varyans analiz sonuçları

Table 3 Results of analysis of variance applied to forage yield and some geomorphological characteristics *of pearl millet cultivars*

Deneme Faktörleri *Source*	**Serbestlik Derecesi** *Degrees of freedom*	**Bitki Boyu** *Plant height*	**Yeşil Ot Verimi** *Green forage yield*	**Kuru Ot Verimi** *Hay yield*	**Yaprak Oranı** *Leaf ratio*	**Sap Çapı** *Stem diameter*	**Kardeş Sayısı** *Number of tillers*
Tekerrür *Replication*	2	241.7	66.6	3.7	1.0	2.4	0.1
Yıl *Year*	1	15919.7**	37153.6**	2680.4**	0.7	66.6**	3.7**
Çeşit *Cultivar*	4	21073.4**	37301.9**	4103.3**	238.4**	4.9**	29.5**
Yıl x Çeşit *Year x cultivar*	4	13736.8**	7863.8**	884.3**	216.7**	3.7*	2.3**
Hata *Error*	18	496.8	226.0	22.8	3.6	0.8	0.4
V.K. (%) *C.V. (%)*		7.74	6.39	8.98	5.78	6.07	13.32

*: p≤0.05, **: p≤0.01

Çizelge 3 izlendiğinde, yıl ve çeşit faktörü ile yıl x çeşit interaksiyonunun inci darı çeşitlerinin bitki boyu, yeşil ot verimi, kuru ot verimi, sap çapı ve kardeş sayısı üzerinde istatistiki olarak önemli derecede fark yarattığı, yaprak oranı üzerine ise çeşit ve yıl x çeşit interaksiyonunun etkisinin istatistiki olarak önemli olduğu ortaya çıkmıştır.

Bitki Boyu (cm)

Araştırmada test edilen inci darı çeşitlerinde saptanan bitki boyu ortalamaları Çizelge 4'te verilmiştir. Çizelge 4'te izlendiği gibi, araştırmanın birinci yılında 312.2±27.2 cm olan bitki boyu ortalaması, araştırmanın ikinci yılında önemli derecede daha düşük değer göstermiş ve 265.0±6.6 cm olarak saptanmıştır. Yıllar arasındaki bu farkın, Heveahri, Yellow ve White çeşitlerinin ikinci yıldaki hasat tarihlerinin, birinci yıla göre 10-15 gün önce yapılmasının neden olduğu söylenebilir.

Araştırmanın yürütüldüğü iki yılda saptanan değerlerin ortalamasına göre, White (354.5±33.4 cm) çeşidi, test edilen diğer inci darı çeşitlerine göre önemli derecede daha yüksek bitki boyu ortalaması gösterirken, Tifleaf 3 (200.3±17.7 cm) çeşidi, test edilen diğer inci darı çeşitlerine göre önemli derecede daha düşük bitki boyu ortalaması göstermiştir. Heveahri (318.4±32.0 cm) ve Yellow (321.5±17.9 cm) çeşitlerinde ise birbirinden istatistiksel olarak önemli derecede farklı olmayan bitki boyu ortalamaları saptanmıştır. Araştırmadan elde edilen bitki boyu değerleri, Izge ve ark. (2007)'nın (137.43-218.71 cm), Govindaraj ve ark. (2011)'nın (149.00-250.00 cm) ve Shah ve ark. (2012)'nın (143-227 cm) elde ettikleri değerlerden daha yüksek bulunmuştur. Araştırıcıların elde ettikleri bulguların, araştırmadan elde edilen bulgulardan farklı olması, araştırmalarda kullanılan çeşit ve genotiplerin farklı genetik yapıda olması, ekolojik faktörlerin farklı olması ve yetiştirme tekniklerinin farklılığı ile açıklanabilir.

Araştırmada, yıl x çeşit interaksiyonu bitki boyu üzerinde önemli derecede fark yaratmıştır. Nitekim, Ashana ve Tifleaf 3 inci darı çeşitlerinin bitki boyu ortalaması birinci yıla göre ikinci yılda önemli derecede daha yüksek değer göstermesine karşılık, söz konusu inci darı çeşitleri dışındaki çeşitlerin bitki boyu ortalamalarının birinci yıla göre, ikinci yılda önemli derecede daha düşük değer gösterdiği ortaya çıkmıştır. Santos ve ark. (2020), inci darının hasat zamanı geciktikçe bitki boyunun günlük olarak

yaklaşık 2.4 cm arttığını bildirmişlerdir. Bu bağlamda, araştırmada test edilen inci darı çeşitlerinin farklı tarihlerde çiçeklenmeye ulaşmaları nedeniyle hasat tarihlerinin yıldan yıla farklı olması (Çizelge 2), çeşitlerin yıllara göre bitki boyu ortalamalarının farklılık göstermesine neden olduğu söylenebilir.

Çizelge 4. İnci darı çeşitlerinde saptanan bitki boyu (cm) ve yeşil ot verimi (t ha^{-1}) ortalamaları
Table 4 Means of plant height (cm) and green forage yield (t ha^{-1}) of pearl millet cultivars

Çeşitler *Cultivars*	Bitki Boyu (cm) *Plant height (cm)*			Yeşil Ot Verimi (t ha^{-1}) *Green forage yield (t ha^{-1})*		
	2018	2019	Ortalama *Average*	2018	2019	Ortalama *Average*
Ashana	227.2±5.4 f[2]	269.4±8.9 de	248.3±10.5 C[1]	50.4±0.8 d[2]	50.5±0.3 d	50.5±0.4 C[1]
Heveahri	389.6±5.1 b	247.3±3.9 ef	318.4±32.0 B	91.9±2.2 a	73.0±0.9 c	82.5±4.3 AB
White	429.2±2.7 a	279.7±0.4 d	354.5±33.4 A	95.2±3.4 a	72.8±0.6 c	84.0±5.2 A
Yellow	352.0±15.6 c	291.1±20.7 d	321.5±17.9 B	86.2±1.8 b	73.5±1.6 c	79.8±3.0 B
Tifleaf 3	163.2±13.8 g	237.4±0.7 f	200.3±17.7 D	35.5±2.2 e	46.6±0.7 d	41.1±2.7 D
Ortalama *Average*	312.2±27.2 A[+]	265.0±6.6 B		71.8±6.5 A[+]	63.3±3.2 B	

[+] Benzer harflerle gösterilen "Yıl" ortalamalar arasında istatistiki olarak fark yoktur
[1,2] Benzer harflerle gösterilen ortalamalar arasında Duncan testine göre P≤0.05 hata sınırları içinde istatistiki olarak fark yoktur

Yeşil Ot Verimi (t ha^{-1})

İki yıl sürdürülen araştırmada saptanan inci darı çeşitlerinin yeşil ot verimi ortalamaları Çizelge 4'te verilmiştir. Çizelge 4'te görüldüğü üzere, yeşil ot verimi ortalaması araştırmanın yürütüldüğü birinci yılda 71.8±6.5 t ha^{-1} olarak gerçekleşirken, ikinci yılda birinci yıla göre önemli derecede daha düşük değer göstermiş ve 63.3±3.2 t ha^{-1} olarak gerçekleşmiştir. Yıllar arasındaki bu farklılığa, araştırmanın birinci yılında saptanan bitki boyunun, araştırmanın ikinci yılına göre daha yüksek olmasının neden olduğu söylenebilir. Nitekim bitki boyu ile yeşil ot verimi arasında önemli ve olumlu ilişkilerin olduğu görülmektedir (Çizelge 7). Benzer bulgular Aswini ve ark. (2023) tarafından da bildirilmektedir.

İki yıllık ortalamaya göre White çeşidinde (84.0±5.2 t ha^{-1}), Heveahri çeşidi (82.5±4.3 t ha^{-1}) hariç, test edilen diğer inci darı çeşitlerine göre önemli derecede daha yüksek yeşil ot verimi ortalaması tespit edilmiştir. Tifleaf 3 çeşidinde (41.1±2.7 t ha^{-1}) ise, test edilen diğer çeşitlere göre önemli derecede daha düşük yeşil ot verimi ortalaması saptanmıştır. Araştırmada saptanan yeşil ot verimi değerleri, bazı araştırıcıların (Abd El-Lattief, 2011; Kaur & Goyal, 2019) saptadıkları yeşil ot verimi değerlerine kısmen benzer, bazı araştırıcıların (Shekara ve ark., 2019; Talasila ve ark., 2019) saptadıkları yeşil ot verimi değerlerinden ise yüksek olduğu ortaya çıkmıştır. Araştırmadan elde edilen yeşil ot verimi değerlerinin, araştırıcıların elde ettikleri yeşil ot verimi değerlerinden farklı olması, araştırmalarda kullanılan inci darı çeşitlerinin farklı genetik yapıda olması, yetiştirme tekniklerinin farklılık göstermesi ve ekolojik farklılıklar ile açıklanabilir.

İki yıl sürdürülen araştırmada, yıl x çeşit interaksiyonunun istatistiki olarak önemli olduğu ortaya çıkmıştır. Nitekim, Heveahri, White ve Yellow çeşitlerinin yeşil ot verimi ortalaması birinci yıla göre ikinci yılda önemli derecede daha düşük değer göstermesine karşılık, Tifleaf 3 çeşidinin yeşil ot verimi ortalaması birinci yıla göre ikinci yılda önemli derecede daha yüksek değer göstermiş, Ashana çeşidinin yeşil ot verimi ortalaması ise iki deneme yılında birbirinden istatistiki olarak önemli derecede farklılık göstermemiştir. İnci darının fotoperiyoda duyarlı kısa gün bitkisi olması (Soler ve ark., 2008; Salama ve ark., 2020; Yücel & Yücel, 2022) ve çeşitlerin, asimilatların daha etkili kullanımı için olanak sağlayan ve bitkiyi destekleyen farklı fotoperiyot dönemine maruz kalması (Maas ve ark., 2007; Mason ve ark., 2015), inci darı çeşitlerinin yıllara göre farklı yeşil ot verimi değeri göstermelerinin nedeni olarak gösterilebilir.

Kuru Ot Verimi (t ha^{-1})

İkinci ürün şartlarında tesis edilen denemeden saptanan inci darı çeşitlerinin kuru ot verimi ortalamaları Çizelge 5'te verilmiştir. Çizelge 5 incelendiğinde, inci darı çeşitlerinin kuru ot verimi ortalamasının birinci yıla (16.0±2.2 t ha^{-1}) göre, ikinci yılda (13.8±1.1 t ha^{-1}) önemli derecede daha düşük değer gösterdiği ortaya çıkmıştır. Araştırmanın birinci yılına göre, ikinci yılındaki yeşil ot verimi ortalamalarının düşük olması, kuru ot verimi ortalamalarının da araştırmanın birinci yılına göre ikinci yılında düşük olmasına neden olmuştur. Yeşil ot verimi ile kuru ot verimi arasında önemli ve olumlu ilişkilerin olduğu Çizelge 7'de görülmektedir. Benzer bulgular Aswini ve ark. (2023) tarafından da bildirilmektedir.

İki yıl sürdürülen araştırmada, White (20.3±2.1 t ha^{-1}) çeşidi, Heveahri çeşidi (19.9±0.8 t ha^{-1}) hariç, test

edilen diğer çeşitlere göre önemli derecede daha yüksek kuru ot verimi ortalaması göstermiştir. Yellow çeşidinde (19.5±0.7 t ha^{-1}) ise Heveahri çeşidi ile birbirinden istatistiki olarak önemli derecede farklı olmayan, ancak Ashana (7.9±0.2 t ha^{-1}) ve Tifleaf 3 (6.9±1.1 t ha^{-1}) çeşidinden istatistiki olarak önemli derecede daha yüksek kuru ot verimi ortalaması tespit edilmiştir. Araştırmadan tespit edilen kuru ot verimi değerleri, Shahin ve ark. (2013) (3.78-10.71 t ha^{-1}), Choudhary ve ark. (2017) (7.29-12.05 t ha^{-1}), Makarana ve ark. (2018) (9.78-14.83 t ha^{-1}) ve Noor ve ark. (2018)'nın (11.57-13.67 t ha^{-1}) tespit ettikleri kuru ot verimi değerleriyle kısmen uyum içerisinde olduğu görülmektedir.

İnci darı çeşitlerinin ikinci ürün olarak tesis edildiği araştırmada, yıl x çeşit interaksiyonunun kuru ot veriminde önemli derecede fark yarattığı ortaya çıkmıştır. Nitekim, Ashana çeşidinin kuru ot verimi ortalaması deneme yıllarına bağlı olarak önemli derecede farklılık göstermemesine karşılık, Heveahri, White ve Yellow çeşitlerinin kuru ot verimi ortalaması denemenin birinci yılına göre ikinci yılında önemli derecede daha düşük değer göstermiştir. Tifleaf 3 çeşidi ise ikinci yılda birinci yıla göre önemli derecede daha yüksek kuru ot verimi vermiştir. Araştırmada, test edilen çeşitlerin kuru ot verimi değerlerinin yıllara bağlı olarak değişimi, yeşil ot verimindeki değişimle uyumlu olmuştur.

Çizelge 5. İnci darı çeşitlerinde saptanan kuru ot verimi (t ha^{-1}) ve yaprak oranı (%) ortalamaları
Table 5 Means of hay yield (t ha^{-1}) and leaf ratio (%) of pearl millet cultivars

Çeşitler *Cultivars*	**Kuru Ot Verimi (t ha^{-1})** *Hay yield (t ha^{-1})*			**Yaprak Oranı (%)** *Leaf ratio (%)*		
	2018	**2019**	**Ortalama** *Average*	**2018**	**2019**	**Ortalama** *Average*
Ashana	7.8±0.3 f[2]	8.1±0.2 f	7.9±0.2 C[1]	41.8±0.7 b[2]	36.0±1.0 c	38.9±1.4 B[1]
Heveahri	21.8±0.1 b	18.0±0.2 c	19.9±0.8 AB	23.6±0.1 f	31.9±0.5 d	27.7±1.9 C
White	24.9±0.5 a	15.7±0.3 d	20.3±2.1 A	25.7±0.5 e	32.1±1.0 d	28.9±1.5 C
Yellow	21.0±0.5 b	17.9±0.2 c	19.5±0.7 B	22.3±0.6 f	33.0±0.6 d	27.7±2.4 C
Tifleaf 3	4.4±0.5 g	9.4±0.2 e	6.9±1.1 D	50.6±0.8 a	31.3±0.7 d	41.0±4.3 A
Ortalama *Average*	16.0±2.2 A[+]	13.8±1.1 B		32.8±3.0	32.9±0.5	

[1,2] Benzer harflerle gösterilen ortalamalar arasında Duncan testine göre P≤0.05 hata sınırları içinde istatistiki olarak fark yoktur

Yaprak Oranı (%)

İkinci ürün koşullarında iki yıl süreyle tesis edilen denemelerden saptanan inci darı çeşitlerinin yaprak oranı ortalamaları Çizelge 5'te verilmiştir. Çizelge 5 izlendiğinde, 2018 yılında yaprak oranı ortalamasının %32.8±3.0 olarak, 2019 yılında ise %32.9±0.5 olarak gerçekleştiği ve yaprak oranı ortalamasının deneme yıllarına göre istatistiki olarak birbirinden önemli derecede farklılık göstermediği ortaya çıkmıştır.

İki yıl sürdürülen araştırmada, Tifleaf 3 çeşidi %41.0±4.3 yaprak oranı ortalaması ile, test edilen diğer çeşitlere göre önemli derecede daha yüksek değer gösterirken, söz konusu çeşidi %38.9±1.4 yaprak oranı ortalaması ile Ashana çeşidi izlemiştir. Tifleaf3 çeşidinin bitki başına yaprak sayısının fazla olması, aynı bitkinin kardeş sayısının fazla olmasından kaynaklanmış olabilir (Çizelge 6). White, Heveahri ve Yellow çeşitleri ise istatistiki olarak birbirinden farklı olmayan yaprak oranı ortalaması göstermişlerdir. Ancak, yaprak oranı açısından yıl x çeşit interaksiyonunun istatistiki olarak önemli çıkması, yılların yaprak oranı üzerindeki etkisinin çeşitlere bağlı olarak önemli derecede farklılık gösterdiğini ortaya koymaktadır. Nitekim, Ashana ve Tifleaf 3 inci darı çeşitlerinin yaprak oranı ortalaması 2018 yılına göre 2019 yılında önemli derecede daha düşük değer göstermesine karşılık, Heveahri, White ve Yellow inci darı çeşitlerinin 2018 yılına göre 2019 yılında önemli derecede daha yüksek yaprak oranı değerleri gösterdikleri ortaya çıkmıştır. Craufurd ve Bidinger (1988), inci darının vejetatif aşama süresi uzadıkça ana sap gelişiminin arttığını ancak ana sap gelişiminin artmasıyla gölge oluşumunun da artarak yaprak üretiminin durmasına neden olduğunu bildirmişlerdir. Bu bağlamda, araştırmada test edilen inci darı çeşitlerinin vejetatif aşama sürelerinin yıllara göre farklı olması, çeşitlerin yaprak oranı değerlerinin yıllara göre farklılık göstermesine neden olduğu söylenebilir.

Sap Çapı (mm)

İkinci ürün koşullarında iki yıl tesis edilen denemede inci darı çeşitlerinden saptanan sap çapı ortalamaları Çizelge 6'da verilmiştir. Çizelgede izlendiği üzere, çeşitlerin sap çapı ortalaması 2018 yılında 16.2±0.4 mm olurken, 2019 yılında sap çapı ortalaması 2018 yılına göre önemli derecede daha düşük değer göstermiş ve 13.4±0.4 mm olarak gerçekleşmiştir.

İki yıllık ortalama değerlere göre, Ashana çeşidi (16.1±0.9 mm), test edilen diğer çeşitlere göre önemli derecede daha yüksek sap çapı değeri gösterirken, Yellow çeşidinin (13.7±0.2 mm), Tifleaf 3 çeşidi (14.3±1.1 mm) hariç, test edilen diğer çeşitlere göre önemli derecede daha düşük sap çapı değeri gösterdiği

tespit edilmiştir. Araştırmadan elde edilen sap çapı değerlerinin, bazı araştırıcıların (Hassan ve ark., 2014; Uhunomwan Omoregie ve ark., 2020) elde ettikleri sap çapı değerleri ile kısmen uyum içerisinde olduğu, bazı araştırıcıların (Piri & Tavassoli, 2012; Noor ve ark., 2018) elde ettikleri sap çapı değerlerinden ise yüksek olduğu anlaşılmıştır. Araştırmadan elde edilen sap çapı değerlerinin, araştırıcıların elde ettikleri sap çapı değerlerinden farklı olması, araştırmalarda kullanılan çeşitlerin farklı genetik yapıda olması, yetiştirme tekniklerinin farklı olması ve ekolojik koşulların farklılık göstermesi ile açıklanabilir.

Araştırmada, yıl x çeşit interaksiyonunun sap çapı üzerinde önemli derecede fark yarattığı ortaya çıkmıştır. Nitekim, Heveahri ve Yellow inci darı çeşitlerinin sap çapı ortalaması deneme yıllarına bağlı olarak önemli derecede farklılık göstermemesine karşılık, Ashana, White ve Tifleaf 3 inci darı çeşitlerinin sap çapı ortalaması 2018 yılına göre, 2019 yılında istatistiki olarak önemli derecede daha düşük değer göstermiştir. Bu durum, inci darı çeşitlerinin yıllar itibariyle vejetatif aşama sürelerinin farklı olması ile açıklanabilir.

Çizelge 6. İnci darı çeşitlerinde saptanan sap çapı (mm) ve kardeş sayısı (adet/bitki) ortalamaları
Table 6 Means of main stem diameter (mm) and number of tiller (number/plant) of pearl millet cultivars

Çeşitler *Cultivars*	**Sap Çapı (mm)** *Stem diameter (mm)*			**Kardeş Sayısı (adet/bitki)** *Number of tillers (number/plant)*		
	2018	**2019**	**Ortalama** *Average*	**2018**	**2019**	**Ortalama** *Average*
Ashana	17.9±0.2 a[2]	14.4±0.8 cd	16.1±0.9 A[1]	4.4±0.2 bc[2]	3.8±0.1 cd	4.1±0.2 BC[1]
Heveahri	15.5±0.2 bc	14.6±1.1 cd	15.1±0.5 B	4.8±0.3 bc	2.6±0.2 e	3.7±0.5 C
White	17.1±0.1 a	12.9±0.4 ef	15.0±1.0 B	4.1±0.1 c	5.4±0.6 b	4.8±0.4 B
Yellow	13.9±0.3 de	13.4±0.1 de	13.7±0.2 C	3.8±0.1 cd	3.0±0.2 de	3.4±0.2 C
Tifleaf 3	16.7±0.1 ab	11.8±0.4 f	14.3±1.1 BC	9.4±0.1 a	8.5±0.6 a	9.0±0.3 A
Ortalama *Average*	16.2±0.4 A[+]	13.4±0.4 B		5.3±0.6 A[+]	4.7±0.7 B	

[+] Benzer harflerle gösterilen "Yıl" ortalamalar arasında istatistiki olarak fark yoktur
[1,2] Benzer harflerle gösterilen ortalamalar arasında Duncan testine göre P≤0.05 hata sınırları içinde istatistiki olarak fark yoktur

Kardeş Sayısı (adet/bitki)

İkinci ürün koşullarında yetiştirilen farklı inci darı çeşitlerinde saptanan kardeş sayısı ortalamaları Çizelge 6'da verilmiştir. Çizelge 6'da görüldüğü üzere, çeşitlerin kardeş sayısı ortalamasının 2018 yılında 5.3±0.6 adet/bitki olarak gerçekleştiği, 2019 yılında kardeş sayısı ortalamasının 2018 yılına göre istatistiki olarak önemli derecede daha düşük değer gösterdiği ve 4.7±0.7 adet/bitki olarak gerçekleştiği ortaya çıkmıştır.

Araştırmada, Tifleaf 3 inci darı çeşidinde (9.0±0.3 adet/bitki), test edilen diğer inci darı çeşitlerine göre önemli derecede daha yüksek kardeş sayısı ortalaması tespit edilmiştir. Yellow çeşidinde (3.4±0.2 adet/bitki) ise, Heveahri (3.7±0.5 adet/bitki) ve Ashana (4.1±0.2 adet/bitki) çeşitleri hariç, diğer çeşitlere göre önemli derecede daha düşük kardeş sayısı ortalaması tespit edilmiştir. Ancak, kardeş sayısı açısından yıl x çeşit interaksiyonunun önemli olması, yılların kardeş sayısı üzerindeki etkisinin çeşitlere bağlı olarak önemli derecede farklılık gösterdiğini ortaya koymaktadır. Nitekim, Ashana, Yellow ve Tifleaf 3 çeşitlerinin kardeş sayısı ortalaması deneme yılına bağlı olarak önemli derecede farklılık göstermezken, Heveahri çeşidinin 2019 yılında kardeş sayısı ortalamasının 2018 yılına göre önemli derecede daha düşük değer gösterdiği, White çeşidinin ise 2019 yılında kardeş sayısı ortalamasının 2018 yılına göre önemli derecede daha yüksek değer gösterdiği ortaya çıkmıştır.

Araştırmadan elde edilen kardeş sayısı değerleri, bazı araştırıcıların (Bouchard ve ark., 2011; Obeng ve ark., 2012; Shah ve ark., 2012) elde ettikleri kardeş sayısı değerlerinden yüksek olduğu ortaya çıkmıştır. Araştırmadan elde edilen kardeş sayısı değerlerinin, araştırıcıların elde ettikleri kardeş sayısı değerlerinden yüksek bulunması, araştırmalarda kullanılan inci darı çeşitlerinin farklı genetik yapıda olması, yetiştirme tekniğinin farklı olması ve ekolojik koşulların farklılık göstermesi ile açıklanabilir.

İncelenen Özellikler Arasındaki İlişkiler

İkinci ürün koşullarında yetiştirilen inci darı çeşitlerinde incelenen özellikler arasındaki korelasyon katsayıları, Çizelge 7'de verilmiştir.

Çizelge 7'de izlendiği gibi, bitki boyu ile yeşil ot verimi (0.9773**) arasında çok önemli ve olumlu ve kuru ot verimi (0.9562*) arasında önemli ve olumlu ilişki olduğu ortaya çıkmıştır. Bu durum, bitki boyunun artmasıyla yeşil ot ve kuru ot veriminin arttığını ortaya koymaktadır. Bitki boyu ile yaprak oranı (-0.9417*) arasında önemli ve olumsuz ilişki olduğu tespit edilmiştir. Bu sonuç, bitki boyu arttıkça gölge oluşumunun artmasından dolayı yaprak üretiminin durduğunu bildiren Craufurd ve Bidinger (1988)'in

Çizelge 7. İnci darı çeşitlerinde incelenen özellikler arasındaki korelasyon katsayıları
Table 7 Correlation coefficients between the traits examined in pearl millet cultivars

	Bitki Boyu *Plant height*	**Yeşil Ot Verimi** *Green forage yield*	**Kuru Ot Verimi** *Hay yield*	**Yaprak Oranı** *Leaf ratio*	**Sap Çapı** *Stem diameter*	**Kardeş Sayısı** *Number of tillers*
Bitki Boyu *Plant height*	1.0000					
Yeşil Ot Verimi *Green forage yield*	0.9773**	1.0000				
Kuru Ot Verimi *Hay yield*	0.9562*	0.9932**	1.0000			
Yaprak Oranı *Leaf ratio*	-0.9417*	-0.9889**	-0.9916**	1.0000		
Sap Çapı *Stem diameter*	-0.0887	-0.1970	-0.3003	0.3003	1.0000	
Kardeş Sayısı *Number of tillers*	-0.7368	-0.7197	-0.6494	0.7162	-0.2277	1.0000

*: p≤0.05, **: p≤0.01

bulgularını destekler niteliktedir. Yeşil ot verimi ile kuru ot verimi (0.9932**) arasında çok önemli ve olumlu ilişki, yeşil ot verimi ile yaprak oranı (-0.9889**) arasında çok önemli ve olumsuz ilişki olduğu ortaya çıkmıştır. Yeşil ot verimi ile kuru ot verimindeki artışın paralellik göstermesi beklenen bir durumdur. Kuru ot verimi ile yaprak oranı (-0.9916**) arasında çok önemli ve negatif ilişki olduğu saptanmıştır. Araştırmadan elde edilen bu bulguların, Vidyadhar ve ark. (2007), Kumar ve ark. (2014) ve Kumawat ve ark. (2019)'nın bulguları ile uyum içerisinde olduğu ortaya çıkmıştır.

SONUÇ ve ÖNERİLER

İkinci ürün koşullarında iki yıl sürdürülen araştırmanın sonucunda, test edilen inci darı çeşitlerinin, Akdeniz ikliminin hüküm sürdüğü sahil bölgelerinde yaz döneminde kaba yem kaynağı olabileceği, sorgum ve mısıra alternatif olarak değerlendirilebileceği ortaya çıkmıştır. Bunun yanında, White ve Heveahri inci darı çeşitlerinin ot verimi bakımında diğer çeşitlerden üstün olduğu anlaşılmıştır. Mevcut bulguların yanında, incelenen çeşitlerin ot kaliteleri ve silaj verimlerinin belirlenerek yeni araştırmaların yapılması gerektiği sonucuna varılmıştır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

Abd El-Lattief, E. A. (2011). Growth and fodder yield of forage pearl millet in newly cultivated land as affected by date of planting and integrated use of mineral and organic fertilizers. *Asian Journal of Crop Science, 3* (1), 35-42. https://doi.org/10.3923/ajcs.2011.35.42.

Açıkgöz, E. (2021). *Yem Bitkileri (IV. Baskı).* T.C. Tarım Orman Bakanlığı Eğitim ve Yayın Dairesi Başkanlığı, Ankara.

Alçiçek, A. (2021). *Türkiye Kaba Yem İhtiyacının Hesaplanması.* Türkiye Hayvancılığında Kaba Yem Sorunları ve Çözüm Yolları Çalıştayı, Muş Alparslan Üniversitesi, 21-22 Haziran 2021, ss. 67.

Anonim, (2019). T.C. Çevre, Şehircilik ve İklim Değişikliği Bakanlığı Meteorolojik Veri Bilgi Sunum ve Satış Sistemi. https://mevbis.mgm.gov.tr/mevbis/ui/index.html#/Workspace. (Alınma Tarihi: 07.12.2021).

Aswini, M. S., Ganesan, K. N., & Ezhilarasi, T. (2023). The correlation between green fodder yield and fodder quality traits in hybrids of pearl millet [*Pennisetum glaucus* (L.) R. Br.]. *International Journal of Plant & Soil Science, 35* (19), 1975-1983.

Benek, A., & Geren, H. (2023). İnci darısı (*Pennisetum glaucum*)'nın tane verimi ve bazı verim unsurlarına farklı azot ve fosfor seviyelerinin etkisi üzerine bir ön çalışma. *ISPEC Tarım Bilimleri Dergisi, 7* (1), 36-44.

Bouchard, A., Vanasse, A., Seguin, P., & Bélanger, G. (2011). Yield and composition of sweet pearl millet as affected by row spacing and seeding rate. *Agronomy Journal, 103* (4), 995-1001. https://doi.org/10.2134/agronj2010.0492.

Choudhary, S., Chopra, N. K., Chopra, N. K., Singh, M., Kumar, R., & Kushwaha, M. (2017). Influence of nitrogen levels and weed management practices on yield and quality of forage pearl millet (*Pennisetum glaucum* L.). *Indian Journal of Animal*

Nutrition, 34 (1), 64-69. doi:10.5958/2231-6744.2017.00010.X.

Craufurd, P. Q., & Bidinger, F. R. (1988). Effect of the duration of the vegetative phase on shoot growth, development, and yield in pearl millet (*Pennisetum americanum* (L.) Leeke). *Journal of Experimental Botany, 39* (1), 124-139. https://doi.org/10.1093/jxb/39.1.124.

Dumanoğlu, Z., Özdemir, S., & Kökten, K. (2022). Farklı inci darısı (*Pennisetum glaucum* (L.) R. Br.) genotiplerine ait tohumların bazı morfolojik ve fizyolojik özelliklerinin belirlenmesi. *ISPEC Tarım Bilimleri Dergisi, 6* (1), 64-71.

Dönmez, H. B. (2022). *Akdeniz İklim Koşullarında Koruyucu Bitki Türü, Tohumluk Miktarı ve Hasat Zamanının Çokyıllık Suni Mera Karışımının Ot Verimi ve Kalitesine Etkileri (Tez no 770916)*. [Doktora Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Govindaraj, M., Selvi, B., Rajarathinam, S., & Sumathi, P. (2011). genetic variability and heritability of grain yield components and grain mineral concentration in India's pearl millet (*Pennisetum glaucum* (L) R. Br.) accessions. *African Journal of Food, Agriculture, Nutrition and Development, 11* (3), 4758-4771. https://doi.org/10.4314/ajfand.v11i3.66627.

Hassan, M. U., Ahmad, A. H., Zamir, S. I., Haq, I., Khalid, F., Rasool, T., & Hussain, A. (2014). Growth, yield, and quality performance of pearl millet (*Pennisetum americanum L.*) varieties under Faisalabad conditions, Pakistan. *American Journal of Plant Sciences, 5* (15), 2215-2223. https://doi.org/10.4236/ajps.2014.515235.

Hatipoğlu, R., & Tükel, T. (2009). *Darılar*. In R. Avcıoğlu, R. Hatipoğlu, & Y. Karadağ (Eds.), *Yembitkileri (Buğdaygil ve Diğer Familyalardan Yembitkileri)*. T.C. Tarım ve Köyişleri Bakanlığı Tarımsal Üretim ve Geliştirme Genel Müdürlüğü.

Hatipoğlu, R., Serbester, U., Avcı, M., & Dönmez, H. B. (2020). Adana ilinde kaba yem üretim durumu ve geliştirilme olanakları. *Turkish Journal of Agriculture-Food Science and Technology, 8* (7), 1497-1501. https://doi.org/10.24925/turjaf.v8i7.1497-1501.3383.

Izge, A. U., Kadams, A. M., & Sajo, A. A. (2007). Agronomic performance of selected cultivars of pearl millet (*Pennisetum glaucum* L.R.Br.) and their hybrids in North-Eastern Nigeria. *Journal of Agronomy, 6* (2), 344-349. https://doi.org/10.3923/ja.2007.344.349.

Jukanti, A. K., Gowda, C. L. L., Rai, K. N., Manga, V. K., & Bhatt, R. K. (2016). Crops that feed the world 11. Pearl Millet (*Pennisetum glaucum* L.): an important source of food security, nutrition, and health in the arid and semi-arid tropics. *Food Security, 8*, 307-329. Doi: 10.1007/s12571-016-0557-y.

Kaur, M., & Goyal, M. (2019). Influence of different nitrogen levels on growth, yield, and quality of forage pearl millet (*Pennisetum glaucum* L.) genotypes. *Forage Research, 45* (1), 43-46.

Kızılkaya, R. (2020). *Toprak Analiz Sonuçlarının Değerlendirilmesi.* Neris Yatırım Gıda İnş. San. ve Tic. A.Ş, Ankara, 13 sy.

Kumar, Y., Lamba, R. A. S., Yadav, H. P., Kumar, R., & Vart, D. (2014). Studies on variability and character association under rainfed conditions in pearl millet (*Pennisetum glaucum* L.) hybrids. *Forage Research, 39* (4), 175-178.

Kumawat, K. R., Sharma, N. K. & Sharma, N. (2019). Genetic variability and character association analysis in pearl millet single cross hybrids under dry conditions of Rajasthan. *Electronic Journal of Plant Breeding, 10* (3), 1067-1070.

Lemus, R. (2015). Pearl millet: Pinpoint Forage for Summer Grazing. *Forage news. Mississippi State University Extension Service, 8* (4), 1-2.

Maas, A. L., Hanna, W. W., & Mullinix, B. G. (2007). Planting date and row spacing affect grain yield and height of pearl millet Tifgrain 102 in the Southeastern coastal plain of the United States. *Journal of SAT Agricultural Research, 5* (1), 1-4.

Makarana, G., Yadav, R. K., Kumar, R., Kumar, A., Soni, P. G., Kar, S., & Rajvaidya, S. K. (2018). Fodder and grain quality of Pearl millet (*Pennisetum glaucum* L.) undercutting management in saline irrigation water. *Journal of Pharmacognosy and Phytochemistry, 7* (3), 1251-1257.

Mason, S. C., Maman, N., & Palé, S. (2015). Pearl millet production practices in semi-arid west Africa: a review. *Experimental Agriculture, 51* (4), 501-521. https://doi.org/10.1017/S0014479714000441.

Noor, M. A., Fiaz, S., Nawaz, A., & Nawaz, M. M. (2018). The effects of cutting interval on agro-qualitative traits of different millet (*Pennisetum americanum* L.) cultivars. *Journal of the Saudi Society of Agricultural Sciences, 17* (3), 317-322. https://doi.org/10.1016/j.jssas.2016.07.002.

Obeng, E., Cebert, E., Singh, B. P., Ward, R., Nyochembeng, L. M., & Mays, D. A. (2012). Growth and grain yield of pearl millet (*Pennisetum glaucum*) genotypes at different levels of nitrogen fertilization in the Southeastern United States. *Journal of Agricultural Science, 4* (12), 155-163. https://doi.org/10.5539/jas.v4n12p155.

Piri, I., & Tavassoli, A. (2012). Determining the best management of nitrogen fertilizer consumption and harvest time of forage yield of pearl millet (*Pennisetum glaucum*) in Shirvan region. *African Journal of Microbiology Research, 6* (10), 2287-2293. https://doi.org/10.5897/ajmr11.918.

Salama, H. S. A., Shaalan, A. M., & Nasser, M. E. A. (2020). Forage performance of pearl millet (*Pennisetum glaucum* [L.] R. Br.) in arid regions: yield and quality assessment of new genotypes on different sowing dates. *Chilean Journal of Agricultural Research, 80* (4), 572-584. https://doi.org/10.4067/S0718-58392020000400572.

Santos, R. D., Neves, A. L. A., Pereira, L. G. R., Sollenberger, L. E., Muniz, E. N., Souza, E. Y. B., Sobral, A. J. S., Costa, N. V., & Gonçalves, L. C. (2020). Performance, agronomic traits, availability, and nutritive value of pearl millet cultivar harvested at different growth stages. *Journal of Agricultural Science, 158* (3), 225-232. https://doi.org/10.1017/S0021859620000222.

Shah, I. A., Rahman, H. U., Shah, S. M. A., Shah, Z., Rahman, S., Noor, I., & Noor, M. (2012). Characterization of pearl millet germplasm for various morphological and fodder yield parameters. *Pakistan Journal of Botany, 44* (1), 273-279.

Shahin, M. G., Abdrabou, R. T., Abdelmoemn, W. R., & Hamada, M. M. (2013). Response of growth and forage yield of pearl millet (*Pennisetum glaucum*) to nitrogen fertilization rates and cutting height. *Annals of Agricultural Sciences, 58* (2), 153-162. https://doi.org/10.1016/j.aoas.2013.07.009.

Shekara, B. G., Mahadevu, P., Chikkarugi, N. M., & Manasa, N. (2019). Response of pearl millet (*Pennisetum glaucum* L.) varieties to nitrogen levels for higher green forage yield and quality in the southern dry zone of Karnataka. *Forage Research, 45* (3), 232-234.

Silungwe, D. (2011). *Evaluation of forage yield and quality of sorghum, sudangrass, and pearl millet cultivars in Manawatu.* [Master Thesis, Massey University, New Zealand].

Soler, C. M. T., Maman, N., Zhang, X., Mason, S. C., & Hoogenboom, G. (2008). Determining optimum planting dates for pearl millet for two contrasting environments using a modeling approach. *Journal of Agricultural Science, 146*, 445-459.

Talasila, V., Singh, R., Kishore, C. R., & Singh, A. C. (2019). Effect of planting density and nitrogen levels on growth and yield of fodder pearl millet (*Pennisetum glaucum* L.). *International Journal of Current Microbiology and Applied Sciences, 8* (7), 312-318.

Uhunomwan Omoregie, A., Ebonka Nwajei, S., & Efebuede Iredia, B. (2020). Effects of planting density on the growth and forage yield of two varieties of millet (*Pennisetum typhoides* Burm. F.) grown in Ekpoma, Nigeria. *Sustainability, Agri, Food and Environmental Research, 8* (2), 118-128. http://dx.doi.org/10.7770/safer-V0N0-art1737.

Vidyadhar, B., Chand, P., Devi, I. S., Reddy, M. V. S., & Ramachandraiah D. (2007). Genetic variability and character association in pearl millet [*Pennisetum glaucum* (L.) R. Br.] and their implications in selection. *Indian Journal of Agricultural Research, 41* (2), 150-153.

Yücel, C., & Yücel, D. (2022). *İnci Darı* (*Pennisetum glaucum* (L.) R. Br.). In S. Doğan & N. Kalender (Ed.), *Tarımda Değişen Yapılar ve Beklentiler*. İksad Yayınevi.

Yurtsever, N. (2011). *Deneysel İstatistik Metotları*. Tarım ve Köy İşleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü Toprak Gübre ve Su Kaynakları Merkez Araştırma Enstitüsü Yayınları Genel Yayın No: 121 Teknik Yayın No 56.

KSÜ Tarım ve Doğa Derg 27 (4), 920-928, 2024
KSU J. Agric Nat 27 (4), 920-928, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1230303

Yüksek Rakımlı Bir Merada Kuru Ot Verimi ile Nispi Yem Değerinin Yöneylere Göre Değişimi

İbrahim Hakan KARABULUT[1]✉, Binali ÇOMAKLI[2]
[1]Tarım ve Kırsal Kalkınmayı Destekleme Kurumu, Erzurum İl Koordinatörlüğü, Erzurum, [2]Atatürk Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Erzurum
[1]https://orcid.org/0000-0002-9740-9085, [2]https://orcid.org/0000-0001-6393-3869
✉: karabulut.hakn@gmail.com

ÖZET

Bu araştırma Erzurum, Erzincan ve Bayburt illerinin kesişiminde yer alan Kop Dağı meralarında 2019 ve 2020 yıllarında yürütülmüştür. Çalışma ile Kop Dağının 1871-2468 m yüksekliğinde yer alan mera kesimlerinin doğu, batı, kuzey ve güney yöneylerine ait kuru ot verimleri ve nispi yem değerleri tespit edilerek, verim ve kalite açısından yöneylere göre karşılaştırılması hedeflenmiştir. Araştırma sonuçlarına göre en yüksek kuru ot verimi (342.17 kg da-1) güney yöneyde, en düşük verim (202.67 kg da-1) ise doğu yöneyinde tespit edilmiştir. En yüksek nispi yem değeri (143.73) kuzey yöneyde ortaya çıkmış, rakım faktörü nispi yem değeri bakımından önemsiz bulunmuştur. Sonuç olarak etek kesimler başta olmak üzere dağ geneli meraların amenajman ilkelerine göre kullanılmaları gerektiği ve özellikle otlatma mevsimi ve otlatılacak hayvan cinsinin dikkate alındığı ve otlatma başlangıcında dağın sırt kesimleri ile batı ve güney yöney mera kesimlerinin öncelendiği bir otlatma programının oluşturulmasının meraların geleceği açısından önem arz ettiği kanaatine varılmıştır. Ayrıca bütün mera kesimleri için ıslah amaçlı araştırma ve uygulama çalışmalarının yapılmasının faydalı olacağı düşünülmektedir.

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 06.01.2023
Kabul Tarihi : 12.01.2024

Anahtar Kelimeler
Mera
Rakım
Yöney
Kuru ot verimi
Nispi yem değeri

Variation of Hay Yield and Relative Feed Value According to Direction in a Highland Rangeland

ABSTRACT

This research was carried out in Kop Mountain's rangelands, located at the intersection of Erzurum, Erzincan, and Bayburt provinces, in the 2019 and 2020 years. The study was aimed at determining the hay yields and relative feed values of the east, west, north, and south directions of the rangeland sites of Kop Mountain at an altitude of 1871–2468 m and comparing them in terms of yield and quality. According to the results of the research, the highest hay yield (342.17 kg da-1) was found in the south, and the lowest (202.67 kg da-1) was found in the east. The highest relative feed value (143.73) was found in the north, and the altitude factor was found to be insignificant in terms of relative feed value. As a result, it is concluded that the rangeland sites, especially on the footslope, should be used by the management principles and that it is important for the future of the rangelands to establish a grazing program that takes into account the grazing season and the type of animals to be grazed, prioritizing the backslope sites of the mountain and western and southern rangeland sites at the beginning of grazing. In addition, it is believed that it would be beneficial to carry out research and application studies for improvement at all the sites of the rangeland.

Field Crops

Research Article

Article History
Received : 06.01.2023
Accepted : 12.01.2024

Keywords
Rangeland
Direction
Altitude
Hay yield
Relative feed value

Atıf Şekli: Karabulut, İ.H., & Çomaklı, B., (2024) Yüksek Rakımlı Bir Merada Kuru Ot Verimi ile Nispi Yem Değerinin Yöneylere Göre Değişimi. *KSÜ Tarım ve Doğa Derg 27*(4), 920-928. https://doi.org/10.18016/ksutarimdoga. vi. 1230303

To Cite : Karabulut, İ.H., & Çomkalı, B., (2024). Variation of Hay Yield and Relative Feed Value According to Direction in a Highland Rangeland. *KSU J. Agric Nat 27*(4), 920-928. https://doi.org/10.18016/ksutarimdoga.vi.1230303

GİRİŞ

Hayvansal üretimin en önemli girdi kalemi yemlerdir. Sürdürülebilir üretim açısından en ucuz kaba yem ise çayır ve meralardan elde edilmektedir. Dünya genelinde hayvansal üretim için ihtiyaç duyulan kaba yemin %70'ine yakın kısmı meralardan elde

edilmektedir (Lund, 2007). Günümüzde nüfus artışı, iklim değişiminin etkisi ile oluşan ekolojik dengesizlik ve öngörülemeyen ekonomik değişkenler neticesinde çayır ve meralardan elde edilebilecek kaliteli kaba yeme duyulan ihtiyaç artmaktadır. Ancak özellikle amaç dışı kullanımları ve aşırı otlatılmaya maruz kalmaları meralardan faydalanma oranını sınırlamaktadır. Zira kurak ve yarı kurak meralardaki tahribatın başlıca sebeplerinden birisi olan aşırı otlatma (Snyman, 2005; Holechek et al., 2011) verimi düşürdüğü gibi, toprağın fiziksel ve kimyasal özellikleri üzerinde olumsuz etkilere neden olmakta (Beukes & Cowling, 2003) ve bu durum meraların zayıflaması ile erozyona açık alanların oluşmasına da neden olmaktadır. Bununla beraber aşırı otlatmanın meralarda istenmeyen türlerin çoğalması, bitki ile kaplı alan ve biokütlenin azalması (Tongway et al., 2003; Çomaklı et al., 2012) gibi olumsuz sonuçları da bulunmaktadır.

Meradan elde edilen kaba yemin verim ve kalitesi, hayvansal üretim açısından bu alanlardan elde edilebilecek faydanın temel belirleyici unsurlarıdır (Heitschmidt et al., 1995). Bu anlamda meralardan sürdürülebilir biçimde faydalanabilmek için, amenajman ilkelerine uygun olarak kullanılmaları gereklidir. Ayrıca mera popülasyonlarının korunması ve riskli bölgelerde ıslah amaçlı uygulamaların gerçekleştirilmesi gerekmektedir. Bu hedeflere ancak mera durumunun tespiti ve meraların zayıflamasına yol açan hususların doğru bir şekilde tespit edilmesi ile ulaşılabilir.

Meralarda otlatma faktörünün baskın etkisinin yanı sıra rakım, yöney gibi topoğrafik faktörler verim ve kaliteye etki etmektedir. Taşdelen ve Özyazıcı (2022) tarafından doğal bir merada farklı yüksekliklerin verim ve botanik kompozisyon üzerine etkilerinin incelendiği araştırmada; yükseklik artışı ile toprağı kaplama oranının arttığı ve üç farklı yükseklik (620, 770 ve 920 m) dikkate alınarak yapılan değerlendirmede, yükseklik arttıkça kuru ot veriminin de arttığı tespit edilmiştir. Bir başka faktör olarak yöney, meralarda bitki örtüsünün zenginliği ve botanik kompozisyonun çeşitliliği üzerinde önemli etkiye sahiptir (Mahdavi et al., 2012). Farklı rakım ile yöneylerin mera vejetasyonuna etkilerinin değerlendirildiği bir araştırmada; rakım arttıkça toprağı kaplama oranının azaldığı ve kuzey yöneyde yer alan mera kesiminin en verimli alan olduğu tespit edilmiştir (Çaçan & Başbağ, 2016). Tutar ve Kökten (2019) tarafından yapılan bir başka araştırmada ise kuru ot veriminin mera yöneylerine göre 23,2-129,3 kg da^{-1} arasında değişiklik gösterdiği, kuru ot veriminin araştırma yöneylerine göre farklılık arz ettiği ve en yüksek nispi yem değerinin doğu yöneyde (109,4) tespit edildiği bildirilmiştir.

Bu araştırmayla Doğu Anadolu ile Doğu Karadeniz arasındaki geçit kuşağında yer alan yüksek rakımlı Kop Dağı üzerinde yer alan mera kesimlerinin, yöneylere göre verim ve kalite açısından durumlarının tespit edilmesi ve benzer ekolojik bölgelerdeki meraların ıslahına temel teşkil edecek bilgilerin elde edilmesi hedeflenmiştir..

MATERYAL ve METOD

Bu çalışma Doğu Karadeniz Dağları sisteminin bir parçası olan ve Bayburt il merkezinin güneyiyle Çoruh Nehri ile Karasu Nehri'nin vadileri arasında uzanmakta olan Kop Dağı meralarında yürütülmüştür. Araştırma 2019 ve 2020 yıllarında Kop Dağının yüksek rakımlı bölgelerinde tespit edilen mera kesimlerinde örnek alma, laboratuvar çalışmaları ve istatistik analizlerin uygulanması şeklinde yürütülmüştür. Arazi çalışmaları sonucunda araştırma sahası olarak seçilen mera kesimlerinin koordinat, rakım ve ortalama eğim değerleri Tablo 1'de sunulmuştur.

Deneme alanı Kop Dağında yer alan mera kesimlerinin doğu, batı, kuzey ve güney yöneyleri dikkate alınarak tespit edilmiş, bu kesimlerden yüksekliği 1871-1985 m arasında olan alanlar "etek", 2372-2468 m arasında olan alanlar ise "sırt" olarak isimlendirilmiştir. Sonuç olarak çalışmalar dağ genelini temsil eden toplam 8 mera kesiminde yürütülmüştür.

Araştırma sahası için iklim verileri Meteoroloji 12. Bölge Müdürlüğü aracılığı ile araştırma alanına en yakın konumdaki Aşkale meteoroloji istasyonundan elde edilmiş ve Tablo 2'de paylaşılmıştır.

İklim verileri incelendiğinde; Aşkale meteoroloji istasyonunda uzun yıllar (2013-2020) için ortalama sıcaklığının 7.6 ^{o}C, toplam yağış 386.95 mm ve ortalama nispi nem değerinin ise %64.7 olduğu görülmektedir. Araştırma yıllarında ise ortalama sıcaklık değerinin 2019'da 7.4 ^{o}C, 2020'de 7.9 ^{o}C olduğu ve nispi nem değerlerinin ilk yıl için %66.9, ikinci yıl için %61.6 olduğu tespit edilirken; en yüksek sıcaklık değeri (20.9 ^{o}C) 2019 yılında ağustos ayında, 2020 yılında temmuz ayında (20.5 ^{o}C), yıllar ortalamasına göre ise ağustosta (21.6 ^{o}C) ölçülmüştür. Yağış verileri bakımından hem 2019 (373.1 mm) hem de 2020 (342.2 mm) değerlerinin uzun yıllar ortalamasına göre daha düşük olduğu tespit edilmiştir. En fazla yağış araştırmanın ilk yılında 64.2 mm ile mayıs ayında, ikinci yılında ise 120.9 mm değeri ile yine mayıs ayında görülmüştür. Yıllar ortalaması açısından en fazla yağış 84.46 mm ile mayıs ayında gerçekleşmiştir.

Araştırma konusu mera kesimlerinden alınan 8 toprak numunesi Atatürk Üniversitesi Ziraat Fakültesi Toprak Bölümü laboratuvarlarında analiz edilmiştir. Sonuçlara göre kuzey yöneyin kumlu killi tınlı, diğer yöneylerin ise kumlu tın tekstürde olduğu; toprağın nötr karakterli (pH 6.45), organik madde içeriğinin % 7.59 (zengin) ve EC (tuzluluk) oranının "çok hafif

tuzlu" olduğu, fosfor oranının 35.76 kg P_2O_5 da^{-1}, potasyum oranının ise 2.40 me 100 g^{-1} olduğu tespit edilmiştir.

Tablo 1. Mera kesimlerine ilişkin yükseklik, koordinat ve eğim bilgileri
Table 1. Height, coordinate and slope informations of the rangeland sites

Yöney *Direction*	**Yükseklik (m)** *Altitude (m)*	**Koordinat** *Coordinate*	**Eğim (%)** *Slope (%)*
Doğu etek	1871	D.0375571 K.4429235	20-25
Batı etek	1908	D.0374924 K.4429543	10-15
Kuzey etek	1970	D.0367711 K.4436539	35-40
Güney etek	1985	D.0368964 K.4436856	25-30
Doğu sırt	2372	D.0371467 K.4434376	45-50
Batı sırt	2468	D.0372042 K.4433219	25-30
Kuzey sırt	2434	D.0372904 K.4434600	15-20
Güney sırt	2449	D.0371816 K.4433285	50-55

Tablo 2. Erzurum ili Aşkale ilçesine ait 2019, 2020 yılı ve uzun yıllar (2013-2020) ortalamasına (UYO) ait bazı iklim değerleri*
Table 2. Some climate data for 2019, 2020, and years average (2013-2020) for the Aşkale district in Erzurum province

Aylar *Months*	**Aylık Toplam Yağış (mm)** *Total precipitation monthly (mm)*			**Aylık Ortalama Sıcaklık** *Average temperature monthly (°C)*			**Aylık Ortalama Nispi Nem(%)** *Average relative humidity monthly (%)*		
	2019	2020	UYO	2019	2020	UYO	2019	2020	UYO
Ocak	11.8	6.4	18.41	-6.7	-6.4	-7.9	87.6	77.1	82.1
Şubat	25.2	18.8	22.54	-6.1	-5.4	-5.5	91.7	77.1	82.7
Mart	21.2	56.5	40.51	-1.7	3.0	1.6	85.4	76.3	74.2
Nisan	62.7	45.6	42.25	5.0	6.4	7.2	80.9	63.7	64.3
Mayıs	64.2	120.9	84.46	12.7	11.5	12	61.0	60.7	66.3
Haziran	49.1	20.3	50.21	18.3	16.4	16.9	56.3	55.3	58.8
Temmuz	25.2	11.2	10.79	19.5	20.5	20.9	48.4	50.1	46.5
Ağustos	29.8	0.2	15.45	20.9	19.9	21.6	44.8	40.9	41.9
Eylül	29.9	17.0	20.55	15.3	18.5	16.6	50.2	39.5	45.4
Ekim	19.5	6.3	36.25	11.3	11.5	9.8	53.7	45.5	60.6
Kasım	15.7	29.9	20.68	2.1	3.6	2.3	60.0	70.6	70.8
Aralık	18.8	9.1	24.85	-1.6	-4.6	-4.9	83.2	81.9	83.0
Toplam/Ort. *Total/mean*	**373.1**	**342.2**	**386.95**	**7.4**	**7.9**	**7.6**	**66.9**	**61.6**	**64.7**

*Tarım ve Orman Bakanlığı Meteoroloji 12. Bölge Müdürlüğü verilerinden alınmıştır.

Mera kesimlerinde ot örneklerinin temini amacıyla her bir mera kesiminde 0.25'er m^2'lik 3 adet çerçeve alanı bölgede mera vejetasyonunun çiçeklenme dönemi olan temmuz ayında hasat edilmiştir. Meraların otlatmaya açık olması sebebi ile örnek alınacak noktalar belirlenirken korunaklı ve mera genelini temsil eden örnekleme alanları tercih edilmiştir. Dağ genelinde meraların etek ve sırt kesimlerinde 4 farklı yöneyden 3 tekerrürlü olarak temmuz ayının aynı gününde alınan toplam 24 (2 yükseklik x 4 yöney x 3 tekerrür) ot numunesi etiketlenerek bez torbalarda muhafaza edilmiştir. Sera ortamında ön kurutmaya tabi tutulan ot örnekleri, 70°C'de sabit ağırlığa ulaşana kadar kurutulmuştur. Kurutma sonrası ot numuneleri tartılarak yöneylere göre kuru ot verimleri hesaplanmıştır. Nispi yem değerinin tespit edilmesi için sindirilebilir kuru madde oranı ile kuru madde

alım yüzdesinden istifade edilmiştir (Van Dyke & Anderson, 2000). Bu verilere ulaşabilmek için öğütülen ot numunelerinin ADF ve NDF analizleri ANKOM Fiber teknolojisinden (Ankom, 2020) faydalanılarak Van Soest et al. (1991) tarafından geliştirilen filtre torba yöntemi ile gerçekleştirilmiştir.

Analizler sonucu kuru ot verimi ile nispi yem değerleri 3 tekerrürlü tesadüf bloklarında 3 faktörlü (yöney x rakım x yıl) deneme desenine göre analiz edilmiştir (Yıldız ve Bircan, 1994). İstatistik analizler 8 farklı mera kesiminden elde edilen oransal verilere Arc Sinüs transformasyonu uygulandıktan sonra SPSS yazılımı (Versiyon 20) kullanılarak yapılmıştır. Varyans analiz sonuçlarına göre istatistik olarak önemli bulunan faktör ortalamaları Duncan testi ile karşılaştırılmıştır.

BULGULAR ve TARTIŞMA

Araştırma konusu mera kesimlerine ait kuru ot verimi (kg da^{-1}) ile nispi yem değerlerinin rakım ve yöneye göre değişimi ile bu değerlere ait varyans analiz sonuçları Tablo 3'te yer almaktadır.

Tablo 3. Kuru ot verimi (kg da^{-1}),nispi yem değeri, NDF (%) ve ADF (%)'nin yöney ve rakıma göre değişimi ile varyans analiz sonuçları

Table 3. The variation of variance analysis results of hay yield (kg da^{-1}), relative forage value, NDF (%) and ADF (%) according to the direction and altitude

	Kuru Ot verimi (kg da^{-1}) *(Hay Yield (kg da^{-1}))*			**Nispi Yem Değeri** *(Relative Feed Value)*			**Nötral Ortamda Çözünemeyen Lif (%)** *NDF (Neutral Detergent Fiber (%))*			**Asit Ortamda Çözünemeyen Lif (%)** *ADF (Acid Detergent Fiber (%))*		
Yöney *Direction*	**2019**	**2020**	**Birleşik Analiz** *(CA)*	**2019**	**2020**	**Birleşik Analiz** *(CA)*	**2019**	**2020**	**Birleşik Analiz** *(CA)*	**2019**	**2020**	**Birleşik Analiz** *(CA)*
Doğu	226.00 C	179.33C	202.67±33D	107.87C	117.81B	112.84±7.03B	54.59 A	52.74 B	53.67 ±1.31 A	35.29 a	30.45 A	32.87 ±3.42 A
Batı	287.67 B	251.50B	269.58±25.58B	120.02B	106.92B	113.47±9.26B	49.53BC	56.77 A	53.15 ±5.12 A	34.50 a	32.10 A	33.30 ±1.70 A
Kuzey	231.17 C	233.33B	232.25 ±1.53 C	135.52A	151.94A	143.73±11.6A	47.05 C	44.66 C	45.86 ±1.69 B	30.14 b	25.18 B	27.66 ±3.51 B
Güney	375.00 A	309.33A	342.17 ±46.44 A	121.08B	116.23B	118.65±3.43B	50.72 B	53.39 B	52.06 ±1.89 A	32.51 ab	31.95 A	32.23 ±0.40 A
Ortalama *(Mean)*	**279.96 ±69.24 A**	**243.37± 53.60 B**	**261.67 ±60.25**	**121.12 ±11.32**	**123.22 ±19.74**	**122.17 ±14.61**	**50.47 ±3.14**	**51.89 ±5.13**	**51.18 ±3.61**	**33.11 ±2.30A**	**29.92 ±3.25B**	**31.51 ±2.61**
Rakım *(Altitude)*												
Etek	226.08 B	235.57	230.83 ±6.71 B	122.51	124.04	123.28 ±1.08	50.26	50.76	50.51 ±0.35	31.83 b	29.77	30.80 ±1.46
Sırt	333.83 A	251.17	292.50 ±58.45 A	119.74	122.41	121.07 ±1.89	50.68	53.02	51.85 ±1.65	34.39 a	30.07	32.23 ±3.05
Ortalama *(Mean)*	**279.96 ±76.19 A**	**243.37 ±11.03 B**	**261.67 ±25.87**	**121.12 ±1.96**	**123.22 ±1.15**	**122.17 ±1.48**	**50.47 ±0.30**	**51.89 ±1.60**	**51.18 ±1.00**	**33.11 ±1.81 A**	**29.92 ±0.21 B**	**31.51 ±2.26**
Yöney	0.000 **	0.000 **	0.000**	0.000 **	0.000 **	0.000**	0.000 **	0.000 **	0.000**	0.013*	0.000 **	0.000**
Rakım	0.000 **	0.188	0.000**	0.507	0.723	0.477	0.713	0.063	0.108	0.032*	0.782	0.072
Yöney x Rakım	0.000 **	0.001 **	0.000**	0.110	0.231	0.055	0.106	0.096	0.018*	0.030*	0.981	0.226
Yıl	-	-	0.001**	-	-	0.498	-	-	0.090	-	-	0.000**
Yıl x Yöney	-	-	0.117	-	-	0.004*	-	-	0.000**	-	-	0.151
Yılx Rakım	-	-	0.000**	-	-	0.854	-	-	0.270	-	-	0.153
Yılx Rakımx Yöney	-	-	0.001**	-	-	0.440	-	-	0.460	-	-	0.108

*****5%'de önemli ******1%'de önemli CA: Combined Analysis

Kuru ot verimi (kg da^{-1})

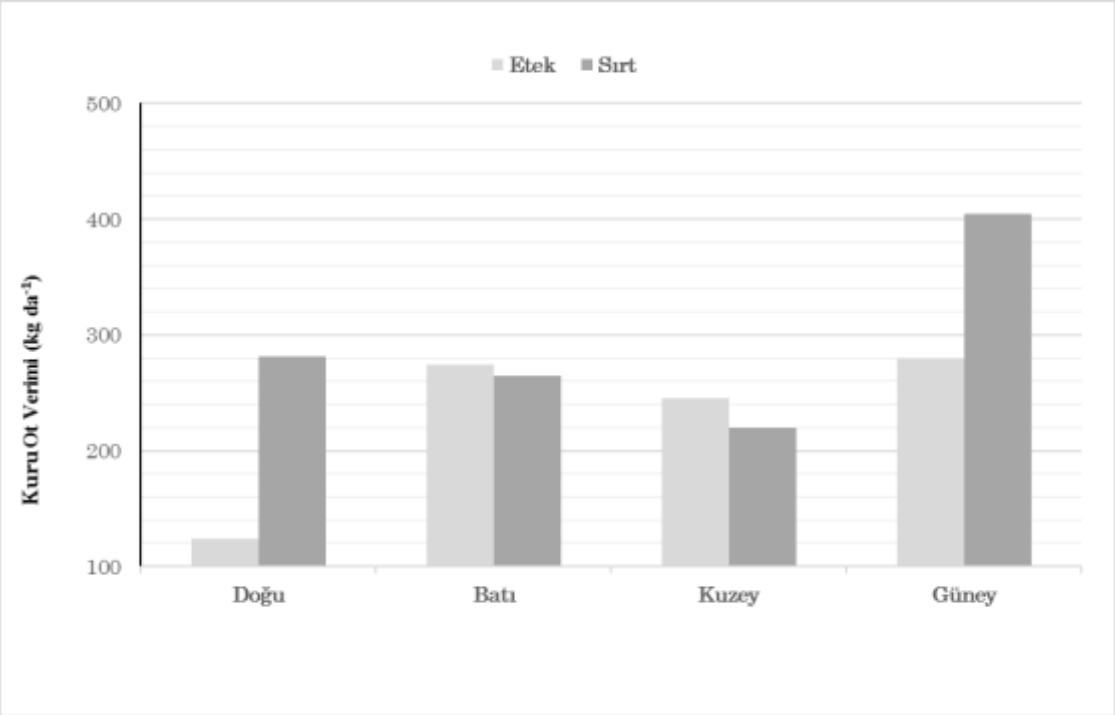
Araştırma konusu mera kesimlerinden elde edilen kuru ot verimlerinin yöney ve rakıma göre değişimi ile varyans analiz sonuçları Tablo 3'te yer almaktadır. Veriler değerlendirildiğinde deneme yıllarındaki değişimin istatistiki olarak çok önemli (p=0.001**) olduğu, yöney açısından kuru ot miktarındaki değişimin hem araştırma yılları hem de yılların birleşik analizinde %1 düzeyinde önemli bulunduğu görülmektedir. Rakım açısından ise 2019 yılı ile birleşik analiz sonuçları çok önemli (p=0.001**) değişiklik gösterirken, 2020 yılı önemsiz bulunmuştur. Bunun yanı sıra yöney x rakım, yıl x rakım ve yıl x yöney x rakım interaksiyonlarının da istatistiki olarak çok önemli olduğu tespit edilmiştir.

Mera yöneylerine göre kuru ot veriminin değişimine bakıldığında; 2019'da en yüksek kuru ot veriminin (375.00 kg da^{-1}) güneyde, en düşük kuru ot veriminin (226.00 kg da^{-1}) ise doğuda bulunduğu tespit edilmiş, doğu ve kuzey yöneyler istatistiki olarak aynı grupta yer almıştır. Araştırmanın ikinci yılında en yüksek kuru ot verimi 309.33 kg da^{-1} değeri ile güney yöneyde tespit edilmiş, bu yöneyi istatistik olarak aynı grupta yer alan batı (251.50 kg da^{-1}) ve kuzey (233.33 kg da^{-1}) takip etmiştir. En düşük kuru o verimi ise doğuda (179.33 kg da^{-1}) bulunmuştur. Yılların birleşik analizine göre en yüksek verim 342.17 kg da^{-1} ile güney kesimde, en düşük verim ise 202.67 kg da^{-1} ile doğu kesimde ortaya çıkmıştır.

Rakımın kuru ot verimi üzerine etkisine bakıldığında 2019'da sırt kesimlerin (333.83 kg da^{-1}) etekten (226.08 kg da^{-1}) daha yüksek kuru ot miktarına sahip olduğu görülmektedir. 2020 yılı ise istatistiki olarak önemli bulunmamıştır. Yılların birleşik analizine göre sırt kesimlerde verim 292.50 kg da^{-1} ile etek kesimlerden (230.83 kg da^{-1}) daha yüksek olmuştur. Araştırma yıllarının ortalaması dikkate alındığında kuru ot

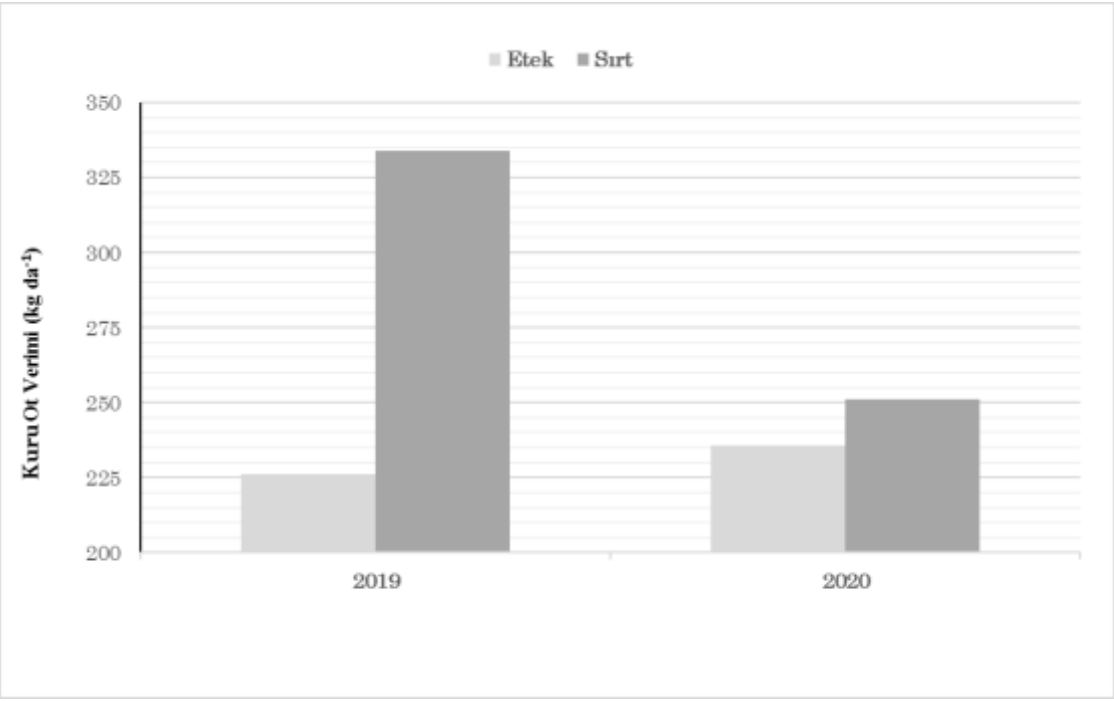
verimi 261.67 kg da^{-1} olarak tespit edilmiştir. Birleşik analize göre kuru ot verimi istatistiki manada %1 düzeyinde önemli bulunmuştur.

Birleşik analiz sonuçlarına göre batı ve kuzey yöneylere ait kuru ot verimleri rakıma göre önemli derece farklılık göstermezken, güney ve doğu yöneylerde kuru ot verimi sırt kesimlerde daha yüksek olmuştur. Kuru ot veriminde meydana gelen bu farklılık neticesinde yöney x rakım interaksiyonu çok önemli bulunmuştur (Şekil 1).



Şekil 1. Birleşik analize göre kuru ot veriminin yöney x rakım interaksiyonu değerleri (kg da^{-1})
Figure 1. Direction x altitude interaction of hay yield according to the combined analysis (kg da^{-1})

Birleşik analize göre ilk yıl sırt araştırma konusu mera kesimleri belirgin bir biçimde daha fazla ot verirken, ikinci yılda büyük bir farklılık gözlenmemiştir. Sonbahar yağışlarından kaynaklandığı düşünülen bu durum, yıl x rakım interaksiyonunun çok önemli olmasına neden olmuştur (Şekil 2).



Şekil 2. Birleşik analize göre kuru ot miktarının yıl x rakım interaksiyonu değerleri (kg da^{-1})
Figure 2. Year x altitude interaction of hay yield according to the combined analysis (kg da^{-1})

Araştırma yıllarında kuru ot verimi çok önemli düzeyde değişiklik göstermiştir. Araştırmanın ilk yılında kuru ot verimi (279.96 kg da^{-1}) ikinci yıldan (243.37 kg da^{-1}) daha yüksek bulunmuştur. Bu durum araştırma yılları arasındaki yağış miktarı ile sıcaklık farklılığından kaynaklanmış olabilir. Zira 2019 yılı için toplam yağış miktarı 373.1 mm yıl^{-1} ve sıcaklık ortalaması 7.4 ^{o}C olurken; 2020 yılı için yağış miktarı 342.2 mm yıl^{-1} ve yıllık sıcaklık ortalaması 7.9 ^{o}C olarak gerçekleşmiştir (Tablo 2). Rakıma göre kuru ot verimi değişimi istatistik açıdan 2020 yılı için önemsiz bulunurken, 2019 yılı ve yılların birleşik analizi bakımında çok önemli (p=0.000**) farklılık göstermiştir. Tablo 3'te yer alan veriler incelendiğinde kuru ot veriminin sırt kesimlerde (292.50 kg da^{-1}) etek kesimlerden (230.83 kg da^{-1}) daha yüksek olduğu görülmektedir. Etek kesimlerde otlatılmaya daha erken başlanılması bu durumun oluşmasında etkili olabilir. Çünkü sırt kesimler bölgenin yerleşim yerlerinden daha uzakta yer almaktadır. Bu nedenle otlatılmanın yerleşim yerlerine daha yakın meralardan daha uzak meralara doğru yapılması neticesinde, sırt kesim meralarda otlatma baskısının daha düşük olması verimi etkilemiş olabilir. Daha fazla otlatılmış meralarda ot miktarının düşmesi de beklenen bir durumdur (Milchunas et al., 1994; Reis et al., 2001; Gökkuş ve ark., 2015).

Kuru ot veriminde tespit edilen değerler benzer çalışmalarda; Tükel ve ark. (2001) tarafından bildirilen 292.7 kg da^{-1}; Altın ve ark. (2010) tarafından tespit edilen 240-342 kg da^{-1}; Bilgin (2010) tarafından tespit edilen 196.67 kg da^{-1}; Güllap (2010) tarafından tespit edilen 161.1-223.9 kg da^{-1}, Ağın (2012) tarafından bildirilen 210.3-279.2 kg da^{-1}; Aydın (2014) tarafından tespit edilen 229.94 kg da^{-1}; Yıldız ve Özyazıcı (2017) tarafından bildirilen 278.1 kg da^{-1}; Sürmen ve Kara (2018) tarafından tespit edilen 114.54-223.03 kg da^{-1} sonuçlarından bir kısmı ile paralellik gösterirken bazı çalışma sonuçları ile farklılık arz etmiştir. Bu durumun oluşmasında ekolojik koşullardaki değişim, mera vejetasyon farklılıkları ile araştırma yöntemlerinde ki uygulama farklılıklarının etkili olduğu söylenebilir. Örneğin Sürmen ve Kara (2018) tarafından yapılan araştırmada kuru ot verimindeki değişimde mera kesimlerindeki eğim farklılığının etkisi ortaya koyulmuştur. Yıldız ve Özyazıcı (2017) tarafından Van ilinde, 2195 m rakımlı ve benzer ekolojik koşullara sahip bir merada yapılan araştırmada ise kuru ot veriminin yöneylere göre değişimi araştırma sonuçları ile benzerlik göstermiş ve en yüksek kuru ot verimi sırasıyla güney, batı ve kuzey yöneylerde ortaya çıkmıştır. Literatüre göre ortaya çıkan söz konusu bu benzerlik ve farklılıkların meraların toprak yapısı, topoğrafya, iklim ve yağış gibi faktörlerin değişiminden kaynaklandığı düşünülmektedir.

Nispi yem değeri

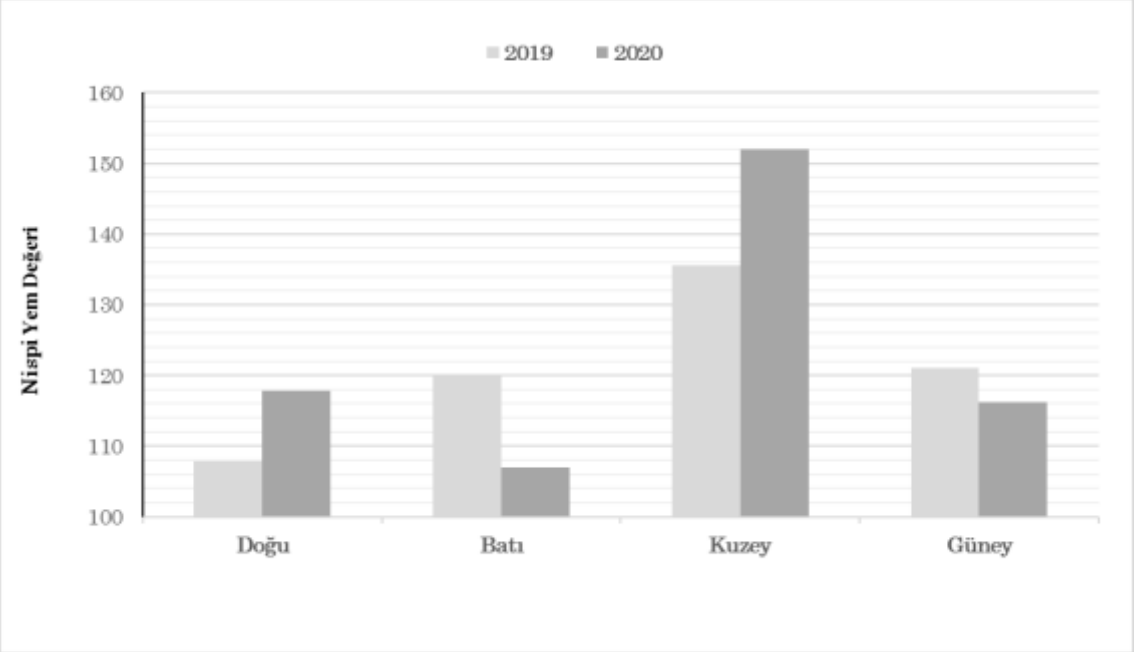
Çalışma ile mera kesimlerinden elde edilen nispi yem değerlerinin yöney ve rakıma göre değişimi ile varyans analiz sonuçları Tablo 3'te yer almaktadır. Varyans analiz sonuçlarına göre rakım ve yıl faktörleri önemsiz bulunurken yöney %1 düzeyinde önemli olmuştur. İnteraksiyonlar bakımından yalnızca yıl x yöney önemli olurken, yöney x rakım ile yıl x rakım ikili interaksiyonu ve yıl x rakım x yöney üçlü interaksiyonu önemsiz bulunmuştur.

Mera kesimlerinde nispi yem değerinin yöneylere göre değişimi incelendiğinde (Tablo 3) araştırmanın ilk yılında en yüksek nispi yem değerinin (135.52) kuzey yöneyde olduğu, batı ve güney yöneyin istatistik olarak aynı grupta yer aldığı ve en düşük nispi yem değerinin (107.87) doğu yöneyde ortaya çıktığı görülmektedir. 2020'de ise en yüksek nispi yem değeri (151.94) yine kuzey yöneyde tespit edilmiş, doğu, batı ve güney yöneyler istatistik olarak aynı grupta yer almıştır. Birleşik analiz bakımından ise en yüksek nispi yem değeri (143.73) yine kuzey yöneyde tespit edilmiştir. En düşük nispi yem değeri (112.84) ise doğu yöneyde ortaya çıkmış ve doğu, batı ve güney yöneyler istatistik olarak aynı grupta yer almıştır. Rakımın nispi yem değişimine etkisi istatistik olarak önemli olmamakla birlikte hem araştırma yıllarında hem de birleşik analiz bakımından etek kesimlerde tespit edilen nispi yem değeri sırt kesimlerden daha yüksek olmuştur.

Birleşik analiz sonuçlarına göre nispi yem değeri araştırmanın ikinci yılında farklılık arz etmiş, 2020'de batı yöneye ait değer önceki yıla göre önemli ölçüde düşüş göstermiştir. Bu değişim neticesinde yıl x yöney interaksiyonu ortaya çıkmıştır (Şekil 3).

Nispi yem değeri yem kalitesinin tespiti bakımından bir gösterge olarak kullanılmakta; bu değerin 100'den büyük olması yem kalitesinin yüksek, 100'den küçük olması ise yem kalitesinin düşük olduğu anlamına gelmektedir (Moore & Undersander, 2002; Kaya, 2008; Canbolat & Karaman, 2009). Nispi yem değerinin 100 olması için ADF oranının %41, NDF oranının ise %53 olması gerekmektedir (Redfearn et al., 2004). Bu nedenle nispi yem değerinde ADF ve NDF oranları etkili olmaktadır. Kuzey yöneyin nispi yem değeri bakımından en verimli yöney olarak tespit edilmesi, bu yöneyde ADF ve NDF oranlarının diğer yöneylere göre daha düşük olmasından kaynaklanmaktadır. Kuzey yöneyde ADF ve NDF değerlerinin daha düşük olması, bu yöneyde baklagil oranının daha yüksek olmasından kaynaklanmış olabilir. Rakıma göre nispi yem değeri istatistiki anlamda önemli bulunmamakla birlikte, etek kesimlerin sırt kesimlere oranla daha yüksek nispi yem değerine sahip olduğu tespit edilmiştir. Bununla beraber etek ve sırt kesimlerde tespit edilen nispi yem değerleri birbirine yakın olmuştur. Bu durum araştırma kapsamında Kop Dağı meralarının etek ve sırt kesimler olarak ayrılmasına rağmen dağ

genelinde benzer ekolojik koşulların mevcudiyeti, rakım farkının az ve kullanım geçmişinin benzer olmasından kaynaklanmış olabilir.



Şekil 3. Birleşik analize göre nispi yem değerinin yıl x yöney interaksiyonu değerleri
Figure 3. Year x direction interaction of relative feed value according to the combined analysis

Araştırma kapsamında elde edilen nispi yem değerleri yapılan benzer bazı araştırma sonuçları (Şahinoğlu (2010) 113-138; Aydın (2014) 137.71; Çaçan ve Başbağ (2016) 113.51) ile paralellik arz etmiştir. Diğer taraftan araştırma ile tespit edilen ham protein oranlarının; Yıldız ve Özyazıcı (2017) tarafından tespit edilen 92.7-107.2; Sürmen ve Kara (2018) tarafından tespit edilen 101.35 ve Tutar ve Kökten (2019) tarafından bildirilen 91.8-109.4 değerlerinden ise düşük olduğu görülmektedir. Ham protein oranlarında gözlenen bu benzerlik ve farklılıkların, botanik kompozisyonda yer alan bitki türlerindeki değişimden kaynakladığı, özellikle baklagil oranının bu durum üzerinde etkili olduğu düşünülmektedir.

SONUÇ ve ÖNERİLER

Araştırma sonuçlarına göre meraların kuru ot verimi yöneylere göre farklılık göstermiş, en yüksek verim güney yöneyde (342.17 kg da^{-1}), en düşük verim ise doğu yöneyde (202.67 kg da^{-1}) ortaya çıkmış, sırt kesimlerde kuru ot verimi etek kesimlerden yüksek olmuştur. Nispi yem değeri yılların birleşik analizi bakımından yöneyler arasında birbirine yakın değerler göstermekle birlikte yalnız kuzey yöney 143.73 değeri ile diğer yöneylerden ayrılmıştır. Rakım faktörü nispi yem değeri bakımından istatistik anlamda önemsiz bulunmuştur. Birleşik analize göre NDF ve ADF değerleri %1 düzeyinde önemli olmuş ve kuzey yöney dışındaki yöneyler istatistik olarak aynı grupta yer almıştır. Bu sonuçlar ışığında Kop Dağı meralarının başta etek kesimler olmak üzere amenajman ilkelerine göre kullanılmaları gerektiği ve özellikle otlatma mevsimi ve otlatılacak hayvan cinsinin dikkate alınarak, otlatma başlangıcında dağın sırt kesimleri ile batı ve güney yöney mera kesimlerinin öncelendiği bir otlatma programının oluşturulmasının meraların geleceği açısından önem arz ettiği kanaatine varılmıştır. Ayrıca dağ genelinde yer alan bütün mera kesimleri için ıslah amaçlı araştırma ve uygulama çalışmalarının yapılmasının faydalı olacağı düşünülmektedir.

TEŞEKKÜR

Bu çalışma ilk yazarın doktora tezinin bir kısmından oluşmaktadır ve araştırma Atatürk Üniversitesi Bilimsel Araştırma Projeleri (BAP) Koordinasyon Birimi tarafından (Proje no: 7276) desteklenmiştir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

Ağın Ö (2012). Bingöl İli Yedisu İlçesi Karapolat Köyü Merasının Verim ve Botanik Kompozisyonunun Saptanması (Tez no 330583). (Yüksek Lisans Tezi, Bingöl Üniversitesi Fen Bilimleri Enstitüsü Tarla

Bitkileri Anabilim Dalı). Yükseköğretim Kurulu Ulusal Tez Merkezi.

Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Çukurova Bölgesi İkinci Ürün Koşullarında Bazı İnci Darı (Pennisetum glaucum (L.) R. Br.) Çeşitlerinin Ot Verimleri ve Agromorfolojik Özellikleri Üzerinde Bir Araştırma. *KSÜ Tarım ve Doğa Dergisi, 27*(4), 901-910. https://doi.org/10.18016/ksutarimdoga.vi.1395888

Altın, M., Tuna, C., & Gürr, M. (2010). Tekirdağ Taban ve Kıraç Meralarının Verim ve Botanik Kompozisyonuna Gübrelemenin Etkisi. *Tekirdağ Ziraat Fakültesi Dergisi, 7*(2), 191-198.

Ankom Technology. (2020). The Ankom 200 Fiber Analyzer. Fairport, NY. Erişim tarihi: 15.05.2019, http://www.ankom.com

Aydın A (2014). Karacadağ'ın Farklı Yükseltilerindeki Meralarında Bitki Tür ve Kompozisyonları İle Ot Verim ve Kalitelerinin Belirlenmesi (Tez no 355605). (Doktora Tezi), Dicle Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Beukes, P. C., & Cowling, R. M. (2003). Non-selective grazing impacts on soil properties of the Nama Karoo. *Journal of Range Management, 56*, 547-552.

Bilgin, F. (2010). Artvin Ardanuç-Aydın Köyü Yaylası Mera Vejetasyonu ile Bazı Toprak Özelliklerinin Yükseltiye Göre Değişiminin İrdelenmesi (Tez no 276264). (Yüksek Lisans Tezi, Artvin Çoruh Üniversitesi Fen Bilimleri Enstitütüsü Orman Mühendisliği Anabilim Dalı). Yükseköğretim Kurulu Ulusal Tez Merkezi.

Canbolat, Ö., & Karaman, Ş. (2009). Bazı Baklagil Kaba Yemlerinin in vitro Gaz Üretimi, Organik madde Sindirilebilirliği, Nispi Yem Değeri ve Metabolik Enerji İçeriklerinin Karşılaştırılması. *Tarım Bilimleri Dergisi, 15*(2), 188-195. https://doi.org/10.1501/Tarimbil_0000001090

Çaçan, E., & Başbağ, M. (2016). Bingöl İli Merkez İlçesi Yelesen-Dikme Köylerinin Farklı Yöney ve Yükseltilerde Yer Alan Mera Kesimlerinde Botanik Kompozisyon ve Ot Veriminin Değişimi. *Ege Üniversitesi Ziraat Fakültesi Dergisi, 53*(1), 1-9. https://doi.org/10.20289/zfdergi.179864

Çomaklı, B., Fayetörbay, D., & Daşcı, M. (2012). Changing of Botanical Composition and Canopy Coverage Ratio in Rangelands at Different Altitudes. *Journal of Agricultural Faculty of Atatürk University, 43*(1), 17-21.

Gökkuş, A., Koç, A., & Çomaklı, B. (2015). Çayır-Mera uygulama kılavuzu. (Geliştirilmiş 4. Baskı). Atatürk Üniversitesi Ziraat Fakültesi Yayınları, Yayın No:142, Atatürk Üniversitesi Ziraat Fakültesi Ofset Tesisi, Erzurum.

Güllap, M. K. (2010). Kargapazarı Dağında (Erzurum) Farklı Otlatma Sistemi Uygulamalarının Mera Bitki Örtüsüne Etkisi (Tez no 270739). (Doktora Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı). Yükseköğretim Kurulu Ulusal Tez Merkezi.

Heitschmidt, R. K., Grings, E. E., Haferkamp, M. R., & Karl, M. G. (1995). Herbage dynamics on two northern Great Plains range sites. *Journal of Range Management, 48*, 211-217.

Holechek, J. L., Pieper, R. D., & Herbel, C. H. (2011). Range management principles and practices. Sixth edition. Prentice Hall, Upper Saddle River, New Jersey, USA, 444 sy.

Kaya, Ş. (2008). Kaba yemlerin değerlendirilmesinde göreceli yem değeri ve göreceli kaba yem kalite indeksi. *Türk Bilimsel Derlemeler Dergisi, 1*(1), 59-64.

Lund, H. G. (2007). Accounting for the world's rangelands. *Rangelands, 29*(1), 3-10. https://doi.org/10.2111/1551-501X(2007) 29 [3:AFTWR]2.0.CO;2

Mahdavi, A., Eshaghi Rad, J., & Jamshidifard, M. (2012). The effect of altitude and aspect on rangeland plant diversity (Case study: Dashte Zahab, Kermanshah, Iran). *Journal of Rangeland Science, 3*(1), 11-20.

Milchunas, D. G., Forwood, J. R., & Lavenroth, W. C. (1994). Productivity of long-term grazing treatments in response to seasonal precipitation. *Journal of Range Management, 47*, 133-139. https://doi.org/10.2307/4002821

Moore, J. E., & Undersander, D. J. (2002). Relative forage quality: Alternative to relative feed value and quality index. In *Proceedings 13th Annual Florida Ruminant Nutrition Symposium* (s. 16-32).

Redfearn, D. D., Zhang, H., & Caddel, J. L. (2004). Forage Quality Interpretations. USA: Division of Agricultural Sciences and Natural Resources, Oklahoma State University.

Reis, J. C. L., Alfaya, H., Siquare, O. J. W., & Farias, C. B. (2001). Seasonal botanical composition and available forage of natural grassland in the southeastern range region of Rio Grande do Sul, Brazil. In *Proc. XIX. I.G.C, Sao Paulo, Brasil* (ID:0116).

Snyman, H. A. (2005). Rangeland degradation in a semi-arid South Africa. I: influence on seasonal root distribution, root/shoot ratios, and water-use efficiency. *Journal of Arid Environments, 60*, 457-481. https://doi.org/10.1016/j.jaridenv.2004.06.006

Sürmen, M., & Kara, E. (2018). Aydın ili ekolojik koşullarında farklı eğimlerdeki mera vejetasyonlarının verim ve kalite özellikleri. *Derim, 2018/35*(1), 67-72. https://doi.org/10.16882/derim.2018.343428

Şahinoğlu, O. (2010). Bafra İlçesi Koşu Köyü Merasında Uygulanan Farklı Islah Yöntemlerinin Meranın Ot Verimi, Yem Kalitesi ve Botanik Kompozisyonu Üzerine Etkileri (Tez no 276670).

(Doktora Tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı). Yükseköğretim Kurulu Ulusal Tez Merkezi.

Taşdelen, S. S., & Özyazıcı, M. A. (2022). Doğal Bir Meranın Farklı Yükseltilerine Göre Verim ve Botanik Kompozisyonunun Değişimi: Türkiye, Siirt ili Merkez ilçesi Doluharman köyü merası. *Turkish Journal of Forestry, 23*(2), 106-115. https://doi.org/10.18182/tjf.1061956

Tongway, D. J., Sparrow, A. D., & Friedel, M. H. (2003). Degradation and recovery processes in arid grazing lands of central Australia. Part I: Soil and Land Resources. *Journal of Arid Environments, 55*, 302-326.

Tutar, H., & Kökten, K. (2019). Mera Vejetasyon Özelliklerinin Farklı Yöneylere Göre Değişimi. *Turkish Journal of Agriculture Research, 6*(3), 312-318. https://doi.org/10.19159/tutad.614351

Tükel, T., Hatipoğlu, R., Özbek, H., Alados, C. L., Çeliktaş, N., & Kökten, K. (2001). İçel İli Çamlıyayla İlçesinde Bulunan Sığır Yaylasındaki Tipik Bir Akdeniz Orman İçi Mera Ekosisteminin Vejetasyon Yapısı ve Verim Gücünün Saptanması Üzerinde Bir Araştırma. In *Türkiye 4. Tarla Bitkileri Kongresi, Tekirdağ, 17-21 Eylül 2001* (s. 37-42).

Van Dyke, N. J., & Anderson, P. M. (2000). Interpreting a Forage Analysis. Alabama cooperative extension. Circular ANR-890.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides concerning animal nutrition. *Journal of Dairy Science, 74*, 3583-3597.

Yıldız, A., & Özyacı, M. A. (2017). Karasal İklim Kuşağında Bulunan Bir Meranın Farklı Yöneylerinde Botanik Kompozisyonun, Ot Verimi ve Ot Kalitesinin Belirlenmesi. *Turkish Journal of Agriculture Research, 4*(3), 218-231. https://doi.org/10.19159/tutad.292549

Yıldız, N., & Bircan, H. (1994). Uygulamalı İstatistik (4. Baskı). Atatürk Üniversitesi Ziraat Fakültesi Yayınları.

KSÜ Tarım ve Doğa Derg 27 (4), 929-939, 2024
KSU J. Agric Nat 27 (4), 929-939, 2024
DOI:10.18016/ksutarimdoga.vi.1323445

Using the GlutoPeak Tester in Determining the Quality Characteristics of Some Bread Wheat Varieties

Sadi GÜR[1] , Harun DIRAMAN[2]
[1]Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü, Akabe Mah. Şehit Burak Aydoğan Cad. No: 3E Karatay/Konya, [2]Afyon Kocatepe Üniversitesi, Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Ahmet Necdet Sezer Kampüsü/03200 Afyonkarahisar
[1]https://orcid.org/0000-0002-1857-8359, [2]https://orcid.org/0000-0002-7431-7524
: sdgr82@hotmail.com

ABSTRACT
This study aims to determine the physical, chemical and technological, rheological properties of 10 registered bread wheat varieties developed by Bahri Dağdaş International Agricultural Research Institute and their quality status in bread analysis and GutoPeak analysis. At the same time, it aims to investigate the relationships between physical, chemical, technological, rheological, and bread analyses with GlutoPeak quality parameters and to reveal the potential of the varieties. In the study, some quality parameters and significance levels between varieties were determined. Also, the results obtained from the GlutoPeak analysis are explained by comparing them with other quality parameters. Protein ratio, wet gluten, and Zeleny sedimentation values were found to be highly correlated with GlutoPeak AM, BEM, AGGRE, PM, GPRT, GW, and GWA. In addition, it was determined that there was a high correlation between harmonograph water absorption and GlutoPeak AGGRE, AM, BEM, GGLT, GPRT, GW, GWA, and PM values. Autograph W value was positively correlated with GlutoPeak AM, BEM, PM, GPRT, GGLT, GW, GWA, and AGGRE values and negatively correlated with PMT. The results obtained in terms of the examined characteristics in this study show that some varieties stand out in terms of different quality characteristics. With the results of this study, it was determined that the GlutoPeak device can detect the quality of wheat flour with fewer samples and in a short time, therefore, GlutoPeak analysis will be useful in variety development and similar studies in bread wheat.

Field Crops

Research Article

Article History
Received : 06.07.2023
Accepted : 16.01.2024

Keywords
Bread wheat
Quality properties
GlutoPeak

Bazı Ekmeklik Buğday Çeşitlerinin Kalite Özelliklerinin Belirlenmesinde Glutopik Test Cihazının Kullanılması

ÖZET
Bu çalışmanın amacı Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Müdürlüğü tarafından geliştirilen tescilli 10 ekmeklik buğday çeşidinin bazı fiziksel, kimyasal ve teknolojik, reolojik özellikleri ve ekmek analizleri ile Glutopik analizlerinde kalite durumlarının tespit edilmesidir. Aynı zamanda fiziksel, kimyasal, teknolojik, reolojik ve ekmek analizlerinin, Glutopik kalite parametreleri ile arasındaki ilişkilerin araştırılması ve çeşitlerin potansiyellerinin ortaya konulması amaçlanmıştır. Araştırmada, bazı kalite parametreleri ve çeşitler arasında önemlilik düzeyleri belirlenmiştir. Ayrıca, Glutopik analizinden elde edilen sonuçlar diğer kalite parametreleri ile kıyaslanarak açıklanmıştır. Protein oranı, yaş gluten ve Zeleny sedimentasyon değerlerinin Glutopik AM, BEM, AGGRE, PM, GPRT, GW ve GWA ile yüksek ilişkili olduğu tespit edilmiştir. Bunun yanında farinograf su absorbsiyonu ile Glutopik AGGRE, AM, BEM, GGLT, GPRT, GW, GWA ve PM değerleri arasında yüksek bir korelasyon olduğu belirlenmiştir. Alveograf W değerinin Glutopik AM, BEM, PM, GPRT, GGLT, GW, GWA ve AGGRE değerleri ile pozitif düzeyde önemli, PMT ile negatif seviyede önemli ilişkili olduğu tespit edilmiştir. Bu çalışmada incelenen özellikler yönünden elde edilen sonuçlar, farklı kalite özellikleri bakımından bazı

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş tarihi : 06.07.2023
Kabul tarihi : 16.01.2024

Anahtar Kelimeler
Ekmeklik buğday
Kalite özellikleri
GlutoPeak

çeşitlerin öne çıktığını göstermektedir. Bu çalışmanın sonuçları ile Glutopik cihazının, az örnekle ve kısa sürede buğday unu kalitesini tespit edebileceği, bu sebeple Glutopik analizinin ekmeklik buğdayda çeşit geliştirme ve benzeri çalışmalarda faydalı olacağı belirlenmiştir.

To Cite : Gür, S., & Diraman, H., (2024). Using the GlutoPeak Tester in Determining the Quality Characteristics of Some Bread Wheat Varieties. *KSU J. Agric Nat 27*(4), 929-939. https://doi.org/ 10.18016/ ksutarimdoga.vi.1323445.

Atıf İçin: Gür, S., & Diraman, H., (2024). Bazı Ekmeklik Buğday Çeşitlerinin Kalite Özelliklerinin Belirlenmesinde Glutopik Test Cihazının Kullanılması. *KSÜ Tarım ve Doğa Derg 27*(4), 929-939. https://doi.org/ 10.18016/ ksutarimdoga.vi.1323445.

INTRODUCTION

Wheat is one of the most produced cereals in Türkiye due to its high adaptability, meeting a significant part of the daily calories and protein required for human nutrition and being a staple food (Kün, 1996). In parallel with the increasing population, the demand for wheat is also increasing. Türkiye is one of the countries with the highest annual grain consumption per capita. In Türkiye, wheat consumption per capita was 179.4 kg on average in the 2018-2019 period (Güneş & Turmuş 2020). In developed countries, wheat consumption per capita is behind the level of developing countries (FAO, 2020). In Türkiye, where wheat is consumed approximately 2.5 times the world average as human food, it is a necessity that the wheat is of high quality. In determining the quality of wheat, primarily physical properties are taken into account. Hectoliter weight and thousand kernel weight are the most basic analyses in determining wheat quality and are widely used for selection in breeding studies. The parameters commonly used to determine bread wheat quality are Zeleny sedimentation value, protein content, gluten index, wet gluten, and dry gluten values. Many processes such as agricultural applications, genetic structure, milling, and baking processes contribute to the final product quality of wheat (Güçbilmez et al., 2019). Gluten is the main storage protein that defines the baking quality of wheat by providing water absorption capacity, viscosity, and elasticity to the dough (Wieser, 2007). Gliadin and glutenin protein are two components of gluten that form the gluten network during dough development and determine dough strength (Sharma et al., 2020). The appropriate combination of the two gluten components affects the visco-elastic properties of the dough and eventually the quality of the final products. Since gluten is the main determinant of quality in wheat, gluten content was used as one of the criteria in the selection of varieties and determining the baking quality of flour samples in the breeding program (Güçbilmez et al., 2019). Various quality testing procedures such as allograph, chronograph, and cooking tests continue to be applied at present to characterize wheat for different end uses (Huen et al., 2018). Rheological measurements such as chronographs and micrographs are widely used to evaluate the gluten strength of dough and the overall baking functionality of wheat flour (Wang et al., 2017). However, such rheological analyses and baking quality tests are often labor-intensive and time-consuming (Bouachra et al., 2017). In such cases, analyses with shorter durations may be more useful. GlutoPeak test has started to be used as a rapid quality test that requires fewer samples and measures the properties of gluten aggregation, especially gluten strength and aggregation rate (Huen et al., 2018). Studies have shown that GlutoPeak parameters can be used to differentiate wheat flours based on gluten aggregation and dough rheological properties (Marti et al., 2015).

Within the scope of this study, some physical, chemical, technological, and rheological properties, bread analyses, and quality status in GlutoPeak analyses of 10 registered bread wheat varieties developed by Bahri Dağdaş International Agricultural Research Institute were determined in detail. At the same time, the relationships between the physical, chemical, and technological analyses and the rheological and bread analyses between the GlutoPeak quality parameters were investigated and the potentials of the varieties were tried to be revealed.

MATERIALS and METHODS

In this research, the seeds obtained from the trial carried out in the 2020-2021 period with two replications in randomized blocks experimental design of 10 bread wheat varieties (Bayındır, Bozkır, Şehzade, İkonya, Meke, Selçuklu, Ekiz, Taner, Tuğra and Yavuz) grown in irrigated conditions in Bahri Dağdaş International Agricultural Research Institute Konya/Türkiye land were used as material. To obtain flour from wheat samples in the research, the AACC methods 26-95 and 26-50 were used with slight modifications (AACC, 2000). One kg of cleaned seed was taken, annealed on a moisture basis of 14.5% (w w^{-1}), and then kept for 12 hours, then ground in Yucebaş YM1 (Yücebaş Machinery Analytical Equipment İzmir, Turkey) flour mill.

Physical, Chemical, and Technological Analyzes

The thousand-grain weight of samples was determined according to Williams et al., (2008) with a Pfeuffer Contador brand device (model 75072, Kitzingen/Germany). Test weight was determined according to the standard method of AACCI (No: 55-10.01) (AACCI, 2010). The grain hardness of bread

wheat samples was determined in a NIR (Foss DS2500 F) device calibrated according to AACC 55-31 method using a single kernel characterization system (SKCS; AACC, 2000). Protein ratio was made according to the Dumas method using the LECO FP 528 (Leco Inc, St Joseph, MI) nitrogen determination device (nitrogen ratio x 5.70) by weighing 0.20-0.25 g of ground sample (AOAC 992.23, 2000). Zeleny sedimentation values of flour samples were determined according to ICC (International Association for Cereal Science and Technology) Standard No.116/1 (ICC, 2008). The wet gluten content of the flour samples was determined according to AACC Method No: 38-12A (AACC, 2000). Gluten index values of flour samples were determined according to AACC Method No: 38-12A (AACC, 2000). The time for the wheat starch to lose its viscosity feature was determined by the falling number device (Yücebaş Makine, model 2016-No Y120033 İzmir/Türkiye) according to AACC Method No: 56-81B (AACC, 2000).

Rheological Analyzes

Farinograph analysis was determined by a chronograph device (Farinograf-AT, Brabender Germany) according to ICC Standard Method No: 115/1 (ICC, 2008). Alveograph analysis was made using the Chopin Alveograph (Model Alveograph NG, Chopin, France) device according to the ICC-Standard No:121 method (ICC, 2008). GlutoPeak analyses were performed with a Brabender GlutoPeak device (803400 model, Brabender GmbH&Co KG, Duisburg, Germany). Nine g flour sample was mixed with 9 g distilled water at a speed of 2750 rpm at 36 °C, and the test material was evaluated using the Rapid Flour Check method specified by Wiertz (2018). Measurements made by GlutoPeak were recorded by the device's software program (GlutoPeakR version 2.2.0) and AM (Torque 15 seconds Before Maximum Torque, GPU), BEM (Maximum Torque of Gluten, GPU), PMT (the time passed until the Maximum Torque, sec), PM (Torque 15 sec After Maximum Torque, GPU), GPRT (GlutoPeak Protein Ratio, %), GGLT (GlutoPeak Wet Gluten Value, %), GW (GlutoPeak Energy Value, $x10^{-4}$ J), GWA (GlutoPeak Water Absorption Capacity, % v w^{-1}), AGGRE (Aggregation Energy Value, GPU) values were obtained.

Bread Analyzes

Straight-Dough Bread-Making method (AACC 10-10B) modified according to Turkish style bread was used in bread-making studies. For this, based on 100 g flour, 1.5% g/g table salt, and 3% g/g yeast were added, and then 2 units more water (cc) than the previously determined farinograph values were added and kneaded until a mature dough was formed. The obtained doughs were left to mass fermentation at 30 °C and 70-80% relative humidity 2 times for 30 minutes and at the end of these periods, they were folded and aerated. At the end of this process, the bread dough was given its final shape and left for final fermentation at 30 °C for 55 minutes, and after fermentation, the doughs were baked at 230 °C for 15-20 minutes (Elgün et al., 2014). Bread weights (g) were determined by weighing the bread with laboratory-type scales at least 1 hour after baking (Elgün et al., 2002). Bread volume was measured by the rapeseed displacement method and the bread volumes of each variety were determined in cm^3 (Elgün et al., 2002).

Statistical Analysis

In the evaluation of the data obtained as a result of the study, variance analysis was performed and the mean values of the features with significant differences were grouped according to the LSD (0.05) test. JMP statistics program (version 5.0.1, SAS Institute Inc., USA) was used in data analysis (JMP, 2003).

RESULTS and DISCUSSION

Evaluation of Analysis Findings of Bread Wheat Varieties

The mean square results of the analysis of variance obtained from the analysis results of the quality parameters of 10 bread wheat varieties used in the research are given in Tables 1 and 2, and the mean values and significance groups are given in Tables 3 and 4. As it can be understood from the examination of the tables, the differences between the varieties in all the parameters obtained from the analyses were found to be statistically significant at the level of $P<0.01$.

Evaluation of Findings Related to Physical, Chemical, and Technological Analyzes

In determining the quality of wheat, first of all, its physical properties are taken into account. Thousand-grain weight and test weight are the most basic analyses in determining wheat quality and are widely used for selection in a variety of development studies (Özkaya & Özkaya, 2005).

The shape and size of the grain, as well as the absence of wrinkles and cracks, are the most important physical grain characteristics that affect the thousand-grain weight and directly affect the flour yield (Tyagi et al., 2015). The thousand-grain weight gives information about the endosperm ratio in the seed. Since the endosperm ratios of varieties with a high thousand-grain weight are generally high, flour yields are high (Posner, 2009). Elgün et al., (2001) reported that thousand-grain weights ranged between 26-36 g in soft wheat and 35-46 g in hard wheat. In the study, the mean value of thousand-grain weight was determined as 33.0 g, and this value varied between 27.5 and 41.5 g. The highest thousand-grain weight was obtained from the Ekiz variety (41.5 g), followed by Şehzade (39.9 g), Meke (36.2 g), Bozkır (34.1 g).

Taner (33.0 g) and Bayındır (32.0 g) varieties were found to have average values (Table 3). Aydoğan & Soylu (2017), in a similar study conducted on 14 bread wheat varieties under Konya conditions, found that the thousand-grain weight of the varieties ranged between 30.90 g and 46.46 g and the mean value of the trial was 38.32 g.

The test weight gives information about the unit volume density, shape, and size of the grain. The high test weight value is desirable for bread wheat varieties. The fact that this value is 80 kg hl^{-1} and above is especially desired by the wheat industrialists. Elgün et al., (2001) reported that test weights ranged between 74-82 kg hl^{-1} in soft wheats and 78-82 kg hl^{-1} in hard wheats. The mean value of test weights obtained in the study was determined as 75.8 kg hl^{-1}, and this value varied between 71.9-80.6 kg hl^{-1}. While the Ekiz variety had the highest value with 80.6 kg hl^{-1}, the Bayındır variety had the lowest value (71.9 kg hl^{-1}) (Table 3). Şahin et al., (2017), determined the test weights between 70.97-77.43 kg hl^{-1} and the mean value as 75.18 kg hl^{-1} in the study made on bread wheat varieties.

Many methods have been developed to measure wheat grain hardness and SKCS has been widely used recently. The bread quality of hard wheat is generally high. In general, hard wheats are suitable for bread making and soft wheats are suitable for biscuits (Giroux & Morris, 1998). During the conversion of very hard wheat into flour, energy consumption is high or in very soft wheat, the flour yield is low because it is difficult to separate the bran from the flour (Elgün et al., 2001). The SKCS hardness values obtained in the study were determined as 68.2% on average and this value varied between 44.4-87.2%. In the study conducted by Şahin et al., (2019), it was determined the hardness values of bread wheat genotypes, consisting of 20 varieties and breeding lines, between 29.78 and 87.66%.

Protein ratio is one of the important quality criteria considered in the study. It has been reported that to classify wheat and characterize wheat flour, it is necessary to measure the protein and gluten content together with the sedimentation value for wheat flour characterization (Başlar & Ertugay, 2011). Although the amount of protein is one of the most influential criteria from climatic conditions and agronomic applications (Aktan, 1992), it is one of the most effective parameters in determining the quality of wheat varieties (Williams et al., 1986). Protein ratios obtained from the study varied between 11.7% and 16.8%. Selçuklu variety had the highest protein content with 16.8%, followed by Bozkır (16.0%), Bayındır, İkonya (15.4%) and Yavuz (15.1%). While the protein ratios of Tuğra (14.3%), Meke (14.1%), Taner (13.9%), and Şehzade (13.1%) varieties were low, the protein ratio of the Ekiz variety (11.7%) was determined to be the lowest (Table 3). Egesel et al. (2009) determined the protein ratio between 10.9% and 13.1% in the study they carried out for two years in 10 bread wheat varieties. Şahin et al. (2019) evaluated the quality and technological characteristics of bread wheat genotypes consisting of 20 varieties and lines and found that the protein ratios varied between 12.29-14.10%.

Wet gluten is an elastic substance formed by the gliadin and glutenin proteins in the wheat composition by absorbing water and swelling. The amount of wet gluten helps to determine the gluten quality (gluten structure, flour strength). The fact that the wet gluten ratio is over 28% in the flour to be used in bread making allows the production of good quality dough (Erekul et al., 2005). The wet gluten mean values obtained in the study were determined as 41.6%. This value varied between 32.2-49.2%. While the Selçuklu variety had the highest wet gluten value at 49.2%, wet gluten values of Meke (35.6%), Şehzade (32.6%), and Ekiz (32.2%) varieties were found low (Table 3). In the study of Keçeli & İkikarakaya (2013) conducted for two years on 4 different bread wheat varieties, it was determined the mean value of the wet gluten ratio was 28.0% in the first year and 27.0% in the second year. Okur (2017) reported that for 57 samples milled as flour and whole wheat flour, the mean value of the wet gluten analysis values in red wheat was determined as 34.51 and 28.07%, and the mean value of the wet gluten analysis values in white wheat was determined as 31.27 and 27.08%, respectively.

Table 1. The mean square results of the variance analysis of the glutopic analysis values.
Çizelge 1. *Glutopik analiz değerlerine ait varyans analizi kareler ortalaması sonuçları.*

VS	SD	AM	BEM	PMT	PM	GPRT	GGLT	GW	GWA	AGREE
Variety *(Çeşit)*	9	100.9**	239.8**	566.6**	215.1**	4.1**	36.2**	37706**	44.1**	208915**
Recurrence *(Tekerrür)*	1	0.8	39.2	7.2	1.25	0.061	0.578	460.8	5.832	1828.8
Error *(Hata)*	9	5.35	22.86	13.42	3.47	0.19	1.81	366.13	5.57	9394.04
General *(Genel)*	19									

** (P<0.01), VS: Variation Sources, SD: Degree of Freedom, AM: Torque 15 sec Before Maximum Torque, BEM: Maximum Torque, PMT: Peak Maximum Time, PM: Torque 15 s After Maximum Torque, GPRT: GlutoPeak Protein Ratio, GGLT: GlutoPeak Wet Gluten Value, GW: GlutoPeak Energy Value, GWA: GlutoPeak Water Absorption Capacity, AGGRE: GlutoPeak Aggregation Energy Value

Table 2: Mean square results of variance analysis of some quality values of bread wheat.
Çizelge 2. *Ekmeklik buğdayların bazı kalite değerlerine ait varyans analizi kareler ortalaması sonuçları.*

VS	SD	Thousand Grain Weight (Bin Tane Ağırlığı)	Test Weight (Hektolitre Ağırlığı) (kg hl-1)	SKCS Hardness (SKCS Sertlik) (%)	Protein Ratio (Protein Oranı) (%)	Zeleny Sedimentation (Zeleny Sedimentasyon)(ml)	Wet Gluten (Yaş Gluten) (%)	Gluten Index (Gluten İndeks) (%)	Falling Number (Düşme Sayısı) (sn)	Farinograph Water Absorption (Farinograf Su Absorbsiyonu)	Alveograph Energy Value (Alveograf Enerji	Bread Weight (Ekmek Ağırlığı) (g)	Bread Volume (Ekmek
Variety	9	44.815**	11.44**	434.329**	4.335**	135.12**	70.8**	251.56**	10060.72*	31.82**	12771.689**	17.05**	1223.7
Recurrence	1	0.722	0.007	0.2668	0.328	6.05	5.398	21.487	1170.45	0.025	897.8	8.039	4.05
Error	9	0.674	0.182	3.908	0.807	1.828	6.594	12.53	130.228	0.512	218.356	0.852	70.72
General	19												

** (P<0.01), VS: Variation Sources, SD: Degree of Freedom

Table 3. Mean values of some quality parameters in bread wheat varieties.
Çizelge 3. *Ekmeklik buğday çeşitlerinde bazı kalite parametrelerine ait ortalama değerler.*

Variety (Çeşit)	Thousand Grain Weight (Bin Tane Ağırlığı) (g)	Test Weight (Hektolitre Ağırlığı) (kg hl-1)	SKCS Hardness (SKCS Sertlik) (%)	Protein Ratio (Protein Oranı) (%)	Zeleny Sedimentation (Zeleny Sedimentasyon) (ml)	Wet Gluten (Yaş Gluten) (%)	Gluten Index (Gluten İndeks) (%)	Falling Number (Düşme Sayısı) (sn)	Farinograph Water Absorption (Farinograf Su Absorbsiyonu)	Alveograph Energy Value (Alveograf Enerji Değeri) (x10-4 J)	Bread Weight (Ekmek Ağırlığı)) (g)	Bread Volume (Ekmek Hacmi) (cm³)
Bayındır	32.0^{de}±0.	71.9^{g}±0.7	87.2^{a}±1.	15.4^{abc}±0	31.0^{f}±1.0	44.6^{ab}±1	53.2^{e}±4.	507^{ab}±3	70.2^{a}±0.4	389.0^{e}±40.	150^{b}±1.	465^{b}±7.
Bozkır	34.1^{c}±0.	76.3^{cd}±0.	54.5^{g}±2.	16.0^{ab}±0.	46.5^{b}±0.7	45.7^{ab}±1	67.8^{d}±3.	348^{f}±1	65.5^{c}±0.1	214.5^{cd}±19	148^{bc}±0	430^{c}±14
Ekiz	41.5^{a}±0.	80.6^{a}±0.1	76.1^{bc}±4	11.7^{g}±0.2	26.5^{g}±0.7	32.2^{c}±1.	65.7^{d}±2.	435^{d}±5	62.0^{f}±0.4	127.5^{ef}±5.0	146^{c}±1.	439^{c}±5.
İkonya	28.1^{fg}±0.	77.4^{b}±0.2	78.0^{b}±1.	15.4^{abc}±0	40.5^{d}±0.7	45.7^{ab}±1	78.1^{bc}±2.	435^{d}±2	63.7^{de}±0.2	191.5^{de}±7.	148^{bc}±0	465^{b}±7.
Meke	36.2^{b}±0.	75.1^{e}±0.2	45.9^{f}±3.	14.1^{bcd}±0	42.5^{cd}±2.1	35.6^{e}±1.	91.0^{a}±4.	377^{e}±2	62.5^{ef}±1.0	220.5^{c}±16.	148^{c}±2.	469^{b}±1.
Selçuklu	27.5^{h}±0.	72.9^{f}±0.2	78.3^{b}±0.	16.8^{a}±0.3	56.0^{a}±2.8	49.2^{a}±0.	85.3^{ab}±1	532^{a}±2	68.6^{b}±0.1	244.0^{b}±14.	153^{a}±0.	469^{b}±1.
Şehzade	39.9^{a}±0.	76.3^{cd}±1.	44.4^{f}±2.	13.1^{de}±0.	35.5^{e}±0.7	32.6^{c}±5.	85.0^{ab}±5	311^{g}±1	55.6^{g}±0.5	115.5^{f}±10.	141^{d}±1.	410^{d}±7.
Taner	33.0^{cd}±0.	75.5^{de}±0.	76.2^{bc}±1	13.9^{cd}±2.	42.0^{cd}±1.4	43.5^{ab}±3	70.0^{d}±4.	481^{c}±6	65.6^{c}±1.4	282.0^{b}±42.	148^{bc}±1	490^{a}±14
Tuğra	30.9^{ef}±1.	76.9^{bc}±0.	68.5^{d}±0.	14.3^{bcd}±0	44.0^{bc}±1.4	44.5^{ab}±2	78.9^{bc}±4.	482^{bc}±2	62.9^{def}±0.5	157.0^{de}±2.	148^{bc}±1	482^{ab}±2.
Yavuz	29.6^{fg}±0.	75.2^{e}±0.1	72.7^{cd}±0	15.1^{ed}±0.	42.0^{cd}±1.4	42.8^{b}±2.	72.2^{cd}±0.	429^{d}±2	64.4^{cd}±0.8	195.5^{de}±7.	147^{c}±0.	445^{c}±7.
Mean(Ortalam	33.0	75.8	68.2	15.0	41.0	41.6	75.0	434	64.1	213.7	147.7	456
CV (%)	2.9	0.6	2.9	6.2	3.3	6.2	4.7	2.6	1.1	6.9	0.6	1.8
$LSD_{0.05}$	2.19	0.96	4.47	2.03	3.1	5.81	8.0	25.8	1.6	33.4	2.1	19.0

CV: Coefficient of Variation, LSD: Least Significant Differences, $^{(de)}$: Different superscripts in the same column indicate statistically significant differences between the means ($P<0.05$).

The parameters commonly used to determine the quality of bread wheat are protein ratio, Zeleny sedimentation value, wet gluten, dry gluten, and gluten index values (Menderis et al., 2008). Gluten index value is used to determine gluten quality and it is required to be between 60-90% in bread flour (Elgün et al., 2001). The gluten index values obtained in the study varied between 53.2-91.0%. Make variety had the highest gluten index value with 91.0%, followed by Selçuklu (85.3%), Şehzade (85.0%), Tuğra (78.9%) and İkonya (78.1%). While the gluten index values of Yavuz (72.2%), Taner (70.0%), Bozkır (67.8%), and Ekiz (65.7%) varieties were below the average value, the Bayındır variety had the lowest value with 53.2% (Table 3). Egesel et al., (2009) determined the gluten index value between 14.0 and 77.8% in a study conducted for two years on 10 bread wheat varieties.

The time for the wheat starch to lose its viscosity with the activity of the α and ß amylase enzymes in the flour gives the falling number. The falling number determines the activity of the amylase enzyme in the flour. The value of a falling number over 300 seconds is an indicator of low amylase activity. If amylase is not added to flours with low amylase activity, bread volume becomes low and bread crumbs become dry. The falling number values obtained in the study were determined as 434 seconds on average. This value varied between 311-532 sec. (Table 3). Kara et al., (2020) determined the falling number values of bread wheat between 262.5 and 882.0 sec. in different grain sizes. It was determined that all varieties had low amylase activity in terms of falling number values.

Evaluation of Findings Related to Rheological Analyzes

In determining the quality of wheat for bread making, physical and physicochemical properties do not provide complete and precise information, so it is necessary to determine the rheological properties of the dough. The rheological properties of the dough give information about the visco-elastic structure of the dough. The visco-elastic structure of the dough shows the bread quality. The visco-elastic structure allows the dough to keep its shape. After the deformation formed in the dough by a force applied to the dough, the dough tries to return to its previous state. This is the most important property of dough (Patel & Chakrabarti-Bell, 2013). The visco-elastic properties of the dough can be measured with some devices. One of the devices developed for this purpose is the chronograph. Farinograph determines the amount of water required for the flour to become a normal dough and provides information about the development, stability, and softening degree of the dough (Elgün et al., 2001).

Farinograph water absorption is the amount of water required to be added to the flour to obtain a dough of a certain consistency, and it is desired that the amount of water to be used in bread making is high. High water absorption is a feature desired by bakers. When flours with high water absorption are kneaded, more dough is obtained. The mean values of water absorption values obtained in the study were determined as 64.1% (Table 3). Al-Saleh & Brennan (2012) reported that the water absorption value varied between 56.30% and 64.05% in a study they conducted with bread wheat genotypes under irrigated conditions.

Alveograph energy (W) value is one of the reliable data to reveal the quality of wheat flour and has a key role in the evaluation of the quality of wheat for bread making among all allograph parameters. Abu Hammad et al. (2012) classified the allograph energy values as weak (<100 $x10^{-4}$ J), moderately weak (101-150 $x10^{-4}$ J), moderately strong (151-200 $x10^{-4}$ J), and strong (201-250 $x10^{-4}$ J), and very strong (>250 $x10^{-4}$ J). In the study, the W value varied between 115.5-389.0 $x10^{-4}$ J. While Bayındır variety has the highest W value with 389.0 $x10^{-4}$ J, Yavuz (195.5 $x10^{-4}$ J), İkonya (191.5 $x10^{-4}$ J), Tugra (157.0 $x10^{-4}$ J), Ekiz (127.5 $x10^{-4}$ J) and Şehzade (115.5 $x10^{-4}$ J) varieties had lower than average W values (Table 3). Kristensen et al., (2019) found the W value between 40-293 $x10^{-4}$ J and the mean value of W value as 134.2 $x10^{-4}$ J in their study. According to Pomeranz (1987), the W value of standard flour is around 141 x 10^{-4} J. Some other researchers have suggested that the W value of standard flour is characterized in the range of 160-200 x 10^{-4} J (Bordes et al., 2008). Considering the literature information, it was determined that the W values of the majority of the varieties examined in this study were almost in the standard range or higher.

Evaluation of Findings Related to GlutoPeak Analysis

The gluten qualities of bread wheat varieties must be suitable for the end product.
To determine the gluten quality, information about water absorption, energy value, and tolerance values against kneading is obtained with devices such as micrographs, allographs, chronographs, and stenographs. These methods require large amounts of samples and take a long time. In recent years, it has been stated that the Glutopik device, which gives results in a shorter time with fewer samples, has been used to measure gluten quality (Güçbilmez et al., 2019). GlutoPeak measures the aggregation of wheat gluten proteins in a flour/water slurry under high-speed shearing (Melnyk et al., 2011). Studies show that GlutoPeak parameters can be used to differentiate wheat flour according to gluten aggregation and dough rheological properties (Malegori et al., 2018; Zawieja et al., 2020).

In the research, the mean value of AM (GPU), which is expressed as torque 15 seconds Before Maximum

Torque, was determined as 25.6 GPU, and this value varied between 17.5 and 42.0 GPU (Table 4). Güçbilmez et al. (2019) and Şahin et al. (2020) reported that AM values varied between 14-36 GPU and 19.5-43.8 GPU respectively, in their study of bread wheat flour.

When the BEM value of the GlutoPeak diagram is examined, the Bayındır variety has the highest BEM value of 89 GPU. Daba et al., (2021) determined the BEM value between 53.5-81.5 GPU, with a mean value of 64.8 GPU in their study.

The PMT value is expressed as the time (sec) from the beginning of the GlutoPeak diagram to the maximum torque. Varieties with strong gluten give lower PMT and higher BEM values, while the opposite is true for varieties with weak gluten (Güçbilmez et al., 2019). In the study, the mean value of PMT was determined as 60.8 seconds, and this value varied between 45.5-87.0 seconds. Wang et al., (2018) stated that the PMT value varied between 41.3 and 92.3 seconds in the GlutoPeak analysis studies on bread wheat.

PM value is measured as the torque 15 sec after maximum torque. Bayındır variety in the study had the highest PM value with 69.5 GPU, followed by Selçuklu (66 GPU), Taner (58 GPU), Yavuz (56 GPU), Bozkır (55 GPU), Tuğra (54 GPU), İkonya (52 GPU), Meke (49 GPU), Ekiz (39.5 GPU) and Şehzade (36 GPU) (Table 4). Daba et al., (2021) are also in agreement with this study in terms of PM value (43.0-67.0 GPU).

In the study, the mean value of GPRT calculated by the GlutoPeak was determined as 13.2%, and this value varied between 10.8 and 15.7%. The Bayındır variety in the study had the highest protein content with 15.7%. Şahin et al., (2020) found the mean of GPRT values as 12.8% in their study on bread wheat.

Table 4. Mean values of GlutoPeak parameters of bread wheat varieties.
Çizelge 4. *Ekmeklik buğday çeşitlerinde glutopik parametrelerine ait ortalama değerler.*

Variety *(Çeşit)*	AM (GPU)	BEM (GPU)	PMT (sn)	PM (GPU)	GPRT (%)	GGLT (%)	GW (x10^{-4} J)	GWA (% v w^{-1})	AGGRE (GPU)
Bayındır	42.0^{a}±14.9	89.0^{a}±1.4	28.0^{g}±0.0	69.5^{a}±2.1	15.7^{a}±0.1	32.4^{bc}±0.6	670^{a}±16.9	72.3^{a}±0.6	2173^{a}±57.1
Bozkır	28.0^{bc}±2.1	68.5^{bc}±0.7	67.0^{bc}±0.0	55.0^{bc}±1.4	13.7^{bc}±0.3	31.6^{cd}±0.0	419^{cd}±8.4	66.4^{b}±0.6	1819^{b}±38.5
Ekiz	17.5^{ef}±3.5	59.0^{cd}±7.1	54.0^{e}±5.7	39.5^{e}±4.9	11.4^{e}±0.6	25.3^{e}±1.9	303^{e}±86.2	60.0^{cd}±2.6	1279^{d}±177.9
İkonya	27.0^{bcd}±4.2	73.0^{b}±5.7	55.5^{de}±13.4	52.0^{cd}±0.7	13.5^{cd}±0.0	32.2^{bc}±1.5	450^{bc}±17.6	64.1^{bc}±0.8	1577^{c}±128.6
Meke	24.0^{cd}±0.7	66.0^{bc}±2.8	80.0^{a}±9.9	49.0^{d}±4.9	12.9^{cd}±0.1	29.8^{cd}±0.6	389^{d}±34.6	63.3^{bc}±1.8	1772^{bc}±26.9
Selçuklu	30.0^{b}±2.9	84.0^{a}±11.3	59.5^{cde}±14.8	66.0^{a}±9.9	14.7^{b}±0.8	35.0^{b}±2.3	631^{a}±35.3	72.9^{a}±6.1	2128^{a}±14.6
Şehzade	16.5^{f}±0.7	51.5^{d}±2.1	87.0^{a}±12.8	36.0^{e}±1.4	10.8^{e}±0.1	23.6^{e}±0.6	212^{f}±26.1	57.9^{d}±0.7	1164^{d}±43.0
Taner	27.5^{bc}±0.7	71.0^{b}±1.4	45.5^{f}±7.8	58.0^{b}±0.7	13.8^{bc}±0.5	38.2^{a}±0.8	474^{b}±69.2	66.8^{bc}±2.4	1768^{bc}±3.8
Tuğra	22.0^{de}±1.4	67.0^{bc}±0.0	69.0^{b}±1.4	54.0^{bc}±1.4	13.4^{cd}±0.1	31.3^{cd}±0.1	401^{d}±0.0	65.1^{bc}±0.2	1567^{c}±75.0
Yavuz	23.0^{cd}±1.4	65.0^{bc}±4.2	62.5^{bcd}±10.6	56.0^{b}±4.2	12.7^{d}±0.6	29.2^{d}±1.9	377^{d}±51.6	66.1^{b}±0.4	1812^{b}±158.1
Ortalama	25.6	69.4	60.8	53.4	13.2	30.8	432.4	65.5	1706
CV (%)	9.0	6.9	6.0	3.5	3.3	4.4	4.4	3.6	5.7
$LSD_{0.05}$	5.2	10.8	8.3	4.2	1.0	3.0	43.3	5.3	219.3

CV: Coefficient of Variation, LSD: Least Significant Differences, $^{(a)}$; Different superscripts in the same column indicate statistically significant differences between the means (P<0.05). AM: Torque 15 sec Before Maximum Torque, BEM: Maximum Torque, PMT: Peak Maximum Time, PM: Torque 15 s After Maximum Torque, GPRT: GlutoPeak Protein Ratio, GGLT: GlutoPeak Wet Gluten Value, GW: GlutoPeak Energy Value, GWA: GlutoPeak Water Absorption Capacity, AGGRE: GlutoPeak Aggregation Energy Value

The GGLT values, which express the wet gluten ratio calculated by the GlutoPeak device, were found to be 30.8% on average. Taner variety had the highest GGLT value with 38.2% followed by Selçuklu (35.0%), Bayındır (32.4%), İkonya (32.2%), Bozkır (31.6%), Tuğra (31.3%), Meke (29.8%), Yavuz (29.8%). 29.2), Ekiz (25.3%) and Şehzade (23.6%) varieties (Table 4). Şahin et al., (2020) found the the mean of GGLT values as 30.5% in their study.

The mean value GW was determined as 432.4x10^{-4} J, and this value varied between 212-670x10^{-4} J. The Bayındır variety included in the study had the highest GW value with 670x10^{-4} J (Table 4). Şahin et al., (2020) determined the average GW value as 392.7x10^{-4} J.

GWA values varied between 57.9-72.9 % v w^{-1}. While the Selçuklu variety had the highest GWA value with 72.9 % v w^{-1}, the Şehzade variety had the lowest GWA value with 57.9 % v w^{-1} in the study. Güçbilmez et al., (2019) determined the GWA value in the range of 52.8-67.1% v w^{-1} and found the mean value as 61.9% v w^{-1} in their study on bread wheat flour.

While the mean value of AGGRE obtained from the GlutoPeak data was determined as 1706 GPU, the Bayındır variety gave the highest AGGRE value with 2173 GPU. Daba et al., (2021) found the mean value of AGGRE as 1794.7 GPU in their studies on dough rheological properties and baking quality of wheat.

Evaluation of Findings Related to Bread Analyzes

While the mean value of bread weight obtained in the

study was 147.7 g, this value varied between 141-153 g among varieties. In addition, the mean value of bread volume was 456 cm^3, and this value changed in the range of 410-490 cm^3. Aydoğan (2016) determined the bread weight between 141.6-149.5 g and the mean value as 146.0 g in the study made on bread wheat varieties grown under irrigated conditions. Also, the bread volume was determined between 368-485 cm^3, and the mean value was 452.3 cm^3 in the research.

Evaluation of Relationships Between GlutoPeak Data and Other Quality Parameters

The data obtained by using classical methods in 10 bread wheat varieties were compared with the data calculated by the GlutoPeak method. The correlation coefficients between the GlutoPeak parameters obtained in the study and other parameters, and their statistical significances are given in Table 5.

Table 5. Correlation coefficients between GlutoPeak and other quality analyses (r) (n=10)
Çizelge 5. *Glutopik ile diğer kalite analizleri arasındaki korelasyon katsayıları (r) (n=10)*

	Thousand Grain Weight *(Bin Tane Ağırlığı)* (g)	Test Weight *(Hektolitre Ağırlığı)* *(kg hl-1)*	SKCS Hardness *(SKCS Sertlik)* (%)	Protein Ratio *(Protein Oranı)* *(%)*	Zeleny Sedimentation *(Zeleny Sedimentasyon)* (ml)	Wet Gluten *(Yaş Gluten)* *(%)*	Gluten Index *(Gluten İndeks)* *(%)*	Falling Number *(Düşme Sayısı)* *(sn)*	Farinograph Water Absorption *(Farinograf Su Absorbsiyonu)* (%)	Alveograph Energy Value *(Alveograf Enerji Değeri)* *(x10-4 J)*	Bread Weight *(Ekmek Ağırlığı)* (g)	Bread Volume *(Ekmek Hacmi)* *(cm^3)*
AM	-0.54	-0.76*	0.55	0.66*	0.12	0.66*	-0.52	0.55	0.87**	0.95**	0.70*	0.43
BEM	-0.69*	-0.72*	0.67*	0.72*	0.30	0.77**	-0.36	0.76*	0.93**	0.86**	0.88**	0.58
PMT	0.33	0.29	-0.89**	-0.20	0.27	-0.44	0.81*	-0.71*	-0.77**	-0.74*	-0.53	-0.44
PM	-0.77**	-0.78**	0.62	0.78**	0.44	0.85**	-0.36	0.74*	0.94**	0.85**	0.86**	0.60
GPRT	-0.70*	-0.73*	0.58	0.76*	0.37	0.82**	-0.38	0.69*	0.93**	0.88**	0.85**	0.62
GGLT	-0.69*	-0.50	0.49	0.59	0.56	0.79**	-0.14	0.65*	0.74*	0.67*	0.75*	0.79**
GW	-0.67*	-0.74*	0.67*	0.71*	0.33	0.76*	-0.35	0.78**	0.94**	0.87**	0.89**	0.60
GWA	-0.74*	-0.79**	0.60	0.79**	0.48	0.83**	-0.32	0.74*	0.94**	0.82**	0.89**	0.54
AGREE	-0.67*	-0.83**	0.45	0.79**	0.48	0.74*	-0.26	0.60	0.92**	0.85**	0.86**	0.50

*(P<0.05) significant at 5% level, ** (P<0.01) significant at 1% level AM: Torque 15 sec Before Maximum Torque, BEM: Maximum Torque, PMT: Peak Maximum Time, PM: Torque 15 s After Maximum Torque, GPRT: GlutoPeak Protein Ratio, GGLT: GlutoPeak Wet Gluten Value, GW: GlutoPeak Energy Value, GWA: GlutoPeak Water Absorption Capacity, AGGRE: GlutoPeak Aggregation Energy Value

Relationships Between GlutoPeak Analysis and Physical Analyzes

It was determined that there was a negative significant correlation between thousand-grain weight and BEM (r=-0.69 P<0.05), PM (r=-0.77 P<0.01), GPRT (r=-0.70 P<0.05), GGLT (r=-0.69 P<0.05), GW (r=-0.67 P<0.05), GWA (r=-0.74 P<0.05) ve AGGRE (r=-0.67 P<0.05). A negative significant correlation between test weight and AM (r=-0.76 P<0.05), BEM (r=-0.72 P<0.05), PM (r=-0.78 P<0.01), GPRT (r=-0.73 P<0.05), GW (r=-0.74 P<0.05), GWA (r=-0.79 P<0.01), AGGRE (r=-0.83 P<0.01) were observed. SKCS had a positive significant correlation with BEM (r=0.67 P<0.05) and GW (r=0.67 P<0.05), and a negative significant correlation with PMT (r=-0.89 P<0.01). Güçbilmez et al., (2019) reported that they found a significant correlation (r=0.7607 P<0.01) between BEM value and hardness value in their study. These findings are compatible with Güçbilmez et al., (2019) in terms of the correlations between BEM and hardness value (r=0.7607 P<0.01).

Relationships Between GlutoPeak Analysis and Chemical and Technological Analyzes

The correlation coefficients between the GlutoPeak parameters obtained in the study and the chemical and technological parameters and their statistical significance are given in Table 5. No statistically significant correlation was found between Zeleny sedimentation values and GlutoPeak parameters. Positive significant correlations were determined between protein ratio and AM (r=0.66 P<0.05), BEM (r=0.72 P<0.05), PM (r=0.78 P<0.01), GW (r=0.71 P<0.05), GWA (r=0.79 P<0.01) and AGGRE (r=0.79 P<0.01). Positive significant correlations between wet gluten and AM (r=0.66 P<0.05), BEM (r=0.77 P<0.01), PM (r=0.85 P<0.01), GPRT (r=0.82 P<0.01), GW (r=0.76 P<0.05), GWA (r=0.83 P<0.01), AGGRE (r=0.74 P<0.01) values were observed. In addition, a positive correlation was found between gluten index value and PMT (r=0.81 P<0.05). When the protein ratio values of the samples made by the Dumas method and GPRT values were evaluated together; the differences between varieties were determined as significant (r=0.76 P<0.05).

Also, when the samples were compared in terms of wet gluten values; it was determined that the differences between varieties were significant (r=0.79 P<0.01). Positive significant correlations were determined between the falling number and BEM (r=0.76 P<0.05), PM (r=0,74 P<0,05), GPRT (r=0,69 P<0,05), GGLT (r=0,65 P<0,05), GW (r=0,78 P< 0.01), GWA (r=0.74 P<0.05) and negative significant correlations were determined with PMT (r=-0.71 P<0.05). Bouchra et al., (2017) stated the positive significant correlation between AM value and gluten quality, BEM value, and

protein ratio.

Relationships Between GlutoPeak Analysis and Farinograph Analysis

When the correlation coefficients and statistical significance between the glutopic parameters and farinograph water absorption were examined, positive correlations between farinograph water absorption and AM (r=0.87 P<0.01), BEM (r=0.93 P<0.01), PM (r=0.94 P<0.01), GPRT (r=0.93 P<0.01), GGLT (r=0.74 P<0.05), GW (r=0.94 P<0.01), GWA (r=0.94 P<0.01), and AGGRE (r=0.92 P<0.01)) and negative correlations with PMT (r=-0.77 P<0.01) were obtained (Table 4). The chronograph water absorption made with the classical method and GWA were evaluated together and it was determined that the differences between genotypes were significant at the P<0.05 level in both. The mean value of water absorption results made with the classical method was found to be 65.5%, and the average GWA value was determined as 64.1%. Similar to this study, Şahin et al., (2020) and Güçbilmez et al., (2019) reported a significant correlation between the values obtained with both devices at the level of r=0.8280 P<0.01 and r=0.9158, P<0.01, respectively.

Relationships Between GlutoPeak Analysis and Alveograph Analysis

The autograph W value was evaluated with the obtained GlutoPeak parameters. It was determined that there were positive correlations between AM (r=0.95 P<0.01), BEM (r=0.86 P<0.01), PM (r=0.85 P<0.01), GPRT (r=0.88 P<0.01), GGLT (r=0.67 P<0.05), GWA (r=0.82 P<0.01) and AGGRE (r=0.85 P<0.01), and negative correlations with PMT (r=-0.74 P<0.05).

Relationships Between GlutoPeak Analysis and Bread Analyzes

When the correlation coefficients and statistical significance between the GlutoPeak parameters and the technological parameters obtained from the study were examined, positive correlations were found between the bread weight and AM (r=0.70 P<0.05), BEM (r=0.88 P<0.01), PM (r=0.86 P<0.01), GPRT (r=0.85 P<0.01), GGLT (r=0.75 P<0.05), GW (r=0.89 P<0.01), GWA (r=0.89 P<0.01), AGGRE (r=0.86 P<0.01). Also, positive correlations were obtained between bread volume and GGLT (r=0.79 P<0.01) (Table 4).

CONCLUSION

According to the data obtained as a result of the analysis performed with the GlutoPeak device developed to evaluate the gluten quality in bread wheat, the high AM, BEM, PM, and AGGRE values indicate high gluten quality, while the high PMT value indicates late aggregation and weak gluten.

A high correlation between GWA and water absorption values obtained from farinograph analysis (r= 0.94, P<0.01) was obtained. In addition, it was determined that there was a significant correlation between GPRT and protein ratio (r= 0.76, P< 0.01), GGLT and wet gluten (r=0.79, P<0.01), and GW and allograph W value (r=0.87, P<0.01).

Rheological measuring devices such as allograph, chronograph, and stenograph are widely used around the world to determine dough properties and bread-making properties of flour. However, bread wheat breeding studies take a long time (13-15 years). Especially in the F1-F3 stages after crossing, the amount of seeds is low (20-25 g), and breeders are curious about the quality values of the wheat lines they will develop at these stages. Therefore, there is a need for analyzers that provide information about technological analyses using fewer samples. Since 9 g of sample is used in the GlutoPeak device, it is considered to be suitable for this purpose. The use of GlutoPeak device data in breeding stages can provide a scientifically remarkable contribution to breeders in the selection of bread wheat in terms of technological properties. Comparison of GlutoPeak parameters with allograph, farinograph, or chemical analysis in advanced stages or variety trials where the sample amount is high; may be required to make accurate assessments in terms of results. Considering the analysis findings of this study (flour water absorption, flour protein ratio, wet gluten ratio, and dough alveograph energy value [W]), it was concluded that GlutoPeak analysis can be used in variety development and similar studies in wheat because it gives results in a short time with few samples.

ACKNOWLEDGMENT

This study was produced from the Master's thesis. I thank my colleagues for their contribution.

Contribution Rate Statement Summary of Researchers

The authors' contribution to the study is equal.

Statement of Conflict of Interest

The authors have no conflict of interest related to the study.

REFERENCES

AACC, (2000). Approved Methods of American Association of Cereal Chemists.10th ed., methods: 10-10, 26-50, 55-31, 26-95, 38-12A, 56-81B, 44-15A, Minnesota, USA.

AACCI, (2010). Approved Methods of the American Association of Cereal Chemists, AACCI method 55-10.01, Approved methods of analyses 11th edition, The Association: St. Paul, MN.

Aktan B, (1992). *Farklı Azot Uygulamasının Makarnalık Buğday Kalitesine Etkisi (Tez no 22811)*. [Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü Gıda Bilimi ve Teknolojisi Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Al-Saleh, A., & Brennan, C. S., (2012). Bread wheat quality: some physical, chemical and rheological characteristics of Syrian and English bread wheat samples. *Foods*, *1*(1), 3-17. https://doi.org/10.3390/foods1010003.

AOAC, (2000). Official Methods of Analysis of Association of Official Analytical Chemists, 17th ed. method 992.23, Gaithersburg, MD.

Aydoğan S, (2016). *Kuru ve Sulu Yetiştirme Şartlarının Ekmeklik Buğday Çeşitlerinin Verim Ve Kalitesine Etkisinin Belirlenmesi (Tez no 430342)*. [Yüksek Lisans Tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Aydoğan, S., & Soylu, S. (2017). Ekmeklik Buğday Çeşitlerinin Verim ve Verim Öğeleri ile Bazı Kalite Özelliklerinin Belirlenmesi. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, *26*(1), 24-30.

Başlar, M. & Ertugay, M. F. (2011). Determination of protein and gluten quality-related parameters of wheat flour using near-ınfrared reflectance spectroscopy(NIRS), *Turkish Journal of Agriculture and Forestry*, *35*, 139-144. https://doi.org/10.3906/tar-0912-507.

Bordes, J., Branlard, G., Oury, F. X., Charmet, G., & Balfourier, F. (2008). Agronomic characteristics, grain quality, ty and flour rheology of 372 bread wheat in a worldwide core collection. *Journal of Cereal Science*, *48* (3), 569-579. .https://doi.org/10.1016/j.jcs.2008.05.005.

Bouachra, S., Begemann, J., Aarab, L., & Hüsken, A. (2017). Prediction of bread wheat baking quality using an optimized GlutoPeak®-test method. *Journal of Cereal Science*, *76*, 8–16. https://doi.org/10.1016/j.jcs.2017.05.006.

Daba, S. D., Simsek, S., & Green, A. J. (2021). Predictive ability of four small-scale quality tests for dough rheological properties and baking quality in hard red spring wheat. *Cereal Chemistry*, *98*(3), 660-672. https://doi.org/10.1002/cche.10410.

Egesel, C. Ö., Kahriman, F., Tayyar, Ş,. & Baytekin, H. (2009). Ekmeklik Buğdayda Un Kalite Özellikleri ile Dane Veriminin Karşılıklı Etkileşimleri ve Uygun Çeşit Seçimi. *Anadolu Tarım ve Bilim Dergisi*, *24*(2), 76-83.

Elgün, A., Ertugay, Z., Certel, M., & Kotancılar, H. G. (2002). *Tahıl ve ürünlerinde analitik kalite kontrolü ve laboratuvar uygulama kılavuzu*, Atatürk Üniversitesi, Ziraat Fakültesi Ofset Tesisleri, 867, 245s, Erzurum.

Elgün, A., Türker, S., & Bilinçli, N. (2001). Tahıl ve ürünlerinde analitik kalite kontrolü, Konya Ticaret Borsası, No: 2, 112 s, Konya.

Erekul, O., Oncan, F., Erekul, A., Yava, İ., Engün, B., & Koca, Y. O. (2005). İleri ekmeklik buğday hatlarında verim ve bazı kalite özelliklerinin belirlenmesi, Türkiye VI. Tarla Bitkileri Kongresi, 5(9), 111-116.

FAO, (2020). Cereal supply and demand brief. http://www.fao.org/worldfoodsituation/csdb/en.

Giroux, M. J., & Morris, C. F. (1998). Wheat grain hardness results from highly conserved mutations in the friable components puroindoline a and b. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(11), 6262-6266. https://doi.org/10.1073/pnas.95.11.6262.

Güçbilmez, Ç. M., Şahin, M., Akçacık, A. G., Aydoğan, S., Demir, B., Hamzaoğlu, S., Gür, S., & Yakışır, E. (2019). Evaluation of GlutoPeak test for prediction of bread wheat flour quality, rheological properties, and baking performance. *Journal of Cereal Science*, *90*, 1-9. https://doi.org/10.1016/j.jcs.2019.102827.

Güneş, E., & Turmuş, E. (2020). Dünyada ve Türkiye'de Gıda Güvenliği/Güvencesinin Hububat Sektörü Yönüyle Değerlendirilmesi. *Türkiye Biyoetik Dergisi*, *7*(3), 124-143. doi: 10.5505/tjob.2020.36449.

Huen, J., Börsmann, J., Matullat, I., Böhm, L., Stukenborg, F., Heitmann, M., Emanuele, Z., & Elle, K. A. (2018). Wheat flour quality evaluation from 'Baker's perspective: Comparative assessment of 18 analytical methods. *European Food Research and Technology*, *244*(3), 535–545. https://doi.org/10.1007/s00217-017-2974-3.

ICC, (2008). International Association for Cereal Science and Technology, Standart No.115/1, 116/1, 121, Vien.

JMP, (2003). JMP 5.0.1, A business unit of SAS, Cary, NC.

Kara, B., Acun, S., & Gül, H. (2020). Ekmeklik Buğdayda (Triticum aestivum L.) Tane İriliğinin Unda Bazı Kalite Özelliklere Etkisi. *Black Sea Journal of Agriculture*, *3*, 246-252.

Keçeli, A., & İkincikarakaya S. Ü. (2013). Bazı Ekmeklik Buğday (Triticum aestivum L.) Çeşitlerinde Farklı Ön Bitki Uygulamalarının Kalite Özellikleri Üzerine Etkileri. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, *22*(2), 41-58.

Kristensen, P. S., Jense, J., Andersen, J. R., Guzman, C., Orbabi, J., & Jahoor, A. (2019). Genomic prediction and genome-wide association studies of flour yield and allograph quality traits using advanced winter wheat breeding material, *Genes*, *10*, 669. https://doi.org/10.3390/genes10090669

Kün, E. (1996). *Tahıllar-I (Serin İklim Tahılları)*. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Yayın No: 1451, Ders kitabı 431, Ankara.

Malegori, C., Grassi, S., Ohm, J., Anderson, J., & Marti, A. (2018). GlutoPeak profile analysis for wheat classification: skipping the refinement

process. *Journal of Cereal Science*, *79*, 73-79. https://doi.org/10.1016/j.jcs.2017.09.005.

Marti, A., Augst, E., Cox, S., & Koehler, P. (2015). Correlations between gluten aggregation properties defined by the gluteal test and content of quality-related protein fractions of winter wheat flour. *Journal of Cereal Science*, *66*, 89–95. https://doi.org/10.1016/j.jcs.2015.10.010.

Melnyk, J. P., Dreisoerner, J., Bonomi, F., Marcone, M. F., & Seetharaman, K. (2011). Effect of the Hofmeister series on gluten aggregation measured using a high shear-based technique. *Food Research International*, *44*(4), 893-896. https://doi.org/ 10.1016/j.foodres.2011.01.053.

Menderis, M., Atlı, A., Köten, M., & Kılıç, H. (2008). Gluten Indeks Değeri ve Yaş Gluten/Protein Oranı ile Ekmeklik Buğday Kalite Değerlendirmesi. *Harran Üniversitesi Ziraat Fakültesi Dergisi*, *12*(3), 57-64.

Okur, Y. (2017). *Ekmeklik buğday kalitesini değerlendirmede kullanılan kimyasal ve fiziksel özelliklerin incelenmesi (Tez no 455717).* [Yüksek Lisans Tezi, Hacettepe Üniversitesi Fen Bilimleri Enstitüsü Kimya Mühendisliği Anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Özkaya, H., Özkaya, B. (2005). *Öğütme Teknolojisi,* Gıda Teknolojisi Derneği Yayınları. 30, 757s, Ankara.

Patel, M. J., & Chakrabarti-Bell, S. (2013). Flour quality and dough elasticity: Dough sheetability. *Journal of Food Engineering*. *115*, 371-383. https://doi.org/10.1016/j.jfoodeng.2012.10.038.

Pomeranz, Y. (1987). *Modern Cereal Science and Technology.* 486, VGH Publishers, Inc.

Posner, E. S. (2009). *Wheat Chemistry and Technology*, AACC International Inc. 466s, 119-152, USA.

Sharma, A., Garg, S., Sheikh, I., Vyas, P., & Dhaliwal, H. S. (2020). Effect of wheat grain protein composition on end-use quality. *Journal of Food Science and Technology*, *57*, 2771–2785. https://doi.org/10.1007/s13197-019-04222-6.

Şahin, M., Akçacık, A. G., Aydoğan, S., Demir, B., Güçbilmez, Ç. M., Hamzaoğlu, S., Gür, S., & Yıldırım, T. (2020). Ekmeklik buğday (Triticum aestivum) genotiplerinin gluten kalitesinin glutopik cihazı ile değerlendirilmesi. *Harran Tarım ve Gıda Bilimleri Dergisi 24* (2), 151-164.

Şahin, M., Akçacık, A. G., Aydoğan, S., Demir, B., Hamzaoğlu, S., Güçbilmez, Ç. M., Gür, S., & Yakışır, E. (2019). Kuru ve sulu şartlarda yetiştirilen ekmeklik buğday genotiplerinin farklı reolojik analiz cihazları ile kalite ve teknolojik özelliklerinin değerlendirilmesi. *Bahri Dağdaş Bitkisel Araştırma Dergisi 8*(2), 216-231.

Şahin, M., Akçacık, A. G., Aydoğan, S., Hamzaoğlu, S., Demir, B., & Yakışır, E. (2017). Kışlık ekmeklik buğday çeşitlerinde zeleny sedimantasyon ile verim ve bazı kalite özellikleri arasındaki ilişkilerin incelenmesi. *Bahri Dağdaş Bitkisel Araştırma Dergisi 6*(1), 10-21.

Tyagi, S., Mir, R. R., Balyan, H. S., & Gupta, P. K. (2015). Interval mapping and meta-qtl analysis of grain traits in common wheat (Triticum aestivum L.). *Euphytica*, *201*, 367-380. https://doi.org/ 10.1007/s10681-014-1217-y.

Wang, K., Dupuis, B., & Fu, B. X. (2017). Gluten aggregation behavior in high-shear- based GlutoPeak test: Impact of flour water absorption and strength. *Cereal Chemistry*, *94*(5), 909–915. https://doi.org/10.1094/cchem-05-17-0084-r.

Wang, J., Hou, G. G., Liu, T., Wang, N., & Bock, J. (2018). GlutoPeak method improvement for gluten aggregation measurement of whole wheat flour, *Food Science and Technology 90*, 8-14. https://doi.org/10.1016/j.lwt.2017.11.059.

Wiertz, J. (2018). GlutoPeak methods- RFC. In: Wiertz, J. (Ed.), Brabender® GmbH & Co. KG-sales seminar 2018 GlutoPeak methods- a quick overview, 7-13, Germany.

Wieser, H. (2007). Chemistry of gluten proteins. 3rd International symposium on sourdough, 24(2), 115–119.

Williams, P. C., El-Haramein, F. J., Nakkoul, H., & Riwhawi, S. (1986). Crop quality evaluation methods and guidelines. ICARDA, Aleppo, 142, Syria.

Williams, R. M., O'Brien, L., Eagles, H. A., Solah, V. A., & Jayasena, V. (2008). The influence of genotype, environment, and genotype x environment interaction on wheat quality. *Australian Journal of Agricultural Research 59* (2), 95-111. https://doi.org/10.1071/ar07185.

Zaweija, B., Makowska, A., Gutsche, M. (2020). Prediction of selected rheological characteristics of wheat based on gluteal test parameters. *Journal of Cereal Science 91*, 102898. https://doi.org/ 10.1016/j.jcs.2019.102898.

KSÜ Tarım ve Doğa Derg 27 (4), 940-948, 2024
KSU J. Agric Nat 27 (4), 940-948, 2024
DOI:10.18016/ksutarimdoga.1325976

Psyllium (*Plantago ovata*) Müsilaj Tozu ve Probiyotik *Saccharomyces Boulardii* İçeren Sodyum Kazeinat Bazlı Yenilebilir Biyoaktif Filmler

Huriye Gözde CEYLAN[1✉], Dilek ASLAN-KAYA[2], Ahmet Ferit ATASOY[3]
[1]Adıyaman Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Adıyaman, Türkiye, [2]Harran Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Şanlıurfa, Türkiye, [3]Harran Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Şanlıurfa, Türkiye
[1]https://orcid.org/ 0000-0001-7363-554X, [2]https://orcid.org/0009-0002-8960-1445, [3]https://orcid.org/ 0000-0002-3390-1177
✉: hgyildiz@adiyaman.edu.tr

ÖZET

Bu çalışmada, sodyum kazeinat esaslı *S. boulardii* ve farklı oranlarda psyllium müsilaj tozu (PMP; %0, %0.125, %0.25 ve %0.5 (a/h)) içeren biyoaktif filmlerin geliştirilmesi amaçlanmıştır. Elde edilen filmler kalınlık, nem içeriği, suda çözünürlük (SÇ), su buharı geçirgenliği (SBG), opaklık, renk ve kurutma sonrası probiyotik canlılığı açısından incelenmiştir. PMP ilavesinin filmlerin kalınlık, nem ve SBG değerleri üzerindeki etkisi önemli ($p<0.05$) bulunmuştur. %0.5 PMP içeren filmlerin kalınlık, nem ve SBG değerlerinde önemli bir artış tespit edilmiştir. PMP konsantrasyonunun film opaklığı üzerindeki etkisi önemli ($p>0.05$) bulunmamıştır. Ancak, PMP konsantrasyonunun renk değerleri üzerindeki etkisi önemli ($p<0.05$) bulunmuştur. PMP ilavesi, probiyotiğin kuruma stabilitesini önemli ölçüde artırmıştır ($p<0.05$) ve en yüksek canlılık oranı %0.25 PMP içeren örnekte tespit edilmiştir. Çalışma sonuçlarımız, psyllium müsilajı içeren sodyum kazeinat filmlerin probiotik *S. boulardii* için taşıyıcı olarak umut verici bir potansiyele sahip olduğunu desteklemektedir.

Gıda Bilimi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 11.07.2023
Kabul Tarihi : 28.11.2023

Anahtar Kelimeler
Psyllium
Müsilaj
Probiyotik maya
S. boulardii
Biyoaktif yenilebilir filmler

Sodium Caseinate-Based Edible Bioactive Films With Psyllium (*Plantago ovata*) Mucilage Powder And Probiotic *Saccharomyces boulardii*

ABSTRACT

This study aimed to develop sodium caseinate-based films incorporating *S. boulardii* and different amounts of psyllium mucilage powder (PMP; 0%, 0.125%, 0.25%, and 0.5% (w/v)). The obtained films were characterized for thickness, moisture content (MC), water solubility (WS), water vapor permeability (WVP), opacity, color, and probiotic viability after the drying process. The addition of PMP had a significant effect ($p<0.05$) on the thickness, MC, and WVP values of the films. The incorporation of 0.5% PMP led to a significant increase in the thickness, MC, and WVP values of films. While the addition of PMP did not result in a statistically significant impact on film opacity ($p>0.05$), it did have a significant effect on color values ($p<0.05$). The incorporation of PMP significantly ($p<0.05$) increased the drying stability of the probiotic, with the highest viability observed in the sample containing 0.25% PMP. Our study results support the promising potential of sodium caseinate films incorporating psyllium mucilage as carriers for the probiotic *S. boulardii*.

Food Science

Research Article

Article History
Received : 11.07.2023
Accepted : 28.11.2023

Keywords
Psyllium
Mucilage
Probiotic yeast
S. boulardii
Bioactive edible films

Atıf İçin : Ceylan, H. G., Aslan-Kaya, D., & Atasoy, A. F. (2024). Psyllium (*Plantago Ovata*) Müsilaj Tozu ve Probiyotik *Saccharomyces Boulardii* İçeren Sodyum Kazeinat Bazlı Yenilebilir Biyoaktif Filmler. *KSÜ Tarım ve Doğa Dergisi, 27*(4), 940-948. DOI:10.18016/ksutarimdoga.1325976

To Cite: Ceylan, H. G., Aslan-Kaya, D., & Atasoy, A. F. (2024). Sodium Caseinate-Based Edible Bioactive Films With Psyllium (*Plantago Ovata*) Mucilage Powder And Probiotic *Saccharomyces Boulardii*. *KSU Journal of Agriculture and Nature, 27*(4), 940-948. https://doi.org/10.18016/ksutarimdoga.1325976

INTRODUCTION

Probiotic is a term used to define "live microorganisms that provide health benefits to the host when consumed in adequate amounts." (FAO/WHO, 2006). However, food products containing probiotics face several limiting factors during their production

process, including the structure of the food, temperature, osmotic and mechanical stress, and acid-base changes. Additionally, stress factors such as lack of nutrients, oxygen and temperature requirements, pH, and competitive microorganisms restrict the viability of probiotics in the final product (Zoghi et al., 2020). Therefore, various methods have been developed to preserve the biological activities of probiotics during food processing and storage stages (Espitia et al., 2016). One recent advancement in this field is the use of edible films as potential carriers for probiotics (Ceylan & Atasoy, 2023).

Lactobacillus and *Bifidobacterium* species are commercially widely used probiotics. *Saccharomyces boulardii* is the only yeast with probiotic properties in the market due to its bio-therapeutic effects (Khodaei et al., 2020; Goktas et al., 2022). The therapeutic effects of *S. boulardii* strains are increasing the demand for products containing *S. boulardii* in the probiotic market (Goktas et al., 2022). Although *S. boulardii* has been the subject of much research (Menezes et al., 2018; Santana et al., 2020) in recent years, there are limited studies (Khodaei et al., 2020; de Oliveira Filho et al., 2023) on the use of this yeast in edible packaging formulations containing probiotics.

The health issues and advancements in research are encouraging researchers to explore and evaluate new prebiotics that have the potential to contribute to human health. Psyllium (*Plantago ovata*) is a widely used prebiotic. Its seeds are recognized as a powerful dietary fiber that improves intestinal performance. Psyllium seeds contain a high proportion of various monosaccharides, including arabinose and xylose. Psyllium seeds are rich in bioactive compounds including minerals such as potassium, sodium and phosphorus, fatty acids, amino acids, polyphenols, and flavonoids. For all these reasons, the importance of products containing fibers obtained from psyllium as a prebiotic and probiotics is becoming increasingly important (Martellet et al., 2022; Martellet et al., 2023). In recent years, psyllium seed mucilage has been the subject of some research as a new sustainable film source due to its low cost, biodegradability, and gel-forming properties (Hajivand et al., 2020; Halász et al., 2022).

There are limited studies on the use of plant seed mucilages in edible packaging formulations containing probiotics (Davachi et al., 2021; Rodrigues et al., 2018; Semwal et al., 2022). In these studies, it has been reported that plant seed mucilages increase probiotic viability in edible films and coatings (Davachi et al., 2021; Semwal et al., 2022). However, no study could be found on the use of psyllium (*Plantago ovata*) mucilage in edible films with probiotics. For these reasons, the objective of this study was to develop films containing *S. boulardii* with different amounts of psyllium seed mucilage. The study aimed to investigate the physicochemical and optical properties of the films, as well as the viability of probiotics after the drying process.

MATERIALS and METHOD

Materials

Sodium caseinate and Psyllium seed were purchased from Kimbiotek A.Ş. (Türkiye, Istanbul) and TOS Grup (Türkiye, Antalya), respectively. *S. boulardi* was isolated from Reflor (lyophilized powder; Biocodex, Gentilly, France). Sodium chloride, glycerol phosphate, and Dichloran Rose Bengal agar (DRBC) were purchased from Merck (Darmstadt, Germany) and YPD broth was obtained from Condalab (Madrid, Spain).

Obtaining psyllium mucilage powder (PMP)

Psyllium seeds were diluted 1:10 and mixed with a magnetic stirrer for 2 h at room temperature. Then, to increase the mucilage yield, it was transferred to 50 mL centrifuge tubes and homogenized in a sonicator (WiseStir, HS-30D, Korea) at 75% power for 2 min. It was then centrifuged at 7471 for 40 min. at 4 °C. The separated supernatant (water) was decanted and the mucilage in the middle layer was separated. The remaining seeds were rehydrated and the same procedures were repeated 2 times. The mucilages were frozen at -20 °C for 72 h and then dried in a lyophilizer. The resulting dry mucilage was homogenized with a grinder and kept at -20 °C until film production.

Obtaining *S. boulardii* pellets

S. boulardii cells were activated in YPD broth (Yeast extract, peptone, dextrose) by modifying the method proposed by Goktas et al. (2022). For this, a capsule containing *S. boulardii* was transferred to 10 mL of YPB broth and incubated at 30°C for 24 h. Then, 3ml of culture was transferred to 150ml of YPD broth and incubated at 30°C for 48 h. At the end of the incubation, the culture was centrifuged in 50 mL tubes at 4500 rpm at 4 °C for 10 min and the pellet was twice swashed with sterile physiological saline (0.85%, w v^{-1}).

Film production

Film production was carried out according to the steps in Fig. 1 based on the method suggested by Ceylan & Atasoy (2022a). In total four film-forming solutions were manufactured which were; control (P-0, without addition of PMP), film containing 0.125% PMP (P-0.125), film containing 0.25% PMP (P-0.250), and film containing 0.5% PMP (P-0.500). Pellets of the harvested cells (corresponding to 150 mL of culture) were added to the film-forming solution to reach a concentration of 10^8 cfu mL^{-1}. Aseptically, 8.5 mL of solution was transferred into sterile Petri dishes (inner

diameter 8.5 cm) and they dried at room conditions for 16 h in a laminar flow cabinet at room temperature. The dry films were carefully removed from the Petri dishes and stored in sterile zippered bags at 4 °C until probiotic cell count.

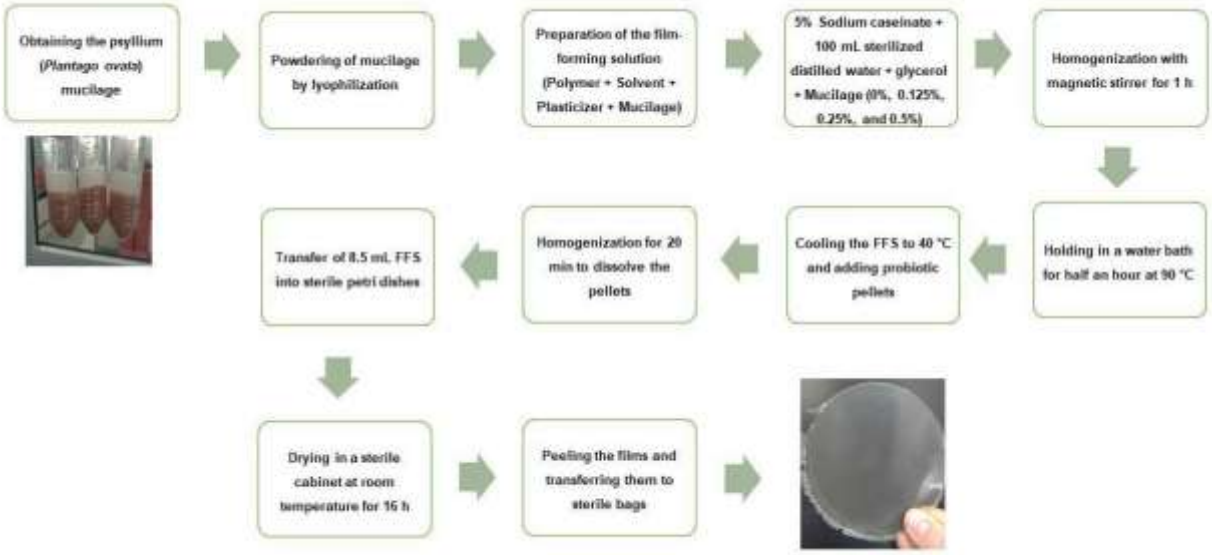


Figure 1. Flow chart of edible bioactive film production
Şekil 1. Yenilebilir biyoaktif film üretimi akış şeması

Determination of probiotic viability after drying process

The probiotics viability in films after the drying process was based on a method proposed by Khodaei et al. (2020) with modifications. One mL of film-forming solution was suspended in sterile 9 mL of NaCI (0.85%, w v^{-1}) and then serial dilutions were prepared. One gram of film samples was dissolved in sterile 99 mL of NACI at 37 °C and then serial dilutions were prepared. The number of colonies was counted on DRBC agar for yeast cells (incubation at 30 °C for 48 h). Additionally, the stability of probiotics in films after the drying process was calculated. The viability of the probiotics was calculated following the drying procedure of the film-forming solution using Eq. 1.

$$Viability\ (\%) = 100 \times N/N_0 \quad (1)$$

where N and N_0 represent the number of viable cells in the film and the film solution, respectively.

Physicochemical analysis of films

The thickness, moisture content (MC, %), and water solubility (WS, %) values of the film samples were determined according to the method described by Ceylan & Atasoy (2022a). The thickness of the films was measured from at least 6 points randomly with a digital micrometer with the precision of 0.001 mm and the average film thickness was calculated. For MC and WS analysis, the films cut in 45 mm * 10 mm size were dried at 105 °C for 12 h. The moisture content was calculated using the percent weight loss after drying. To determine the WS value, the dry film was stirred in distilled water at room temperature for 3 min. And then the film was dried at 105 °C. The final weight of film was measured and the WS was calculated as a percentage based on the initial weight.

Water vapor permeability

For the water vapor permeability (WVP) of the films, silica gel dried at 105 °C was transferred into special containers and the mouth of the container was sealed with a film. Then, the samples were placed at 25 °C in a desiccator containing distilled water, and the weight changes were recorded hourly for 8 h. WVP values of films were calculated according to the ASTM E 96 (1995) procedure.

Opacity and color analysis of films

L^*, a^*, and b^* values were determined in the films placed on white paper based on the CIE Lab color measurement system by colorimeter (Minolta CR-400, Japan). Also, the ΔE value of films was calculated using Eq. 2 (Khodaei et al., 2020). As a standard, the L*, a*, and b* values on the surface of the white paper were 90.04, 1.05, and -1.29, respectively.

$$\Delta E = \sqrt{(L^*_f - L^*_s)^2 + (a^*_f - a_s)^2 + (b^*_f - b^*_s)^2} \quad (2)$$

Opacity analysis was performed with a UV/Visible spectrophotometer (Biochrom Libra, S60, Cambridge, UK) according to the method specified by Al-Hassan &

Norziah (2012). The opacity value was calculated by dividing absorbance at 600 nm wavelength by thickness (mm).

Statistical analysis

The obtained data were subjected to a one-way analysis of variance (ANOVA) using SPSS software (IBM Corp, Armonk, NY, USA). Differences between samples were determined using the Duncan multiple comparison test at a 95% confidence level. Edible bioactive film samples containing different levels of PMP were compared in terms of tested properties. The experiments were conducted in triplicate. The results were presented as mean ± standard deviation.

RESULTS and DISCUSSION

Physical properties of edible bioactive films

Fig. 2 presents the thicknesses, moisture content, and water solubility values of the bioactive edible films containing different concentrations of PMP. Thickness is one of the most important parameters affecting the optical, mechanical, and barrier properties of films (Ceylan & Atasoy, 2022b). The thickness range of the edible films was 0.068–0.094 mm. It was observed that the thickness of the edible films increased with the addition of PMP. The thickness value of the P-0.050 film was found to be significantly higher ($p<0.05$) than the control film. This was probably due to the amount of solid materials in the film-forming solution (Daei et al., 2022). Film thickness can be affected by the interaction between the ingredients as well as the content and amounts of the components in the film formulation (Zhang et al., 2020). Similar observations were reported by different researchers (Krystyjan et al., 2017; Daei et al., 2022) for edible films containing PMP.

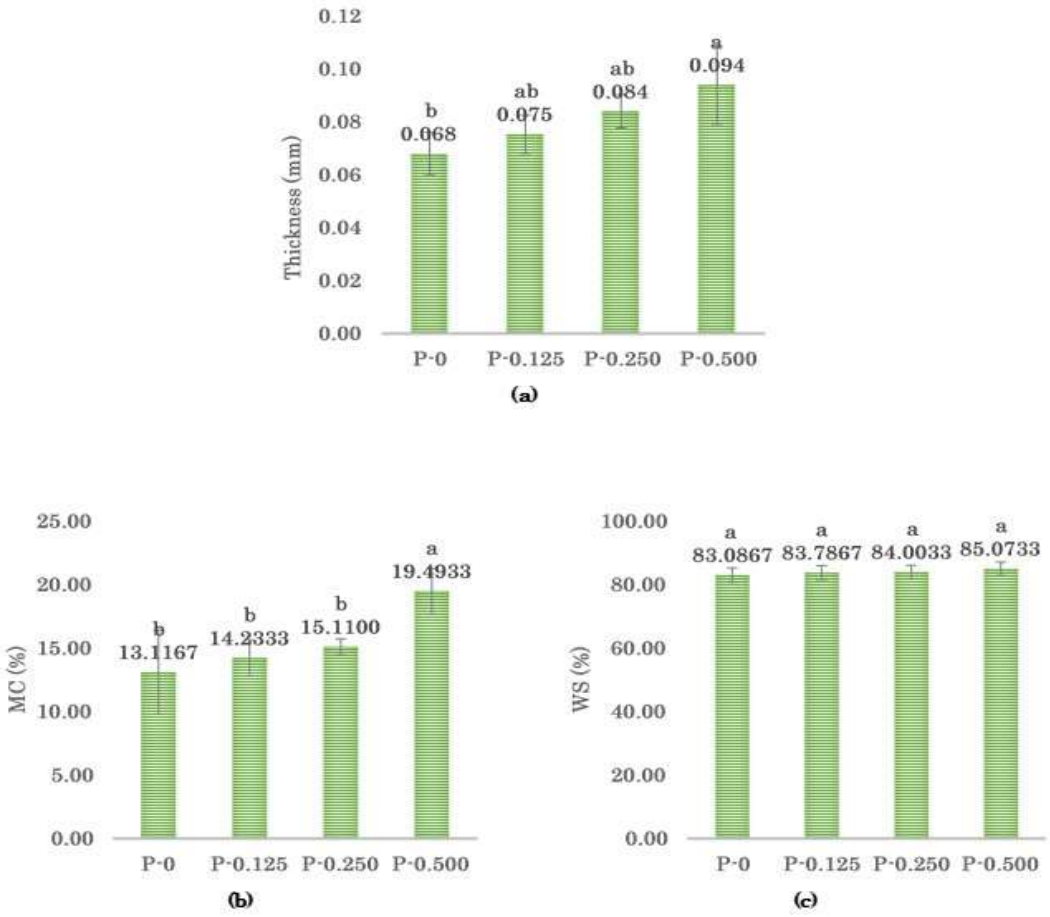


Figure 2. Physical properties of edible bioactive films

Şekil 2. Yenilebilir biyoaktif filmlerin fizikokimyasal özellikleri

Results are the as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences ($p<0.05$) between samples. MC, moisture content; WS, water solubility, P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

The melting of edible films is affected by factors such as moisture content (MC) and water solubility (WS) (Todhanakasem et al., 2022). In addition, the MC of the films after drying is of critical importance, as it affects the viability of probiotics in films (Davachi et al., 2021). As seen in Fig. 2, the MC value of films increased with the increase of the PMP content. The MC value of pure sodium caseinate film was the lowest (13.12%), while the MC value of the film containing 0.5% PMP was the highest (19.49%). However, the MC values of the films containing 0.125% and 0.25% PMP were not significantly different ($p>0.05$) from the control film. The addition of PMP causes an increase in the number of available active sites for water binding due to the hydrophilic nature of the mucilage (Badreddine et al., 2022). This can result in edible films becoming more hydrophilic and an increase in MC. Zhang et al. (2020) reported that the MC value increased depending on the PMP content in films containing different ratios of whey protein isolate and PMP.

Water solubility (WC) is a property that plays a significant role in determining the applicability of edible films in both the food and pharmaceutical industries. The solubility in water is influenced by the chemical composition of the films and serves as an indicator of their stability when exposed to water (Ceylan & Atasoy, 2022b). Furthermore, the solubility of probiotic films influences the release of probiotic cells, and higher WS leads to increased cell release (Kalantarmahdavi et al., 2021). The solubility of bioactive films ranged from 83.09% to 85.07% (Fig. 2). Semwal et al. (2022) reported the WS of probiotic films containing sodium caseinate and chia mucilage in the range of 35% and 92%. In another study (Krystyjan et al., 2017), the WS values of the films containing different ratios of psyllium mucilage and starch were found to be between 16.76% and 22.85%. The differences can be related to the polymers and their concentrations in the film formulation, as well as the differences in film preparation methods. In addition, the high solubility of bioactive films in this study may be attributed to the utilization of sonication during the production of psyllium mucilage. Sonication has the potential to decrease the molecular weight and increase the polydispersity to some extent, thereby enhancing solubility (Halász et al., 2022). When the film or coating is utilized as an edible covering and consumed along with the food, having a high level of water solubility can prove to be beneficial (Halász et al., 2022). While the WS values of the films increased with the increasing PMP content, the WS values of the films did not differ significantly ($p>0.05$). Previous studies (Krystyjan et al., 2017; Zhang et al., 2020) showed that psyllium mucilage added to edible films made from different polymers increased the water solubility of the films.

Water vapor permeability of edible bioactive films

The water vapor barrier (WVP) property of edible films is crucial for preserving food quality due to the significant role of water in food deterioration (Badreddine et al., 2022). Fig. 3 shows the WVP values found for films containing different concentrations of PMP. Bioactive films have a low permeability and WVP values were found in the range of 0.43 to 0.56 g mm m^{-2} h^{-1} kPa^{-1}. Zhang et al. (2020) reported the WVP values of films containing different ratios of WPI/psyllium mucilage in the range of 1.27 to 2.43 g m^{-1} s^{-1} Pa^{-1}. A low permeability implies resistance against water vapor interactions and hindered passage due to structural uniformity (Araújo et al. 2018). As the concentration of psyllium in the formulation increased, the water vapor permeability (WVP) generally increased. Films containing 0.5% (P-0.500 sample) psyllium had significantly higher ($p<0.05$) WVP values compared to the control film. However, films containing 0.125% and 0.25% psyllium had WVP values similar ($p>0.05$) to the control film. Water vapor transfer generally occurs along the hydrophilic portion of the film, and therefore, it varies depending on the hydrophilic-hydrophobic ratio of the film components (Rojas-Graü et al., 2006). Furthermore, the water vapor permeability (WVP) value of edible films is significantly influenced by factors such as film thickness, moisture content, biopolymer structure, and extraction method (Halász et al., 2022). Considering the influence of thickness and moisture on permeability, the thickness and MC values of the films containing 0.5% psyllium were found to be consistent with the WVP results.

Optical properties of edible bioactive films

Optical properties are crucial for edible films and food packaging applications, as they not only affect consumer preferences but also the quality of products (Davachi et al., 2021). The measured opacity values of the films ranged from 2.011 to 2.416. The opacity values of the prepared bioactive films were found to be lower than low-density polyethylene (LDPE, 4.264) films commonly used as commercial packaging materials and higher than oriented polypropylene (OPP, 1.566) films (Guerrero et al., 2011). Halász et al. (2022) determined the opacity values of pure psyllium mucilage films containing different proportions of glycerol in the range of 2.45-3.95. In another study (Krystyjan et al., 2017), the transparency values of starch-based films supplemented with psyllium mucilage ranged from 0.81 to 2.42. There was no significant difference in opacity among the films with different concentrations of PMP ($p>0.05$). This can be interpreted as the homogeneous distribution of PMP within the film matrix and its compatibility with the components present in the formulation. The light transmittance of films is dependent on the

microstructural properties of the film and the homogeneous distribution of the components comprising the film. Weak compatibility among the components in the film matrix increases opacity by causing scattering or reflection of light at the interfaces (Zhang et al., 2020). Contrary to our findings, Krystyjan et al. (2017) reported that the addition of psyllium mucilage to starch-based films resulted in a more transparent appearance. Zhang et al. (2020) found that the light transmittance of films prepared with different ratios of WPI/psyllium mucilage increases depending on the mucilage content.

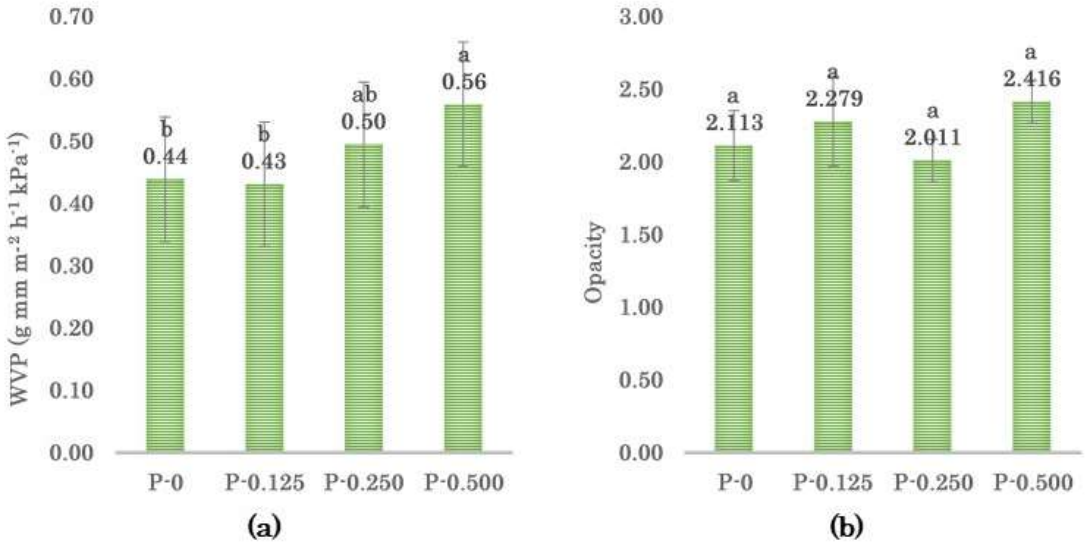


Figure 3. Water vapor permeability (a) and light barrier properties (b) of edible bioactive films
Şekil 3. Yenilebilir biyoaktif filmlerin su buharı geçirgenliği (a) ve ışık bariyer özellikleri (b)

Results are the same as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences ($p<0.05$) between samples. WVP, water vapor permeability, P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

The L*, a*, b*, and ΔE values of the bioactive films are shown in Table 1. The L*, a*, b*, and ΔE values were significantly ($p<0.05$) affected by the PMP concentration in the formulation. The film containing 0.5% PMP was found to have significantly ($p<0.05$) lower brightness compared to the control film. The a* values of the films decreased with increasing PMP content, while the b* values decreased. The highest ΔE values were observed in the films containing 0.25% and 0.500% PMP. The changes in the color values of the bioactive films indicate that the addition of PMP results in a shift toward darkness, greenness, and yellowness in the film. In contrast to our findings, Zhang et al. (2020) reported that the mucilage in WPI/psyllium mucilage-based films led to an increase in the *L* value and a decrease in the *a*, *b*, and ΔE values of the films. The reason for this discrepancy could be the variety and concentration of materials included in the formulation, as well as the film production technique.

Table 1. Colour characteristics of edible bioactive films
Çizelge 1. Yenilebilir biyoaktif filmlerin renk karakteristikleri

Film	L*	a*	b*	ΔE
P-0	88.10±0.20^{a}	0.40±0.09^{a}	2.01±0.52^{b}	3.88±0.56^{c}
P-0.125	87.62±0.54^{ab}	0.20±0.09^{b}	2.09±0.25^{b}	4.27±0.11^{cb}
P-0.250	87.70±0.06^{ab}	0.01±0.04^{c}	3.00±0.31^{a}	5.00±0.29^{a}
P-0.500	87.42±0.36^{b}	0.03±0.10^{c}	3.46±0.41^{a}	4.82±0.26^{ab}

Results are the same as mean ± standard deviation (n=3). Different lowercase letters in the same column indicate statistically significant differences ($p<0.05$) between samples. P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

Probiotic viability after drying of edible bioactive films

Figure 4 demonstrates the probiotic viability after the drying process in films containing different concentrations of PMP. There was a decrease in the viable cell count during the drying stage of the film-forming solutions. Khodaei et al. (2020) reported that the number of *S. boulardii* cells decreased during the drying of film-forming solutions in gelatin and low methoxyl pectin-based films. Similarly, de Oliveira

Filho et al. (2023) reported that drying of the filmogenic solution resulted in a reduction in the number of probiotics in films containing alginate, mangaba pulp, and *S. boulardii*.

The probiotic viability in the films after drying was found to be between 76.61% and 94.87%. The drying process did not have an acute toxic effect on the viability of probiotic cells. Semwal et al. (2022) reported that the drying process in films supplemented with chia mucilage based on sodium caseinate had no toxic effect on probiotic cells. This result may be associated with the moderate temperature application during drying, allowing the removal of water from probiotic cells without subjecting them to any significant thermal stress (Semwal et al., 2022). Additionally, the presence of sodium caseinate in bioactive film formulations may have contributed to the survival of probiotic cells under osmotic stress during drying. In protein-based films, proteins play a role in preserving cell membrane integrity by capturing free radicals and providing micronutrients (Semwal et al., 2022).

The effect of PMP on the drying stability of the probiotic was found to be significant ($p<0.05$). The film containing 0.25% PMP exhibited the highest probiotic viability, while the control film showed the lowest probiotic viability. The cell-protective effect of the mucilage during the drying process may be attributed to its chemical composition. Psyllium seeds are considered a notable source of prebiotics due to their composition, which includes various monosaccharides like arabinose and xylose (Martellet et al., 2022). These polysaccharides play a protective role in the survival of probiotics by interacting with cell membrane phospholipids (Semwal et al., 2022). Similarly, Semwal et al. (2022) discovered that incorporating chia mucilage based on sodium caseinate in probiotic films influenced the inactivation rates of *L. fermentum* and *L. brevis*.

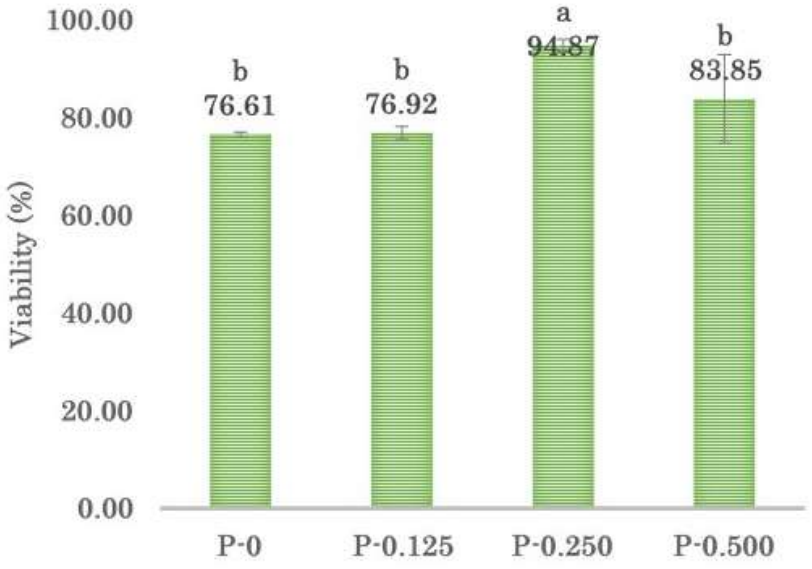


Figure 4. Viability of probiotics after the drying process
Şekil 4. Kuruma prosesi sonrası probiyotik canlılığı
Results are the same as mean ± standard deviation (n=3). Different lowercase letters indicate statistically significant differences ($p<0.05$) between samples. P-0: control, P-0.125: film containing 0.125% PMP, P-0.250: film containing 0.25% PMP, P-0.500: film containing 0.5% PMP.

CONCLUSION

In this study, bioactive edible films on sodium caseinate-based incorporating *S. boulardii* and different amounts of psyllium mucilage powder (PMP; 0%, 0.125%, 0.25%, and 0.5%) were developed. The physicochemical, barrier, and color properties of the obtained films, as well as the viability of probiotic cells after the drying process, were investigated. The effect of film formulations was significant on the tested properties of the films, except for opacity and water solubility. The highest thickness, moisture content, and water vapor permeability were observed in the samples containing 0.5% PMP. Furthermore, it was found that PMP led to the formation of darker, greener, and more yellowish films. During the drying process, the film formulations did not have a toxic effect on *S. boulardii*. Additionally, PMP exhibited a cell-protective effect during the drying process. The highest viability after drying was observed in the film containing 0.25%.

The obtained results demonstrate that the tested film formulations were suitable carriers for *S. boulardii* cells, and the addition of PMP to sodium caseinate films enhances the film properties and drying stability of the probiotic. Our plans regarding the development of bioactive films containing psyllium mucilage and probiotics include the utilization of other probiotic strains, examination of mechanical, SEM, and FTIR analyses, as well as the investigation of probiotic viability during storage. Furthermore, it is recommended that future studies systematically investigate the applicability of the developed film formulations for coating various food materials, thereby expanding our understanding of their potential in food preservation and packaging. The current findings demonstrate that the tested formulations serve as suitable carriers for *S. boulardii* and that PMP enhances the film properties and drying stability of the probiotic. Therefore, bioactive films incorporating psyllium mucilage and *S. boulardii* could present a novel alternative for the probiotic food market.

Author's Contributions

Dr. Ceylan designed the research plan, conducted laboratory experiments, performed statistical analyses, and wrote this article. Mrs. Aslan Kaya carried out the laboratory experiments. Prof. Dr. Atasoy conceptualized the study, provided financial support, and performed the writing-editing of the manuscript. All authors read and approved the final manuscript.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Al-Hassan, A.A., & Norziah, M.H. (2012). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids, 26,* 108e117.

Araújo, A., Galvão, A., Silva Filho, C., Mendes, F., Oliveira, M., Barbosa, F., ... & Bastos, M. (2018). Okra mucilage and corn starch bio-based film to be applied in food. *Polymer Testing, 71,* 352-361.

ASTM, (1995). Standard test methods for water vapor transmission of materials. *American Society for Testing and Materials*, E 96/E 96M.

Badreddine, M., Abdellah, R., Laid, G., & Ali, R. (2022). Development and characterization of edible biofilms based on mucilage of Opuntia ficus-indica and Locust Bean Gum from Tissemsilt region in Algeria. *South Asian Journal of Experimental Biology, 12*(1), 117-127.

Ceylan, H.G., & Atasoy, A.F. (2022a). Optimization and characterization of prebiotic concentration of edible films containing *Bifidobacterium animalis* subsp. *lactic* BB-12® and its application to block-type processed cheese. *International Dairy Journal, 134,* 105443.

Ceylan, H.G., & Atasoy, A.F. (2022b). Characterization of edible films containing *Lactobacillus rhamnosus* GG and *Bifidobacterium bifidum* BB-12. *Adıyaman University Journal of Engineering Sciences, 9*(16), 194-203.

Ceylan, H.G., & Atasoy, A.F. (2023). New Bioactive Edible Packing Systems: Synbiotic Edible Films/Coatings as Carries of Probiotics and Prebiotics, *Food and Bioprocess Technology, 1*(16), 1413–1428.

Daei, S., Mohtarami, F., & Pirsa, S. (2022). A biodegradable film based on carrageenan gum/Plantago psyllium mucilage/red beet extract: physicochemical properties, biodegradability and water absorption kinetic. *Polymer Bulletin, 79*(12), 11317-11338.

Davachi, S.M., Pottackal, N., Torabi, H., & Abbaspourrad, A. (2021). Development and characterization of probiotic mucilage-based edible films for the preservation of fruits and vegetables, *Scientific Reports, 11*(1), 16608.

de Oliveira Filho, J.G., de Sousa, T.L., Bertolo, M.R.V., Junior, S.B., Mattoso, L.H.C., Pimentel, T.C., & Egea, M.B. (2023). Next-generation food packaging: Edible bioactive films with alginate, mangaba pulp (*Hancornia speciosa*), and *Saccharomyces boulardii*. *Food Bioscience,* 102799.

Espitia, P.J.P., Batista, R.A., Azeredo, H.M.C., & Otoni, C.G. (2016). Probiotics and their potential applications in active edible films and coatings, *Food Research International, 90,* 42-52.

FAO/WHO, 2006. *Probiotics in food: Health and nutritional properties and guidelines for evaluation*. Food and Nutrition Paper 85. Food and Agriculture Organization of the United Nations/World Health Organization. Rome.

Goktas, H., Dikmen, H., Bekiroglu, H., Cebi, N., Dertli, E., & Sagdic, O. (2022). Characteristics of functional ice cream produced with probiotic *Saccharomyces boulardii* in combination with *Lactobacillus rhamnosus* GG. *LWT – Food Science and Technology, 153*, 112489.

Guerrero, P., Hanani, Z.N., Kerry, J.P., & De La Caba, K. (2011). Characterization of soy protein-based films prepared with acids and oils by compression. *Journal of Food Engineering, 107*(1), 41-49.

Hajivand, P., Aryanejad, S., Akbari, I., & Hemmati, A. (2020). Fabrication and characterization of a promising oregano-extract/psyllium-seed mucilage edible film for food packaging, *Journal of Food Science, 85*(8), 2481-2490.

Halász, K., Tóth, A., Börcsök, Z., & Preklet, E. (2022). Edible, antioxidant films from ultrasonically extracted Plantago psyllium seed husk flour mucilage, *Journal of Polymers and the*

Environment, 30(7), 2685-2694.

Kalantarmahdavi, M., Khanzadi, S., & Salari, A. (2021). Edible films incorporating *Lactobacillus plantarum* based on sourdough, wheat flour, and gelatin: films characterization and cell viability during storage and simulated gastrointestinal condition. *Starch-Stärke, 73*(9-10), 2000268.

Khodaei, D., Hamidi-Esfahani, Z., & Lacroix, M. (2020). Gelatin and low methoxyl pectin films containing probiotics: Film characterization and cell viability. *Food Bioscience, 36,* 100660.

Krystyjan, M., Khachatryan, G., Ciesielski, W., Buksa, K., & Sikora, M. (2017). Preparation and characteristics of mechanical and functional properties of starch/Plantago psyllium seeds mucilage films. *Starch-Stärke, 69*(11-12), 1700014.

Martellet, M.C., Majolo, F., Ducati, R.G., de Souza, C.F.V., & Goettert, M.I. (2022). Probiotic applications associated with Psyllium fiber as prebiotics geared to a healthy intestinal microbiota: A review, *Nutrition,* 111772.

Martellet, M.C., Majolo, F., Cima, L., Goettert, M.I., & de Souza, C.F.V. (2023). Microencapsulation of Kluyveromyces marxianus and Plantago ovata in cheese whey particles: Protection of sensitive cells to simulated gastrointestinal conditions, *Food Bioscience, 52,* 102474.

Menezes, A.G.T., Ramos, C.L., Dias, D.R., & Schwan, R.F. (2018). Combination of probiotic yeast and lactic acid bacteria as starter culture to produce maize-based beverages. *Food Research International, 111*, 187-197.

Rodrigues, F.J., Cedran, M.F., & Garcia, S. (2018). Influence of linseed mucilage incorporated into an alginate-base edible coating containing probiotic bacteria on shelf-life of fresh-cut yacon (*Smallanthus sonchifolius*), Food and Bioprocess Technology, 11(8), 1605–1614.

Rojas-Graü, M.A., Avena-Bustillos, R.J., Friedman, M., Henika, P.R., Martín-Belloso, O., & McHugh, T.H. (2006). Mechanical, barrier, and antimicrobial properties of apple puree edible films containing plant essential oils. *Journal of Agricultural and Food Chemistry, 54*(24), 9262-9267.

Semwal, A., Ambatipudi, K., & Navani, N.K. (2022). Development and characterization of sodium caseinate based probiotic edible film with chia mucilage as a protectant for the safe delivery of probiotics in functional bakery, *Food Hydrocolloids for Health, 2,* 100065.

Santana, R.V., dos Santos, D.C., Santana, A.C.A., de Oliveira Filho, J.G., de Almeida, A.B., de Lima, T.M., ... & Egea, M.B. (2020). Quality parameters and sensorial profile of clarified "Cerrado" cashew juice supplemented with *Saccharomyces boulardii* and different sweeteners. *LWT – Food Science and Technology, 128,* 109319.

Todhanakasem, T., Boonchuai, P., Itsarangkoon Na Ayutthaya, P., Suwapanich, R., Hararak, B., Wu, B., & Young, B.M. (2022). Development of bioactive Opuntia ficus-indica edible films containing probiotics as a coating for fresh-cut fruit. *Polymers, 14*(22), 5018.

Zhang, X., Zhao, Y., Li, Y., Zhu, L., Fang, Z., & Shi, Q. (2020). Physicochemical, mechanical, and structural properties of composite edible films based on whey protein isolate/psyllium seed gum. *International Journal of Biological Macromolecules, 153,* 892-901.

Zoghi, A. Khosravi-Darani, K. Mohammadi, R. 2020. Application of edible films containing probiotics in food products, *Journal of Consumer Protection and Food Safety, 15*(4), 307-320.

KSÜ Tarım ve Doğa Derg 27 (4), 949-957, 2024
KSU J. Agric Nat 27 (4), 949-957, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1331388

Determination of Phenolic Contents and Antioxidant Activities in Different Solvents of Hatila Valley and Macahel Bee Products in Artvin, Turkey

Tuğba Nigar BOZKUŞ[1]✍
[1]Laboratory Technology Program, Artvin Vocational School, Artvin Coruh University, Artvin, Türkiye
[1]https://orcid.org/0000-0001-9613-6911
✉: tubisco@hotmail.com

ABSTRACT

This study determines the extracts of bee products prepared with different solvents in terms of phenolic content and antioxidant activity of the Hatila Valley and Macahel region of Artvin, Turkey. For this purpose, water and ethanol extracts of royal jelly and honey; water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol extracts of pollen and propolis have been analyzed by total polyphenol and flavonoid content and ferric-reducing power. The results of the analysis indicated that Macahel honey and propolis had higher phenolic content and antioxidant activity than Hatila honey and propolis. The propolis of both regions, whose mixtures were prepared, was determined as Macahel propolis > Hatila + Macahel propolis > Hatila propolis in terms of polyphenol and flavonoid content and ferric reducing power. Although propolis samples with 70% ethanol and 70% propylene glycol had higher solubility, the lowest solubility was in water. These differences vary depending on the geographical location, botanical variations of the region where the bee products are collected, and the solvent used for the extraction.

Food Science

Research Article

Article History
Received : 24.07.2023
Accepted : 03.12.2023

Keywords
Artvin
Bee products
Phenolic content
Antioxidant activity

Türkiye Artvin İli Hatila Vadisi ve Macahel Arı Ürünlerinin Farklı Çözücülerdeki Fenolik İçeriklerinin ve Antioksidan Aktivitelerinin Belirlenmesi

ÖZET

Bu çalışma, Türkiye'nin Artvin ili Hatila Vadisi ve Macahel yöresinin farklı çözücülerle hazırlanan arı ürünlerinin ekstraktlarını fenolik içerik ve antioksidan aktivite yönünden belirlemektedir. Bu amaçla arı sütü ve balın su ve etanol ekstraktları; polen ve propolisin su, etanol, %70 etanol, propilen glikol ve %70 propilen glikol ekstraktları, toplam polifenol ve flavonoid içerik ve demir indirgeyici güç açısından analiz edilmiştir. Analiz sonuçları Macahel balı ve propolisinin Hatila balı ve propolisine göre daha yüksek fenolik içeriğe ve antioksidan aktiviteye sahip olduğunu göstermiştir. Karışımları da hazırlanan her iki yörenin propolisi polifenol ve flavonoid içerik ve demir indirgeyici güç bakımından Macahel propolisi > Hatila + Macahel propolisi > Hatila propolisi olarak belirlenmiştir. %70 etanol ve %70 propilen glikollü propolis örnekleri daha yüksek çözünürlüğe sahip olmakla birlikte en düşük çözünürlük suda olmuştur. Bu farklılıklar, arı ürünlerinin toplandığı bölgenin coğrafi konumu, botanik çeşitliliği ve ekstraksiyon için kullanılan çözücüye bağlı olarak değişmektedir.

Gıda Bilimi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 24.07.2023
Kabul Tarihi : 03.12.2023

Anahtar Kelimeler
Artvin
Arı ürünleri
Fenolik içerik
Antioksidan aktivite

To Cite : Bozkuş, T.N., (2024). Determination of Phenolic Contents and Antioxidant Activities in Different Solvents of Hatila Valley and Macahel Bee Products in Artvin, Turkey. *KSU J. Agric Nat 27*(4), 949-957. https://doi.org/10.18016/ksutarimdoga.vi.1331388

Atıf Şekli: Bozkuş, T.N., (2024) Türkiye Artvin İli Hatila Vadisi ve Macahel Arı Ürünlerinin Farklı Çözücülerdeki Fenolik İçeriklerinin ve Antioksidan Aktivitelerinin Belirlenmesi. *KSÜ Tarım ve Doğa Derg 27* (4), 949-957. https://doi.org/10.18016/ksutarimdoga.vi.1331388

INTRODUCTION

Considering that natural products can help protect human health, they are in high demand by consumers, the food industry, and researchers today (Agüero et al., 2011). Bee products are particularly notable because of their increasing importance among these natural products and their widespread use in various sectors. Bee products are royal jelly and bee venom, which the bee secretes directly from its body, and honey, pollen,

and propolis, which are products that the bee collects from plants and partially adds to their body secretions (Rita Elkins, 2011). The biological properties of bee products vary depending on the vegetation and geographical characteristics of the region where they are collected (Kaskoniene, 2010). Bee products can be used to prevent pathological conditions such as inflammation, diabetes, cancer, and heart diseases and to protect human health due to their antioxidant, antimicrobial, antibacterial, antiviral, anti-inflammatory, and anticancer properties (Kumazawa et al., 2004; Korkmaz, 2008; Premratanachai & Chanchao, 2014). Natural bee products with high antioxidant capacity contain pharmacologically effective flavonoids, and various phenolic and aromatic compounds (Buratti et al., 2007; Olszewski et al., 2010; Nori et al., 2011).

Honey is a natural sweet substance that honeybees produce from the nectar of flowers, the secretions of living parts of plants, or the excrement of plant-sucking insects on living parts of plants, which honeybees collect, transform, and combine with certain substances (Mendes et al., 1998; Kahraman et al., 2010). While honey contains approximately 70-80% carbohydrates, 18-20% water, and 1-2% protein, organic acids, phenolic compounds, and mineral substances, it contains more than 200 chemical components (Saxena et al., 2010; Otmani et al., 2021). Bee pollen is formed because of mixing flower pollen collected by bees with nectar and secretions from the hypopharyngeal glands (Oliveira et al., 2013). Bee pollen has a very rich content in terms of proteins, various amino acids, carbohydrates, saturated/unsaturated fatty acids, lipids, sterols, vitamins, terpenes, phenolic substances, enzymes, and minerals (Villanueva et al., 2002; Campos et al., 2010; Fuenmayor et al., 2014; Conte et al., 2017). Propolis is a sticky, resinous natural substance collected by honeybees from various plant sources. Bees use propolis to seal holes in honeycombs, smooth the inner walls of the hive, and protect the entrance from outsiders (Burdock, 1998). Propolis contains more than 300 chemical components such as polyphenols (flavonoid aglycones, phenolic acids and esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, and inorganic compounds (Khalil, 2006). Royal jelly is a thick and milky secretion secreted from the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera* L.) and is used to feed larvae (Isidorova et al., 2009). Royal jelly generally consists of 60-70% water, 9-18% protein, 7-18% carbohydrates, 3-8% lipids and minerals, vitamins, and essential amino acids (Sabatini et al., 2009).

Artvin is one of the most valuable gene centers that preserve and house the pure Caucasian bee breed in Turkey's beekeeping sector. Thanks to its rich plant diversity and ecosystem, the Macahel (Camili) region, which was declared the first "Biosphere Reserve" of Turkey by UNESCO, and the Hatila Valley, which hosts around 1300 rich and diverse plant species with endemic characteristics, are among the most important regions of Artvin in terms of beekeeping (Eminağaoğlu, 2015; Anonymus, 2021).

It has been determined that there are a limited number of studies conducted by different researchers on bee products from the Artvin region in the literature (Popova et al., 2005; Silici et al., 2005; Girgin et al., 2009; Silici et al., 2010; Aliyazıcıoglu et al., 2013; Keskin et al., 2019). In addition, while there are no studies on the bee products of Hatila Valley, one of the two important regions of Artvin, there are very few studies on the Macahel (Camili) region (Özen et al., 2010; Temiz et al., 2013; Sarıkahya et al., 2021). However, none of these studies included a study in which bee product samples from Hatila Valley and Macahel were investigated in terms of phenolic content and antioxidant activity.

The extraction solvent is as important as the extraction method in determining the phenolic content and antioxidant activities of bee products (Cunha et al., 2004; Sforcin, 2007; Bozkuş & Değer, 2022). Extraction methods can affect the activity of bee products, as different solvents can dissolve and extract different components in other bee products (royal jelly, honey, pollen) just like in propolis (Sforcin, 2007). Therefore, in this study, water, ethanol, and propylene glycol were preferred as solvents for the extraction of bee products, which have not been proven to be harmful to health. In the literature, there is no study in which bee product extracts dissolve best among these solvents and the difference between these solvents is revealed. This study aimed to determine the amounts of total polyphenol and total flavonoid content and antioxidant activities of water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol extracts from bee product samples (honey, pollen, royal jelly, propolis) obtained from Hatila Valley and Macahel and to determine the best solvent for the samples of propolis. At the same time, the comparison of the phenolic content and antioxidant activities of all Hatila Valley bee products, the evaluation of Hatila Valley and Macahel propolis, and their mixtures in terms of phenolic content and antioxidant activity are among the objectives of this study. In this sense, this study is the first research in this field.

MATERIAL and METHOD

Preparation of Bee Product Extracts

Extracts of natural bee product samples obtained from the Macahel and Hatila Valley of Artvin province in Turkey were prepared as follows;

Hatila royal jelly: 15 g of Hatila royal jelly sample was weighed and 25 mL of pure water and ethanol were

added to it. These samples were coded as HRJ_W and HRJ_E.

Hatila honey: 15 g of Hatila honey sample was weighed and 25 mL of pure water and ethanol was added. These samples were coded as HH_W and HH_E.

Macahel honey: 15 g of Macahel honey sample was weighed and 25 mL of pure water and ethanol were added to it. These samples were coded as MH_W and MH_E.

Hatila pollen: 2.5 g of Hatila pollen sample was weighed and 25 mL of pure water, ethanol, and propylene glycol were added each. These samples were coded as $HPol_W$, $HPol_E$, and $HPol_{PG}$.

Hatila propolis: 2.5 g of Hatila propolis sample was weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added to it. These samples were coded as $HPro_W$, $HPro_E$, $HPro_{PG}$, $HPro_E$(70%), $HPro_{PG}$(70%).

Macahel propolis: 2.5 g of Macahel propolis sample was weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added. These samples were coded as MProW, MProE, MProPG, $MPro_E$(70%), $MPro_{PG}$(70%).

Hatila + Macahel propolis: 1.25 g of Hatila propolis sample and 1.25 g of Macahel propolis sample were weighed and 25 mL of pure water, ethanol, propylene glycol, 70% ethanol, and 70% propylene glycol were added. These samples were coded as $H+MPro_W$, $H+MPro_E$, $H+MPro_{PG}$, $H+MPro_E$(70%), $H+MPro_{PG}$(70%).

Royal jelly and honey samples were vortexed, kept in an ultrasonic bath for 10 minutes, and left to incubate in a shaker incubator at 25°C at 150 rpm for 24 hours with continuous shaking to dissolve. Pollen and propolis samples were vortexed and incubated in a shaker incubator at 60°C at 150 rpm for 24 hours with continuous shaking to dissolve. After 24 hours of incubation, royal jelly, honey, pollen, and propolis extracts were centrifuged at 2057 g for 10 minutes and filtered with filter paper. Thus, 600 mg/mL water and ethanol stock royal jelly and honey extracts, 100 mg/mL water, ethanol and propylene glycol stock pollen extracts, and 100 mg/mL water, ethanol, 70% ethanol, propylene glycol, and 70% propylene glycol stock propolis extracts were prepared. The extracts were stored in the refrigerator at +4 °C in the dark to be used in the necessary experiments.

Determination of the Total Polyphenol Content

The total polyphenol content of the extracts was determined spectrophotometrically according to the modified Folin-Ciocalteu method (Horzic et al., 2009). According to this method, 12.5 µL of bee product extract, 62.5 µL of 1:10 diluted Folin-Ciocalteu reagent, and 125 µL of 20% sodium carbonate solution (Na_2CO_3) were added to a 96-well microplate. After 30 minutes of incubation at room temperature and in the dark, absorbance was measured at 700 nm on a microplate reader. The total polyphenol content was calculated using a calibration curve constructed with gallic acid as standard. The results are given in mg gallic acid (GA) / g sample.

Determination of the Total Flavonoid Content

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric method (Chang et al., 2002). According to this method, 20 µL of bee product extract, 172 µL of 80% ethanol, 4 µL of 10% aluminum chloride ($AlCl_3$), and 4 µL of 1 M potassium acetate (KCH_3COO) solution were added to a 96-well microplate. After 40 minutes of incubation at room temperature and in the dark, the absorbance was measured at 415 nm in a microplate reader. The total flavonoid content was calculated using a calibration curve constructed with quercetin as standard. Results are given as mg quercetin (Q) / g sample.

Determination of Ferric (Fe^{3+}) Reducing Antioxidant Power

Antioxidant activity with the ferric (Fe^{3+}) reducing power method was determined by modifying the method proposed by (Moreira et al., 2008). 40 µL of bee product extract, 100 µL of 0.2 M phosphate buffer (pH: 6.6), and 100 µL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] were added to each of 1.5 mL microtubes and incubated at 50 °C for 20 minutes in the dark. After incubation, the microtubes were cooled. 100 µL of 10% trichloroacetic acid (TCA) was added to the mixtures in the microtube and this mixture was centrifuged at 3000 g for 10 minutes. 100 µL of the upper phases of the centrifuged samples were taken from the 96-well microplate and 100 µL of distilled water and 20 µL of iron (III) chloride ($FeCl_3$) were added to them. The final mixture was incubated for 5 minutes at room temperature and in the dark. After incubation, the absorbance was measured at 700 nm in a microplate reader. The ferric (Fe^{3+}) reducing antioxidant power was calculated using a calibration curve generated with trolox as standard. Results are given as mg trolox (T) / g sample.

Statistical Analysis

The results were expressed in the form of arithmetic mean ± standard deviation (S.D); n = 4. Data were statistically evaluated using the R program (Version 4.3). To reveal the relationship between the groups, normality analysis was performed, and it was seen that the data were normally distributed. Data were evaluated with one-way analysis of variance (ANOVA) and t-test. Based on the results of this analysis, the Games-Howell post-hoc test analysis was used among the significant groups, and those with $p<0.05$ were considered significant.

RESULTS and DISCUSSION

In this study, bee product samples obtained from Hatila Valley and Macahel were prepared in different solvents and analyzed to evaluate their polyphenol and flavonoid content and antioxidant activities. The results of the determination of the total polyphenol content of the extracts are given as mg GA/g sample in Table 1, the results of the determination of the total flavonoid content are given as mg Q/g sample in Table 2 and the results of the determination of the ferric (Fe^{3+}) reducing antioxidant power are given as mg T/g sample in Table 3.

Table 1. Total polyphenol content of bee product extracts (mg GA/g sample)
Çizelge 1. Arı ürünü ekstraktlarının toplam polifenol içeriği (mg GA/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.50 ± 0.02	0.43 ± 0.01			
HH	0.12 ± 0.02	0.15 ± 0.04			
MH	0.20 ± 0.01	0.19 ± 0.02			
HPol	4.76 ± 0.09	17.24 ± 0.28	18.93 ± 0.63		
HPro	6.59 ± 0.13	21.11 ± 0.07	29.41 ± 0.46	24.24 ± 0.64^{a}	27.01 ± 0.76^{a}
MPro	10.07 ± 0.24^{b}	76.04 ± 0.88^{b}	74.06 ± 1.58^{b}	94.04 ± 1.84^{a,b}	99.12 ± 2.75^{a,b}
H+MPro	8.59 ± 0.15^{b}	67.06 ± 1.54^{b}	66.65 ± 0.92^{b}	68.76 ± 2.11^{a,b}	72.31 ± 3.78^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, GA: Gallic acid. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p<0.05$). b: It differs significantly in terms of location ($p<0.05$).

Table 2. Total flavonoid content of bee product extracts (mg Q/g sample)
Çizelge 2. Arı ürünü ekstraktlarının toplam flavonoid içeriği (mg Q/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.16 ± 0.03	0.05 ± 0.02			
HH	0.02 ± 0.01	Not detected			
MH	0.03 ± 0.00	Not detected			
HPol	0.79 ± 0.13	2.31 ± 0.03	2.56 ± 0.11		
HPro	0.67 ± 0.13	6.52 ± 0.28	7.98 ± 0.13	5.38 ± 0.26^{a}	4.52 ± 0.30^{a}
MPro	0.43 ± 0.06^{b}	15.51 ± 0.28^{b}	17.59 ± 0.38^{b}	25.93 ± 0.79^{a,b}	16.81 ± 0.59^{a,b}
H+MPro	0.54 ± 0.10^{b}	10.63 ± 0.77^{b}	11.32 ± 0.55^{b}	16.59 ± 0.62^{a,b}	11.49 ± 0.31^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, Q: Quercetin. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p < 0.05$). b: It differs significantly in terms of location ($p < 0.05$).

Table 3. Ferric (Fe^{3+}) reducing antioxidant power of bee product extracts (mg T/g sample)
Çizelge 3. Arı ürünü ekstraktlarının demir (Fe^{3+}) indirgeyici antioksidan gücü (mg T/g örnek)

Sample Code	Water Extract	Ethanol Extract	Propylene Glycol Extract	70% Ethanol Extract	70% Propylene Glycol Extract
HRJ	0.38 ± 0.04	0.26 ± 0.01			
HH	0.60 ± 0.03	0.13 ± 0.04			
MH	0.69 ± 0.04	0.23 ± 0.01			
HPol	7.96 ± 0.26	36.24 ± 1.12	47.01 ± 0.81		
HPro	11.10 ± 0.15	20.12 ± 0.21	33.43 ± 0.21	25.97 ± 0.42^{a}	33.36 ± 0.62^{a}
MPro	17.82 ± 0.25^{b}	141.74 ± 0.93^{b}	174.76 ± 0.42^{b}	125.21 ± 1.89^{a,b}	170.48 ± 1.80^{a,b}
H+MPro	14.40 ± 0.21^{b}	88.74 ±0.87^{b}	132.82 ± 0.50^{b}	92.27 ± 0.97^{a,b}	107.88 ± 0.69^{a,b}

HRJ: Hatila royal jelly, HH: Hatila honey, MH: Macahel honey, HPol: Hatila pollen, HPro: Hatila propolis, MPro: Macahel propolis, H+MPro: Hatila+Macahel propolis, T: Trolox. Each value is expressed as mean ± S.D., n =4. a: It differs significantly in terms of solvent ($p < 0.05$). b: It differs significantly in terms of location ($p < 0.05$).

Although it varies depending on the solvent used in the study, it was detected in the range of the total polyphenol content of honey samples is 0.12 ± 0.02 – 0.20 ± 0.01 mg GA/g honey, the total flavonoid content is 0.02 ± 0.01 – 0.03 ± 0.00 mg Q/g honey, and the amount of ferric reducing antioxidant power is 0.13 ± 0.04 – 0.69 ± 0.04 mg T/g honey. It was determined that between linden honey obtained from Hatila Valley and chestnut honey obtained from Macahel, chestnut honey has higher phenolic content and antioxidant activity compared to linden honey. Likewise, although it varies depending on the extraction solvent used, it was determined the total polyphenol content of Hatila, Macahel and Hatila + Macahel propolis samples is 6.59

± 0.13 – 99.12 ± 2.75 mg GA/g propolis, the total flavonoid content is 0.43 ± 0.06 – 25.93 ± 0.79 mg Q/g propolis, and the amount of ferric reducing antioxidant power between 11.10 ± 0.15 – 174.76 ± 0.42 mg T/g propolis. Depending on the solvent used, it was determined in the range the total polyphenol content of pollen samples is 4.76 ± 0.09 – 18.93 ± 0.63 mg GA/g pollen, the total flavonoid content is 0.79 ± 0.13 – 2.56 ± 0.11 mg Q/g pollen, and the amount of ferric reducing antioxidant power is 7.96 ± 0.26 – 47.01 ± 0.81 mg T/g pollen, while the highest amounts were detected in the propylene glycol extract. It was determined that the total polyphenol, total flavonoid, and ferric-reducing antioxidant power of royal jelly samples were higher in the water extract than in the ethanolic extract and varied according to the extraction solvent, respectively: 0.43 ± 0.01 – 0.50 ± 0.02 mg GA/g royal jelly, 0.05 ± 0.02 – 0.16 ± 0.03 mg Q/g royal jelly was between 0.26 ± 0.01 – 0.38 ± 0.04 mg T/g royal jelly.

In terms of supporting this study, in various studies with different types of honey, it has been reported that the amount of polyphenolic substances in chestnut honey is higher than in other honey (Al-Mamary et al., 2002; Aljadi & Kamaruddin, 2004; Küçük et al., 2007). Tezcan et al. (2011) determined the total phenolic content of 10 different honey samples obtained from the Black Sea region of Turkey in the range of 0.36 ± 0.02 – 1.14 ± 0.02 mg GA/g honey. From this study, it was concluded that dark-colored honey such as chestnut honey show higher antioxidant values depending on their total phenolic compound content. In another study conducted by Al et al. (2009) using water extracts of 24 different kinds of honey in Romania, they determined that the total polyphenol content of the honey was in the range of 2.00 – 125.00 mg GA/100 g honey and the total flavonoid content was in the range of 0.91 – 28.25 mg Q/100 g honey. Furthermore, they reported that the highest flavonoid content was found in honey with multiple floral sources. In another study conducted by Bertoncelj et al. (2007) with the 7 most common types of honey in Slovenia, it was found that all honey types contain phenolic compounds and have antioxidant activity. The total phenolic content and antioxidant activity showed great differences between different types of honey. It was determined that the total amount of phenolic content and antioxidant activity was the lowest in light-colored acacia and linden honey, and the highest in darker honeys such as fir, spruce, and forest honey. Phenolic compounds have been observed to be responsible for the antioxidant activity of honey and it has been determined that there is a significant relationship between antioxidant activity and phenolic content (Bertoncelj et al., 2007).

Raw propolis is not an easily consumed mixture due to the resin- and wax-like substances in its structure. For this reason, it is necessary to reveal the biologically active components in its structure with the applied extraction method (Pietta et al., 2002; Özkök et al., 2021). Solvents such as ethanol, glycerol, polyethylene/polypropylene glycol, glycerol, and water are used in the extraction process (Özkök et al., 2021). Since different solvents can dissolve and extract different components in propolis, propolis extraction methods can affect propolis activity (Sforcin, 2007). In the study conducted by Bozkuş and Değer (2022), Turkish propolis was collected from different provinces of Turkey and mixed. The water, ethanol, glycerol, dimethyl sulfoxide (DMS, O), and acetone extracts of Turkish propolis were found to have total polyphenol content in the range of 19.7 ± 0.29 – 141.2 ± 9.99 mg GA/g propolis, total flavonoid content in the range of 1.3 ± 0.12 – 55.3 ± 6.63 mg Q/g, and ferric reducing power in the range of 26.2 ± 8.57 – 273.8 ± 11.62 mg T/g propolis. Depending on the amount of phenolic content and antioxidant activity, it was determined that propolis dissolved best in DMSO, followed by ethanol, acetone, glycerol, and water. As in the previous study, in this study, the ethanol extract of propolis was found to be higher in terms of phenolic content and antioxidant activity than the water extract. In a study by Silva et al. (2012) conducted in three different regions of Portugal, hydroalcoholic extracts of propolis were found to have significantly higher polyphenol and flavonoid content compared to methanol and water extracts. Findings like the study conducted by Silva et al. (2012) were also determined in this study, and it was found that 70% ethanol and 70% propylene glycol extracts of propolis generally had higher phenolic content and antioxidant activity than ethanol, propylene glycol, and water. The total polyphenol content ranged from 87.15 ± 4.80 – 277.17 ± 7.50 mg GA/g propolis in hydroalcoholic extracts, 58.61 ± 3.10 – 181.31 ± 4.71 mg GA/g propolis in methanol extracts, and 18.52 ± 1.35 – 72.15 ± 1.20 mg GA/g propolis in water extracts. The total flavonoid content ranged from 25.15 ± 2.53 – 142.32 ± 4.52 mg Q/g propolis in hydroalcoholic extracts, 13.62 ± 2.49 – 135.51 ± 4.18 mg Q/g propolis in methanol extracts, and 6.34 ± 0.55 – 42.30 ± 2.10 mg Q/g propolis in water extracts.

The results obtained from this study were found to be compatible with each other when compared with the data in the literature in terms of the amount of polyphenol and flavonoid content. In addition, since different modified methods have been applied in the literature in terms of ferric-reducing power, it was seen that the results were in line with the literature data when compared only with propolis.

In the study, when Hatila Valley propolis, Macahel propolis, and Hatila + Macahel propolis mixture were compared in terms of polyphenol and flavonoid content and ferric reducing power, it was determined that the order was Macahel propolis > Hatila + Macahel

propolis > Hatila propolis. In addition, it was determined that there was a statistically significant difference between 70% ethanol and 70% propylene glycol and water, and the lowest solubility was in water. Only water, ethanol, and propylene glycol solvents were used in this study. However, the use of different solvents or solvent combinations can increase or alter the extraction of different components, which can affect the antioxidant activity and phenolic content. Therefore, this study is important for the optimization of extraction procedures and the most effective use of bee products, depending on the effects of different solvents on the phenolic content and antioxidant activity of bee products.

Kroyer and Hegedus (2001) found the total polyphenol content of the pollen samples collected from various places and prepared as water, ethanol, and methanol-water (1:1) extracts between 7.4 ± 0.2 – 9.7 ± 0.3 mg GA/g pollen. The total polyphenol content of the pollen mixture was determined as 8.2 mg GA/g pollen. When this study is compared with the study conducted by Kroyer and Hegedus (2001), the total polyphenol content of the aqueous and ethanol extracts of pollen was determined in the range of 4.76 ± 0.09 – 17.24 ± 0.28 mg GA/g pollen, so the results were different.

Although there are many studies on honey, pollen, and propolis in the literature, there are not many studies on the amount of phenolic content and antioxidant activity of royal jelly. Nagai and Inoue (2004) stated that royal jelly is a rich mixture of protein and fatty acids, and the amount of phenolic substances is not very high. In another study, six samples of royal jelly were collected from the Mediterranean region (Morocco, Portugal, and Spain), and the total polyphenol content in their water extracts ranged from 3.0 ± 0.1 –9.0 ± 0.8 mg GA/g royal jelly. The total flavonoid content ranged from 0.1 ± 0.0 – 0.5 ± 0.0 mg Q/g royal jelly (El-Guendouz et al., 2020). When the values in this study were compared with the study conducted by El-Goundez et al. (2020), the polyphenol content of the water extract of royal jelly was determined as 0.50 ± 0.02 mg GA/g royal jelly, while the flavonoid content amount was determined as 0.43 ± 0.01 mg Q/g royal jelly, and the results were found to be compatible with each other.

In this study, when Hatila Valley bee product samples were compared in terms of polyphenol and flavonoid content and antioxidant activity, it was determined that the order was propolis > pollen > royal jelly > honey. There are many studies in the literature showing that propolis has a high amount of polyphenols (Kumazawa et al., 2004; Choi et al., 2006; Ahn et al., 2007; Moreira et al., 2008; Kalogeropoulos et al., 2009). Therefore, it is expected that the phenolic content of propolis is high, and the values obtained were found to be compatible with the literature. Furthermore, this study focused only on the Hatila Valley and Macahel bee products. Therefore, the antioxidant activities and phenolic content of the bee products obtained from other regions and different climatic conditions will be different.

The main components responsible for the antioxidant activity of bee products are flavonoids and phenolic compounds, and these antioxidant effects are closely related to their free radical scavenging activities (Eraslan et al., 2008; Kanbur et al., 2009; Hegazi, 2012). Their composition, which varies according to botanical origin, is also responsible for high levels of antioxidant activity (Kanbur et al., 2009). Therefore, both in the studies conducted in the literature and in this study, it was determined that as the amount of phenolic substance increased, the total antioxidant activity increased in parallel.

This study shows that bee products have phenolic content and antioxidant activity, revealing the potential health benefits of these natural products. This can make a significant contribution to existing knowledge in the field of health and nutrition. In addition, this study highlights the potential of bee products for wider use in diet and health applications.

CONCLUSION and RECOMMENDATIONS

Today, bee products are widely consumed as nutritional supplements and are used in the food, pharmaceutical, and cosmetic industries. It is known that honeybee products such as honey, pollen, propolis, and royal jelly are rich in antioxidants and have various antioxidant effects and beneficial functions in the human body.

In this study, when the Hatila Valley bee product samples were compared in terms of polyphenol and flavonoid content and antioxidant activity, it was determined that the order was propolis > pollen > royal jelly > honey and the values obtained were consistent with the literature. Therefore, for bee products, it is possible to say that as the amount of phenolic substance increases, the total antioxidant activity increases in parallel. Additionally, when Hatila Valley propolis, Macahel propolis, and Hatila + Macahel propolis were compared in terms of phenolic content and antioxidant activity, it was determined that the order was Macahel propolis > Hatila + Macahel propolis > Hatila propolis. In addition, it was determined that there was a significant difference between 70% ethanol and 70% propylene glycol and water, and while the solubility of these solvents was higher, the solvent with the lowest solubility was water. It can be concluded from this study that extracts with less potency are mixed with more active extracts, resulting in lower activity than extracts with high activity, but higher activity than extracts with low activity. This strengthens the idea that such propolis mixtures can be suitable for different applications in terms of various sectors such as the food, medical and

cosmetic industries. Therefore, because of the mixtures obtained, it will be possible to provide maximum benefit from this natural product, as well as increase its value both biologically and economically.

Since Hatila Valley and Macahel bee products have a rich phenolic content and therefore high antioxidant activity, they can potentially be used in applications such as food additives, nutritional supplements, or functional foods in terms of food science and technology. This study also shows that the phenolic content and antioxidant activity of bee products can vary depending on the type of solvent. This can be an important factor in the selection of solvents used in the extraction and processing of bee products, not only in the food industry but also in various sectors such as pharmaceuticals and cosmetics.

In conclusion, this study is important for the conservation and sustainability of local ecosystems, as it provides important information on the local biodiversity of certain regions such as the Hatila Valley and Macahel.

ACKNOWLEDGEMENTS

This study was supported by Artvin Coruh University Scientific Research Projects Coordinators (AÇÜ BAP), Türkiye under Grant No. 2021.M90.02.03. The author would like to thank MACAHEL Beekeeping Tourism Transport and Trade Inc., which helped in the supply of bee product samples, and Artvin Coruh University Science-Technology Application and Research Center and Artvin Coruh University Medicinal-Aromatic Plants Application and Research Center for laboratory use.

Author's Contributions

The article has a single author.

Conflict of Interest Statement

The author declares that there is no conflict of interest in this study.

REFERENCES

Agüero, M. B., Svetaz, L., Sanchez, M., Luna, L., Lima, B., Lopez, M. L., Zacchino, S., Palermo, J., Wunderlin, D., Feresin, G. E., & Tapia, A. (2011). Argentinean Andean propolis associated with the medicinal plant Larrea nitida Cav. (Zygophyllaceae). HPLC-MS and GC-MS characterization and antifungal Activity. *Food and Chemical Toxicology, 49*(9), 1970-1978. https://doi.org/10.1016/j.fct.2011.05.008.

Ahn, M. R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F., & Nakayama, T. (2007). Antioxidant Activity and Constituents of Propolis Collected in Various Areas of China. *Food Chemistry, 101*(4), 1383-1392. https://doi.org/10.1016/j.foodchem.2006.03.045.

Al, M. L., Daniel, D., Moise, A., Bobis, O., Laslo, L., & Bogdanov, S. (2009). Physico-chemical and Bioactive Properties of Different Floral Origin Honeys from Romania. *Food Chemistry, 112*(4), 863-867. https://doi.org/10.1016/j.foodchem.2008.06.055.

Aliyazıcıoglu, R, Sahin, H, Erturk, O, Ulusoy, E, & Kolayli, S. (2013). Properties of Phenolic Composition and Biological Activity of Propolis from Turkey. *International Journal of Food Properties, 16*(2), 277-287. https://doi.org/10.1080/10942912.2010.551312.

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of The Phenolic Contents and Antioxidant Capacities of Two Malaysian Floral Honeys. *Food Chemistry, 85*(4), 513-518. https://doi.org/10.1016/S0308-8146(02)00596-4.

Al-Mamary, M., Al-Meeri A., & Al-Habori, M. (2002). Antioxidant Activities and Total Phenolics of Different Types of Honey. *Nutrition Research, 22*(9), 1041-1047. https://doi.org/10.1016/S0271-5317(02)00406-2.

Anonymus, (2021). Macahel Camili Biyosfer Rezervi. https://www.macahel.com (Alınma Tarihi: 20.09.2021).

Bertoncelj, J., Dobersek, U., Jamnik, M., & Golob, T. (2007). Evaluation of The Phenolic Content, Antioxidant Activity, and Colour of Slovenian Honey. *Food Chemistry, 105*(2), 822-828. https://doi.org/10.1016/j.foodchem.2007.01.060.

Bozkuş, T. N., & Değer, O. (2022). Comparison of total phenolic contents and antioxidant activities of propolis in different solvents. *Food and Health, 8*(2), 111-117. https://doi.org/10.3153/FH22011.

Buratti, S., Benedetti, S., & Cosio, M. S. (2007). Evaluation of the antioxidant power of honey, propolis, and royal jelly by amperometric flow injection analysis. *Talanta, 71*(3), 1387-1392. https://doi.org/10.1016/j.talanta.2006.07.006.

Burdock, G. A. (1998). Review of the biological properties and toxicity of bee propolis (Propolis). *Food and Chemical Toxicology, 36*(4), 347-363. https://doi.org/10.1016/S0278-6915(97)00145-2.

Campos, M. G. R., Frigerio C., Lopes, J., & Bogdanov, S. (2010). What is the Future of Bee Pollen? *Journal of ApiProduct and ApiMedical Science, 2*(4), 131-144. https://doi.org/10.3896/IBRA.4.02.4.01.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178-182. https://doi.org/10.38212/2224-6614.2748.

Choi, Y. M., Noh, D. O., Cho, S. Y., Suh, H. J., Kim, K. M., & Kim, J. M. (2006). Antioxidant and Antimicrobial Activities of Propolis from Several Regions of Korea. LWT - *Food Science and Technology, 39*(7), 756-761. https://doi.org/10.1016/

j.lwt.2005.05.015.

Conte, G., Benelli, G., Serra, A., Signorini, F., Bientinesi, M., Nicolella, C., Mele, M., & Canale, A. (2017). Lipid Characterization of Chestnut and Willow Honeybee-collected Pollen: Impact of Freeze-drying and Microwave-assisted Drying. *Journal of Food Composition and Analysis,* 55, 12-19. https://doi.org/10.1016/j.jfca.2016.11.001.

Cunha, I. B. S., Sawaya, A. C. H. F., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., Povia, G. S., & Carvalho, P. O. (2004). Factors that influence the yield and composition of Brazilian propolis extracts. *Journal of the Brazilian Chemical Society, 15*(6), 964-970. https://doi.org/10.1590/S0103-50532004000600026.

El-Guendouz, S., Machado, A. M., Aazza, S., Lyoussi, B., Miguel, M. G., Mateus, M. C., & Figueiredo, A. C. (2020). Chemical Characterization and Biological Properties of Royal Jelly Samples from the Mediterranean Area. *Natural Product Communications, 15*(2). https://doi.org/10.1177/1934578X20908080.

Eminağaoğlu, Ö. (2015). *Artvin'in Doğal Bitkileri. PROMAT Basım Yayın San. ve Tic. A.Ş.,* İstanbul.

Eraslan, G., Kanbur, M., & Silici, S. (2008). Effect of Carbaryl on Some Biochemical Changes in Rats: The Ameliorative Effect of Bee Polen. *Food and Chemical Toxicology, 47*(1), 86-91. https://doi.org/10.1016/j.fct.2008.10.013.

Fuenmayor, B., Zuluaga, D., Diaz, M., Quicazán de C, Cosio, M., & Mannino, S. (2014). Evaluation of the Physicochemical and Functional Properties of Colombian Bee Pollen. *Revista MVZ Cordoba, 19*(1), 4003-4014. https://doi.org/10.21897/rmvz.120.

Girgin, G., Baydar, T., Ledochowski, M., Schennach, H., Bolukbasi, D. N., Sorkun, K., Salih, B., Sahin, G., & Fuchs, D. (2009). Immunomodulatory effects of Turkish propolis: Changes in neopterin release and tryptophan degradation. *Immunobiology, 214*(2), 129-134. https://doi.org/10.1016/j.imbio.2008.07.002.

Hegazi, A. G. (2012). The medical importance of bee products. *Uludag Bee Journal, 12*(4), 136-146.

Horzic, D., Komes, D., Belscak, A., Ganic, K. K., Ivekovic, D., & Karlovic, D. (2009). The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chemistry, 115*(2), 441-448. https://doi.org/10.1016/j.foodchem.2008.12.022.

Isidorova, V. A., Czyzewskaa, U., Isidorovab, A. G., & Bakier, S. (2009). Gas chromatographic and mass spectrometric characterization of the organic acids extracted from some preparations containing lyophilized royal jelly. *Journal of Chromatography. B, 877*(29), 3776-3780. https://doi.org/10.1016/j.jchromb.2009.09.016.

Kahraman, T., Buyukunal, S. K., Vural, A., & Sandıkcı Altunatmaz, S. (2010). Physico-chemical properties in honey from different regions of Turkey. *Food Chemistry, 123*(1), 41-44. https://doi.org/10.1016/j.foodchem.2010.03.12.

Kalogeropoulos, N. Konteles, S. J., Troullidou, E., Mourtzinos, I., & Karathanos, V. T. (2009). Chemical Composition, Antioxidant Activity and Antimicrobial Properties of Propolis Extracts from Greece and Cyprus. *Food Chemistry, 116*(2), 452-461. https://doi.org/10.1016/j.foodchem.2009.02.060

Kanbur, M., Eraslan, G., Beyaz, L., Silici, S., Liman, B. C., Altinordulu, S., & Atasever, A. (2009). The Effects of Royal Jelly on Liver Damage Induced by Paracetamol in Mice. *Experimental and Toxicologic Pathology, 61*(2), 123-132. https://doi.org/10.1016/j.etp.2008.06.003.

Kaskoniene, V. (2010). Floral Markers in Honey of Various Botanical and Geographic Origins: A review. *Comprehensive Reviews in Food Science and Food Safety, 9*(6), 620-634. https://doi.org/10.1111/j.1541-4337.2010.00130.x.

Keskin, M., Keskin, Ş., & Kolayli, S. (2019). Preparation of alcohol-free propolis-alginate microcapsules, characterization, and release properties. *LWT-Food Science and Technology, 108*, 89-96. https://doi.org/10.1016/j.lwt.2019.03.036.

Khalil, M. L. (2006). Biological activity of bee propolis in health and disease. *Asian Pacific Journal of Cancer Prevention, 7*, 22-31.

Korkmaz, A. (2008). *Bal. T.C. Samsun Valiliği, İl Tarım Müdürlüğü,* Samsun.

Kroyer, G., & Hegedus, N. (2001). Evaluation of Bioactive Properties of Pollen Extracts as Functional Dietary Food Supplement. *Innovative Food Science & Emerging Technologies, 2*(3), 171-174. https://doi.org/10.1016/S1466-8564(01)00039-X.

Küçük, M., Kolayli, S., Karaoglu, Ş., Ulusoy., E., Baltacı, C., & Candan, F. (2007). Biological Activities and Chemical Composition of Three Honeys of Different Types from Anatolia. *Food Chemistry, 100*(2), 526-534. https://doi.org/10.1016/j.foodchem.2005.10.010.

Kumazawa, S., Hamasaka, T., & Nakayama, T. (2004). Antioxidant activity of propolis of various geographic origins. *Food Chemistry, 84*(3), 329-339. https://doi.org/10.1016/S0308-8146(03)00216-4.

Mendes, E., Brojo, P. E., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers, 37*(3), 219–223. https://doi.org/10.1016/S0144-8617 (98) 00063-0.

Moreira, L., Dias, L. G., Pereira, J. A., & Estevinho, L. (2008). Antioxidant properties, total phenols, and pollen analysis of propolis samples from Portugal. *Food and Chemical Toxicology, 46*(11), 3482-3485. https://doi.org/10.1016/j.fct.2008.08.025.

Nagai, T., & Inoue, R. (2004). Preparation and Functional Properties of Water Extract and Alkaline Extract of Royal Jelly. *Food Chemistry,*

84(2), 181-186. https://doi.org/10.1016/S0308-8146(03)00198-5.

Nori, M. P., Favaro-Trindade, C. S., Alencar, S. M., Thomazini, M., Balieiro, J. C. C., & Castillo, C. J. C. (2011). Microencapsulation of propolis extract by complex coacervation. *LWT- Food Science and Technology, 44*(2), 429-435. https://doi.org/10.1016/j.lwt.2010.09.010.

Oliveira, M. C., Silva, D. M., Loch, F. C., Martins, P. C., Dias, D. M. B., & Simon, G. A. (2013). Effect of bee pollen on the immunity and Tibia characteristics in broilers. *Brazilian Journal of Poultry Science, 15*(4), 323-328. https://doi.org/10.1590/ S1516-635X2013000400006.

Olszewski, T. K., Bomont, C., Coutrot, P., & Grison, C. (2010). Lithiated anions derived from (alkenyl) pentamethyl phosphoric triamides: Useful synthons for the stereoselective synthesis of 9-oxo- and 10-hydroxy-2(E)-decanoic acids, important components of queen substance and royal jelly of honeybee *Apis mellifera*. *Journal of Organometallic Chemistry, 695*(21), 2354-2358. https://doi.org/10.1016/j.jorganchem.2010.06.020.

Otmani, A., Amessis-Ouchemoukh, N., Birinci, C., Yahiaoui, S., Kolayli, S., Rodriguez-Flores, M. S., Escuredo, O., Seijo, M. C., & Ouchemoukh, S. (2021). Phenolic compounds and antioxidant and antibacterial activities of Algerian honey. *Food Bioscience, 42*, 101070. https://doi.org/10.1016/j.fbio.2021.101070.

Özen, T., Kılıç, A., Bedir., O., Koru, Ö., Sorkun, K., Tanyüksel, M., Kılıç, S., Gençay, Ö., Yıldız., O., & Baysallar, M. (2010). *In Vitro* Activity of Turkish Propolis Samples Against Anaerobic Bacteria Causing Oral Cavity Infections. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16*(2), 293-298. https://doi.org/10.9775/kvfd.2009.707.

Özkök, A., Keskin, M., Tanuğur Samancı, A. E., Yorulmaz Önder, E., & Takma, Ç. (2021). Characterization of Propolis Extracts Prepared Using Different Solvents at the Different Concentrations. *Progress in Nutrition, 23*(3), 2021108. https://doi.org/10.23751/pn.v23i3.10795.

Pietta, P. G., Gardana, C., & Pietta, A. M. (2002). Analytical methods for quality control of propolis. *Fitoterapia, 73*(1), 7-20. https://doi.org/10.1016/S0367-326X(02)00186-7.

Popova, M., Silici, S., Kaftanoglu, O., & Bankova, V. (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine, 12*(3), 221-228. https://doi.org/10.1016/j.phymed.2003.09.007.

Premratanachai, P., & Chanchao, C. (2014). Review of the anticancer activities of bee products. *Asian Pacific Journal of Tropical Biomedicine, 4*(5), 337-344. https://doi.org/10.12980/APJTB.4.2014C1262

Rita Elkins, M. H. (2011). Bee pollen, royal jelly, propolis and honey, *Woodland Publishing,* Incorporated, London, 36 p.

Sabatini, A. G., Marcazzan, G. L., Caboni, M. F., Bogdanov, S., & Almeida-Muradian, L. B. D. (2009). Quality and standardization of royal jelly. *Journal of ApiProduct and ApiMedical Science, 1*(1), 1-6. https://doi.org/10.3896/IBRA.4.01.1.04.

Sarıkahya, N. B., Gören, A. C., Okkalı, G. S., Çöven, F. O., Orman, B., Kırcı, D., Yücel, B., Kışla, D., Demirci, B., Altun, M., Önem, A. N., & Nalbantsoy, A. (2021). Chemical composition and biological activities of propolis samples from different geographical regions of Turkey. *Phytochemistry Letters, 44*, 129-136. https://doi.org/10.1016/j.phytol.2021.06.008.

Saxena, S., Gautam, S., & Sharma, A. (2010). Physical, Biochemical and Antioxidant Properties of Some Indian Honeys. *Food Chemistry, 118*(2), 391-397. https://doi.org/10.1016/j.foodchem.2009.05.001.

Sforcin, J. M. (2007). Propolis and the immune system: a review. *Journal of Ethnopharmacology, 113*(1), 1-14. https://doi.org/10.1016/j.jep.2007.05.012.

Silici, S., Koç, N. A., Ayangil, D., & Çankaya, S. (2005). Antifungal Activities of Propolis Collected by Different Races of Honeybees Against Yeasts Isolated from Patients with Superficial Mycoses. *Journal of Pharmacological Sciences, 99*(1), 39-44. https://doi.org/10.1254/jphs.fpe05002x.

Silici, S., Sagdic, O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant, and antimicrobial activities of Rhododendron honeys. *Food Chemistry, 121*(1), 238-243. https://doi.org/10.1016/j.foodchem.2009.11.078.

Silva, J. C., Rodrigues, S., Feas, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile, and role in the inflammation of propolis. *Food and Chemical Toxicology, 50*(5), 1790-1795. https://doi.org/10.1016/j.fct.2012.02.097.

Temiz, A., Şener Mumcu, A., Özkök Tüylü, A., Sorkun, K., & Salih, B. (2013). Antifungal Activity of Propolis Samples Collected from Different Geographical Regions of Turkey Against Two Food-Related Molds, Aspergillus versicolor and Penicillium aurantiogriseum. *Gıda, 38*(3), 135-142. https://doi.org/10.5505/gida.2013.10820.

Tezcan, F., Kolaylı, S., Sahin, H., Ulusoy, E., & Erim, B. F. (2011). Evaluation of Organic Acid, Saccharide Composition and Antioxidant Properties of Some Authentic Turkish Honeys. *Journal of Food and Nutrition Research, 50*(1), 33-40.

Villanueva, M. T. O., Marquina, A. D., Serrano, R. B., & Abellan, G. B. (2002). The Importance of Bee-Collected Pollen in the Diet: a Study of Its Composition. *International Journal of Food Sciences and Nutrition, 53*(3), 217-224. https://doi.org/10.1080/09637480220132832.

KSÜ Tarım ve Doğa Derg 27 (4), 958-966, 2024
KSU J. Agric Nat 27 (4), 958-966, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1353230

Gaziantep İlinde Baharatlık Kırmızıbiberin (Maraş Biberi) Üretim Maliyeti ve Pazarlama Durumu

Elife YAVUZ[1], Vedat DAĞDEMİR[2]
[1] Atatürk Üniversitesi Ziraat Fakültesi Tarım Ekonomisi Bölümü, 25240, Erzurum. [2]Atatürk Üniversitesi Ziraat Fakültesi Tarım Ekonomisi Bölümü, 25240, Erzurum.
[1]https://orcid.org/0000-0002-2798-5263, [2] https://orcid.org/0000-0002-2293-9460
✉: : dagdemir@atauni.edu.tr

ÖZET

Bu çalışmada Gaziantep ilinde baharatlık kırmızıbiber üreten işletmelerin üretim maliyetinin hesaplanması ve pazarlama yapısının ortaya konulması açlanmıştır. Gaziantep ilinin İslahiye ve Nurdağı ilçelerinde baharatlık kırmızıbiber üretimi yapan 162 tarım işletmesi üzerinde gerçekleştirilmiş olan bu araştırmada, işletmelerin üretim maliyeti hesaplamaları, karlılık analizi ve pazarlama yapıları incelenmiştir. Tek ürün bütçe analiz yöntemi kullanılan çalışmada incelenen tarım işletmelerinde ortalama olarak 45.41 dekar alanda baharatlık kırmızıbiber üretimi gerçekleştirildiği, baharatlık kırmızıbiber üretiminde dekara ortalama değişken masrafların 1788.20 ₺ da-1, sabit masrafların 434.10 ₺ da-1 ve toplam üretim masrafının 2222.30 ₺ da-1 olduğu belirlenmiştir. Baharatlık yaş kırmızıbiber verimi ortalama 1656.80 kg da-1, baharatlık kırmızıbiber üretimiyle 3810.64 ₺ da-1 gayrisafi üretim değeri elde edilmiştir. 1 kg baharatlık kırmızıbiber üretim maliyeti ortalama 1.34 ₺ kg-1 olarak tespit edilmiştir. Pazara ulaşan kırmızıbiberin maliyeti 1.37 ₺ kg-1 olarak belirlenmiştir. Pazarlama masrafları dikkate alındığında, 1 dekar alandan elde edilen net kar ortalama 1532.76 ₺, brüt kar ise ortalama 1967.04 ₺ olarak hesaplanmıştır. Çalışmanın sonuçlarına göre, araştırma bölgesindeki baharatlık kırmızıbiber üreticilerinin 1 ₺'lik üretim maliyetine karşılık elde ettikleri oransal kar ortalaması üretim ve pazarlama aşamaları için sırasıyla 1.72 ve 1.68 olarak hesaplanmıştır. Tüketicinin 1 kg kuru kırmızıbibere ödediği paranın %17.69'u üretici eline geçerken, %82.31'i aracıların eline geçmektedir.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 31.08.2023
Kabul Tarihi : 24.01.2024

Anahtar Kelimeler
Kırmızıbiber
Maliyet
Net kar
Pazarlama marjı
Gaziantep

Production Cost and Marketing Situation of Spice Red Pepper (Maras Pepper) in Gaziantep Province

ABSTRACT

This study aimed to calculate the production cost and reveal the marketing structure of the enterprises producing spicy red pepper in Gaziantep province. This research was conducted on 162 agricultural enterprises producing spice red pepper in the İslahiye and Nurdağ districts of Gaziantep province. The study examined the production cost calculations, profitability analysis, and marketing structures of these enterprises. Using the single-crop budget analysis method, the study found that on average, spice red pepper was produced on 45.41 acres of land. The average variable costs per acre for spice red pepper production were determined as 1788.20 ₺ acre-1, fixed costs were 434.10 ₺ acre-1, and the total production cost was 2222.30 ₺ acre-1. The average yield of spice fresh red pepper was 1656.80 kg acre-1, resulting in a gross production value of 3810.64 ₺ acre-1. The average production cost of 1 kg of spice red pepper was determined as 1.34 ₺ kg-1. The cost of red pepper reaching the market was determined as 1.37 ₺ kg-1. Taking marketing expenses into account, the average net profit per acre obtained was 1532.76 ₺, and the average gross profit was 1967.04 ₺. According to the results of the study, the average proportional profit obtained by spice red pepper producers in the research area was calculated as 1.72 for production and 1.68 for marketing, for every 1 ₺ of production cost. While

Agricultural Economics

Research Article

Article History
Received : 31.08.2023
Accepted : 24.01.2024

Keywords
Red pepper
Cost
Net profit
Marketing margin
Gaziantep

17.69% of the money paid by the consumer for 1 kg of dried red pepper goes to the producer, 82.31% goes to intermediaries.

Atıf Şekli: Yavuz, E., & Dağdemir, V., (2024). Gaziantep İlinde Baharatlık Kırmızıbiberin (Maraş Biberi) Üretim Maliyeti ve Pazarlama Durumu. *KSÜ Tarım ve Doğa Derg 27* (4), 958-966. https://doi.org/10.18016/ksutarimdoga.vi.1353230

To Cite : Yavuz, E., & Dağdemir, V., (2024). Production Cost and Marketing Situation of Spice Red Pepper (Maras Pepper) in Gaziantep Province. *KSU J. Agric Nat 27* (4), 958-966. https://doi.org/10.18016/ksutarimdoga.vi1353230

GİRİŞ

Tarım sektörü; dünya nüfusunun beslenmesine ve sanayi sektörünün hammadde ihtiyacını karşılaması yanında dış ticaret, istihdam, sermaye tedariki ve gelir teminine katkı sağlaması ile dünya ekonomisi üzerinde büyük bir öneme sahiptir. Nüfus artışıyla beraber tarım ve gıda sektöründeki arz ve talep de buna bağlı olarak artmaktadır. Bu artış trendi içerisinde arz ve talebi artan tarım ürünlerinden biri de baharatlık kırmızıbiberdir (Maraş Biberi).

Kırmızıbiber otsu bir sebze olup hem dünyada hem de Türkiyede açıkta ve tek yıllık olarak üretimi yapılmaktadır (Verit ve ark., 2001). İnsan işgücüne ihtiyaç duyularak tarladan toplanılan tam olgunlaşmış bu sebzelerin önce tekniğine uygun bir şekilde sapları temizleyerek kurutulması ve öğütülmesi sonucu baharatlık kırmızıbiber elde edilmektedir. Elde edilen kırmızıbiberlerin tüm dünyada olduğu gibi Türkiye'de de kullanım alanı çok geniş olup genellikle gıda maddesi olarak yemeklere lezzet, tat, aroma ve renk vermesi için kullanılmaktadır (Çoksöyler, 1995). Eski zamandan bu yana şifa kaynağı olarak bilinen kırmızıbiber, geleneksel ve modern tıp alanında önemli bir yere sahiptir. Halk arasında romatizma, soğuk algınlığı, grip vb. rahatsızlıklarda faydalı olduğuna inanılmakta olup kalp ve damar ile kanser gibi birçok hastalığı önleyici ya da tedavi edici özelliği olduğu da bilinmektedir (Beis, 1990; Perucka & Materska, 2001).

Dünyadaki baharatlık kırmızıbiber üretimi 2019 yılı verilerine göre 4.2 milyon tondur. Hindistan dünya üretiminin %41.00'ını, Tayland %8.20'sini, Çin %7.70'ini, Etiyopya %7.40'ını, diğer ülkeler ise %35.70'lik kısmını karşılamaktadır. Türkiye 15 bin ton kırmızıbiber üretimi ile dünya üretiminin %0.36'sını karşılamaktadır (FAO, 2020).

Dünyada dış ticarete konu olan kırmızıbiber miktarı 2019 yılı verilerine göre yaklaşık 920 bin ton civarında olup ithalatçı ülkeler arasında ilk iki sırada Çin (%19.00) ve Amerika Birleşik Devletleri (%16.20) yer almaktadır. Dünyadaki baharatlık kırmızıbiber ihracatçısı ülkeler ise sırasıyla; Hindistan, Çin, İspanya, Meksika ve Peru'dur. Hindistan %49.00'ıuk bir oranla ilk sırada yer alırken aynı zamanda ithalatçı olan Çin %22.30'lük bir oranla ikinci sırada yer almaktadır. Türkiye'nin toplam ihracatı yaklaşık 2786 ton olup ihracattaki payı %0.30'dur (FAO, 2020).

Türkiye'de 2020 yılında baharatlık kırmızıbiberin 119869 da ekili alanda 256735 ton üretimi yapılmıştır. Türkiye'de kırmızıbiber üretimi yapan iller üretim miktarlarına göre sırasıyla Şanlıurfa, Gaziantep, Kilis, Kahramanmaraş, Bursa, Aydın, Hatay ve Muğla'dır. Ekili alanın %35.87'sini karşılayan Gaziantep, toplam üretimin %25.94'ünü karşılamaktadır. Gaziantep'te 2020 yılında 43 bin dekar alanda baharatlık kırmızıbiber ekimi yapılmış olup 66603 ton üretim gerçekleşmiştir (TÜİK, 2020).

Üretim maliyetleri, piyasa fiyatları ve verim gibi faktörlerin kırmızıbiber yetiştiriciliğinin karlılığını etkilediği ve karlılığın belirli koşullara bağlı olduğu yapılan bazı çalışmalar ile ortaya konulmuştur (Akbay ve ark., 2012; Candemir ve ark., 2012; Çitak, 2015; Ilić ve ark., 2017; Aytop, 2018; Dessie ve ark., 2019; Telek ve ark., 2019; Hastuti & Sari, 2020; Hayran & Gül, 2020).

Çalışma konusu olarak belirlenen kırmızıbibere Kahramanmaraş ilinde 14.04.2002 tarihinde "Maraş Biberi" ismiyle menşe işaretli olarak coğrafi işaret tescili verilmiştir (Anonim, 2002).

Baharatlık kırmızıbiberin üretim aşamasında yoğun işgücüne ihtiyaç duyması, çeşitli tarıma dayalı sanayi işletmelerinde hammadde olarak kullanılması, iç ve dış pazarın taleplerine cevap vermesi ile ülke ve bölge ekonomisinde önemli bir etkiye sahiptir. Tarım sektöründe önemli bir yeri olan baharatlık kırmızıbiber üretiminde makine işgücünden çok insan işgücüne ihtiyaç duyulmakta ve üretim aşamasında girdi maliyetleri artmaktadır. Serbest piyasa koşullarında girdi maliyetlerinin yüksek olması üreticileri olumsuz etkilemektedir. Üretim aşamasında ne kadar masrafla üretimin organize edilebileceğine karar verebilen üreticiler, üretmiş oldukları ürünün fiyatını belirlemede aynı esnekliğe sahip değillerdir. Bunlardan dolayı üreticiler için üretim ve pazarlama masraflarının ayrıntılı bir şekilde belirlenmesi büyük önem teşkil etmektedir.

Mevcut araştırmada, Gaziantep İli İslahiye ve Nurdağı İlçelerinde baharatlık kırmızıbiber üreten işletmelerin üretim maliyetinin hesaplanması, karlılığın ölçülmesi, pazarlama yapısının ortaya konulması, baharatlık kırmızıbiberin üretimi ve pazarlanmasında yaşanılan sorunların belirlenmesi ve bunlara yönelik çözüm önerilerinin geliştirilmesi amaçlanmıştır.

MATERYAL ve METOD

Materyal

Çalışmada temin edilen veriler, birincil ve ikincil verilerden oluşmuştur. Gaziantep'in İslahiye ve Nurdağı ilçelerinde baharatlık kırmızıbiber (Maraş Biberi) üreticiliği yapan işletmelerle yüz yüze yapılan anketlerden elde edilen veriler birincil verileri meydana getirmiştir. İkincil veriler ise Türkiye İstatistik Kurumu (TÜİK), Food and Agriculture Organization of The United Nations (FAO) tarafından yayımlanan istatistik verileri, Tarım ve Orman Bakanlığı kayıtları, konu ile ilgili ulusal ve uluslararası düzeyde yapılmış tez, makale ve proje raporlarından elde edilmiştir. Çalışma 2020 yılı üretim dönemini kapsamaktadır.

Metotlar

Örnekleme aşamasında kullanılan yöntem

Tarım ekonomisi araştırmalarında tesadüfi örnekleme yöntemleri daha çok tercih edilmektedir. Bu yöntemin ayrıcalığı, örnekten elde edilen istatistiklerin popülasyon parametrelerini, belirli bir güven aralığında ve bilinen bir hata payı ile temsil edilebilmesi olarak belirtilmiştir.

Araştırmada en sağlıklı verilere ulaşmada, 2020 yılı Gaziantep Tarım ve Orman Bakanlığı İl ve İlçe Müdürlüklerinde Çiftçi Kayıt Sisteminden (ÇKS) alınan bilgiler doğrultusunda baharatlık kırmızıbiber işletmelerinin ekim alanlarının parçalı ve dağınık olmasından dolayı aynı işletmelere ait ekilen alanlar birleştirilerek, toplam üretim miktarları hesaba katılmıştır. Ekilen alanların birleştirilmesi sonucunda İslahiye ve Nurdağı ilçelerinde toplam 972 baharatlık kırmızıbiber üreten tarım işletmesinin mevcut olduğu saptanmıştır. Bu tarım işletmelerinin 566'sı (%58.20) İslahiye ilçesinde, 406'sı (%41.80) ise Nurdağı ilçesindedir. Çalışma bölgesindeki tarım işletmelerinin arazi büyüklükleri $2 \leq n \leq 200$ dekar olarak belirlenmiştir. Bu ölçek kapsamında 2 da altındaki işetmelerin sadece öz tüketim için üretim yapması, 200 da üzerindeki işletmelerin çok büyük ve nispetlerinin çok düşük olmasından dolayı popülasyonu temsil etme niteliği taşımadığı düşünülerek ana kitleye dahil edilmemiştir.

Bu nedenle örnek işletmelerin seçiminde Basit Tesadüfi Örnekleme Yöntemi tercih edilmiş olup örneklem kitlesini belirleyecek formül ve hesaplama Eşitlik 1'de gösterilmiştir (Dağdemir & Aşkan, 2004).

$$n = \frac{N * S^2 * z^2}{(N-1) * d^2 + S^2 * z^2}$$

$$= \frac{972 * 1238,49 * 2,706}{(972-1) * 17,214 + 1238,49 * 2,706} = 162 \qquad (1)$$

Burada;

n = Örnek hacmi
S^2 = Varyans (1234,49)
z = % 90 güven aralığında z cetvel değeri (1.645)
N = Ana kitleye ait toplam birim sayısı (972)
d = Kabul edilebilir hata payı (d = X * 0.10) (d = 41.49 * 0.10 = 4.149 → d2 = 17.214)
X = İşletme başına ortalama arazi miktarı (41.49 da) %90 güven sınırında (z =1.645) ve ortalamadan %10 sapma ile örnek işletmelerin hacimleri belirlenmiştir.

Eşitlik 1'deki veriler dikkate alındığı zaman toplam anket sayısı 162 olarak bulunmuştur. Gerek anketör gerekse katılımcılardan kaynaklanabilecek hatalı anketlerin yerine ikame edilmek üzere %10 yedek anket yapılmış olup analizler 162 anket üzerinden yapılmıştır. 162 anketin 94 tanesi İslahiye, 68 tanesi ise Nurdağı ilçesindeki işletmelerle yapılmıştır.

Baharatlık kırmızıbiber üretim maliyet analizi

Tarımda veya diğer sektörlerde işletmeler her zaman farklı üretim faktörlerini bir araya getirerek, toplumun ihtiyaçlarını karşılamaya yarayacak biçim, nitelik ve miktarda ürün üretirler. Bu ürünler mal ve hizmet şeklinde olabilmektedir. Her işletmenin kendi faaliyet konusunu oluşturan mal ve hizmetleri elde edebilmek için harcadığı farklı üretim girdilerinin para ile ölçülen değerine, o ürünün maliyeti denilmektedir (Dağdemir, 2018).

Maliyet analizindeki veriler baharatlık kırmızıbiber için 1 dekar alana yapılan üretim masraflarını gösterecek şekilde düzenlenmiştir. Çalışmada elde edilen tüm veriler tartılı ortalamaları ifade edecek şekilde değerlendirilmiştir. Baharatlık kırmızıbiber üretiminde, biber tohumunu ekmek için toprak hazırlığının yapıldığı aşamadan başlayıp ürünün hasat edilip depolanmasından pazarlanmasına kadarki süreçte olan masrafların tamamı üretime yapılan maliyetleri ifade etmektedir.

Üretim masrafları sabit ve değişen masraflar olarak iki grupta incelenmiştir. Sabit masraflar, üretim miktarına bağlı olarak değişmeyen, diğer bir deyişle yapılsa da yapılmasa da ortaya çıkan masraflardır. Arazi kirası ve genel idare giderleri (değişen masrafların %3'ü) sabit masraf olarak dikkate alınmıştır (Kızıloğlu & Dağdemir, 2010). Değişen masraflar ise üretim faaliyetinin başlamasına olanak sağlayan, üretim hacmine bağlı olarak artan ya da azalan masraflardır. Değişen masraf kalemleri, işçilik (çapalama, gübreleme, ilaçlama, sulama, hasat) ve işlemler için gerekli olan üretim girdileri (tohum, gübre, ilaç) ile üretimde kullanılan makine çeki gücü masraflarının tamamı toplanmış ve döner sermaye faizinin yarısı da (%12/2) eklenerek değişen masraflar toplamı hesaplanmıştır. İşgücü ve çeki gücü masrafı hesaplanırken çalışmanın yapıldığı sahada göz önünde tutulan işgücü ücretleri ve makine çeki güçlerine ait kira bedelleri ele alınarak değerlendirilmiştir. Üretim

masrafları toplamının dekara verime bölünmesi ile 1 kg kırmızıbiber maliyeti hesaplanmıştır.

İşletmelerin aile işgücü; cinsiyet, yaş ve çalıştıkları süreler göz önünde tutularak hesaplanmıştır. Cinsiyet ve yaş farklılıkları dikkate alınarak mevcut olan işgücü EİB'ne çevrilip mevcut iş gücü varlığı bulunmuştur. EİB'ye çevrilen aile ve yabancı işgücü, üretim dönemi içerisindeki toplam çalışma süresi ve günlük işgücü ücretiyle çarpılarak işgücü gideri hesaplanmıştır (Dağdemir, 2005)

Baharatlık kırmızıbiber üretiminden elde edilen GSÜD, net kar ve brüt kar formülleri aşağıda belirtilmiştir.

GSÜD (kg da-1) = Verim * 1 Kg Kırmızıbiber Satış Fiyatı

Net Kar (₺ da-1) = GSÜD – Toplam Üretim Masrafları

Brüt Kar (₺ da-1) = GSÜD – Değişen Masraflar Toplamı

Pazarlama marjının analizi

Pazarlama marjı; tüketicinin üretilmiş olan herhangi bir ürünün bir kg'ı için ödediği fiyat ile aynı miktardaki ürüne benzer olan ilk ürün için çiftçinin eline geçen fiyat arasındaki fark olarak tanımlanabilir (Gülten, 1971; Dağdemir ve Aşkan, 2004).

Mutlak Pazarlama Marjı = $P_r - P_f$

P_f = 1 kg kırmızıbiber için üreticinin eline geçen fiyat

P_r = 1 kg kırmızıbibere tüketicinin ödediği fiyat

BULGULAR ve TARTIŞMA

Kırmızıbiber Üretiminde Sosyal Sermaye

İslahiye ve Nurdağı ilçesinde üretim yapan baharatlık kırmızıbiber üreticilerinin ankete cevap verenlerin tamamı erkek olup yaş ortalaması 50.35 yıl olarak bulunmuştur. Yaş dağılımına göre ise üreticilerin 35 yaş ve altı %10.49, 35 ile 55 yaş aralığında %51.85 ve 55 yaş üzerinde ise %37.65 oranında olduğu belirlenmiştir. Bölgede önceki yıllarda yapılan çalışmalar incelendiğinde son on yıllık süreçte üretici yaşının düzenli bir şekilde yükseldiği görülmekte olup 2010 yıllarının başında ortalama 45 olan üretici yaşı 2015 yılından sonra 47'ye yükselmiştir (Akbay ve ark., 2012; Boz, 2013; Aytop, 2018). Bölgede üreticilerin yaş ortalamasının yükseldiği görülmektedir. Bu durumun ülkede uzayan insan ömrü ve gençlerin tarımsal faaliyetlerden uzaklaşması ile ilişkili olduğu düşünülmektedir.

Üreticilerin eğitim düzeyleri incelendiğinde %19.14'ü okuryazar, %16.05'i ilkokul, %19.14'ü ortaokul, %41,98'i lise ve %3,70'inin ise üniversite mezunu olduğu tespit edilmiştir. Üreticilerin %93.83'ünün sosyal güvencesinin olduğu, %6.17'sinin ise olmadığı belirlenmiştir. Üreticilerin %42.59'unun tarım dışı faaliyetlerde de bulundukları, %57.41'ininde sadece tarımsal faaliyette bulundukları tespit edilmiştir.

Kırmızıbiber Üretiminde Arazi Sermayesi

İşleteme arazisi olarak çiftçiler tarafından işlenen toplam tarım arazisi; mülk arazi, kiraya ve ortağa tutulan arazilerin toplamından oluşmaktadır. Ortağa verilen arazi bulunmamaktadır. Arazi tasarruf şekli Çizelge 1'de verilmiştir.

Çizelge 1. Baharatlık Kırmızıbiber Üreten İşletmelerin Arazi Tasarruf Şekli
Table 1. Land Savings of Enterprises Producing Spicy Red Pepper

Arazi Tasarruf Şekli	İşletme Başına Ortalama Arazi Varlığı (da)
Kiraya Tutulan	8.37
Ortağa Tutulan	5.89
Mülk Arazisi	85.31
Toplam İşlenen Arazi Varlığı	**99.57**

Araştırma sonuçlarına göre işletmelerin ortalama arazi genişliği 99.57 dekardır. Toplam işlenen arazi varlığının 45.45 dekarında kırmızıbiber üretimi yapılmaktadır. İşletmelerde ortakçılık çok kullanılmamakta olup ortakçılık yapan işletme sahiplerinin de genellikle akrabalar arasında olduğu gözlemlenmiştir. Kiraya tutulan arazi ortağa tutulan arazi oranından daha fazla tercih edilmekte olduğu tespit edilmiştir. Üreticilerin arazileri genel olarak nadasa bırakılmayıp, toprağı hem dinlendirecek hem de zenginleştirecek şekilde değerlendirme yapıp kullandıkları tespit edilmiş ve kullanılan arazilerin tamamının sulu olduğu bildirilmiştir.

Bölgede yapılan benzer çalışmalarda elde edilen mülkiyet yapısının geçen sürede değişmediği ve üreticilerin arazi kullanımlarının aynı şekilde devam ettiği görülmektedir. (Akbay ve ark., 2012; Aytop, 2018). Çalışma kapsamında 200 dekar arazinin üst sınır olarak kabul edilmesi nedeni ile ortalama kullanılan arazi miktarı önceki çalışmaların biraz altında bulunmuştur.

Kırmızıbiber Üretim Masrafları ve Maliyet

Baharatlık kırmızıbiber üretiminde dekara yapılan üretim masrafları incelendiğinde üretim masraflarının %80.47'ini değişen masrafların, %19.53'ünü sabit masrafların oluşturduğu tespit edilmiştir. Değişen masraflar içerisinde en fazla paya

işgücü ve materyal masrafları sahipken en az paya ise çeki gücü masraflarının sahip olduğu tespit edilmiştir. Sabit masraflar içerisinde yer alan arazi kirası üretim masraflarının %17.12'sini oluşturmaktadır (Çizelge 2)

Çizelge 2. Birim Alana Üretim Masrafları ve Dağılımı
Table 2. Production Costs and Distribution per Unit Area

	Değer (₺ da^{-1})	Oran (%)
Değişen Masraflar Toplamı	**1788.20**	**80.47**
İşgücü Masrafı	*785.30*	*35.34*
Çeki Gücü Masrafı	*252.70*	*11.37*
Materyal Masrafı	*649.00*	*29.20*
Döner Sermaye Masrafı	*101.20*	*4.55*
Sabit Masraflar Toplamı	**434.05**	**19.53**
Genel İdare Gideri	*53.65*	*2.41*
Arazi Kirası	*380.40*	*17.12*
Üretim Masrafları Toplamı	**2222.25**	**100.00**

Biber yetiştiriciliğinde değişen masrafların toplam masraflardaki oranı farklı çalışmalar incelendiğinde, Bayramoğlu ve ark. (2021) %73.73, Başaran ve Engindeniz (2015) %82.39, Ukay (2018) %84.07 ve Aytop (2018) tarafından %86.71 oranında olduğu belirlenmişlerdir. Dünyada en önemli kırmızıbiber üreten ülkelerden olan Hindistan'da Navyasri ve ark. (2021) tarafından yapılan çalışmada konvansiyonel biber yetiştiriciliğinde değişen masraflar %73.00 olarak belirlenmiştir. Değişen masraflar içerisinde işgücü masrafı gerek Türkiye gerekse de diğer ülkelerde yapılan çalışmalarda en önemli masraf unsuru olarak ortaya çıkmıştır. Bu durum biber yetiştiriciliğinin emek yoğun bir üretim dalı olduğu sonucunu ortaya çıkarmaktadır.

Bir dekar baharatlık kırmızıbiber üreten çiftçinin tarladaki ürünü için yapmış olduğu toplam üretim masrafı 2222.25 ₺ da^{-1} olarak hesaplanmıştır. Baharatlık kırmızıbiber verimi 1656.80 kg da^{-1} olarak bulunmuştur. 1 kg baharatlık kırmızıbiber üretim maliyeti bölge ortalamasında 1.34 ₺, pazar maliyeti 1.37 ₺ olarak hesaplanmış olup satış fiyatı 2.30 ₺'dir (Çizelge 3).

Candemir ve ark. (2012) tarafından yapılan çalışmada organik kırmızıbiber veriminin 1300 kg da^{-1}, üretim maliyetinin 1.27 ₺ kg^{-1}, üretici eline geçen ortalama fiyatının 1.34 ₺ kg^{-1} olduğu tespit edilmiştir.

Aytop (2018) tarafından yapılan çalışmaya göre de kırmızıbiber üretiminde değişken masraflar toplamının 1671.94 ₺ da^{-1}, sabit masraflar toplamının 255.70 ₺ da^{-1}, üretim masrafları toplamının ise 1927.64 ₺ da^{-1} olduğu belirtilmiştir. Kırmızıbiber üretiminde elde edilen ortalama verim 1558.01 kg da^{-1} olarak bulunmuştur. Ürün ortalama 1.56 ₺ kg^{-1}'a satılırken, ürünün birim maliyetinin 1.24 ₺ kg^{-1} olduğu tespit edilmiştir.

Kırmızıbiber Üretim ve Pazarlamasında Oransal Kar, Brüt Kar ve Net Kar

Bir dekar baharatlık kırmızıbiber üretiminden ortalama 3810.64 ₺ da^{-1} gayrisafi üretim değeri sağlanırken 1588.39 ₺ da^{-1} net kar, 2022.44 ₺ da^{-1} da brüt kar elde edildiği belirlenmiştir. Üreticilerin üretim için yaptığı 1 ₺'lik masraf karşılığında sağlanan kar, yani üretim maliyetine göre oransal kar ortalama 1.72 olarak hesaplanmıştır (Çizelge 4).

Baharatlık kırmızıbiber pazar toplam masrafı 2277.65 ₺ da^{-1}'dır. 1 Kg baharatlık kırmızıbiber pazar maliyeti ise 1.37 ₺ olarak hesaplanmıştır. Pazar masrafları toplamı dikkate alındığında 1 dekardan sağlanan net kar 1532.76 ₺, brüt kar ise 1967.04 ₺'dir. Pazar maliyetine göre oransal karın ise 1.68 olduğu tespit edilmiştir. Araştırma bölgesinde baharatlık kırmızıbiber üreticisi için söz konusu ürünü üretmenin karlı bir tarımsal faaliyet olduğu anlaşılmaktadır (Çizelge 4).

Candemir ve ark. (2012) tarafından yapılan çalışmada organik kırmızıbiberden elde edilen kar marjının 0.07 ₺ kg^{-1} olduğu tespit edilmiştir. Aytop (2018) tarafından yapılan çalışmaya göre kırmızıbiberden sağlanan kar marjı 0.32, gayrisafi üretim değeri 2430.50 ₺ da^{-1}, brüt kar ise 758.56 ₺ olarak tespit edilmiştir. Kırmızıbiber üretiminde oransal kar 1.26 olarak belirlenmiştir.

Baharatlık Kırmızıbiberin Pazarlama Yapısı

Kırmızıbiber yaygın olarak üretimi yapılan ve çiftçiler için önemli bir geçim kaynağı haline gelen bir bitkidir. Gaziantep'teki biber yetiştiriciliği özellikle kuru kırmızıbiber üretimi için yapılmakta, baharat ürünü olan pul ve toz biber üretiminde kullanılmaktadır. Pazara uygunluk bakımından da kırmızıbiber önemli bir yere sahiptir. Kırmızıbiber, iç pazar için gerekli olduğu kadar, yabancı ülkelere yapılan dış satım potansiyeli açısından da ekonomik bir öneme sahiptir.

Kırmızıbiber üreticilerinin ürünlerini ya direk işleme tesisleri olan fabrikalara ya da toplayıcı tüccarlara satmakta olduğu tespit edilmiştir. İşleme tesisleri ise baharatlık kırmızıbiberleri gerekli işlemler sonucunda toz veya pul biber olarak doğrudan toptancı firmalara pazarlamakta ya da ihracatçı firmalar aracılığıyla dış piyasaya satışı yapmaktadırlar.

Çizelge 3. Baharatlık Kırmızıbiberin Üretim ve Pazar Maliyetinin Hesabı (₺ da^{-1})
Table 3. Calculation of Production and Market Cost of Spicy Red Pepper (₺ da^{-1})

Üretim İşlemleri	İşgücü		Çekigücü		Kullanılan Ekipman	Kullanılan Materyal			Masraflar Toplamı (₺)	Yüzde (%)
	Saat	Tutar (₺)	Saat	Tutar (₺)		Cinsi	Miktar (kg)	Tutar (₺)		
1- Toprak Hazırlığı										
a) 1. Sürüm (Ekim)	0.40		0.40	40.00	Pulluk				40.00	1.80
b) 2. Sürüm (Kasım-Aralık)	0.32		0.32	32.00	Pulluk				32.00	1.44
c) 3. Sürüm	0.23		0.23	24.00					24.00	1.08
d) Ekim (Mart)	0.15		0.15	24.50	Mibzer	Tohum	1.4 kg	24.60	49.10	2.21
2- Bakım										
a) Çapalama (Mayıs-Haziran)	8.00	35.50			Çapa		3-4 defa		35.50	1.60
b) Gübreleme (Mart-Haziran-Temmuz)	2.90		2.90	47.30	Gübre Dağıtıcı	Kim. Gübre Hay. Gübre	95.2 kg 2875 kg	 202.30	369.70	16.63
c) İlaçlama (Mart-Haz.-Temmuz)	1.50		1.50	29.50	Holder	Corojen vb	4 defa	36.40	65.90	2.97
d) Sulama (Mayıs'tan Eylül'e)	46.30	227.00					6-8 defa	231.20	458.20	20.62
3- Hasat (Toplama)										
a) Hasat (Ağustos-Eylül-Ekim)	88.70	522.80			Elle				522.80	23.52
b) Taşıma (Ağustos-Eylül-Ekim)	1.18	1.18		55.40	Römork				55.40	2.49
c) Diğer masraflar (Ağustos-Eylül-Ekim)					Çuval, ip vb			34.40	34.40	1.55
4- Ara Toplam (1+2+3)									***1687.20***	***75.92***
5- Döner Sermaye Faizi (%12/2 = 0.06)									101.20	4.55
6- Değişken Masrafların Toplamı (4+5)									**1788.20**	**80.47**
7- Genel İdare Giderleri (%3)									53.65	2.41
8- Arazi Kirası									380.40	17.12
9- Sabit Masraflar Toplamı (7+8)									**434.05**	**19.53**
10- Üretim Masrafları Toplamı (6+9)									**2222.25**	**100.00**
11- Baharatlık Kırmızıbiber Verimi (kg da^{-1})									1656.80	
12- 1 Kg Baharatlık Kırmızıbiberin üretim Maliyeti (10/11) (₺ kg^{-1})									**1.34**	
13- Kırmızıbiber Taşıma Masrafı									55.40	
14- Kırmızıbiber Pazar Masrafları Toplamı (10+13)									2277.65	
15- 1 Kg Kırmızıbiber Pazar Maliyeti (14/11) (₺ kg^{-1})									**1.37**	
16- 1 Kg Baharatlık Kırmızıbiberin Satış Fiyatı (₺ kg^{-1})									**2.30**	

Çizelge **4.** Kırmızıbiber Üretim ve Pazar Maliyetine Göre Oransal, Brüt ve Net Kar

Table 4. Proportional, Gross and Net Profit According to Red Pepper Production and Market Cost

Değişken Masraflar Toplamı (₺ da^{-1})	(1)	1788.20
Üretim Masrafları Toplamı (₺ da^{-1})	(2)	2222.25
Yaş Kırmızıbiber Verimi (kg da^{-1})	(3)	1656.80
1 Kg Kırmızıbiber Üretim Maliyeti (₺ kg^{-1})	**(4=2/3)**	**1.34**
1 Kg Yaş Kırmızıbiber Satış Fiyatı (₺ kg^{-1})	(5)	2.30
Üretim Maliyetine Göre Oransal Kar	**(6=5/4)**	**1.72**
Gayrisafi Üretim Değeri (₺ da^{-1})	(7=3*5)	3810.64
Üretim Maliyetine Göre Net Kar (₺ da^{-1})	(8=7-2)	1588.39
Üretim Maliyetine Göre Brüt Kar (₺ da^{-1})	(9=7-1)	2022.44
Kırmızıbiber Taşıma Masrafı (₺ da^{-1})	(10)	55.40
1 Kg Kırmızıbiber Taşıma Maliyeti (₺ kg^{-1})	(11=10/3)	0.03
Pazardaki Toplam Masraf (₺ da^{-1})	(12=2+10)	2277.65
1 Kg Kırmızıbiber Pazar Maliyeti (₺ kg^{-1})	**(13=12/3)**	**1.37**
Pazar Maliyetine Göre Oransal Kar	**(14=5/13)**	**1.68**
Pazar Maliyetine Göre Net Kar (₺ da^{-1})	(15=7-12)	1532.99
Pazar Maliyetine Göre Brüt Kar (₺ da^{-1})	(16=7-(1+10))	1967.04

Anketlerden elde edilen verilere göre üreticilerin %88.27'si yaş-kuru kırmızıbiberleri fabrikaya, %11.73'ü ise toptancı-tüccara sattıkları tespit edilmiştir.

Aytop (2018) tarafından yapılan araştırmaya göre üreticilerin %79.41'i kırmızıbiberi fabrikalara, %20.51'i toptancı/tüccara sattıklarını, %9.62'si ise kendilerinin pazarladıklarını bildirmişlerdir.

Baharatlık Kırmızıbiberin Pazarlama Marjı

Anketlerden elde edilen verilere göre ortalama olarak 5 kg yaş kırmızıbiberden 1 kg kuru kırmızıbiber elde edildiği tespit edilmiştir. Yaş kırmızıbiberin satış fiyatı 2.30 ₺ kg^{-1} olarak bulunmuştur. Bu hesaba göre 1 kg kuru kırmızıbiber için üretici eline 11.50 ₺ (5 kg * 2.30 ₺) geçmektedir.

Kuru kırmızıbiber ortalama toptan satış fiyatı 45.00 ₺ kg^{-1}, ortalama perakende satış fiyatı ise 65.00 ₺ kg^{-1} olarak belirlenmiştir. Perakende satış fiyatına göre pazarlama marjı 53.50 (65.00 -11.50) ₺ kg^{-1} olarak hesaplanmıştır. Tüketicinin 1 kg kuru kırmızıbibere ödediği paranın %17,69'u üretici eline geçerken, %82.31'i aracılar eline geçmektedir.

Pazarlama marjının endüstri ürünlerinde ve özellikle ihracata konu olan geleneksel gıdaların üretiminde ürünlerin işleme ile farklılaşması nedeni ile yüksek olduğu öngörülmektedir (Aslan 2022). Kırmızıbiber üretiminde işleme ve ürün farklılaşmalarından dolayı marj yüksek olarak değerlendirilmektedir.

Pazarlamada Karşılaşılan Sorunlar

Pazarlama yapılırken üreticiler farklı sorunlarla karşı karşıya kalmaktadır. Üreticilerin %99.38'inin ürünü pazarlama konusunda yeterli pazarın olmadığının önemli olduğunu, %0.62'si de az önemli olduğunu ve %100'ünün de pazarın güvenilir olmamasının önemli ve gerekli olduğunu savundukları belirlenmiştir. Üretim miktarının az olmasını üreticilerin %93.21 önemli, %5.56'sı az önemli, %1.23'ü de hiç önemli olmadığını ayrıca üreticilerin %86.42'si pazarlamada rekabet gücünün zayıf olduğunun önemli, %9.88'nin az önemli, %3.70'inin de önemli olmadığını savundukları tespit edilmiştir (Çizelge 5).

Üretilen ürünlerin hasattan sonra yapılan kurutma işlemlerini yapılırken gerekli tedbirlerin alınmamasından oluşan küf sorunlarının ve depolamada yanlış kullanılan materyallerden dolayı kalite sorunlarının meydana geldiği üreticiler tarafından ifade edilmiştir. Kalite standartlarının düşük olması üreticilerin %75.92'si tarafından önemli olduğu, %14.20'si az önemi %9.88'i ise önemli olmadığını ifade etmişlerdir. Üreticilerin pazarlamada karşılaştıkları önemli sorunlardan biride ürün satış fiyatlarının düşük olması olup bu sorun tüketicilerin %96.91'i tarafından önemli görülmekte, %3.09'u tarafından ise az önemli olduğu belirtilmiştir. Biberde desteklemenin olmaması biber üreticilerini üretim konusunda teşvik ve destek amacıyla olmasının önemli olduğunu üreticilerin %76.54'ü savunmakta, %16.05'i az önemli olduğunu, %7.41'i ise önemli olmadığını savunduğu tespit edilmiştir. Pazarlamada aracıların çok olması üreticilerin %91.98'i tarafından önemli bir sorun olduğu, %6.79'u bu sorunun az önemli olduğunu, %1.23'ü önemli olmadığını savunduğu belirlenmiştir. Aracıların ürünü ucuza aldıklarını da bir sorun olarak belirten üreticilerin %96.91'i önemli sorun olduğunu, %3.09'u az önemli olduğunu belirtmişlerdir. Diğer önemli sorunlardan biride pazarın uzak olma durumudur ve bu sorunun önemli olduğunu belirten üreticilerin %95.68'i, az önemli olduğunu %3.09'u, üreticilerin %1.23'ü sorun olmadığını belirtmiştir. Ayrıca nakliye masraflarının da önemli bir sorun olduğunu %98.77 önemli, %1.23'ü ise sorun olmadığını savunmuşlardır.

Çizelge 5. Pazarlamada Karşılaşılan Sorunlar (%)
Table 5. Problems Encountered in Marketing (%)

Pazarlamada Karşılaşılan Sorunlar	Önemli	Az önemli	Hiç önemli değil
Yeterli pazarın olmaması	99.38	0.62	0.00
Pazarın güvenilir olmaması	100.00	0.00	0.00
Üretim miktarının az olması	93.21	5.56	1.23
Rekabet gücünün zayıf olması	86.42	9.88	3.70
Kalite standartlarının düşük olması	75.92	14.20	9.88
Ürün satış fiyatlarının düşük olması	96.91	3.09	0.00
Biberde destekleme olmaması	76.54	16.05	7.41
Aracıların çok olması	91.98	6.79	1.23
Aracıların ürünü ucuza alması	96.91	3.09	0.00
Pazarın çok uzak olması	95.68	3.09	1.23
Nakliye masraflarının yüksek olması	98.77	1.23	0.00

Ürün işleme ve değerlendirme açısından yapılabilecek bir yönetim ve işletmecilik anlayışı hem bölge ekonomisine hem de ülke ekonomisine katkı sağlayacaktır. Ayrıca biber kurutma işlemlerinde yaşanılan aflatoksin olayının pazarlamada büyük sorun oluşturduğu gözlemlenmiştir. Bölgede yapılan diğer çalışmalar ile (Akbay ve ark., 2012; Aytop, 2018) benzer sorunların yıllar içerisinde devam ettiği görülmüştür.

SONUÇ ve ÖNERİLER

Gaziantep ilinde baharatlık kırmızıbiber üreten işletmecilerin son on yıllık süreçte ortalama yaşlarının yükseldiği, arazi kullanım yapısının değişmediği belirlenmiştir. Üretici ortalama yaşının sürekli ilerlemesinin genç nüfusun tarıma yönlendirilmesi ile durdurulması sürdürülebilirlik açısından önemlidir. Ayrıca baharatlık kırmızıbiber üretiminin sürdürülebilir olması için üreticinin pazardan aldığı pay arttırılmalıdır. Kurutma işlemlerini daha hijyenik ve daha gelişmiş modern ortamlarda yapılmasının pazarlamada kolaylık sağlayacağı düşünülmektedir. Bu konuda gerekli tedbir ve önemler yetkililer tarafından alınmalıdır.

Reklam ve tanıtımlar arttırılması ile iç ve dış piyasa satışlarının kolay bir şekilde yapılmasına yardımcı olunabilecektir. Baharatlık kırmızıbiberin kullanım alanları detaylı bir şekilde televizyon, radyo vb. araç gereçlerle anlatılarak halkın bu konu hakkında bilgilendirilmeleri önerilebilir. Böylece hem tüketimde hem de pazarlamada artış sağlayacağı düşünülmektedir. Üreticilerin ürünlerini kolay şekilde pazara sunabilecekleri güvenilir alanlar tahsis edilmelidir. Ayrıca pazarlama konusunda meydana gelen fiyatlardaki dalgalanmalarda oluşan sorunlar çözülerek üreticilerin zarar etmeyeceği şekilde fiyatların belirlenmesi için gerekli plan yapılıp kararlar alınmalıdır. Yapılacak teşvikler ve desteklemelerle Gaziantep ve Türkiye genelinde üretimin payı, verimi, ekilen alanların ve ihracatın artış sağlayacağı öngörülmektedir.

TEŞEKKÜR [Century10 bold]

Bu araştırma makalesi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü tarafından onaylanmış Yüksek Lisans Tezinden türetilmiştir.

Araştırmacıların Katkı Oranı Beyan Özeti [Century10 bold]

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı [Century10 bold]

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

Akbay C, Boz İ, Tiryaki GY, Candemir S & Arpacı BB (2012). Kahramanmaraş ve Gaziantep İllerinde Kırmızıbiberin Üretim Yapısı ve Kurutma Yöntemleri. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 15(2), 1-10,* Kahramanmaraş.

Aslan A (2022). *Malatya İlinde Kuru Kayısı Üretim ve Pazarlama Etkinliğinin Belirlenmesi* (Tez no:775470). Doktora Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Ana Bilim Dalı. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Anonim (2002). Coğrafi İşaret Belgesi, Türk Patent Enstitüsü. https://ci.turkpatent.gov.tr/cografi-isaretler/detay/37908. (Alınma Tarihi:15.09.2020)

Aytop Y (2018). *Maraş Biberi'nin Ekonomik Analizi ve Üretici Memnuniyeti* (Tez no:493775). Doktora Tezi, Kahramanmaraş Sütçü İmam Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Ana Bilim Dalı Yükseköğretim Kurulu Ulusal Tez Merkezi. .

Başaran C & Engindeniz S (2015). Sivri Biber Üretiminde Girdi Kullanım Etkinliğinin Analizi:

İzmir Örneği. Tarım Ekonomisi Dergisi, 21(1), 77-84.

Bayramoğlu Z, Karakayacı Z, Ağızan K, Ağızan S & Bozdemir M (2021). *Başlıca sebze ürünlerinde üretim maliyetlerini etkileyen faktörlerin belirlenmesi. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 24*(3), *603-613.*

Beis SH 1990. *Kırmızıbiberden Gıda Boyası Eldes*i (Tez no:13285). Yüksek Lisans Tezi, Anadolu Üniversitesi Sağlık Bilimleri Enstitüsü Eczacılık ve Farmakoloji Ana Bilim Dalı. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Boz İ (2013). Doğu Akdeniz Bölgesi'nde Süt Sığırcılığı Yapan İşletmelerin Yapısı, Sorunları ve Çözüm Önerileri, *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 16(1), 24-32.*

Candemir S, Arpacı BB & Akıncı İE (2012). Organik Kırmızıbiber Üretiminde Uygulanan Üretim Sistemlerinin Ekonomik Performanslarının Karşılaştırılması. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 15(1), 1-6.*

Çitak L (2015). Pazarlama Yatırımlarının Finansal Performans Üzerindeki Etkisi ve Borsa İstanbul KOBİ Sanayi Endeksi Firmalarının Etkinliklerinin Değerlendirilmesi. *Erciyes Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 45*, 49-68. https://doi.org/10.18070/euiibfd.25074

Çoksöyler N (1995). Aflatoksin ve Aflatoksin Oluşumunu Etkileyen Faktörler. Kahramanmaraş Kırmızıbiberinde İhracata Yönelik Kaliteli Yetiştirme, İşleme ve Pazarlamada Karşılaşılan Sorunlara Çözüm Arayışları Paneli Kahramanmaraş Sütçü İmam Üniversitesi Rektörlüğü Yayınları, (11):18-25.

Dağdemir V & Aşkan E (2004). Erzurum merkez ilçede bir ekmeğin pazarlama marjının belirlenmesi üzerine bir çalışma. VI. Tarım Ekonomisi Kongresi, 16-18 Eylül, Tokat, 384–402.

Dağdemir V (2005). Bayburt İli Kop ve Burnaz Dere Havzalarında Hayvancılık Yapan İşletmelerin Genel Durumu ve Kooperatifleşmeye Bakış Açısı. *Türk Kooperatifçilik Kurumu, Üçüncü Sektör Kooperatifçilik, 147*, *48-58.*,

Dağdemir V (2018). "Tarım İşletmelerinin Analizi" Lisansüstü Ders Notları.

FAO (2020). FAOSTAT. Baharatlık Kırmızıbiberin Üretim, Üretim Alanları, İhracat, İthalat Verileri.

Gülten Ş (1971). Erzurum ilinde canlı hayvan ve et üretim, tüketim ve pazarlama analizleri. Atatürk Üniversitesi Yayın No:284, Ziraat Fakültesi No:141, Araştırma No:79, Erzurum.

Dessie AB, Koye TD, Koye AD & Abitew AA (*2019). Analysis of red pepper marketing: evidence from Northwest Ethiopia. Journal* of Economic Structures*, 8(24), 1-14.* https://doi.org/10.1186/s40008-019-0156-0

Hayran S & Gül A (2020). Mersin İlinde bazı önemli sebzelerin karlılığı. *Ziraat Mühendisliği, 370, 45-53.* https://doi.org/10.33724/zm.708295

Hastuti H & Sari AW (2020). Analisis pemasaran komoditas cabai rawit merah di desa brajan, kecamatan prambanan, kabupaten klaten. *Jurnal Ilmu Pertanian Indonesia, 25(4), 524-532.* https://doi.org/10.18343/10.18343/jipi.25.4.524

Ilić ZS, Milenković L, Šunić L, Barać S, Mastilović J, Kevrešan Ž & Fallik E (2017). Effect of shading by colored nets on yield and fruit quality of sweet pepper. *Zemdirbyste-Agriculture, 104(1), 53-62.* Doı: 10.13080/z-a.2017.104.008

Kızıloğlu R & Dağdemir V (2010). Erzurum İlinde Beyaz Lahananın Üretim ve Pazar Maliyeti; Aziziye, Yakutiye ve Pasinler İlçeleri Örneği. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü. Dergisi, 2(2), 57-64.*

Navyasri K, Srilatha C, Suhasini K & Janaiah A (2021). An economic analysis of different methods of irrigation systems in red chili cultivation in Bhadradri Kothagudem district of Telangana state. *The Pharma Innovation, 10(10), 1378-1383.*

Perucka I & Materska M (2001). Phenylalanine Ammonia-Lyase and Antioxidant Activities of Lipophilic Fraction of Fresh Pepper Fruits Capsicum Annuum L. *Innovative Food Science& Emerging Technologies, 2(3), 189-192.*

Telek Ü, Akinci I & Küsek M (2019). The effects of rhizobacteria strains on yield and plant characteristics of red hot pepper (capsicum annuum l.). *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22*(1), *62-70.* https://doi.org/10.18016/ksutarimdoga.vi.448536.

TÜİK (2020). Türkiye İstatistik Kurumu, Bitkisel Üretim İstatistikleri, https://biruni.tuik.gov.tr.

Ukav İ (2018). A Research on the Production Costs and Profitability of Some Vegetables Produced in Adıyaman Province. *Turkish Journal of Agriculture-Food Science and Technology 6 (9), 1285-1289*

Verit A, Yeni E & Ünal D (2001). Tarihten Günümüz Ürolojisine Kırmızı Acı Biber. *Türk Üroloji Dergisi, 27 (4), 399-402.*

KSÜ Tarım ve Doğa Derg 27 (4), 967-972, 2024
KSU J. Agric Nat 27 (4), 967-972, 2024
DOI:10.18016/ksutarimdoga.vi.1368022

COVID-19 The Impact of the Pandemic on Farmers' Use of the Internet for Agricultural Issues

Ahmet Yesevi KOÇYİĞİT[1]✍, Kürşat DEMİRYÜREK[2]
[1]Department of Agricultural Economics, Faculty of Agriculture, Ondokuz Mayıs University, Atakum, Samsun, Türkiye. [2]Department of Agricultural Economics, Faculty of Agriculture, Ondokuz Mayıs University, Atakum, Samsun, Türkiye
[1]https://orcid.org/0000-0002-6193-9957, [2]https://orcid.org/0000-0002-6193-9957
✉: ahmetyesevi.kocyigit@omu.edu.tr

ABSTRACT
To assess the impact of COVID-19 on farmers' use of the internet for agricultural issues. A questionnaire was used to collect data from 188 farmers in Samsun province. For the interpretation of the collected data, a Paired Sample t-test was used using Python programming language. Significant differences were found in the number of mobile gigabytes (GB) owned by farmers, the frequency of attending online agricultural training, the frequency of visiting the websites of the Ministry of Agriculture and private companies, the frequency of searching agricultural issues on the Internet, and the frequency of checking product and input prices on the Internet compared to before the COVID-19 pandemic. On the other hand, there is no significant difference in the frequency of using the Digital Agricultural Market (ditap.gov.tr) website, conducting marketing research on the internet and e-commerce compared to before the COVID-19 pandemic. The study results suggest that challenging crises such as COVID-19 can accelerate farmers' adoption of innovations. The study helps to understand the impacts of crises such as COVID-19 on the diffusion of information. Understanding these changes can be useful for policymakers and sector leaders to develop strategies to support farmers and ensure the continued success of the agricultural sector during and after the pandemic.

Agricultural Economics

Research Article

Article History
Received : 29.09.2023
Accepted : 23.11.2023

Keywords
COVID-19
Internet Use of Farmers
Diffisuon of Innovation
Agricultural Extension

KOVİD-19 Salgının Çiftçilerin Tarımsal Konularda İnternet Kullanımı Üzerindeki Etkisi

ÖZET
KOVİD-19'un çiftçilerin tarımsal konularda internet kullanımı üzerindeki etkisini değerlendirmektir. Samsun ilindeki 188 çiftçiden veri toplamak için bir anket kullanılmıştır. Toplanan verilerin anlamlandırılması için Python programlama dili kullanılarak İlişkili Örneklem t Testi'nden yararlanılmıştır. Çiftçilerin sahip olduğu mobil gigabayt (GB) miktarı, çevrimiçi tarımsal eğitimlere katılma sıklığı, Tarım Bakanlığı ve özel şirketlerin web sitelerini ziyaret etme sıklığı, internette tarımsal konularda arama yapma sıklığı, internette ürün ve girdi fiyatlarını kontrol etme sıklıklarında KOVİD-19 pandemisi öncesine göre anlamlı farklılıklar tespit edilmiştir. Öte yandan, Dijital Tarım Marketi (ditap.gov.tr) web sitesini kullanma sıklığı, internette pazarlama araştırması yapma ve e-ticaret yapma sıklıklarında KOVİD-19 pandemisi öncesine göre anlamlı bir fark bulunmamaktadır. Çalışma sonuçları, COVID-19 gibi zorlu krizlerin çiftçilerin yenilikleri benimsemesini hızlandırabileceğini ortaya koymaktadır. Çalışma, KOVİD-19 gibi krizlerin bilginin yayılımı üzerindeki etkilerinin anlaşılmasına yardımcı olmaktadır. Bu değişikliklerin anlaşılması, politika yapıcıların ve sektör liderlerinin çiftçileri desteklemek ve tarım sektörünün pandemi sırasında ve sonrasında başarısını sürdürmesini sağlamak için stratejiler geliştirmeleri açısından faydalı olabilir.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 29.09.2023
Kabul Tarihi : 23.11.2023

Anahtar Kelimeler
KOVİD-19
Çiftçilerin İnternet Kullanımı
Yeniliklerin Benimsenmesi
Tarımsal Yayım

Atıf Şekli: Koçyiğit, A. Y., & Demiryürek, K., (2024) KOVİD-19 Salgının Çiftçilerin Tarımsal Konularda İnternet Kullanımı Üzerindeki Etkisi. *KSÜ Tarım ve Doğa Derg 27* (4), 967-972. https://doi.org/10.18016/ksutarimdoga.vi.1368022

To Cite : Kocyigit, A. Y., & Demiryurek, K., (2024). COVID-19 The Impact of the Pandemic on Farmers' Use of the Internet for Agricultural Issues. *KSU J. Agric Nat 27* (4), 967-972. https://doi.org/10.18016/ksutarimdoga.vi.1368022

INTRODUCTION

The COVID-19 pandemic has deeply affected the society in almost every aspect (Bostan et al., 2020; Lone & Ahmad, 2020; Phelps & Sperry, 2020; Talevi et al., 2020; Suryasa et al., 2021) and severely affected the agricultural sector (Arumugam & Kanagavalli, 2020; Dev, 2020; Gray, 2020; Siche, 2020; Beckman & Countryman, 2021; Demiryürek et al., 2021). While the COVID-19 pandemic has deeply shaken the agricultural sector, it has also drastically changed the social lives of farmers. It is thought that farmers' use of the Internet for agricultural issues may be among these changes.

The Internet has long been used as a valuable and useful tool for farmers (Just & Just, 2001; Rolfe et al., 2003; Yurtlu et al., 2012; Fernando et al., 2016; Alao et al., 2021). Using the Internet, farmers can better manage the agricultural production process with decision support applications in agricultural production (Rose et al., 2016; Demir et al., 2021), learn weather forecasts in detail (Ray, 2017), access information on market prices more easily (Kızılaslan & Gönültaş, 2011), and share information faster (Yan et al., 2016). Internet use in agriculture also enables farmers to better communicate with other farmers and experts in the field.

It is a fact seen in the literature that the problems experienced by farmers in accessing the internet are not common and that they generally have access to the Internet (Just and Just, 2001; Panda et al., 2019; Derya & Demiryurek, 2020). However, the same literature review on farmers' use of the Internet for agricultural purposes shows that farmers' use of the Internet for agricultural purposes is not common enough (Smith et al., 2004; Erdal & Çallı, 2013). It is also known that with the COVID-19 pandemic, social life has been greatly restricted worldwide, and accordingly, the frequency of people's internet use has increased. (Branscombe, 2020; Pandey & Pal, 2020; Sun et al., 2020; Huang et al., 2021). It is also in the literature that farmers use digital communication tools (Erjavec et al. 2021) and the Internet more frequently for sales (Godrich et al., 2022).

In the literature searches, no study was found to examine the effects of the COVID-19 pandemic on farmers' use of the internet for agricultural issues. Therefore, this study aims to fill this gap in the literature.

The main objective of this study is to reveal the impact of COVID-19 on farmers' use of the internet for agricultural issues. In this context, the main hypothesis of the study is that the COVID-19 pandemic has a significant impact on farmers' use of the Internet for agricultural issues. In addition, this study will also help to understand the impact of crises such as COVID-19 on the diffusion of information in rural areas.

METHODOLOGY

The main material of the study consists of primary data obtained from the survey conducted with farmers (face-to-face, online, or both) in Samsun province. In Samsun province, 188 farmers were interviewed. Districts that can reflect Samsun province were selected for the purpose. While selecting the districts for the purpose, time, personnel, budget constraints as well as the vital threat posed by the COVID-19 pandemic and the bans imposed due to COVID-19 disease were taken into consideration. In this context, the survey was completed with a total of 188 farmers, 81 (43.1%) in Bafra, 52 (27.6%) in Çarşamba, 29 (15.4%) in Tekkeköy, 15 (8%) in Alaçam, 10 (5.3%) in Terme and 1 (0.5%) in Atakum.

Statistical Analysis

To test the difference in the internet usage status of farmers before and after the COVID-19 outbreak, a Paired Sample t-test was conducted through the SPSS Program.

To determine the level of internet use of farmers before and after the COVID-19 pandemic, 13 statements were asked of farmers. Farmers were asked to answer each statement as Disagree=1, Neutral=2, or Agree=3. Farmers could score at least 1 and at most 3 points for each statement. The score for each statement was summed and a score was created for internet use in the agricultural sector. The scores were averaged and a Paired Sample t-test was applied.

Limits and Permissions

Researchers faced many travel restrictions during the data collection period, such as curfews or bans on travel by private vehicle. In addition, several bans were also imposed on farmers, the target audience for data collection, but the government was more flexible towards farmers.

As the impact of the COVID-19 pandemic intensified, the Ministry of Health required all researchers to obtain permission to study COVID-19. According to this requirement, since the thesis topic involved COVID-19, an application was submitted to the Ministry of Health and the necessary permits were obtained for all researchers.

Apart from the official bans, the lethal risks of the disease prevented farmers and researchers from meeting from time to time and shortened the duration

of the research. Despite all these limitations, the study was completed.

Findings

This section presents the results of the analysis of the data collected to answer the research questions and test the hypotheses. Analyses were conducted following the methodological approach of the study. The findings are explained and interpreted.

When the socio-demographic characteristics of the farmers participating in the study are analyzed in Table 1, it is seen that 89.9% of the farmers are male. 17.6% of farmers did not continue their education after primary school. 51.6% of farmers consider the farming profession profitable.

The average age of the 188 farmers participating in the study is 49.77 years. The youngest farmer is 25 years old and the oldest farmer is 75 years old. It was determined that farmers had an average of 21.62 years of agricultural experience. The farmer with the highest agricultural experience is 60 years, while there are also farmers with no experience. Looking at the land assets of the farmers participating in the research; it is seen that the average land owned by the farmers is 69.83 decares and the largest is 4000 decares. The average amount of internet used by the farmers on their smartphones is found to be 9.02 GB per month.

When the internet use of farmers before and after the COVID-19 pandemic was analyzed, significant differences were observed in some transactions performed by farmers. Related findings are presented in Table 3.

Table 1. Descriptive of farmers
Çizelge. Tanımlayıcı bilgiler

Descriptive Statistics	Frequency	Percent
Gender		
Female	19	10.1
Male	169	89.9
Education Level		
Primary School	99	17.6
Middle School	15	2.7
High School	47	8.3
University	27	4.8
Finding a Farming Profession Profitable		
Finds it's Profitable	91	48.4
Not Profitable	97	51.6

Table 2. Age, experience, lands, and mobil gb of farmers
Çizelge 2. Çiftçilerin yaşı, deneyimi, arazileri ve mobil internet kullanımı

	Minimum	Maximum	Mean	Standard Deviation
Age	25	75	49.77	11.358
Agricultural Experience (years)	0	60	21.62	10.452
Land Assets in Ownership	0	4000	69.83	317.926
Amount of Mobile Internet Owned (GB)	0	35	9.02	4.966

As can be seen in Table 3, there have been differences in some transactions of farmers after the COVID-19 pandemic. According to these results, the amount of mobile GB in farmers' smartphones, the frequency of farmers' participation in online agricultural training, the frequency of farmers' banking transactions over the internet, the frequency of farmers' researching the problems they experience during agricultural production over the internet, the frequency of farmers' visiting the websites of companies and unions in the agricultural sector, the frequency of farmers' visiting the website of the Ministry of Agriculture and Forestry, the frequency of farmers' checking product and input prices over the internet increased compared to before the COVID-19 pandemic and these increases were found to be statistically significant.

The first expression which states that there is a significant difference in the amount of mobile GB in farmers' smartphones compared to before the COVID-19 pandemic, is accepted ($t=-13.521$, $p<0.001$). There is an average increase of 2.58 GB in the amount of mobile GB that farmers have on their smartphones. When the literature is examined, there are studies with similar findings (Demiryurek et al., 2021). It is seen that the internet use of farmers has increased with the COVID-19 pandemic.

It is seen that there is no significant change in the frequency with which farmers perform Digital Agricultural Market (ditap.gov.tr) transactions ($t=-1.640$, $p>0.05$). A similar result was found in a study conducted across Türkiye (Demiryurek et al., 2021).

Table 3. Impact of covid-19 on farmers internet use
Çizelge 3. Covid-19'un çiftçilerin internet kullanımı üzerindeki etkisi

Expression	Mean ± SD Before the Pandemic	Mean ± SD After Pandemic	Mean Difference ± ss	t	p
Mobile Internet (GB)	6.44±4.17 GB	9.02±5.49 GB	-2.58±2.6	-13.521	**.000***
Digital Agriculture Market (ditap.gov.tr) density of use	1.03±0.19	1.05±0.25	-0.02±0.17	-1.640	.103
The frequency with which farmers search for new markets to sell their agricultural products	1.06±0.32	1.08±0.35	-0.22±0.46	-1.345	.180
Frequency of farmers' participation in online agricultural education	1.08±0.35	1.16±0.49	-0.08±0.34	-3.204	**.002***
Frequency of farmers' online banking transactions	1.79±0.88	1.88±0.92	-0.08±0.56	-2.084	**.039***
Frequency of farmers researching on the internet to solve the problems they encounter during agricultural production	1.24±0.61	1.39±0.74	-0.14±0.52	-3.879	**.000***
Frequency of farmers visiting the websites of companies and unions in the agriculture sector	1.25±0.51	1.48±0.71	-0.23±0.52	-6.103	**.000***
Frequency of farmers' visits to the website of the Ministry of Agriculture and Forestry	1.32±0.54	1.55±0.72	-0.22±0.46	-6.573	**.000***
Farmers selling agricultural products on the internet	1.07±0.31	1.12±0.39	-0.04±0.30	-1.899	.059
Frequency of farmers checking the prices of agricultural products they can sell on the internet	1.51±0.64	1.85±0.79	-0.34±0.50	-9.193	**.000***
The frequency with which farmers check the prices of inputs required for agricultural production online	1.55±0.62	1.84±0.77	-0.28±0.52	-7.579	**.000***
The frequency with which farmers conduct research about irrigation on the internet	1.15±0.47	1.19±0.52	-0.03±0.36	-1.404	.162
Frequency of farmers obtaining information about the marketing of agricultural products they can sell from the internet	1.14±0.45	1.19±0.51	-0.04±0.37	-1.741	.083

It is seen that farmers are slightly more likely to attend online information meetings on agricultural issues than before the COVID-19 pandemic ($t=-3.204$, $p<0.05$). However, it is seen that this significant increase is one of the least changed transactions when the difference between the other transactions is analyzed. In a study conducted in the literature, it is stated that farmers' participation in agricultural information meetings organized online has increased, but there is still no change in the majority of them (Demiryurek et al., 2021).

Table 4. Test of main hypothesis
Çizelge 4. Ana hipotezin test edilmesi

Hypothesis	Mean ± SD Before the Pandemic	Mean ± SD After Pandemic	Mean Difference± SD	t	p
There is a significant increase in farmers' use of the Internet for agricultural issues compared to before the COVID-19 pandemic.	15.21±4.09	16.77±5.16	1.56±2.37	-9.014	**.000***

A new score was calculated from the sum of the statements in Table 3 and farmers' use of the Internet for agricultural issues was analyzed. In this case, a statistically significant increase is observed and the main hypothesis is accepted ($t=-9.014$, $p<0.001$). It can be said that the COVID-19 pandemic may have had an impact on farmers' use of the internet and its use in agricultural issues.

A review of the literature shows similar results to the findings of the study. A study conducted in Slovenia found that farmers use digital communication tools more frequently (Erjavec et al., 2021). In another study conducted in Costa Rica, it was determined that farmers increased web-based sales with the effect of COVID-19 (Godrich et al., 2022).

CONCLUSIONS and RECOMMENDATIONS

The COVID-19 pandemic may have had a significant impact on farmers' use of the internet for agricultural issues. The pandemic, together with factors such as social distancing and mobility restrictions, may have led farmers to turn more to digital platforms for information access and marketing strategies. In particular, issues such as marketing of agricultural products, exchange of information on agricultural techniques, weather forecasts, and agricultural policies have become easier and faster to follow online. This may have increased the importance of the Internet as a source of information for agricultural activities in rural areas. For example, online agricultural seminars, web-based training programs, and social media groups can help farmers become more aware of agricultural issues.

The findings of this study, in addition to the studies in the literature, showing a higher behavioral intention to adopt technology when individuals are ready for technology (Lin & Hsieh, 2007; Yap et al., 2023), show that individuals can adopt technology even if they are not ready for the technology. The findings suggest that knowledge diffusion may accelerate in the agricultural sector, affected by crises.

It is a matter of curiosity what will happen to the information diffusion that accelerated in the agricultural sector as a result of the COVID-19 crisis once the effects of the COVID-19 crisis subside. It is the authors' recommendation to conduct a study on this issue.

Researchers' Contribution Rate Declaration

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declare that there is no conflict of interest between them.

LITERATURE

Alao, A., Usadolo, S. E., & Brink, R. (2021). Rural Farmers' Perception of the Adoption of Internet-Enabled Computers in the Eastern Cape, South Africa. *Journal of Human Ecology*, *73*(1-3), 1-14.

Arumugam, D. U., & Kanagavalli, D. G. (2020). COVID-19: Impact of agriculture in India. *Aegaeum Journal*, *8*(5).

Beckman, J., & Countryman, A. M. (2021). The importance of agriculture in the economy: impacts from COVID-19. *American journal of agricultural economics*, *103*(5), 1595-1611.

Bostan, S., Erdem, R., Ozturk, Y., Kilic, T., & Yilmaz, A. (2020). The effect of the COVID-19 pandemic on the Turkish society. Electron J Gen Med. 2020; 17 (6): em237. In.

Branscombe, M. (2020). The network impact of the global COVID-19 pandemic. *The New Stack*, *14*.

Demir, Ü., Nihal, K., & Uğurlu, B. (2021). Tarımda Yapay Zekâ Kullanımına Yönelik Karar Destek Modeli Önerisi: Domates Zararlısı Tespiti Örneği. *Lapseki Meslek Yüksekokulu Uygulamalı Araştırmalar Dergisi*, *2*(4), 91-108.

Demiryurek, K., Köksal, Ö., & Kawamorita, H. (2021). *Acceleration of Digital Transformation in Agriculture Sector for Ensuring Sustainable Food Security (in Türkiye)*.

Derya, G., & Demiryurek, K. (2020). Kırsal ve kentsel alanda bilgi ve iletişim teknolojilerinin kullanım durumu: Ankara örneği. *Anadolu Tarım Bilimleri Dergisi*, *35*(3), 339-352.

Dev, S. M. (2020). Addressing COVID-19 impacts on agriculture, food security, and livelihoods in India. *IFPRI book chapters*, 33-35.

Erdal, G., & Çallı, A. (2013). Çiftçilerin Bilgisayar ve İnternet Kullanımı Üzerine Bir Araştırma: Denizli İli Çivril İlçesi Örneği. *Gaziosmanpaşa Bilimsel Araştırma Dergisi*(8), 71-81.

Erjavec, K., Janzekovic, M., Kovač, M., Simčič, M., Mergedus, A., Terčič, D., … & Klopčič, M. (2021). Changes in the use of communication channels by livestock farmers during the COVID-19 pandemic. Sustainability, 13(18), 10064. https://doi.org/10.3390/su131810064

Fernando, E., Assegaff, S., & Rohayani, A. H. (2016). Trends information technology in E-agriculture: A systematic literature review. 2016 3rd International Conference on Information Technology, Computer, and Electrical Engineering (ICITACEE),

Godrich, S., Macau, F. R., Kent, K., Lo, J., & Devine, A. (2022). Food supply impacts and solutions associated with the COVID-19 pandemic: a regional Australian case study. International Journal of Environmental Research and Public Health, 19(7), 4116. https://doi.org/10.3390/ijerph19074116

Gray, R. S. (2020). Agriculture, transportation, and the COVID-19 crisis. *Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie*, *68*(2), 239-243.

Huang, Q., Chen, X., Huang, S., Shao, T., Liao, Z., Lin, S., Li, Y., Qi, J., Cai, Y., & Shen, H. (2021). Substance and Internet use during the COVID-19 pandemic in China. *Translational psychiatry*, *11*(1), 491.

Just, D. R., & Just, R. E. (2001). Harnessing the Internet for farmers. *Choices*, *16*(316-2016-6513).

Kızılaslan, H., & Gönültaş, H. (2011). Bilişim teknolojisinin tarım ürünlerinin pazarlamasındaki konum ve önemi (E-Pazarlama).

Lin, J.-S. C., & Hsieh, P.-L. (2007). The influence of technology readiness on satisfaction and behavioral intentions toward self-service technologies. *Computers in Human Behavior*, *23*(3), 1597-1615. https://doi.org/https://doi.org/10.1016/j.chb.2005.07.006

Lone, S. A., & Ahmad, A. (2020). COVID-19 pandemic–an African perspective. *Emerging microbes & infections*, *9*(1), 1300-1308.

Panda, S., Modak, S., Devi, Y. L., Das, L., Pal, P., & Nain, M. (2019). Access and usage of Information and Communication Technology (ICT) to accelerate farmers' income.

Pandey, N., & Pal, A. (2020). Impact of digital surge during Covid-19 pandemic: A viewpoint on research and practice. *International Journal of Information Management*, *55*, 102171.

Phelps, C., & Sperry, L. L. (2020). Children and the COVID-19 pandemic. *Psychological Trauma: Theory, Research, Practice, and Policy*, *12*(S1), S73.

Ray, P. P. (2017). Internet of things for smart agriculture: Technologies, practices, and future direction. *Journal of Ambient Intelligence and Smart Environments*, *9*(4), 395-420.

Rolfe, J., Gregor, S., & Menzies, D. (2003). Reasons why farmers in Australia adopt the Internet. *Electronic Commerce Research and Applications*, *2*(1), 27-41.

Rose, D. C., Sutherland, W. J., Parker, C., Lobley, M., Winter, M., Morris, C., Twining, S., Ffoulkes, C., Amano, T., & Dicks, L. V. (2016). Decision support tools for agriculture: Towards effective design and delivery. *Agricultural systems*, *149*, 165-174.

Siche, R. (2020). What is the impact of COVID-19 disease on agriculture? *Scientia Agropecuaria*, *11*(1), 3-6.

Smith, A., Goe, W. R., Kenney, M., & Paul, C. J. M. (2004). Computer and Internet use by Great Plains farmers. *Journal of Agricultural and Resource Economics*, 481-500.

Sun, Y., Li, Y., Bao, Y., Meng, S., Sun, Y., Schumann, G., Kosten, T., Strang, J., Lu, L., & Shi, J. (2020). Brief report: increased addictive internet and substance use behavior during the COVID-19 pandemic in China. *The American Journal on Addictions*, *29*(4), 268-270.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2).

Talevi, D., Socci, V., Carai, M., Carnaghi, G., Faleri, S., Trebbi, E., di Bernardo, A., Capelli, F., & Pacitti, F. (2020). Mental health outcomes of the COVID-19 pandemic. *Rivista di psichiatria*, *55*(3), 137-144.

Yan, B., Yan, C., Ke, C., & Tan, X.-C. (2016). Information Sharing in Supply Chain of Agricultural Products Based on the Internet of Things. *Industrial Management & Data Systems*. https://doi.org/10.1108/imds-12-2015-0512

Yap, C. S., Keling, W., Ho, P. L., & Omar, Q. (2023). Technology readiness of farmers in Sarawak: The effect of gender, age, and educational level. *Information Development*, 02666669231194712. https://doi.org/10.1177/02666669231194712

Yurtlu, Y. B., Demiryürek, K., Bozoğlu, M., & Ceyhan, V. (2012). Çiftçilerin tarım makineleri kullanımına ilişkin risk algıları. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, *49*(1), 93-101.

KSÜ Tarım ve Doğa Derg 27 (4), 973-983, 2024
KSU J. Agric Nat 27 (4), 973-983, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1329540

Per Capita Meat Consumption: The Trend and Macroeconomic Determinants in Nigeria

Sunday AKPAN[1]✉, Edet UDOH[2], Veronica NKANT[3]
[1]AKWA Ibom State University, Department of Agricultural Economics and Extension, Obio-Akpa Campus, Nigeria, [2,3]University of Uyo, Department of Agricultural Economics and Extension, Uyo, Nigeria.
[1]https://orcid.org/0000-0002-0458-028X, [2]https://orcid.org/0000-0002-4550-4320, [3]https://orcid.org/ 0000-0001-7035-7978.
✉: sundayakpan@aksu.edu.ng.

ABSTRACT
This study examined trends in per capita consumption of game meat, mutton, pork, and total meat and provided evidence of their correlation with key macroeconomic factors in Nigeria. Data from the Food and Agricultural Organization (FAO), the Central Bank of Nigeria (CBN), and the World Bank (WB) covering the period 1981-2021 were analyzed. The results showed that game meat, mutton, pork, and total meat consumption per capita recorded annual exponential growth rates of -0.87%, 2.05%, 2.08%, and -0.54%, respectively. The empirical results showed that the inflation rate, nominal exchange rate, GDP per capita, credit to the agricultural sector, and the capacity utilization rate in the meat industry influence the per capita consumption of game meat, mutton, pork, and the total meat consume in both the short and long-run periods. To increase the per capita meat consumption in the country, it is strongly recommended that appropriate macroeconomic measures should be adopted to reduce the current inflation rate and the nominal exchange rate (N/$). At the same time, concerted efforts should be made to increase Nigerians purchasing power through an increase in the per capita GDP.

Agricultural Economics

Research Article

Article History
Received : 20.07.2023
Accepted : 18.01.2024

Keywords
Meat
Trend
Macroeconomic
Nigeria

Kişi Başına Et Tüketimi: Nijerya'daki Trend ve Makroekonomik Belirleyiciler

ÖZET
Bu çalışma, av eti, koyun eti, domuz eti ve toplam etin kişi başına tüketimindeki eğilimleri incelemiş ve bunların Nijerya'daki temel makroekonomik faktörlerle korelasyonuna dair kanıtlar sağlamıştır. Gıda ve Tarım Örgütü (FAO), Nijerya Merkez Bankası (CBN) ve Dünya Bankası'nın (WB) 1981-2021 dönemini kapsayan verileri analiz edildi. Sonuçlar, kişi başına av eti, koyun eti, domuz eti ve toplam et tüketiminin sırasıyla -%0,87, %2,05, %2,08 ve -%0,54 oranında yıllık üstel büyüme oranları kaydettiğini gösterdi. Ampirik sonuçlar enflasyon oranının, nominal döviz kurunun, kişi başına düşen GSYH'nin, tarım sektörüne verilen kredinin ve et endüstrisindeki kapasite kullanım oranının her iki ülkede de kişi başına av eti, koyun eti, domuz eti tüketimini ve toplam et tüketimini etkilediğini göstermiştir. kısa ve uzun vadeli dönemler. Ülkede kişi başına et tüketiminin artırılması amacıyla, mevcut enflasyon oranının ve nominal döviz kurunun (N/$) düşürülmesine yönelik uygun makroekonomik önlemlerin alınması önemle tavsiye edilmektedir. Aynı zamanda kişi başına düşen GSYİH'yi artırarak Nijeryalıların satın alma gücünü artırmak için ortak çaba sarf edilmelidir.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 20.07.2023
Kabul Tarihi : 18.01.2024

Anahtar Kelimeler
Et
Akım
Makro ekonomi
Nijerya

Atıf Şekli: Akpan, S. B., Udoh, E. J., & Nkanta, V. S. (2023). Kişi Başına Et Tüketimi: Nijerya'daki Trend ve Makroekonomik Belirleyiciler. *KSÜ Tarım ve Doğa Derg 27* (4), 973-983. https://doi.org/10.18016/ksutarimdoga.vi.1329540.

To Cite : Akpan, S. B., Udoh, E. J., & Nkanta, V. S. (2023). The Per Capita Meat Consumption: The Trend and Macroeconomic Determinants in Nigeria. *KSU J. Agric Nat 27* (4), 973-983. https://doi.org/10.18016/ksutarimdoga.vi.1329540

INTRODUCTION
The Nigerian meat industry has recently been challenged by several factors and its production has been undermined by poor processing facilities and the unregulated market system, among others (Babatunde and Qaim, 2010; Agboola and Balcilar, 2012; Ogbeide, 2015; Akpan, 2022a). Despite the disadvantages, the production and consumption of meat in Nigeria offers

huge potential for both domestic and foreign investment. However, efficient and sustainable production and consumption of meat as well as other agricultural products are anchored, among other things, in a stable and favorable economic environment (Akpan et al., 2014, Akpan and Udo 2021, Akpan and Umoren, 2021). As the OECD (2023) notes, meat consumption is linked not only to macroeconomic uncertainties and GDP shocks, but also to living standards, nutrition, animal production, and consumer prices.

According to recent surveys, the demand for meat in sub-Saharan Africa especially in Nigeria is increasing (Reardon et al. 2021; FAO 2021). The increasing activities in the meat industry in Nigeria have provided food for millions of people and provide excellent livelihoods (OECD, 2023). The increase in youth population, improved human and social capital, personal income, and expanding cities and towns are some of the factors that have contributed to the increase in demand for meat and its derivatives (Akpan 2022a; Akpan and Nkanta 2022; OECD 2023). Despite the boom in meat and demand for meat derivatives in the country, the supply capacity is fundamentally small-scale and has a marginal annual production growth rate (Babatunde and Qaim 2010; Agboola and Balcilar, 2012; Ogbeide, 2015; Odoemena et al., 2020).

Given the country's current population of more than 200 million (NPC, 2023; Akpan and Ebong, 2021), there are enormous challenges in meeting citizens' recommended animal protein requirements. In addition, most of the country's livestock farmers are resource-poor and affected by climate change, conflict, and economic changes, among others (Bamaiyi, 2013; Akpan and Monday 2021). In addition, most of the country's population (which is young) quickly adapts to a diet rich in animal protein and its derivatives (Ogbeide, 2015; Akpan et al., 2015). As a result, the consumption of meat and its by-products in the country is increasing sharply. It is imperative to increase domestic production to prioritize the minimum animal protein consumption needs of Nigerians (Osazuwa-Peters, 2021; Odetokun et al., 2021, Akinsulu, et al., 2019; Arowolo, et al., 2021; Elegbede, et al., 2018).

The adequacy of meat consumption in the country is easily assessed using the index of meat consumption per capita. Meat consumption per capita represents the average amount of meat (in kilograms) available to a person at a given point in time in a well-defined area. It is calculated by dividing the total meat consumption (i.e. the sum of total domestic meat production, total imported quantity, and total game meat in kg) by the total population at a given point in time. The Meat Consumption Index per capita measures the availability of meat for all citizens of a country at any given time. In Nigeria, per capita meat consumption is roughly equal to per capita meat production as the country rarely exports meat products (FAO, 2023).

In Nigeria, the per capita meat consumption index is much lower than in most African countries. Between 2018 and 2020, the average African consumed 12.7 kg of meat per year (OECD/FAO, 2019). Chicken meat had the highest annual consumption on the continent at 5.77 kilograms per capita. Meanwhile, Africa had the lowest meat consumption per capita in the world for the same period. In 2019, according to OECD/FAO (2021a), the Seychelles had the highest per capita amount of meat available for human consumption in any country in Africa. The average meat supply on the African island was 65 kilograms per person. South Africa followed in second place with a supply of almost 64 kilograms per capita. In contrast, Burundi and the Democratic Republic of the Congo had the lowest annual meat stocks, at just 3.8 kilograms and 2.4 kilograms per person, respectively.

Furthermore, according to statistics, Nigeria's per capita meat consumption in 2021 was 8.30 kg per person per year, which is much lower than most African countries (FAO, 2023; OECD/FAO, 2021b). It is also well below the average consumption of 19.0 kg person-1 across the continent and the minimum consumption recommended by the World Health Organization for adults (0.830 g kg-1 body weight per day) (FAO, 2019; OECD/FAO, 2022). To address the problem of animal protein shortages in Nigeria, the three levels of government have implemented specific policies in line with the federal government's Livestock Transformation Framework. The livestock transformation program was designed to increase the production and consumption of animal protein sources to meet the average minimum protein requirement of most Nigerians in 2027 (Williams, 1989; Ojiako and Olayode 2008; Akpan 2022a). However, one of the expected outcomes of the livestock transformation program, which is an increase in meat consumption per capita, depends, among other things, on a sound macroeconomic and political environment (Udah et al., 2015a, Udah et al., 2015b; Akpan et al., 2015; Udoh and Akpan, 2019; Akpan and Umoren, 2021; Ecker and Hatzenbuehler 2022, Akpan 2022b). The literature has provided evidence that agricultural production and food consumption are strongly associated with macroeconomic fundamentals in developing countries (Akpan et al., 2012; Udah et al., 2015b; Udah et al., 2015c; Akpan et al, 2021; Akpan and Udo, 2021; Mekonnen et al. 2021; Ecker and Hatzenbuehler 2022).

Although there is little literature on this core topic, its contributions need to be validated and updated, especially given that unexpected economic downturns have bewildered the country in recent years. From the archive, Simo-Kengna et al. (2018) several factors affecting meat consumption in SEAFO countries. The results showed the price of meat, the country's GDP,

the rate of inflation, the amount of exports, and imports, and the size of urban areas. Similarly, Akpan and Udo (2021) made connections between the gross domestic meat and milk production indices and the macroeconomic fundamentals of Nigeria. The results show that the long-run determinants of milk and meat production are GDP per capita, nominal exchange rate, and land density, while the short-run determinants are income per capita, economic creditworthiness, and land density. In addition, Betru and Kawashima (2009) identified the factors affecting meat consumption in Ethiopia. Their results showed that cities and personal income had a significantly positive impact on meat consumption. James et al. (2009) examined beef demand drivers and opportunities for improvement in the United States. Their results showed that there was a significant positive relationship between consumer spending and beef consumption, and that beef price fluctuations had a negative and inelastic effect on consumer demand. Bascron et al. (2019) investigated red and white meat consumption and production trends in Egypt. The results showed an annual increasing trend of 1.40% and 2.87% per year for both variables. Akpan (2022a) again analyzed the relationship between ruminant meat production and several key macroeconomic factors in Nigeria. The results showed that the inflation rate, personal income, and exchange rate affect domestic meat production. Fatima et al. (2022) conducted a study investigating macroeconomic factors influencing red meat consumption in Saudi Arabia from 1980 to 2020. They used an autoregressive distributed lag model and found a significant negative correlation between domestic red meat consumption and the meat price index.

The literature indicates that there is no particular focus on per capita meat consumption in Nigeria. The importance of meat consumption to the well-being of Nigerians cannot be overstated given that the country is one of the poorest countries in the world. The meat sub-sector therefore needs policy recommendations to close the current consumption deficit gap. Additionally, Nigeria's macroeconomic environment has changed significantly over the past two decades, including the recent global COVID-19 pandemic, necessitating an update of meat consumption trends and their correlation with macroeconomic variables. Therefore, the study specifically examines:

a) the trends in annual per capita consumption of mutton, pork, game meat, and total meat in the country from 1981 to 2021, and
b) establish the relationship between the per capita meat consumption and some key macroeconomic fundamentals.

RESEARCH METHODOLOGY

Study Area and Data Source: The study took place in Nigeria, a country on the Gulf of Guinea in sub-Saharan Africa. Nigeria extends from 40 to 140 degrees north of the equator and from 30 to 150 degrees east of Greenwich. It covers a land mass of 923,769 square kilometers (or approximately 98.3 million hectares) and has 853 kilometers of coastline along the northern edge of the Gulf of Guinea. The country has a population of over 200 million (National Population Commission, 2023) and is rich in agricultural, mineral, marine, and forest resources. More than 60 percent of the country's population is engaged in the production of food crops such as corn, cassava, yam, rice, beans and vegetables, ginger, carrots, legumes, sorghum, onions, tomatoes, and melons. In addition, animal husbandry, aquaculture, and fishing are carried out on a large scale in all regions of the country. The main cash crops grown in Nigeria are cocoa, cotton, peanuts, palm oil, and rubber (Federal Ministry for the Environment, 2021). The study used secondary data from the WB (World Bank), FAO (Food and Agriculture Organization), and CBN (Central Bank of Nigeria). The data/information covered the period from 1981 to 2021.

Model Specification

The trends in per capita meat consumption

The explicit form of an exponential trend equation was adopted to analyze the trends in the per capita meat consumption in Nigeria. The specification is shown in Equation 1:

$$lnTMC_t = \lambda_0 + \lambda_1 t + \varepsilon_t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where TMC_t is the total per capita meat consumption measured in kg person-1, "t" is the annual trend. Note, that the per capita is computed as in equation 2.

$$Per\ capita\ meat\ consumption = \sum_{i=1}^{n} TM_t \Big/ \sum_{i=1}^{n} POP_t \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where TM_t is a specified meat in kg at period t while the POP_t is the country's population in period t. Hence, the dependent variables for this study are defined as:

(a) MMC_t = mutton per capita consumption (kg person-1) (meat from sheep)

(b) KMC_t = game meat per capita consumption (kg person-1) (meat from wild animal animals)

(c) PMC_t = pork per capita consumption (kg person-1) (meat from pig)

(d) TMCt = Total meat per capita consumption (kg person-1)

Therefore, the exponential growth rate from Equation 1 is expressed in Equation 3:

$$(\text{Growth rate}) = \left(e^{\lambda_1} - 1\right) * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

To determine whether the specified per capita meat consumption growth rate suggests an accelerated or decelerated trend, an exponential equation in

quadratic form was explicitly used in equation 4:

$$log_e TMC_t = \varphi_o + \varphi_1 t_1 + \varphi_2 t_1^2 + \varepsilon_t \ldots\ldots\ldots\ldots(4)$$

If the estimated coefficient φ_2 is positive and significant, it implies persistent long-run growth in the per capita meat consumption; but if φ_2 is negative and statistically significant, it means there is a significant long-run deceleration in the per capita meat production. Nevertheless, if φ_2 is not significant, it connotes dormancy or stagnation in the per capita meat consumption in the long run (Ojiako and Olayode 2008; Akpan and Okon 2019; Akpan 2019). Note equations 1-4 were estimated for all dependent variables.

The determinants of the per capita meat consumption

The relationship between the per capita meat consumption and some exogenous factors (i.e. macroeconomic fundamentals) was explicitly stated in a double -log form and is stated as:

$$LnTMC_t = \Psi_0 + +\Psi_1 LnFLT_t + \Psi_2 \mathrm{Ln}INC_t + \Psi_3 \mathrm{Ln}RET_t + \Psi_4 \mathrm{Ln}CRD_t + \Psi_5 \mathrm{Ln}CAU_t + U_t \ldots.(5)$$

Where,

TMC_t = the total meat per capita consumption in kg/person as described in equation 1

FLT_t = annual rate of inflation (%)

INC_t = annual GDP per capita (naira person-1)

RET_t = annual nominal exchange rate (Naira Dollar-1) (%)

CRD_t = annual domestic credit disbursed to the agricultural sector per GDP (%)

CAU_t = capacity utilization rate of the meat industry (%)

U_t = Stochastic error term and $U_t \sim$ IID $(0, \delta^2_U)$

Note Equation 5 was estimated for all the dependent variables listed in Equation 1. The study used the Engle and Granger two-step technique test to verify the presence of a long-run stable relationship between a specific per capita meat consumption and some key macroeconomic variables (Engle and Granger, 1987). The conditions for using the method required that all variables involved be integrated in the same order. The error correction model (ECM) was also estimated for the co-integrating equations. The estimated ECM is shown explicitly in Equation 6.

$$\Delta \mathrm{Ln}TMC_t = \pi_0 + \pi_1 \Delta LnTMC_{t-1} + \pi_2 \Delta LnFLT_t + \pi_3 \Delta LnINC_t + \pi_4 \Delta LnRET_t + \pi_5 \Delta LnCRD_t + \pi_6 \Delta LnCAU_t + \pi_7 ECM_{t-1} + U_t \quad (6)$$

Table 1. Descriptive Statistics of Variables
Çizelge 1. Değişkenlerin Tanımlayıcı İstatistikleri

Variable	Mean	Minimum	Maximum	Std. dev.	CV	Skewness
Total meat/capita (TMC_t)	8.3815	6.7380	10.2820	0.8642	0.1031	0.1373
Game meat/capita (KMC_t)	0.9872	0.8160	1.3302	0.1289	0.1306	1.1547
Mutton/capita (MMC_t)	0.7169	0.4051	1.0593	0.2221	0.3098	-0.0474
Pork/capita ((PMC_t)	1.2223	0.4820	1.4857	0.2890	0.2365	-1.4175
Inflation rate (FLT_t)	18.9490	5.3880	72.836	16.659	0.8792	1.8542
GDP/capita (INC_t)	1683.60	270.03	4471.10	1025.0	0.6088	0.3422
Exchange rate (RET_t)	108.170	0.6177	403.58	110.14	1.0182	0.9842
Domestic credit (CRD_t)	9.2506	4.9575	19.626	3.4678	0.3749	1.1862
Capacity utilization (CAU_t)	45.9710	12.700	75.750	14.450	0.3143	0.1553

Source: Computed by authors.

The coefficients (π_7) measure the deviation from the long-run equilibrium in the previous period. Also, equation 6 was estimated for all dependent variables specified in Equation 1.

RESULTS and DISCUSSION

The descriptive statistics of the given variables are presented in Table 1. The coefficients of variability are 10.31% for total meat consumption per capita, 13.06%, 30.98%, and 23.65% for per capita consumption of game, mutton, and pork, respectively. The results suggest that the Nigerian country experienced little variation in annual meat consumption per capita. The results suggest that annual meat consumption per capita in Nigeria has not changed significantly over the years. The skewness indices showed that the annual per capita meat consumption of individual types of meat changes only slightly. Nevertheless, the mean of the total meat consumption was 8.38 kg per person. This is well below the mean for Africa with 12.77 kg per person in 2019 and 2021. While Seychelles, South Africa, Gabon, Mali, and Ghana had 65.08 kg/person, 63.67 kg person-1, 61.76 kg person-1, 23.35 kg person-1, and 13.63 kg person-1 respectively in 2019. The mean for individual meat consumption is given as 0.99 kg/person for game meat, 0.72 kg/person for mutton, and 1. 22 kg per person-1 indicated for pork. These statistics revealed the great lack of individual meat consumption in the country compared to other countries in Africa. For example, the per capita consumption of poultry in Africa in 2020 was reported at 5.77 kg per person-1, mutton at 2.18 kg per person-1, and beef at 3.68 kg per person-1. However, the average per capita consumption of pork (1.22 kg person-1) in Nigeria was higher than the average reported for Africa (1.08 kg person-1) in 2021.

The descriptive tests performed on the macroeconomic

variables revealed significant fluctuations in the nominal exchange rate (RET), indicating its instability over the indicated study period. The inflation rate also showed large fluctuations and averaged 87.92% per year. On the other hand, the coefficient of variation was lowest for the capacity utilization variable of the meat industry and loans paid to the agricultural sector. This suggests that while financing of the agricultural sector and capacity utilization of the meat industry have evolved positively over the years, they have not changed significantly. The skewness index of macroeconomic fundamentals all showed positive signs, suggesting that they exhibited progressive patterns throughout the period of interest.

The Trends in annual meat per capita consumption in Nigeria

Table 2 shows estimates of exponential and quadratic trend equations for per capita game meat, mutton, and pork consumption and total meat consumption per capita in Nigeria. The results showed that the annual per capita consumption of mutton and pork was positively and significantly related to time. Annual exponential growth rates of approximately 2.05% and 2.08%, respectively, were achieved for mutton and pork. However, the squared-exponential trend estimates showed a slowdown in the growth rates of both meat sources over the long term.

Table 2: The exponential and quadratic trend equations for meat per capita consumption
Çizelge 2. Kişi başına et tüketimi için üstel ve ikinci dereceden trend denklemleri

	Game meat/capita		Mutton/capita		Pork/capita		Total meat/capita	
Exponential trend equation								
Variable	**Coeff.**	**t-value**	**Coeff.**	**t-value**	**Coeff.**	**t-value**	**Coeff.**	**t-value**
Constant	0.1623	7.64***	−0.8101	−11.16***	−0.2705	−4.47***	2.2345	86.00***
Time	−0.0087	−9.89***	0.0203	6.74***	0.0206	8.20***	−0.005	−5.02***
R-square	0.7148		0.5381		0.6328		0.3926	
F- cal. (1, 39)	97.748***		45.425***		67.217***		25.208***	
Exp. GR (%)	**−0.86713**		**2.0497**		**2.0799**		**−0.5410**	
Quadratic exponential trend equation								
Variable	**Coeff.**	**t-value**	**Coeff.**	**t-value**	**Coeff.**	**t-value**	**Coeff.**	**t-value**
Constant	0.2560	9.60***	−1.2353	−17.88***	−0.6300	−11.20***	2.1775	55.99***
Time	−0.0218	−7.44***	0.0796	10.50***	0.0708	11.45***	0.0025	0.59
Time Square	0.0003	4.61***	−0.0014	−8.07***	−0.0012	−8.38***	−0.0002	−1.92*
R-squared	0.8170		0.8297		0.8709		0.4463	
F- cal. (2, 49)	84.842***		92.536***		128.264***		15.313***	

Source: computed by authors. The symbols ***, **, and * represent different levels of statistical significance.

In addition, game meat consumption and total meat consumption per capita increased exponentially by -0.87% and -0.54%, respectively. This suggests that average game meat consumption and total meat consumption per person is decreasing over time. Further studies showed a significant acceleration and deceleration of game meat consumption and total meat consumption per person respectively over a long period. Several phenomena, including uncertainty in the macroeconomic environment, have been linked to the relationship between meat consumption per capita and time (Akpan, 2022). In addition, several studies on agricultural commodities in Nigeria have estimated a similar exponential growth rate, e.g. Ojiako and Olayode (2008), Baskhron et al. (2019), and Akpan (2022).

Figure 1 shows a graphical representation of the trend lines for different meats per capita consumed in Nigeria. The evolution of per capita consumption of mutton showed a gradual increase in per capita consumption of mutton from 1981 to 2010 with a clear trough in 2012 and a subsequent minimal acceleration until 2021. For game meat, there was a downward trend from 1981 to 2009. Beginning in 2010, per capita consumption of game meat assumed an accelerating pattern that later declined through 2021. Pork consumption per capita trend indicates a progressive trend from 1981 to 2021. This means that the consumption level of pork was mounting perhaps with the potential for more investments.

The trend in total meat per capita consumption fluctuates from 1981 to 2021. From 1981 to 1985, the country witnessed an increment in total meat consumption reaching a peak of 10.28kg/person in 1985. From 1986 to 1991 representing the period of the structural adjustment programme (SAP), (Kanayo et al., 2013; Ogbonna, 2012), the total meat consumption declined reaching the mark of 7.57kg/person in 1991. In 1988, an import ban was implemented for fresh and chilled as well as frozen meat to safeguard local producers, but it was unsuccessful following an adverse economic environment (Egwaikhide, 1997). Following the implementation of several agricultural policies to boost animal protein intake, the per capita meat consumption upsurge from 7.85kg/person in 1992 to 8.57kg/person in 2000. From the period 2001 to 2021, the country witnessed progressive declines in total meat per capita, being lower than most African countries. The index deteriorated to 6.87kg/person in 2020 which is far below the WHO recommended index

and average obtained for Africa. During this time, private investment in the meat industry significantly increased, but the sub-sector development was constrained by the rising instability of the underlying macroeconomic conditions (Akpan, et al., 2012; Ejedegba, 2023). In the post-SAP era, lasting from 1993 to 2021, new agricultural policies were implemented, including a restriction on frozen poultry meat imports, currency devaluation, and a significant increase in private participation in meat production, processing, and marketing (Akpan et al., 2012; Kanayo et al., 2013; Ogbonna, 2012). Although these incentives helped increase meat production somewhat, they had little impact on per capita meat consumption in the country given the growing population. This is reflected in the erratic decline in total meat consumption per capita between 2001 and 2021. Also, the emergence of the COVID-19 pandemic in 2020 and the increasing rise in feed prices made it difficult for total meat consumption to increase, thus contributing to the continued downward trend growth.

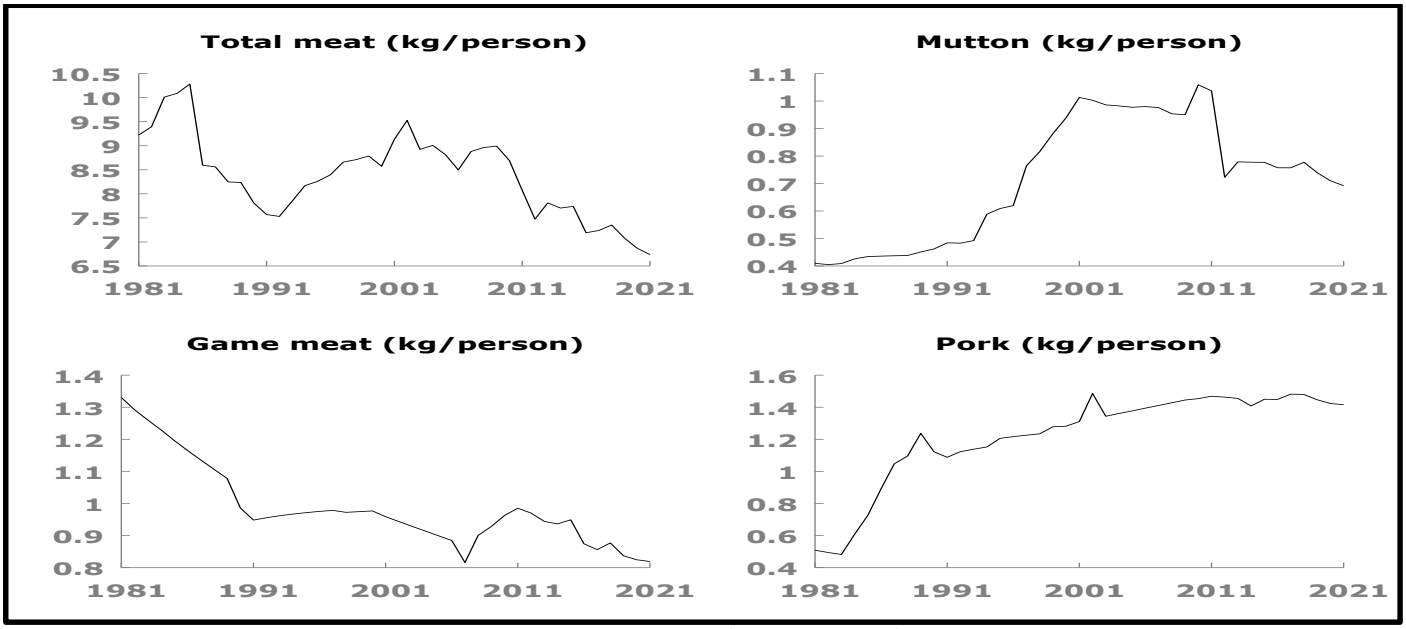


Figure 1. Trends in per capita meat production in Nigeria (1981 to 2021*).*
Şekil 1. Nijeryada kişi başı et tüketimindeki yönelimler (1981-2021)

Unit root test

The augmented Dickey-Fuller test was used in the study to confirm the unit root of the specified variables. The results shown in Table 3 confirm that the variables reported were not stationary at their levels but were at their first difference. This suggests that Engle Granger's two-step method can be used to examine the long-term relationship between the variables (Engle and Granger, 1987). However, to use this method, all variables must be stationary at the same level.

Test of cointegration

Engle Granger's two-stage method was used to investigate whether there was a cointegration between the variables mentioned. The results presented in Table 4 show that all equations presented showed evidence of cointegration. This suggests that there is a stable long-term relationship between some macroeconomic variables and per capita meat consumption of game meat, mutton, pork, and total meat in Nigeria.

The Short and the Long run determinants of per capita meat consumption in Nigeria

The results of analyses for the specified meat per capita consumption are discussed in the following sub-sections.

Determinants of per capita mutton consumption in the short and long runs

Table 5 shows estimates of the equations for per capita consumption of mutton in both the short and long run. The ECM adjustment coefficient is negative and significant, indicating a 28.18% convergence rate toward long-run equilibrium. Additional diagnostic tests confirmed the reliability and sufficiency of the estimates. The empirical results show that the inflation rate has a significant negative relationship with the per capita consumption of mutton in both the short and long term. In addition, there is a positive relationship between per capita income or GDP/capita and per capita mutton consumption in the short run, but a negative relationship in the long run. Furthermore, the capacity utilization coefficient increases with the per capita consumption of mutton in the short and long runs, while the exchange rate has a

positive correlation with the per capita consumption of mutton in the long run. These results are supported by previous studies by Simo-Kengne et al. supported. (2018), Akpan and Umoren (2021), and Akpan (2022).

Table 3. ADF unit root tests on variables
Çizelge 3. Değişkenler üzerinde ADF birim kök testleri

Variables	ADF unit root (without constant)				
	Level	**Lag**	**1st Diff.**	**Lag**	**Decision**
Total meat/capita (TMCt)	-0.5139	0	-5.7771***	0	1(1)
Game meat/capita (KMCt)	-1.7297	0	-5.0280***	0	1(1)
Mutton/capita (MMCt)	-1.8323	0	-5.6027***	0	1(1)
Pork/capita ((PMCt)	-1.7558	0	-3.7646***	0	1(1)
Inflation rate (FLTt)	-0.8074	0	-6.2809***	0	1(1)
GDP/capita (INCt)	-0.1086	0	-6.0466***	0	1(1)
Exchange rate (RETt)	1.9705	0	-4.3109***	0	1(1)
Domestic credit (CRDt)	0.2469	0	-5.7867***	0	1(1)
Capacity utilization (CUTt)	-0.4462	0	-6.5973***	0	1(1)

Source: Author's compilation and asterisks *** indicate a 1% significance level. Note that, variables are expressed in a natural logarithm and the significant level was defined at a 1% level only.

Table 4: Co-integration test of variables
Çizelge 4. Değişkenlerin eş bütünleşme testi

Equation residual	ADF test (without constant)	Order of integration	Remark
Total meat/capita (TMCt)	-2.5692**	1(0)	Co-integration
Mutton/capita (MMCt)	-2.3122**	1(0)	Co-integration
Game meat/capita (KMCt)	-2.8546***	1(0)	Co-integration
Pork/capita ((PMCt)	-2.8665***	1(0)	Co-integration

Source: Author's compilation and asterisks *** indicate a 1% significance level. Note that, variables are expressed in a natural logarithm and the significant level was defined at a 1% level only.

Table 5: Determinants of mutton per capita consumption in Nigeria
Çizelge 5. Nijerya'da kişi başına koyun eti tüketiminin belirleyicileri

ECM of mutton/capita				**Long run equation of mutton/capita**				
Variables				**Variables**				
	Coeff.	Std. error	t-value		Coeff.	Std. error	t-value	VIF
Constant	0.0107	0.0145	0.738	Constant	−0.7198	0.3954	−1.821*	-
Inflation rate	−0.0578	0.0243	−2.382**	Inflation rate	−0.0829	0.0406	−2.044**	1.237
GDP/capita	0.1445	0.0383	3.773***	GDP/capita	−0.0986	0.0372	−2.651**	1.371
Exchange rate	0.0179	0.0445	0.403	Exchange rate	0.1106	0.0194	5.699***	2.500
Credit disbursed	0.0321	0.0729	0.439	Credit disbursed	0.1707	0.1223	1.396	2.934
Capacity utilized	0.0607	0.0192	3.162***	Capacity utilized	0.1322	0.0596	2.218**	1.318
Mutton lag 1	0.2175	0.1249	1.741*					
ECM_{t-1}	**−0.2818**	**0.1036**	**−2.721*** **					
				Diagnostic test				
R^2	0.254			R^2	0.806			
F- cal. (7,31)	6.5073***			F- cal. (5,35)	29.1601***			
Normality test	5.3789 (0.6629)							
RESET test	0.6083 (0.4415)							
Breush-Pagan	2.3359 (0.8429)							
CUSUMSQ test	-1.27509 (0.2121)							
Durbin Watson	1.8755 (0.2697)							

Source: The author performed the computation. Symbols ***, **, and * represent significance levels of 1%, 5%, and 1%, respectively. Variables used in the error correction model (ECM) are expressed in log differences, while those used in the long-run equation are expressed in natural logarithms.

Determinants of short and long runs per capita game meat consumption

Estimates for game meat consumption per capita are presented in Table 6. The ECM coefficient has the appropriate sign and is statistically significant at the 1% probability level. This confirms the existence of a long-run equilibrium in the per capita game meat consumption model. Other statistical tests indicate that the estimated model is adequate. The empirical results showed that in the short-run model, GDP per capita and the previous value of per capita game meat consumption have a positive influence on the per capita game meat consumption, while loans disbursed to the agricultural sector showed a negative relationship. In the long-term model, the study found

a significantly positive correlation between per capita game meat consumption and GDP per capita and a significantly negative correlation with the nominal exchange rate. These results are supported by the work of Simo-Kengne et al. (2018), Akpan and Umoren (2021), and Akpan (2022a).

Table 6. Determinants of Game meat per capita consumption in Nigeria
Çizelge 6. Nijerya'da kişi başına av eti tüketiminin belirleyicileri

	ECM of game meat/capita				Long run equation of game meat/capita			
Variables	Coeff.	Std. error	t-value	**Variables**	Coeff.	Std. error	t-value	VIF
Constant	−0.0051	0.0055	−0.932	Constant	−0.0559	0.1178	−0.475	
Inflation rate	0.0054	0.0077	0.7018	Inflation rate	−0.0011	0.0121	−0.093	1.237
GDP/capita	0.0317	0.0132	2.405***	GDP/capita	0.0187	0.0111	1.692*	1.371
Exchange rate	−0.0139	0.0160	−0.871	Exchange rate	−0.0597	0.0058	−10.33***	2.500
Credit disbursed	−0.0507	0.0272	−1.866*	Credit disbursed	0.0185	0.0365	0.508	2.934
Capacity utilized	0.0189	00.0181	1.043	Capacity utilized	0.0208	0.0237	0.876	1.318
Game meat lag 1	0.2986	0.1551	1.926*					
ECM_{t-1}	−0.4466	0.1231	−3.627***					
R^2	0.422			R^2	0.876			
F- cal. (7,31)	3.2272**			F- cal. (5,35)	49.4499***			
Normality test	7.4404 (0.7518)							
RESET test	1.5835 (0.3039)							
Breush-Pagan	3.1809 (0.7158)							
CUSUMSQ test	0.3192 (0.7518)							
Durbin Watson	1.9968 (0.41084)							

Source: The author performed the computation. Symbols ***, **, and * represent significance levels of 1%, 5%, and 1%, respectively. Variables used in the error correction model (ECM) are expressed in log differences, while those used in the long-run equation are expressed in natural logarithms.

Determinants of short and long runs per capita pork consumption

Estimates for the per capita pork consumption equation are shown in Table 7. The ECM estimates achieve the expected results with a convergence rate to the long-run equilibrium of 25.30%. The empirical results show that the exchange rate, previous pork consumption, and per capita income have a significant positive or positive impact on per capita pork consumption in Nigeria in the short term. On the other hand, the capacity utilization coefficient has a significant negative impact in both the short and long runs. Furthermore, the long-run model shows a strong negative correlation between Nigeria's nominal exchange rate and per capita pork consumption. These results are supported by the findings of Simo-Kengne et al. supported. (2018), Akpan and Umoren (2021), and Akpan (2022a).

Determinants of short and long runs per capita total meat consumption

The results presented in Table 8 show a convergence rate of 31.70% in the long-run for total meat consumption per capita short-run equation. This supports the use of cointegration models. The stability of the estimated model over time is confirmed by the CUSUMSQ value. The error term follows a normal distribution, justifying the use of the ordinary least squares method. The RESET test and the Breush-Pagan test support the structural rigidity of the estimated short-run model and indicate a minimal influence of heteroscedasticity respectively. The error correction model shows a positive short-run relationship between total meat consumption per capita and GDP per capita as well as the capacity utilization of the meat industry in Nigeria. In addition, there is a significant negative short-term relationship between total meat consumption per capita and the inflation rate. In addition, the long-term estimates revealed significant negative correlations between total meat consumption per capita the rate of inflation, and the nominal exchange rate. On the contrary, there is a positive relationship between total meat consumption per capita and capita GDP as well as the capacity utilization of the meat industry. These results are consistent with research by Akpan and Umoren (2021) and Akpan (2022a).

CONCLUSION and RECOMMENDATIONS

The analyses have shown that meat consumption in Nigeria is completely inadequate. The results revealed that the average per capita consumption of game meat, mutton, and total meat in Nigeria is lower than the data for Africa in 2021. However, the pork meat per capita consumption in Nigeria was slightly higher than the average in Africa. The study estimated negative annual growth rates for per capita game meat and total meat, while positive growth rates were estimated for mutton and pork per capita consumption in Nigeria.

This has two implications. First, the country consumed far less animal protein compared to the recommended standard and most African countries. This means that the dietary composition of the majority of Nigerians is deficient in terms of nutrient balance and is therefore well below the minimum standard recommended by the World Health Organization. This is a serious challenge, especially given that the country is at the forefront of poverty, malnutrition, and insecurity.

Secondly, the current meat consumption situation in Nigeria offers numerous opportunities for domestic and foreign investment in the meat industry, benefiting from the large population of over 200 million people. The empirical results showed that changes in per capita income, credit to the agricultural sector, inflation rate, nominal exchange rate, and meat industry capacity utilization have significant effects on annual per capita meat consumption in Nigeria. This underscores the important role of the macroeconomic environment in agricultural commodity consumption. Therefore, based on these findings, it is recommended that the country effectively controls inflation, improves per capita income, implements exchange rate stabilization measures, improves agricultural credit disbursement, and increases resource capacity utilization in the meat industry as complementary strategies to improve meat consumption in the country.

Table 7: Determinants of pork per capita consumption in Nigeria
Çizelge 7. Nijerya'da kişi başına domuz eti tüketiminin belirleyicileri

	ECM of pork/capita				Long run equation of pork/capita			
Variables	Coeff.	Std. error	t-value	**Variables**	Coeff.	Std. error	t-value	VIF
Constant	0.0033	0.0104	0.314	Constant	0.6084	0.2565	2.372	-
Inflation	−0.0138	0.0150	−0.919	Inflation	−0.0161	0.0263	−0.613	1.237
GDP/capita	0.072	0.0262	2.746***	GDP/capita	0.0217	0.0241	0.901	1.371
Exchange rate	0.0933	0.0332	2.811***	Exchange rate	0.1585	0.0126	12.59***	2.500
Credit disbursed.	−0.0099	0.0510	−0.194	Credit disbursed	−0.0901	0.0793	−1.136	2.934
Capacity utilized	−0.0799	0.0344	−2.324**	Capacity utilized	−0.2477	0.0517	−4.795***	1.318
Pork lag 1	0.36433	0.1345	2.709**					
ECM_{t-1}	−0.2527	0.1086	−2.327**					
R^2		0.516		R^2		0.906936		
F- cal. (7,31)		4.7240***		F- cal. (5,35)		68.21673		
Normality test		1.3362 (0.2694)						
RESET test		1.5744 (0.3684)						
Breush-Pagan		9.0484 (0.2492)						
CUSUMSQ test		-1.0002 (0.2546)						
Durbin Watson		2.1219 (0.5526)						

Source: Significance levels are indicated by ***, **, and *, which represent 1%, 5%, and 1% respectively. Variables used for the ECM are expressed in log difference, while those used for the long-run equation are expressed in a natural logarithm.

Table 8: Determinants of total meat per capita consumption in Nigeria
Çizelge 8. Nijerya'da kişi başına toplam et tüketiminin belirleyicileri

	ECM of total meat /capita				Long run equation of total meat/capita			
Variables	Coeff.	Std. error	t-value	**Variables**	Coeff.	Std. error	t-value	VIF
Constant	−0.0005	0.0075	−0.062	Constant	2.0913	0.1992	10.50***	-
Inflation	−0.0343	0.0104	−3.298***	Inflation	−0.0401	0.0104	−3.856***	1.237
GDP/capita	0.0296	0.0090	3.282***	GDP/capita	0.0403	0.0187	2.154**	1.371
Exchange rate	−0.0335	0.0316	−1.058	Exchange rate	−0.0451	0.0098	−4.608***	2.500
Credit disbursed	0.0089	0.0399	0.222	Credit disbursed	0.0998	0.0616	1.620	2.934
Capacity utilized	0.0324	0.0164	1.976*	Capacity utilized	0.0846	0.0401	2.108**	1.318
Total meat lag 1	0.1053	0.1385	0.760					
ECM_{t-1}	−0.3169	0.1252	−2.532**					
R^2		0.224		R^2		0.496		
F- cal. (7,31)		5.2183***		F- cal. (5,35)		6.8872***		
Normality test		2.2309 (0.3731)						
RESET test		2.6263 (0.11557)						
Breush-Pagan		7.7222 (0.3564)						
CUSUMSQ test		-0.9907 (0.3298)						
Durbin Watson		1.9243 (0.3237)						

Source: Computed by the authors. Significance levels is indicated by ***, **, and *, which represent 1%, 5%, and 1% respectively. Variables used for the ECM are expressed in log difference, while those used for the long-run equation are expressed in a natural logarithm.

Researchers' Contribution Rate Declaration

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declare that there is no conflict of interest between them.

REFERENCES

Agboola, M. O., & Balcilar, M. (2012). Impact of Food Security on Urban Poverty: A Case Study of Lagos State, Nigeria. *Procedia - Social and Behavioral Sciences, 65,* 1225-1229.

Akinsulu, A. A., Ajijola, S., Odetola, S. K., & Awoyemi, D. O. (2019). Factors Influencing Meat Consumption in Ijebu-North Local Government

Area of Ogun States, Nigeria. *Journal of Marketing and Consumer Research, 52,* 10-16. DOI: 10.7176/JMCR.

Akpan, S. B. (2019). Oil palm fruit supply function in Nigeria. *Ife Journal of Agriculture, 31*(3), 11–26.

Akpan, S. B. (2022a). Contribution of the crop sub-sectors performance to the economic development of Nigeria: the ARDL model approach. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 22*(4), 39–48.

Akpan, S. B. (2022b). Trends in major ruminant meats production and the roles of the macroeconomic environment in Nigeria. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 22*(4), 49–62.

Akpan, S. B., & Monday I. (2021). Factors Productivity in small scale upland Vegetable Production in the South-South region of Nigeria. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 21*(1), 35–45.

Akpan, S. B., & Nkanta, V. S. (2022). Trend Analysis and Macroeconomic Variable Determinants of Egg Production in Nigeria. *Journal of Agricultural Production, 3*(2), 100-109.

Akpan, S. B., & Okon, U. E. (2019). Vegetable Consumption Paradox: Has Domestic Consumption Match the International Recommended Minimum Standard in Nigeria? *International Journal of Advances in Agriculture Sciences, 4*(3), 1–7.

Akpan, S. B., & Udo, U. J. (2021). Indigenous meat and milk gross production indexes and the dynamic macroeconomic fundamentals in Nigeria: ARDL model approach. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 21*(3), 97–110.

Akpan, S. B., & Umoren, A. A. (2021). Agricultural production indicators and the dynamic macroeconomic variables in Nigeria: ARDL model approach. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 21*(3), 111–124.

Akpan, S. B., & Ebong, V. O. (2021). Agricultural land use and population growth in Nigeria. The need for synergy for sustainable agricultural production. *Journal Agribusiness and Rural Development, 61*(3), 269–278. http://dx.doi.org/10.17306/J.JARD. 2021.01424.

Akpan, S. B., Edet J. Udoh, Ini-mfon V. Patrick, & Udoro J. Udo (2014). Seasonal Festive Periods and Meat Price Transmission and Market Integration in Akwa Ibom State, Southern Nigeria. *Asian Journal of Agricultural Extension, Economics & Sociology, 3*(4), 331–364.

Akpan, S. B., Ini-mfon V., Glory E., & Daniel E. (2012). Agricultural Productivity and Macro-economic Variable Fluctuation in Nigeria. *International Journal of Economics and Finance, 4*(8), 114–125.

Akpan, S. B., Samuel J. Udoka, & Inimfon V. Patrick (2015). Roles of Macroeconomic Variables on Agricultural Diversification in Nigeria. *American Journal of Economics and Business Administration, 7*(2), 77–93. DOI: 10.3844/ajebasp.2015.77.93.

Akpan, S. B., Udoka, S. J., & Patrick, I. V. (2021). Agricultural Sub Sectors' Outputs and Economic Growth in Nigeria: Implication for Agricultural Production Intensification. *AKSU Journal of Agriculture and Food Sciences, 5*(1), 56–68.

Arowolo, O. V., Oladejo, J. A., Oguntoye, T. O., Kabir, G. B., & Salako, B. A. (2021). Analysis of Meat Consumption Pattern in Ogbomoso North Local Government Area, Oyo State, Nigeria. *Appl. Sci. Environ. Manage., 25*(4), 555–559.

Babatunde, R. O., & Qaim, M. (2010). Impact of off-farm income on food security and nutrition in Nigeria. *Food Policy, 35*(4), 303–311.

Bamaiyi, P. H. (2013). Factors Militating against Animal Production in Nigeria. *International Journal of Livestock Research, 3*(2), 54–66.

Baskhron, R., Khalifa, A., Al-Sharqawi, S., & Al-Rasul, A. (2019). Production and consumption of red and white meat in Egypt. *Journal of Agricultural Economics and Social Sciences, 10*(12), 651–656. https://www.academia.edu/42795463/.

Betru, S., & Kawashima, H. (2009). Pattern and determinants of meat consumption in urban and rural Ethiopia. *Livestock Research for Rural Development, 21,* Article #143. Retrieved July 9, 2022, from http://www.lrrd.org/lrrd21/9/betr 21143. htm.

Ecker, O., & Hatzenbuehler, P. L. (2022). Food consumption–production response to agricultural policy and macroeconomic change in Nigeria. *Applied Economic Perspectives and Policy, 44*(2), 982–1002. DOI: 10.1002/aepp.13161.

Egwaikhide, F. O. (1997). Import Substitution Industrialization in Nigeria: A Selective Review. *The Nigerian Journal of Economic and Social Studies, 39*(2), 183–204.

Ejedegba, R. U. (2023). Macroeconomic Instability, Policy Uncertainty, and Investment Performance: The case of Nigeria. *International Review of Economics & Finance, 83,* 1–17.

Elegbede, V. A., Afolami, C. A., & Oyedepo, E. O. (2018). Determinants of meat consumption among students of the Federal University of Agriculture Abeokuta, Ogun State Nigeria. *Nigerian Journal of Agricultural Economics (NJAE), 8*(1), 127–136.

Engle, R. F., & Granger, C. W. J. (1987). Cointegration and error correction: representation, estimation, and testing. *Econometrica, 55,* 251–276.

FAO. (2019). The future of livestock in Nigeria. Opportunities and challenges in the face of uncertainty, African Sustainable Livestock, 2050, Rome.

Fatimah, M. A., Azharia, A. E., & Ishtiag, F. A. (2022). Nexus of Macroeconomic Indicators and Meat Consumption in Saudi Arabia: An ARDL Approach. *Universal Journal of Agricultural Research, 10*(3), 240–248. DOI: 10.13189/ujar.2022.100306.

Federal Ministry of Environment Document (2021). Retrieved on the 19th of February 2022 at: https://environment.gov.ng/.

James, M., Glynn, T., & Ted, S. (2009). U.S. Beef Demand Drivers and Enhancement Opportunities: A Research Summary, Kansas State University, Department of Agricultural Economics. Available at www.agmanager.info.

Kanayo, O., Nancy, S., & Maurice, O. U. (2013). A Review of Value Added in Nigeria's Pre and Post–SAP Agricultural Sector: Background and Issues. *Journal of Economics and Behavioral Studies, 5*(1), 44–56.

Mekonnen, D. A., Trijsburg, L., Achterbosch, T., Brouwer, I., Kennedy, G., Linderhof, V., Ruben, R., & Talsma, E. F. (2021). Food consumption patterns, nutrient adequacy, and the food systems in Nigeria. *Agricultural and Food Economics, 9*(16). https://doi.org/10.1186/s40100-021-00188-2.

National Population Commission document (2023). Retrieved on the 19th of May 2022 at: https://www.nationalpopulation.gov.ng/.

Odetokun, S. M., Borokinni, B. O., Bakare, S. D., Ghali-Mohammed, I., & Alhaji, N. B. (2021). A Cross-Sectional Survey of Consumers' Risk Perception and Hygiene of Retail Meat: A Nigerian Study. *Food Protection Trends, 41*(3), 274–283.

Odoemena, K. G., Walters, J. P., & Kleemann, H. M. (2020). A System Dynamics Model of Supply-Side Issues Influencing Beef Consumption in Nigeria. *Sustainability, 12*(3241), 1–22. http://dx.doi.org/10.3390/su12083241.

OECD/FAO. (2019). *OECD-FAO Agricultural Outlook*, OECD Agriculture statistics (database), http://dx.doi.org/10.1787/agr-outl-data-en.

OECD/FAO. (2021a). *OECD-FAO Agricultural Outlook*, OECD Agriculture statistics (database), http://dx.doi.org/10.1787/agr-outl-data-en.

OECD/FAO. (2021b). *OECD-FAO Agricultural Outlook 2021-2030,* OECD Publishing, Paris, https://doi.org/10.1787/19428846-en.

Ogbeide, O. A. (2015). Meat Industry Development in Nigeria: Implications of the Consumers' Perspective. *Mayfair Journal of Agribusiness Management, 1*(1), 59–75.

Ogbonna, B. C. (2012). Structural Adjustment Program (SAP) in Nigeria: An Empirical Assessment. *Journal of Banking, 6*(1), 19–40.

Ojiako, I. A., & Olayode, G. O. (2008). Analysis of trends in livestock production in Nigeria: 1970-2005. *Journal of Agriculture and Social Research (JASR), 8*(1), 114–120.

Organization for Economic Co-operation and Development (OECD) (2023), Meat consumption (indicator). doi: 10.1787/fa290fd0-en (Accessed on 15 June 2023).

Osazuwa-Peters, M. (2021). The local contexts of meat consumption: analyzing gratification in Nigeria. *Food, Culture & Society, 24*(5), 712–730. DOI: 10.1080/15528014.2021.1936788.

Reardon, T., Tschirley, D., Liverpool-Tasie, L. S., Awokuse, T., Fanzo, J., Minten, B., Vos, R., Dolislager, M., Sauer, C., Dhar, R., Vargas, C., Lartey, A., Raza, A., & Popkin, B. M. (2021). The processed food revolution in African food systems and the double burden of malnutrition. *Global Food Security, 28,* 100466. https://doi.org/10.1016/j.gfs.2020.100466.

Simo-Kengne, B. D., Dikgang, J., & Ofstad, S. P. (2018). Effect of marine protected areas and macroeconomic environment on meat consumption in SEAFO countries. *Agric Econ 6, 12,* 1–17. https://doi.org/10.1186/s40100-018-0105-5.

Udah, S. C., Nwachukwu, I. N., & Akpan, S. B. (2015). Performance Evaluation of Various Policy Regimes Towards Agricultural Growth in Nigeria. *International Journal of Agriculture, Forestry, and Fisheries, 3*(2), 57–63.

Udah, S. C., Nwachukwu, I. N., Nwosu, A. C., Mbanasor, J., & Akpan, S. B. (2015a). Determinants of Agricultural Export Growth in Nigeria. *International Journal of Agriculture, Forestry and Fisheries, 3*(3), 105–109.

Udah, S. C., Nwachukwu, I. N., Nwosu, A. C., Mbanasor, J. A., & Akpan, S. B. (2015c). Analysis of Contribution of Various Agricultural Subsectors to Growth in Nigeria's Agricultural Sector. *International Journal of Agriculture, Forestry and Fisheries, 3*(3), 80–86.

Udoh, E. J., & Akpan, S. B. (2019). Macroeconomic variables affecting fish production in Nigeria. *Asian Journal of Agriculture and Rural Development, 9*(2216–2230).

Williams, T. O. (1989). Livestock development in Nigeria: A survey of the policy issues and options. Network Paper No. 21, International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia.

KSÜ Tarım ve Doğa Derg 27 (4), 984-993, 2024
KSU J. Agric Nat 27 (4), 984-993, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1390318

Su Ürünlerinin Endüstri-İçi Ticaretinin Analizi: Türkiye Örneği

Muhammed Fatih AYDEMİR[1]✍
[1]Bursa Uludağ Üniversitesi, Sosyal Bilimler Meslek Yüksekokulu, Dış Ticaret Bölümü, 16200, Bursa, TÜRKİYE.
[1]https://orcid.org/0000-0002-4248-9057
✉: mfaydemir@uludag.edu.tr

ÖZET
Su ürünleri sektörü; tüketim açısından nüfusun artışının devam etmesi, ülkelerin gelir düzeylerinde görülen artışlar, sağlıklı beslenmeye yönelik artan eğilim, üretim bakımından ise su ürünlerinde depolama ve ulaşım olanaklarının gelişmesi ile ülke ekonomileri açısından önemli bir sektör haline gelmiştir. Türkiye'nin su ürünleri ihracatı başta olmak üzere dış ticaretinde son yıllarda önemli bir büyüme yaşanmıştır. Bu çalışmanın amacı, Türkiye'nin dış ticaret yaptığı ülkeler ile su ürünleri sektörü endüstri-içi ticaretinin analiz edilmesidir. Bu kapsamda TÜİK'ten 2010-2022 dönemi için su ürünleri 4 ve 6 haneli HS (Harmonize Sistem) kodlu ürün grupları dış ticaret verileri, Grubel-Lloyd endeksi ile analiz edilmiştir. Çalışmanın bulgularına göre, Türkiye'nin su ürünleri dış ticaretinde EİT (endüstri-içi ticaret) düzeyinin düşük olduğu saptanmıştır. Dolayısıyla su ürünleri dış ticaretinde, ihracatın ithalattan fazla olduğu dikkate alındığında, ihracat yönlü endüstriler-arası şekli öne çıkmaktadır. Aynı zamanda ham su ürünleri ve işlenmiş su ürünlerinde de EİT seviyesinin düşük olduğu gözlenmiştir. Bu bakımdan ham su ürünlerinde ihracat yönlü, işlenmiş su ürünlerinde ise ithalat yönlü endüstriler-arası ticaret şeklinde dış ticaret yapısının olduğu ortaya çıkmıştır. Sadece ham su ürünleri içinde yer alan kabuklu hayvanlar ve yumuşakçalar ürün gruplarında EİT düzeyinin yüksek olduğu görülmüştür.

Tarım Ekonomisi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 13.11.2023
Kabul Tarihi : 26.01.2024

Anahtar Kelimeler
Su ürünleri
Endüstri-İçi ticaret
Grubel-Lloyd endeksi
İthalat-İhracat analizi

Analysis of Intra-Industry Trade in Aquaculture Products: The Case of Türkiye

ABSTRACT
The aquaculture sector has become an important sector in terms of national economies due to the continuous increase of the population in terms of consumption, the increase of the income level of the countries, the increasing tendency towards healthy nutrition, and the development of storage and transportation facilities in terms of production. Türkiye's foreign trade, especially the export of aquaculture products, has experienced significant growth in recent years. This study aims to analyze Türkiye's intra-industry trade in the aquaculture sector with the countries with which Türkiye has foreign trade. In this context, foreign trade data of aquaculture products with 4 and 6-digit HS (Harmonized System) coded product groups for the period 2010-2022 from TurkStat were analyzed using the Grubel-Lloyd index. According to the results of the study, the level of intra-industry trade (IIT) in Türkiye's foreign trade of aquaculture products is low. Therefore, considering that exports exceed imports in the foreign trade of aquaculture products, the export-oriented inter-industry pattern stands out. At the same time, it is also observed that the IIT is low for raw seafood and processed seafood. In this respect, the export-oriented inter-industry trade in raw seafood products and the import-oriented inter-industry trade in processed seafood products were found to have a foreign trade structure. Only the product groups of crustaceans and mollusks, which are included in the raw seafood products, were found to have a high level of IIT.

Agricultural Economics

Research Article

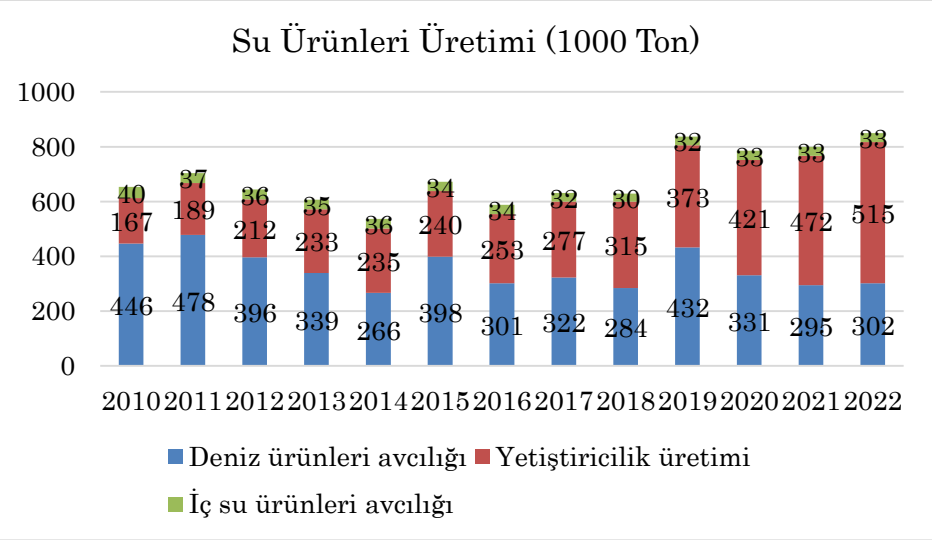
Article History
Received : 13.11.2023
Accepted : 26.01.2024

Keywords
Aquaculture products
Intra-Industry trade
Grubel-Lloyd index
Import-Export analysis

Atıf Şekli: Aydemir, M. F. (2024). Su ürünlerinin endüstri-içi ticaretinin analizi: Türkiye örneği. *KSÜ Tarım ve Doğa Dergisi*, *27*(4), 984-993. https://doi.org/10.18016/ksutarimdoga.vi.1390318
To Cite : Aydemir, M. F. (2024). Analysis of intra-industry trade in aquaculture products: The case of Türkiye. *KSU Journal of Agriculture and Nature*, *27*(4), 984-993. https://doi.org/10.18016/ksutarimdoga.vi.1390318

GİRİŞ

Dünya nüfusunun artmasıyla, gıda tüketiminde artış yaşanmaktadır. Gıda ürünlerinin tüketiminde gelir düzeyi, yaşam kalitesi ve tüketim alışkanlıkları gibi birçok faktör etkili olmaktadır (Ukav, 2023). Gıda tüketiminde önemli bir yeri olan su ürünleri, insanların sağlıklı beslenmesinde önemli bir rol oynamaktadır (Yılmaz Yavuz & Yavuz, 2022). Bu bakımdan son yıllarda sağlıklı beslenmeye gösterilen ilgi, su ürünleri tüketiminin daha da artmasını sağlamıştır. FAO (Anonim, 2022) verilerine göre, 1960'lı yıllarda kişi başı balık tüketimi yaklaşık 9 kilogram iken, 2019 yılında 20 kilogramı geçmiştir. Aynı zamanda ülkelerin gelir düzeyi de balık tüketimi açısından önemli bir role sahiptir (Kuşat, 2020). 2019 yılında gelir düzeyi düşük olan ülkeler kişi başına yaklaşık 5 kg olan balık tüketimi, orta gelirli ülkelerde yaklaşık 15 kilogram, yüksek gelirli ülkelerde 26 kilogramın üzerinde gerçekleşmiştir (Anonim, 2022). Bu açıdan bakıldığında, ülkelerin gelir düzeyi arttıkça su ürünlerine olan talep artmaktadır. Bu faktörlerin dışında su ürünlerinin depolanması ve dağıtımı ile ilgili teknolojik gelişmeler, bu sektördeki üretimi olumlu yönde etkilemiştir. 2050 yılında kişi başı balık tüketiminin 25 kilogramı geçeceği beklentisi (Anonim, 2022), bu sektörün gelecekte daha önemli bir yere sahip olacağına işaret etmektedir. Su ürünlerinin tüketiminde dünya genelinde bu tür gelişmeler yaşanırken, Türkiye'nin su ürünleri tüketiminde kişi başına ortalama 7 kilogram ile önemli bir değişim yaşanmamıştır (Anonim, 2023c). Türkiye'nin su ürünleri tüketiminde yeterince ilerleme sağlanamasa da, üretimde genel bir büyüme eğiliminden bahsetmek mümkündür. Bu açıdan TÜİK (Anonim, 2023a) verileri ile oluşturulan Türkiye su ürünlerine yönelik bin ton bazında üretim miktarlarına Şekil 1'de yer verilmiştir.



Şekil 1. Türkiye Su Ürünleri Üretimi
Figure1. Türkiye's Aquaculture Production

Şekil 1'e bakıldığında, Türkiye su ürünleri üretimi toplamı 2010 yılında yaklaşık 610 bin ton iken, 2014 yılında 537 bin tona düşmüş, daha sonra genel eğilim artış şeklinde olmuş, 2022 yılında 850 bin ton olarak gerçekleşmiştir. Asıl dikkat çekici olan ise su ürünleri yetiştiriciliğinin 2010 yılından 2022 yılına kadar olan süreçte iki kattan fazla artış yaşamasıdır. Başka bir bakış açısıyla, 2010 yılında yetiştiriciliğin toplam su ürünleri içindeki payı %25 iken, bu pay 2022 yılında %60'a yükselmiştir. Avcılık tarafında ise deniz ürünleri avcılığında genel eğilim düşüş şeklinde olmuş ve 2022 yılında 302 bin ton olarak gerçekleşmiştir. İç su ürünleri avcılığının payı ise bütün yıllarda düşük olmuştur. Dolayısıyla avcılığın su ürünleri içindeki payı azalırken, yetiştiriciliğin payında önemli bir artış olmuştur. Su ürünleri üretimi ile birlikte su ürünlerinin dış ticaretinde de gelişmeler yaşanmaktadır. Türkiye'nin su ürünleri dış ticareti yaklaşık %15 büyürken, dünya su ürünleri dış ticareti aynı dönemde %5 civarında büyüme göstermiştir (Dalkıran, 2019). Bu çerçevede, Türkiye'nin su ürünleri dış ticaretindeki büyüme, dünya ortalamasının üzerinde gerçekleşmiştir. Bu kapsamda Türkiye'de su ürünleri sektörünün önemi gün geçtikçe daha fazla artmaktadır. Buna ilişkin olarak Türkiye'nin su ürünleri dış ticaret rakamlarına ve dış ticaretteki payına yer verilmiştir (Çizelge 1).

Çizelge 1. Türkiye'nin dış ticaretinde su ürünlerinin payı
Table 1 Share of aquaculture products in Türkiye's foreign trade

	Su Ürünleri Dış Ticaret Değerleri (1000 $)			Toplam Dış Ticaret Değerleri (1000 $)			Su Ürünlerinin Dış Ticaretteki Payı		
Yıl	İhracat	İthalat	Dış Ticaret Hacmi	İhracat	İthalat	Dış Ticaret Hacmi	İhracat Payı	İthalat Payı	Dış Ticaret Payı
2010	363582	257288	620870	113883219	185544332	299427551	0.003	0.001	0.002
2011	440089	291534	731623	134906869	240841676	375748545	0.003	0.001	0.002
2012	457439	333319	790758	152461737	236545141	389006878	0.003	0.001	0.002
2013	588169	396192	984361	151802637	251661250	403463887	0.004	0.002	0.002
2014	707712	400089	1107801	157610158	242177117	399787275	0.004	0.002	0.003
2015	704598	450090	1154688	143838871	207234359	351073230	0.005	0.002	0.003
2016	805965	409605	1215570	142529584	198618235	341147819	0.006	0.002	0.004
2017	855555	466644	1322199	156992940	233799651	390792591	0.005	0.002	0.003
2018	960971	471674	1432645	167920613	223047094	390967707	0.006	0.002	0.004
2019	1009857	507514	1517371	171464945	202704320	374169265	0.006	0.003	0.004
2020	1092184	454248	1546432	160656652	209534325	370190977	0.007	0.002	0.004
2021	1379327	534119	1913446	213598369	260682217	474280586	0.006	0.002	0.004
2022	1676924	810481	2487405	235247081	342209950	577457031	0.007	0.002	0.004

Kaynak: Anonim, 2023b; Orijinal Hesaplamalar.

Çizelge 1'de, 2010-2022 döneminde Türkiye su ürünleri ihracatı yaklaşık beş kat artarak 1.7 milyar ABD dolarına yaklaşmıştır. Aynı dönemde yapılan su ürünleri ithalatı hemen hemen iki kat artarak 810 milyon dolara ulaşmıştır. Dolayısıyla su ürünleri ihracatı ithalattan çok hızlı artmış, ihracat ithalatın iki katını geçmiştir. Bu açıdan son yıllardaki dış ticaret fazlası da artarak devam etmektedir. Diğer taraftan Türkiye su ürünleri dış ticaretinin toplam dış ticareti içindeki payları ise düşük kalmıştır. İhracat için bu pay binde 7 iken, ithalatta binde 2 ve dış ticaretteki pay ise binde 4 seviyesinde olmuştur. Ayrıca su ürünleri ihracatındaki artış, Türkiye'nin toplam ihracatındaki artıştan daha fazla olmuştur. Bu verilerden hareketle, Şekil 1'de yer alan su ürünleri üretimindeki büyüme ile karşılaştırıldığında, su ürünleri ihracatının daha fazla büyümesi önemli bir gelişme olarak kabul edilebilir. Böylelikle son yıllarda su ürünleri dış ticaretine verilen önemin arttığı anlaşılmaktadır.

Klasik uluslararası ticaret teorileri genel olarak her bir ülkenin karşılaştırmalı üstünlüğe sahip olduğu ürünlerde uzmanlaşmasını ve uzmanlaştığı ürünleri ihraç etmesi gerektiğini ileri sürmektedir. Aynı zamanda ülkelerin uluslararası ticarette karşılaştırmalı olarak dezavantajlı olduğu ürünlerde ise ithalat yapması gerektiği ve böylelikle uluslararası ticaretten ülkelerin daha kazançlı çıkacağı ifade edilmektedir (Atik & Türker, 2011). Böyle bir durumda ülkeler arasındaki ticarette farklı ürünlerin dış ticaretinin daha yoğun olması beklenmektedir. Fakat yapılan ampirik araştırmalar ülkeler arasındaki ticaretin farklı mallar üzerinden değil, daha çok aynı sektörde yer alan farklılaştırılmış ürünlerin uluslararası ticaretinin ağırlıkta olduğunu göstermiştir (Balassa, 1966). Bu durum, endüstri-içi ticaret (EİT) olarak ifade edilmektedir. EİT, en basit ifadeyle aynı endüstride yer alan farklılaştırılmış ürünlerin eş zamanlı olarak ithalatı ve ihracatını ifade etmektedir (Tharakan & Calfat, 1996). Diğer taraftan, karşılaştırmalı üstünlüğe dayalı olarak ortaya çıkan uluslararası ticaret şekli, endüstriler-arası ticaret (EAT) olarak belirtilmektedir (Atik & Türker, 2011).

Greenaway ve Milner (1983), iki nedenden dolayı EİT'in önemli olduğunu savunmaktadır. İlki ülkeler arasında gerçekleştirilen uluslararası ticaretin sadece faktör oranları ile belirtilemeyeceğidir. İkincisi ise EİT'e önem veren ülkelerin uluslararası ticarete daha kolay adapte olabileceğidir (Greenaway & Milner, 1983). Diğer taraftan gelir düzeylerindeki farklılıklardan dolayı gelişmiş ülkeler arasında gerçekleşen EİT'in, gelişmekte olan ülkeler arasındaki EİT'e göre daha yüksek olduğu ortaya çıkmıştır (Başkol, 2009). Son dönemde gelişmekte olan ülkelerin gelir düzeylerinin artması ve ekonomik entegrasyonlara bu ülkelerin daha çok katılımı ve çokuluslulların bu ülkelere yaptıkları yatırımlar sayesinde daha fazla ürün çeşitlendirmesine gitmektedirler. Ürün çeşitlendirmesinin artmasıyla hem gelişmekte olan ülkeler ölçek ekonomilerinden daha fazla yararlanmakta hem de EİT düzeylerinde yükselişler görülmektedir. Diğer taraftan ürün

farklılaştırmasına bağlı olarak fiyat farklılaştırmasının fazla olduğu ürünler, üretim süreci daha uzun olan ve sermaye-yoğun olarak üretilen ürünlerde EİT düzeylerinin daha yüksek olduğu vurgulanmıştır (Pomfret, 1987). Ayrıca monopolcü rekabetin daha yoğun hissedildiği, çokulusluların daha yoğun faaliyet gösterdiği ve dünya ticaretine daha fazla eklemlenen ülkelerde EİT seviyelerinin daha yüksek olduğu ifade edilmiştir (Yılmaz, 2016).

Çizelge 2. Türkiye'nin EİT analizine yönelik bazı çalışmalar
Table 2 Some Studies on Türkiye's IIT Analysis

Çalışma	Yıl	Ülke veya Ülkeler	Sektör veya Ürün grubu	Bulgular
Başkol (2009)	1969-2009	Türkiye ile Dış Ticaret Yaptığı Ülkeler	İmalat Sanayi	Türkiye'nin imalat sanayi EİT düzeyinin düşük olduğu tespit edilirken, EİT düzeyinin zaman içinde arttığı gözlenmiştir.
Küçükefe (2009)	1982-2008	Türkiye ile Dış Ticaret Yaptığı Ülkeler	Bütün Sektörler	Türkiye'nin EİT düzeyinin genel olarak düşük olduğu tespit edilirken, EİT düzeylerinin zaman içinde arttığı gözlenmiştir. Gıda sektöründe EİT oranlarının 0.50 düzeyinde dalgalandığı bulgusuna ulaşılmıştır.
Başkol (2012)	1992-2009	Türkiye ile Orta Asya Türk Cumhuriyetleri	Bütün Sektörler	Türkiye ile Orta Asya Türk Cumhuriyetleri arasındaki dış ticarette EİT oranlarının çok düşük olduğu tespit edilmiştir. Dolayısıyla bu ülkeler arasındaki uluslararası ticaretin EAT şeklinde olduğu anlaşılmıştır.
Bashimov (2017)	2002-2014	Türkiye ile Rusya	Tarım ve Gıda Ürünleri	Tarım ve gıda ürünlerinde EİT'in yüksek olduğu ortaya çıkarken, bitkisel ürünlerdeki EİT oranlarının daha yüksek olduğu tespit edilmiştir.
Mangır ve Fidan (2017)	1996-2016	Türkiye ile Dış Ticaret Yaptığı Ülkeler	Tarım Ürünleri	Gıda ürünlerinde yüksek seviyede EİT ile ham tarım ürünlerinde ise düşük seviyede EİT ile karşılaşılmıştır.
Yurttançıkmaz ve Azgün (2017)	1995-2015	Türkiye ile Komşu Ülkeler	Bütün Sektörler	Genel olarak düşük seviyede EİT ile karşılaşılırken, gıda ve canlı hayvan ürün grubunda bazı komşu ülkeler ile yüksek seviyede EİT değerlerinin olduğu tespit edilmiştir.
Kemer ve Aydemir (2017)	2001-2014	Türkiye ile Dış Ticaret Yaptığı Ülkeler	İmalat Sanayi	2001-2010 döneminde düşük seviyede EİT oranları tespit edilirken, 2010 ve sonrası yüksek düzeyde EİT ile karşılaşılmıştır.
Kurt ve İmren (2018)	2007-2017	Türkiye ile G8 Ülkeleri	Tıbbi ve Aromatik Bitkiler Ürün Grubu	Türkiye'nin dış ticaretinde "kebere, çöven, ıhlamur ve kişniş" ürünlerinde EİT'in hakim olduğu, "defne, kekik, mahlep, nane, rezene ve sumak" ürünlerinde ihracat yönlü EAT, "çörekotu ve zencefil" ürünlerinde ise ithalat EAT olduğu görülmüştür.
Erdal ve Durmuş (2019)	2005-2017	Türkiye ile Dış Ticaret Yaptığı Ülkeler	Tarım Ürünleri	Tarım ürünlerinde yüksek düzeyde EİT ticaret değerleri ile karşılaşılmıştır. Su ürünlerinde 2010-2013 dönemi ve 2015 yüksek düzeyde EİT oranlarına ulaşılırken, diğer yıllarda düşük seviyede EİT olduğu tespit edilmiştir.
Kuşat (2020)	2009-2019	Türkiye ile AB (28) Üyesi Ülkeler	Su Ürünleri	Su ürünlerinde EİT düzeyinin çok düşük olduğu tespit edilirken, Fransa ile EİT oranlarının daha yüksek olduğu bulgusuna ulaşılmıştır.

Çizelge 2'de görüldüğü gibi, Türkiye'nin dış ticaretinin yapısına yönelik EİT analizi içeren birçok çalışma yapılsa da, bu çalışmaların bir kısmı gıda ve tarım ürünlerine yönelik yapılmıştır. Doğrudan su ürünlerini ele alan sadece tek bir çalışma (Kuşat, 2020) olduğu görülmüştür. İlgili çalışmada, Türkiye ile AB ülkeleri arasındaki su ürünleri ticaretinde EİT düzeylerinin oldukça düşük olduğu tespit edilmiştir (Kuşat, 2020).

Su ürünleri dış ticaretinin artan önemi dikkate alındığında, bu çalışmanın amacı Türkiye'nin su ürünleri sektöründe dış ticaret yaptığı ülkeler ile endüstri-içi ticaretinin analiz edilmesidir. Bu çalışma ile su ürünleri sektöründe başta işlenmişlik seviyelerine göre su ürünlerinin EİT'inin analiz edilmesi açısından diğer çalışmalardan ayrışmaktadır.

Bunun ötesinde 4 ve 6 haneli HS kodlarına göre ürün gruplarının EİT analizleri ile çalışma daha detaylı olarak gerçekleştirilmiştir. Bu kapsamda, Türkiye'nin su ürünleri dış ticaretinde EİT'i hesaplamak için TÜİK (Anonim, 2023b) özel ticaret sistemine göre HS (Harmonize Sistem) 4 ve 6 haneli ürün gruplarının verileri kullanılmıştır. 2010-2022 dönemi için elde edilen veriler, Grubel-Lloyd endeksi ile analiz edilmiştir. Grubel-Lloyd endeksi ile öncelikli olarak su ürünleri sektörünün ortalama EİT oranlarına ulaşılmıştır. Daha sonra su ürünleri ham ve işlenmiş su ürünleri olmak üzere işlenmişlik seviyelerinin ortalama EİT seviyelerine için hesaplamalar yapılmıştır. EİT oranlarını daha detaylı görebilmek için HS kodlarına göre alt ürün gruplarına yönelik su ürünlerinin EİT analizleri yapılmıştır.

MATERYAL ve METOD

Materyal

Türkiye'nin su ürünleri dış ticaretinde EİT'i hesaplamak için öncelikle su ürünlerinin kapsamına odaklanılmıştır. Bu çerçevede, su ürünlerinin kapsamını ve sınıflandırmasını belirlemek için Luo ve Han (2018) dikkate alınmıştır. Bu çerçevede Çizelge 3'te işlenmişlik seviyelerine göre su ürünlerine bakıldığında, ham su ürünleri 10 alt ürün grubundan oluşurken, işlenmiş su ürünleri 6 alt ürün grubundan oluşmaktadır. Bu kapsamda, TÜİK (Anonim, 2023b) özel ticaret sistemine göre HS (Harmonize Sistem) kodları açısından ürün gruplarının verileri elde edilmiştir.

Çizelge 3. İşlenmişlik seviyelerine göre su ürünleri HS kodları
Table 3 Aquaculture products seafood HS codes by processing level

Ham Su Ürünleri Grubu		İşlenmiş Su Ürünleri Grubu	
HS Kodu	Kategori İsmi	HS Kodu	Kategori İsmi
0301	Canlı balık	1504	Balıkların veya deniz memelilerinin katı ve sıvı yağları ve bunların fraksiyonları (kimyasal olarak değiştirilmemiş)
0302	Balıklar (taze veya soğutulmuş); 03.04 pozisyonundaki balık filetoları ve diğer balık etleri hariç	1604	Hazırlanmış veya konserve edilmiş balıklar; havyar ve balık yumurtalarından elde edilen havyar yerine kullanılan ürünler
0303	Balıklar (dondurulmuş); 03.04 pozisyonundaki balık filetoları ve diğer balık etleri hariç	1605	Hazırlanmış veya konserve edilmiş kabuklu hayvanlar, yumuşakçalar ve diğer su omurgasızları
0304	Balık filetoları ve diğer balık etleri (taze, soğutulmuş veya dondurulmuş)	051191	Balık, kabuklu hayvan, yumuşakça ve suda yaşayan omurgasızların ürünleri ve suda yaşayan cansız hayvanlar
0305	Balıklar (kurutulmuş, tuzlanmış veya salamura edilmiş), tütsülenmiş balıklar	230120	Balık, kabuklu deniz hayvanı, yumuşakça veya diğer su omurgasızlarının unları, kaba unları ve pelletleri;
0306	Kabuklu hayvanlar (canlı, taze, soğutulmuş, dondurulmuş, kurutulmuş, tuzlanmış, vb. işlem görmüş)	391310	Aljinik asit, tuzları, esterleri (ilk şekillerde)
0307	Yumuşakçalar (canlı, taze, soğutulmuş, dondurulmuş, kurutulmuş, tuzlanmış, salamura veya tütsülenmiş)		
0508	Mercan ve benzeri maddeler, yumuşakça veya kabuklu hayvanların kabukları (işlenmemiş veya basit şekilde hazırlanmış)		
7101	Tabii inci ve kültür incileri; mıhlanmamış veya takılmamış, ipliğe dizilmemiş veya geçici olarak ipliğe dizilmiş		
130231	Agar-agar		

Kaynak: Luo ve Han, 2018.

Metod

EİT'in analizi literatürde birçok farklı yöntem uygulanmıştır. Bu yöntemlerden en çok tercih edilen standart Grubel-Lloyd Endeksi olmuştur. Standart Grubel-Lloyd Endeksi'nin hesaplanma yöntemi aşağıdaki şekilde gösterilebilir (Grubel & Lloyd, 1971):

$$B_i = \frac{[(X_i + M_i) - |X_i - M_i|]}{(X_i + M_i)}.100 \quad ; 0 \leq B_i \leq 1 \qquad (1)$$

X_i: i sektöründeki ihracat değerini,
M_i: i sektöründeki ithalat değerini belirtmektedir.

B_i, 0 ile 100 arasında değerler almaktadır. B_i = 0 olduğunda ülkeler arasındaki ticarette EİT'in olmadığı veya EAT'in en üst düzeyde olduğu anlaşılırken, B_i = 100 olduğunda ise uluslararası ticarette tam EİT'e ulaşılmaktadır. 50 ve üzeri değerler yüksek düzeyde EİT'i gösterirken, 50'nin altında yer alan değerler EİT'in düşük olduğunu ifade etmektedir.

EİT, ürün grupları veya alt sektörler için toplulaştırılmış şekilde de hesaplama yapılabilmektedir. Bu doğrultuda, n sayıda sektörün toplam uluslararası ticaret değeri içinde yer alan ürün gruplarının ihracat ve ithalat oranları ile ağırlıklandırılması ile oluşturulan ortalama EİT, aşağıdaki gibi formüle edilebilmektedir (Grubel & Lloyd, 1971; Grubel & Lloyd, 1975):

$$\overline{B_i} = \frac{\sum_i^n B_i\,(X_i + M_i)}{\sum_i^n (X_i + M_i)}.100 = \frac{\sum_i^n (X_i + M_i) - \sum_i^n |X_i - M_i|}{\sum_i^n (X_i + M_i)}.100 \quad ; 0 \leq B_i \leq 1 \qquad (2)$$

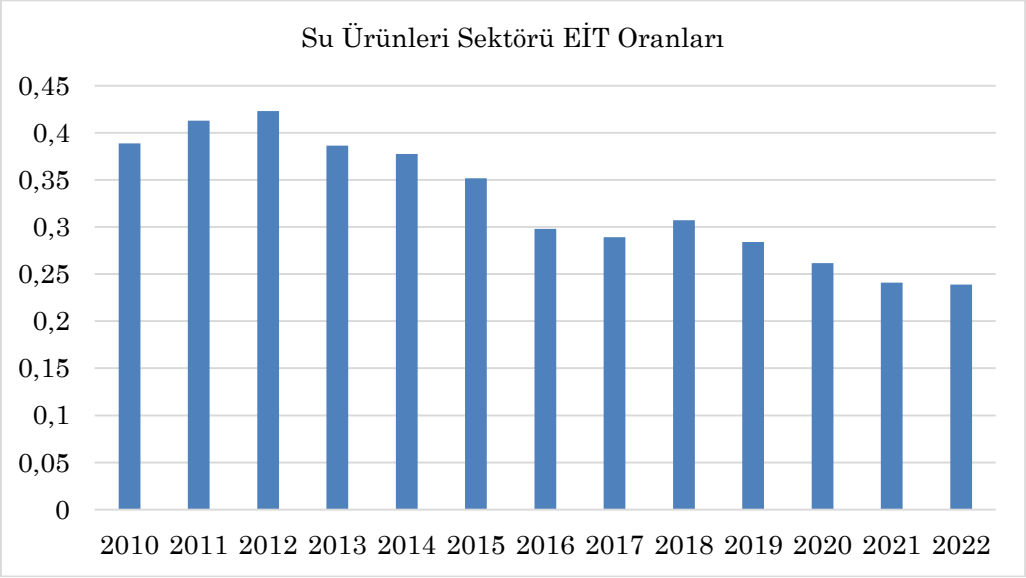
Çalışmada EİT değerleri 100'e bölünmüştür. Böylelikle ortaya çıkan değerlerin, oransal olarak değerlendirilmesi sağlanmıştır. Bulgular, Çizelge 4'e göre yorumlanmıştır. EİT'e yönelik sayısal ifadeler 0 ile 1 arasındaki değerler ile değerlendirilmiştir. 0.50 ve üzerinde yer alan değerler, EİT'in yüksek olduğuna yönelik bilgi vermekte ve uluslararası ticaretin ağırlıklı olarak benzer ürün gruplarında iki yönlü olarak gerçekleştiğine işaret etmektedir. 0.50'nin altında kalan değerler ise ülkeler arasında yapılan ticaretin EAT biçiminde olduğuna işaret etmekte ve uluslararası ticaretin karşılaştırmalı üstünlüklere göre yapıldığını göstermektedir.

Çizelge 4. G-L değer aralıklarına göre EİT derecelendirmeleri
Table 4 IIT Ratings Based on G-L Value Ranges

G-L Değer Aralığı	EİT Derecesi
0<G-L<0.25	Çok düşük
0.25<G-L<0.50	Düşük
0.50<G-L<0.75	Yüksek
0.75<G-L<1	Çok Yüksek

BULGULAR

Bu bölümde Türkiye'nin su ürünleri sektörü dış ticaretine ilişkin verilere yönelik Grubel-Lloyd endeksi ile hesaplanan değerlere yer verilmiştir. Bu kapsamda ilk olarak su ürünleri sektörü için EİT hesaplamaları yapılmıştır. İkinci olarak ham ve işlenmiş su ürünlerine yönelik EİT oranlarına yer verilmiştir. Üçüncü olarak, 4 ve 6 haneli HS kodlarına göre sınıflandırılan ürün gruplarına göre su ürünlerinin EİT analizi yapılmıştır. Su ürünlerinin ağırlıklandırılmış Grubel-Lloyd endeksine göre hesaplanan EİT oranları, Şekil 2'de gösterilmiştir.

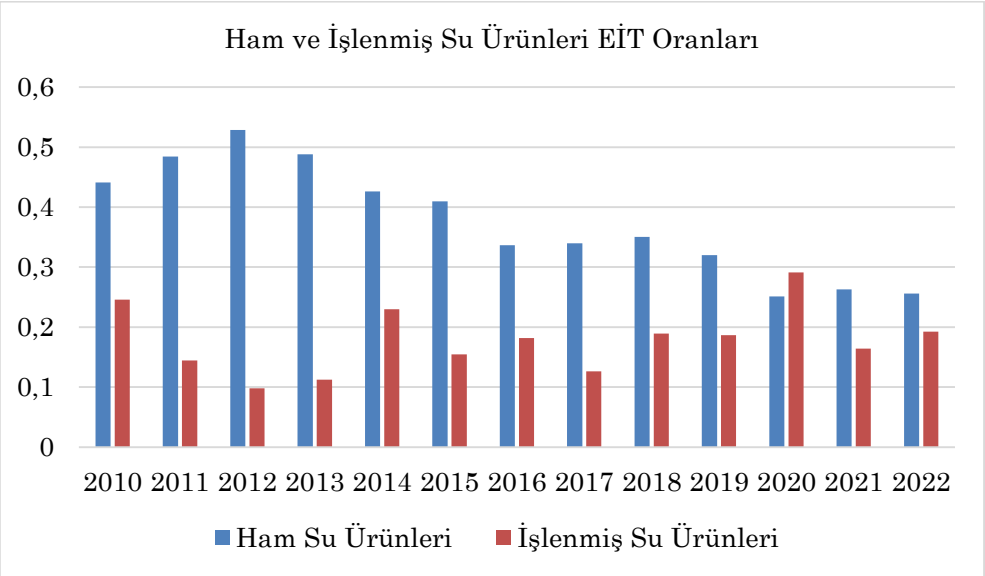


Şekil 2. Su Ürünleri Sektörü EİT Oranları (Ortalama G-L Endeksi)
Figure2. IIT Ratios for the Aquaculture Sector (Average G-L Index)

Şekil 2'ye göre, Türkiye'nin su ürünleri dış ticaretinde 0.42 değeri ile EİT oranının en yüksek olduğu 2012 yılı olmuştur. 2010-2020 döneminde su ürünleri EİT oranlarının düşük olduğu, 2021-2022 döneminde ise

çok düşük olduğu görülmüştür. Türkiye'nin su ürünleri dış ticaretinde EİT'in düşük seviyede olması ve dış ticaret fazlasının olması nedeniyle dış ticaret yapısının daha çok ihracat yönlü EAT şeklinde olduğu anlaşılmıştır.

Su ürünleri dış ticaretine yönelik EİT analizini daha ayrıntılı bir şekilde işlenmişlik seviyelerine göre görebilmek için ham ve işlenmiş su ürünleri ayrımı yapılmıştır. Ham ve işlenmiş su ürünleri ürün grupları için (ağırlıklandırılmış) Grubel-Lloyd endeksi kullanılarak hesaplamalar yapılarak Şekil 3'te gösterilmiştir.



Şekil 3. Ham ve işlenmiş su ürünleri EİT oranları (Ortalama G-L endeksi)
Figure3. IIT Ratios of raw and processed aquaculture products (Average G-L index)

Şekil 3'te, ham su ürünlerinin EİT düzeyinin 2020 yılı dışında diğer bütün yıllarda işlenmiş su ürünlerine göre daha yüksek olduğu görülmektedir. Ham su ürünlerine ilişkin 2012 yılında yüksek düzeyde EİT oranı ile karşılaşılsa da, son yıllarda EİT düzeyinin düşük olduğu gözlenmiştir. İşlenmiş su ürünlerinde ise dalgalı bir seyir olmakla birlikte, 2020 yılı dışında bütün dönemde EİT düzeylerinin düşük olmuştur. Dolayısıyla Türkiye'nin ham ve işlenmiş su ürünlerinin her ikisinde de karşılaştırmalı üstünlüklere göre dış ticaret yapısı öne çıkmaktadır. Bunun ötesinde dış ticaret verileri dikkate alındığında ham su ürünlerinde ihracat yönlü EAT, işlenmiş su ürünlerinde ithalat yönlü EAT dikkat çekmektedir.

Su ürünleri sektörü HS kodlarına göre 16 alt ürün grubundan oluşmaktadır. Ürün gruplarına göre EİT oranlarının gösterildiği Çizelge 5'e bakıldığında, ham su ürünleri içinde bulunan 0301 kodlu "Canlı balık" alt ürün grubunda 2012, 2018, 2020 ve 2021 yıllarda çok yüksek düzeyde EİT oranlarının olduğu görülmüştür. 2016, 2017 ve 2019 yıllarında ise yüksek düzeyde EİT oranlarına ulaşılmıştır. Diğer yıllarda ise EİT oranlarının düşük olduğu bulgusuna ulaşılmıştır. 0302 kodlu "Balıklar (dondurulmuş)" ürün grubunda 2022 yılı dışında yüksek ve çok yüksek EİT oranları ile karşılaşılmıştır. 0304 kodlu "Balık filetoları ve diğer balık etleri (taze, soğutulmuş veya dondurulmuş)" ürün grubunda sadece 2010 yılında yüksek EİT oranı gözlenmiştir. 0306 "Kabuklu hayvanlar" ürün grubunda bütün yıllarda EİT oranlarının yüksek olduğu göze çarpmıştır. 0307 kodlu "Yumuşakçalar" ürün grubunda 2010 yılı dışında EİT oranları yüksek ve çok yüksek olmuştur. 7101 kodlu "Tabii inci ve kültür incileri" ürün grubunda ise sadece 2017 yılında yüksek seviyede EİT ile karşılaşılmıştır. Diğer ham su ürünlerinde ise EİT oranları oldukça düşük kalmış, bu ürünlerin karşılaştırmalı üstünlüğe dayalı dış ticareti öne çıktığı anlaşılmıştır.

İşlenmiş su ürünleri içinde yer alan 1504 kodlu "Balıkların veya deniz memelilerinin katı ve sıvı yağları ve bunların fraksiyonları" ürün grubunda 2010, 2014 ve 2020 yıllarında yüksek EİT olduğu tespit edilmiştir. 1604 kodlu "Hazırlanmış veya konserve edilmiş balıklar; havyar ve balık yumurtalarından elde edilen havyar yerine kullanılan ürünler" ürün grubunda sadece 2020 yılında yüksek EİT değeri ile karşılaşılmıştır. Bu ürün grupları dışında yer alan su ürünlerinde ise EİT oranları düşük kalmıştır. Dolayısıyla işlenmiş su ürünleri dış ticaretinde karşılaştırmalı üstünlüğe dayalı ticaretin hakim olduğu görülmüştür.

TARTIŞMA ve SONUÇ

Su ürünleri sektörü, gıda sektörü içinde önemini her geçen daha fazla arttıran alt sektörlerden birini oluşturmaktadır. Dünya genelinde yaşanan nüfus artışı, daha sağlıklı yaşama isteği ve gelir düzeylerinin artması gibi nedenler su ürünlerine olan talebi

arttırmaktadır. Aynı zamanda üretim, depolama ve ulaşımdaki gelişmeler su ürünleri arzının artmasına destek olmaktadır. Dolayısıyla su ürünlerindeki büyüme eğiliminin devam edeceği beklentisi öne çıkmaktadır.

Tablo 5. Ürün gruplarına göre EİT oranları (G-L endeksi)
Table 5 IIT ratios for product groups (G-L Index)

HS Kodu	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
0301	0.41	0.28	0.90	0.47	0.32	0.33	0.70	0.59	0.88	0.72	0.83	0.96	0.49
0302	0.32	0.37	0.43	0.31	0.26	0.31	0.20	0.18	0.16	0.14	0.10	0.12	0.14
0303	0.69	0.81	0.89	0.99	0.98	0.73	0.88	0.76	0.98	0.78	0.72	0.64	0.47
0304	0.52	0.46	0.40	0.38	0.32	0.31	0.22	0.26	0.18	0.22	0.14	0.05	0.13
0305	0.09	0.04	0.03	0.02	0.02	0.03	0.00	0.03	0.03	0.01	0.01	0.00	0.03
0306	0.76	0.68	0.52	0.93	0.75	0.97	0.69	0.85	0.97	0.96	0.84	0.82	0.53
0307	0.32	0.67	0.74	0.65	0.75	0.96	0.79	0.83	0.68	0.79	0.58	0.94	0.60
0508	0.38	0.16	0.02	0.12	0.23	0.24	0.28	0.16	0.31	0.18	0.01	0.09	0.17
1504	0.64	0.41	0.22	0.28	0.53	0.34	0.47	0.24	0.42	0.27	0.53	0.28	0.42
1604	0.33	0.23	0.23	0.07	0.22	0.27	0.28	0.18	0.08	0.30	0.52	0.36	0.34
1605	0.12	0.03	0.06	0.09	0.15	0.25	0.17	0.29	0.19	0.18	0.22	0.24	0.18
7101	0.24	0.02	0.31	0.12	0.05	0.03	0.08	0.57	0.02	0.05	0.42	0.17	0.06
051191	0.03	0.05	0.08	0.26	0.03	0.05	0.04	0.01	0.04	0.07	0.02	0.02	0.00
130231	0.02	0.06	0.01	0.01	0.03	0.01	0.02	0.07	0.00	0.02	0.02	0.15	0.04
230120	0.09	0.02	0.03	0.00	0.03	0.00	0.03	0.05	0.10	0.14	0.13	0.04	0.03
391310	0.04	0.02	0.06	0.14	0.12	0.15	0.13	0.13	0.09	0.12	0.07	0.12	0.13

Kaynak: Anonim, 2023b; Orijinal Hesaplamalar.

Türkiye'de su ürünlerinin üretiminde özellikle 2019 yılında önemli bir büyüme gerçekleşmiştir. Daha sonraki yıllarda ise benzer üretim miktarları devam ettirilmiştir. Üretimdeki büyüme içinde yer alan yetiştiricilik üretiminin payındaki artış dikkat çekici bir gelişme olarak görülebilir. Diğer taraftan 2010-2022 döneminde su ürünleri ihracatından elde edilen gelir iki kat artarken, ithalattaki artış üç kat olmuştur. Aynı zamanda ihracatın ithalatın iki katına ulaşması, sektördeki dış ticaret fazlasının artarak devam ettiğini göstermektedir. Dolayısıyla su ürünlerinin üretimindeki artışa göre, dış ticaretteki ihracat yönlü büyüme daha fazla olmuştur. Ayrıca bu dönemde su ürünlerinin dış ticaretten aldığı pay sınırlı düzeyde kalsa da, su ürünlerinin üretimi ve ihracatında önemli gelişmeler yaşanmıştır.

Yapılan çalışma neticesinde, su ürünleri dış ticaretinde EİT oranının düşük olduğu görülmüştür. Bu sonuç, ham su ürünleri dış ticaretinin EİT oranlarını analiz eden Erdal ve Durmuş (2019)'un çalışmasından farklılık taşımaktadır. Yine bu çalışma, AB üyeleri ile Türkiye'nin dış ticareti üzerinden EİT analizine odaklanan Kuşat (2020) ile paralellik göstermektedir. İlgili çalışmalardan farklı olarak, bu çalışmada işlenmiş su ürünlerine dikkat çekilmiş ve alt ürün grupları daha detaylı analiz edilmiştir.

Ham su ürünlerinde EİT oranı sadece 2012 yılında yüksek iken diğer yıllarda düşük olmuş, sonraki dönemde ise azalmıştır. Ham su ürünleri kapsamında 0306 ve 0307 kodlu ürün gruplarında yüksek EİT düzeyi dikkat çekmiştir. İşlenmiş su ürünlerinde ele alınan bütün dönemde EİT oranlarının düşük olduğu gözlenmiş, sadece 1504 ve 1604 kodlu ürün gruplarında EİT'e ulaşma potansiyelinin daha yüksek olduğu anlaşılmıştır.

Türkiye'nin işlenmiş su ürünlerinde ihracatın yeterince artmadığı ve daha çok ithalatçı konumda olduğu dikkate alındığında, su ürünlerinde yetiştiriciliğin payı artmasına rağmen, işlenmiş su ürünlerine henüz yeterince önem verilmediği anlaşılmaktadır. Bununla birlikte işlenmiş su ürünlerinde EİT oranlarının düşük olması, Türkiye'nin su ürünleri dış ticaretindeki potansiyeline tam olarak ulaşamadığı yönünde bilgi vermektedir. Başta konserve balık ve balık yağları olmak üzere işlenmiş su ürünlerindeki EİT düzeyinin artması durumunda, Türkiye'nin su ürünleri dış ticaretinden daha fazla fayda elde edebileceği düşünülmektedir. Aynı zamanda işlenmiş su ürünlerinde ürün çeşitlendirme olanağının daha yüksek olması, Türkiye'nin su ürünleri dış ticaretinde daha yüksek EİT'e ulaşma imkânı vermektedir. Bu doğrultuda hem işlenmiş su ürünlerinin daha yüksek katma değer sağlaması hem de raf ömrünün daha uzun olması dikkate alındığında, Türkiye'nin işlenmiş su

ürünlerinin üretimini ve ihracatını arttırması için su ürünleri içerisinde işlenmiş su ürünlerini öncelikleyen teşvik ve destek mekanizmalarına ağırlık vermesi gerekmektedir. Bütün bunlar düşünüldüğünde, gelir seviyesinin daha yüksek olduğu ülkeler ile yapılan ticarette ve işlenmiş su ürünleri başta olmak üzere ham su ürünleri içinde yetiştiriciliğe dayalı ürünlerde EİT'in arttırılması, Türkiye'nin su ürünleri dış ticaretine daha önemli katkı sağlayabilir. Bu bakımdan hem yüksek gelirli ülkeler grubu olarak hem de artan su ürünleri talebinden dolayı AB'ye yapılan su ürünlerinin ihracatına daha fazla önem verilmesi gerekmektedir. Ayrıca, Türkiye'de hala düşük seviyede olan ham ve işlenmiş su ürünleri tüketiminin önemine daha fazla dikkat çekilmeli, böylelikle artan iç talep ile birlikte sağlanacak ölçek ekonomisinin ihracatı destekleyeceği düşünülmektedir. Aynı zamanda literatürde işlenmiş su ürünlerine yönelik çalışmaların az olması nedeniyle bu konuda daha fazla bilimsel yayına ihtiyaç duyulmaktadır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazar çalışmanın tamamını kendisinin yaptığını beyan eder.

Çıkar Çatışması

Yazar bu çalışmada herhangi bir çıkar çatışması olmadığını beyan eder.

KAYNAKLAR

Anonim, (2022). FishStat Plus-Universal software for fishery statistical time series. http://www.fao.org/fishery/statistics/software/fishstat/en (Alınma tarihi: 08.08.2023).

Anonim, (2023a). Su Ürünleri İstatistikleri. https://data.tuik.gov.tr/Bulten/Index?p=Su-%C3%9Cr%C3%BCnleri-2022-49678&dil=1#:~:text=T%C3%9C%C4%B0K%20Kurumsal&text=Su%20%C3%BCr%C3%BCnleri%20%C3%BCretimi%202022%20y%C4%B1l%C4%B1nda%20bir%20%C3%B6nceki%20y%C4%B1la%20g%C3%B6re%20%256,bin%20808%20ton%20olarak%20ger%C3%A7ekle%C5%9Fti. (Alınma tarihi: 15.08.2023).

Anonim, (2023b). Özel ticaret sistemine göre dış ticaret verileri. https://biruni.tuik.gov.tr/disticaretapp/menu.zul (Alınma tarihi: 28.08.2023).

Atik, H. & Türker, O. (2011). Modern Dış Ticaret Kuramları. *Nobel Akademik Yayıncılık*, Ankara, 140 sy.

Balassa, B. (1966). Tariff reductions and trade in manufacturers among the industrial countries. *The American Economic Review, 56*(3), 466-473. https://www.jstor.org/stable/1823779

Bashimov, G. (2017). Tarım ve Gıda Ürünlerinde Endüstri-İçi Ticaretin Analizi: Türkiye ve Rusya Örneği. *Bolu Abant İzzet Baysal Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, 17*(4), 155-167. https://dergipark.org.tr/tr/pub/basbed/issue/38799/459066

Başkol, M. O. (2009). Türkiye'nin endüstri-içi ticaretinin analizi. *Bursa Uludağ Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 28*(2), 1-24. http://www.uludag.edu.tr/dosyalar/iibfdergi/genel-dokuman/2009_2/ASL01.pdf

Başkol, M. O., (2012). Intra-Industry Trade Between Turkey and the Central Asian Countries. *Central Asian Economies in Transition* (pp.170-182), Cambridge Scholars Publishing.

Dalkıran, G. (2019). *Türkiye Su Ürünleri Yetiştiriciliği İşletmelerinin Uluslararası Rekabetçilik Durumu Üzerine Bir Araştırma (Tez no 605898).* [Doktora Tezi, Karabük Üniversitesi Lisansüstü Eğitim Enstitüsü İşletme Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Erdal, G., & Durmuş, H. (2019). Türkiye Tarım Sektöründe Endüstri İçi Ticaretin Analizi. *SETSCI Conference Proceedings, 4*(6), 80-84. https://doi.org/10.36287/setsci.4.6.026

Greenaway, D., & Milner, C. (1983). On the measurement of intra-industry trade. *The Economic Journal, 93*(372), 900-908. https://doi.org/10.2307/2232755

Grubel, H. G. & Lloyd, P. J. (1971). The empirical measurement of intra-industry trade. *Economic record, 47*(4), 494-517. https://doi.org/10.1111/j.1475-4932.1971.tb00772.x

Grubel, H. G. & Lloyd, P. J. (1975). Intra-Industry Trade: The Theory and Measurement of International Trade in Differentiated Products. *The Economic Journal, 85*(339), 646-648. https://doi.org/10.2307/2230917

Kurt, R. & İmren, E. (2018). Türkiye'deki önemli tıbbi ve aromatik bitkilerin endüstri içi ticaret göstergeleri ile statik ve dinamik analizi. *Journal of Bartin Faculty of Forestry, 20*(3), 548-557. https://doi.org/10.24011/barofd.472457

Kuşat, N. (2020). Türkiye-AB 28 ticaretinde su ürünleri sektörünün 'endüstri içi ticaret' analizi. *Acta Aquatica Turcica, 16*(4), 535-544. https://doi.org/10.22392/actaquatr.740830

Luo, X. F., & Han, Y. H. (2018). Analysis of the Characteristics of Aquatic Products Trade between China and ASEAN based on the HS Classification. *Earth and Environmental Science, 153*(3), 032054. https://iopscience.iop.org/article/10.1088/1755-1315/153/3/032054/pdf

Mangır, F., & Fidan, A. (2017). Grubel-Lloyd endeksi ile endüstri-içi ticaret analizi: tarım sektörü Türkiye örneği. *Karamanoğlu Mehmetbey Üniversitesi Sosyal ve Ekonomik Araştırmalar Dergisi, 19*(33), 45-51. https://doi.org/10.18493/kmusekad.400137

Pomfret, R. (1986). On the Division of Labour and International Trade: or, Adam Smith's Explanation

of Intra-Industry Trade. *Journal of Economic Studies, 13*(4), 56-63. https://doi.org/10.1108/eb002633

Tharakan, P. K. M., & Calfat, G. (1996). Empirical analyses of international trade flows (pp. 59-81). *Macmillan Education*. https://doi.org/10.1007/978-1-349-24563-5_4

Ukav, İ. (2023). Türkiye Su Ürünleri Dış Ticareti. *Econharran, 7*(11), 16-22. https://dergipark.org.tr/tr/ pub/econharran/issue/77155/1245137

Yılmaz Yavuz, A., & Yavuz, F. (2022). Denizcilik alanındaki liselerde öğrenim gören öğrencilerin balık tüketim alışkanlıklarının belirlenmesi: Türkiye örneği. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25*(Ek Sayı 2), 515-522. https://doi.org/10.18016/ksutarimdoga.vi.1060871

Yılmaz, Ş. E. (2016). Dış Ticaret Kuramlarının Evrimi. *Efil Yayınevi*, Ankara, 359 sy.

Yurttançıkmaz, Z. & Azgün, S. (2017). Türkiye ve Komşu Ülkeler Arasında Endüstri İçi Ticaretin Düzeyi Ve Sektörel Rekabet Gücü (1995-2015). *Atatürk Üniversitesi İktisadi ve İdari Bilimler Dergisi, 31*(3), 513-528. https://www.acarindex.com/pdfs/530750

KSÜ Tarım ve Doğa Derg 27 (4), 994-1004, 2024
KSU J. Agric Nat 27 (4), 994-1004, 2024
DOI:10.18016/ksutarimdoga.vi.1310574

Investigation of Plant Height, Fresh Weight and Dry Weight of Sorghum with Growth Curve Models

Şenol ÇELİK[1]✉, Erdal GÖNÜLAL[2], Halit TUTAR[3]
[1]Department of Animal Sciences, Biometry and Genetics, Faculty of Agriculture, University of Bingol, Bingol, 12000, Türkiye, [2]Bahri Dağdaş International Agricultural Research Institute, Konya, 42000, Türkiye, [3]Department of Plant and Animal Production, Vocational School of Food, Agriculture and Livestock, University of Bingol, Bingöl, 12000, Türkiye
[1]https://orcid.org/0000-0001-5894-8986, [2]https://orcid.org/0000-0002-1621-0892, [3]https://orcid.org/0000-0002-9341-3503
✉: senolcelik@bingol.edu.tr

ABSTRACT
In this study, the sorghum plant, which is one of the most important plants in the world, was used as material. It was grown in Konya province of Türkiye, which has semi-arid climate conditions. Plant height, fresh weight, and dry weight were determined for 11 weeks during the vegetation period. To determine the shape of the plant growth, some growth models were used and the parameters of the models were tried to be defined. The coefficient of determination (R^2), Pseudo R^2, Mean Squares of Error (MSE), and Akaike Information Criteria (AIC) statistics were taken into account in comparing the performances of the Brody, Gompertz, Von Bertalanffy, Logistic, and Log-Logistic models. The R^2, Pseudo R^2, MSE and AIC values of the Gompertz model found suitable for plant height were found to be 0.998, 0.999, 23.162, and 21.013 respectively. The R^2, Pseudo R^2, MSE, and AIC values of the von Bertalanffy model, which was found suitable for wet weight estimation, were obtained as 0.995, 0.998, 1817.141, and 41.993 respectively. The R^2, Pseudo R^2, MSE, and AIC values of the Log-logistic model, which were found suitable for estimating the dry weight of the plant, were calculated as 0.998, 0.9993, 51.007, and 24.784 respectively. It can be suggested that nonlinear mathematical growth models are useful methods in terms of describing important plant characteristics such as plant height, and fresh and dry weight, calculating maximum plant height and weight, and determining the average growth rate. As a result, the growth curve models showed different results in different characteristics such as plant height, and fresh and dry weight of the plant.

Biometrics

Research Article

Article History
Received : 07.06.2023
Accepted : 19.10.2023

Keywords
Growth curve models
Maturing index
Inflection point
Sorghum
Plant Characteristics

Sorgumun Bitki Boyu, Taze Ağırlığı ve Kuru Ağırlığının Büyüme Eğrisi Modelleri ile Araştırılması

ÖZET
Bu çalışmada dünyanın en önemli bitkilerinden biri olan sorgum bitkisi materyal olarak kullanılmıştır. Bu bitki Türkiye'nin yarı kurak iklim koşullarına sahip Konya ilinde yetiştirilmiştir. Vejetasyon döneminde 11 hafta boyunca bitki boyu, yaş ağırlığı ve kuru ağırlığı ölçülmüştür. Bitkide büyümenin şeklini belirlemek amacıyla bazı büyüme modelleri kullanılmış ve modellerin parametreleri tanımlanmaya çalışılmıştır. Brody, Gompertz, Von Bertalanffy, Logistic ve Log-Logistic modellerinin karşılaştırılmasında belirleme katsayısı (R^2), Pseudo R^2, Hata Kareler Ortalaması ve Akaike Bilgi Kriteri istatistikleri dikkate alınmıştır. Bitki boyu için uygun bulunan Gompertz modelinin R^2, Pseudo R^2, Hata Kareler Ortalaması ve Akaike Bilgi Kriteri değerleri sırasıyla 0.998, 0.999, 23.162 ve 21.013 olarak bulunmuştur. Yaş ağırlık için uygun bulunan Von Bertalanffy modelinin R^2, Pseudo R^2, Hata Kareler Ortalaması ve Akaike Bilgi Kriteri değerleri sırasıyla 0.995, 0.998, 1817.141 ve 41.993 olarak elde edilmiştir. Kuru ağırlık için uygun bulunan Log-Logistik modelinin R^2, Pseudo R^2, Hata Kareler Ortalaması ve Akaike Bilgi Kriteri değerleri sırasıyla 0.998, 0.9993, 51.007 ve 24.784 olarak

Biyometri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 07.06.2023
Kabul Tarihi : 19.10.2023

Anahtar Kelimeler
Büyüme eğrisi modelleri
Olgunlaşma indeksi
Büküm noktası
Sorgum
Bitki karakteristikleri

hesaplanmıştır. Bitki boyu, yaş ve kuru ağırlık gibi önemli bitki özelliklerinin tanımlanması, maksimum bitki boyu ve ağırlığının hesaplanması ve ortalama büyüme hızının belirlenmesi açısından doğrusal olmayan matematiksel büyüme modellerinin faydalı yöntemler olduğu önerilebilir. Sonuç olarak sorgum bitkisinde bitki boyu, bitkinin yaş ve kuru ağırlığı gibi farklı özelliklerinde büyüme eğrisi modelleri farklı sonuçlar göstermiştir.

Atıf İçin : Çelik, Ş., Gönülal, E., & Tutar, H., (2024). Sorgumun Bitki Boyu, Taze Ağırlığı ve Kuru Ağırlığının Büyüme Eğrisi Modelleri ile Araştırılması. *KSÜ Tarım ve Doğa Derg 27* (4), 994-1004. DOI: 10.18016/ksutarimdoga.vi. 1310574.

To Cite: Çelik, Ş., Gönülal, E., & Tutar, H., (2024). Investigation of Plant Height, Fresh, Weight, and Dry Weight of Sorghum with Growth Curve Models. *KSU J. Agric* Nat 27 (4), 994-1004. DOI: 10.18016/ksutarimdoga.vi. 1310574.

INTRODUCTION

Sorghum bicolor (L.) Moench is a plant belonging to the *Poaceae (Gramineae)* family. It is an essential staple food for millions in developing countries, mostly in semi-arid and arid tropical regions (Abreha et al., 2022). Provides fiber, protein-rich and gluten-free nutrition (McCann et al., 2015; Impa et al., 2019). In addition to food use, it is utilized as a raw material source for bioethanol production (Mathur et al., 2017). It is used in the livestock and biofuel industry in America and other developed countries (McLaren et al., 2003). Sorghum is a warm-season grassy C4 plant grown in different ecological conditions. It requires less fertilizer than many economically important plants and is tolerant to drought, high temperature, and salinity (Mastrorilli et al., 1999; Gnansounou et al., 2005; Tesso et al., 2005; Almodares et al., 2007). Its successful cultivation in semi-arid and arid regions makes sorghum an important component (Murungweni et al., 2016; USDA-FAS, 2018).

Sorghum is the fifth most important cereal crop in the world after rice, wheat, corn, and barley. According to FAO reports, in 2021, 40.9 million hectares of land and 61.3 million tons of production were made worldwide. In terms of production share, Africa (42.8%) ranks first, followed by America (38.5%), Asia (14.2%), Oceania (2.7%) and Europe (1.9%). In terms of production, the USA (11.3 million tons) is in the first place, followed by Nigeria (6.7 million tons), India (4.8 million tons), Ethiopia (4.4 million tons) and Mexico (4.3 million tons) (FAO, 2022).

The use of mathematical growth models to describe growth is common in the agricultural sciences (Sari et al., 2019). The growth models can be used to describe a biological process, such as seed germination (Sousa et al., 2014) and plant growth (Bem et al., 2017, 2018). In addition, nonlinear regression and growth models are still little used when statistical analysis is made in field crop trials, and when used, growth models are mostly adjusted according to accumulated production data. Nonlinear mathematical growth models have been used in some plants (Jane et al., 2020; Lacasa et al., 2021; Rahemi-Karizaki et al., 2021; Liu et al., 2021; Alam et al., 2022; Karizaki et al., 2022).

Up until the harvest point for ensiling, linear and nonlinear modeling techniques such as linear, quadratic, and Wood models were used to characterize the chemical composition and evaluate the biometric characteristics of pearl millet, corn, and sorghum. Growth models were used in the maize, pearl millet, and sorghum crops to characterize leaf growth up to the harvest point for ensiling (Chrisostomo et al., 2022). Non-linear regression analysis was applied to data on the dry weight of sorghum biomass that was gathered throughout the growing season in Italy (Pannacci and Bartolini 2016).

In this study, fresh weight, dry weight, and plant height parameters of the sorghum plant, which is one of the most important plants in the world, were measured for 11 weeks. It is aimed to model the obtained data by comparing it with 5 different growth curve methods.

MATERIAL and METHOD

The research was conducted in the province of Konya, located in the Central Anatolia Region of Türkiye, in 2020. Early Sumac variety of sorghum plant was used as material. The area where the study was carried out; is an area with a clay loam structure, not very rich in organic matter, high lime content, pH value between 7.6 - 8.3, and no salt problem (Table 1).

The plant vegetation period is May-August. When Table 2 is examined; according to the climate data of the study area for long years, the average temperature was 20.7 °C and the highest average temperature was 23.5 °C (July). According to long years, the average temperature in the sorghum growing period was 15.9 °C in May, 20.1 °C in June, 23.5 °C in July and 23.3 °C in August. Considering the precipitation data, it was seen that the average for long years was 82.4 mm. In the study year, the average temperature of the sorghum vegetation period was 21.5 °C, while the total precipitation amount was 74.3 mm.

In the study, the plots were formed in 4 rows, 5 cm on the row, 45 cm between the rows, and 5 m in length. The parcel dimensions are arranged as 1.8 m x 5 m = 9

m². In the study, sowing was done on 12 May 2020, and 50 mm of water was given to all plots. After soil preparation, phosphorus fertilizer (9 kg/da) was given and a total of 18 kg N was given throughout the period, taking into account the soil analysis with planting. All of the phosphorus fertilizer and 3 kg/da of nitrogen fertilizer were given with the planting, and the remaining part of the nitrogen fertilizer was distributed equally to the plots by drip irrigation in the form of 4 parts (15 kg/da). In weed control, both mechanical and drug control methods were applied. In the study, irrigation was done with a drip irrigation system, and a total of 480 mm of irrigation water was given.

Table 1. Some soil properties of the experimental area
Çizelge 1. Deneme alanına ait bazı toprak özellikleri

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	(structure)	Field capacity %)	Wilting point %)	Volume weight (g/cm³)	pH	EC (dSm⁻¹)	Lime %)	Organic Matter (%)	P_2O_5 (kg da⁻¹)	K_2O (kg da⁻¹)
0-30	9.6	29.4	61.0	CL	26.2	16.8	1.24	7.9	0.65	45.6	1.4	12.7	85
30-60	10.4	30.7	58.9	CL	27.3	17.3	1.35	8.3	0.49	35.9	1.2	11.6	63
60-90	9.1	28.4	62.5	CL	28.4	17.7	1.33	8.4	0.42	39.4	1.1	10.8	44

Table 2. Climate data of the study area (1929-2019, 2020)
*Çizelge 2. Çalışma alanın iklim verileri (*1929-2019, 2020)

Years		May	June	July	August	Average/Total
Long Years (1929-2019)	Avr. Tem. (°C)	15.9	20.1	23.5	23.3	20.7
	Max. Tem.(°C)	22.4	26.7	36.6	30.2	29.0
	Min. Tem.(°C)	8.6	12.6	15.9	15.6	13.2
	Precipitation (mm)	43.4	25.7	7.0	6.3	82.4
2020	Avr. Tem. (°C)	16.2	20.3	25.5	23.8	21.5
	Max. Tem.(°C)	34.5	34.4	36.2	36.3	35.4
	Min. Tem.(°C)	0.3	5.8	11.5	8.3	6.5
	Precipitation (mm)	41.7	20.1	7.5	5.0	74.3

In the study; From 22 June until 1 September, plant height, fresh weight, and dry weight measurements were made weekly for a total of 11 weeks. Data were obtained from 3 randomly selected plants each week. Brody, Gompertz, Logistic, Von Bertalanffy, and Log-Logistic non-linear functions were used in the study. Table 3 lists the formulas, growth rates, and inflection point locations for these functions. The asymptotic weight or length is referred to as the A parameter in all models, and all other parameters are described as constants relating to the shape and instant growth rates of the growth curve. B is the integration constant and k is the maturity rate (Winsor, 1932; Brody, 1945; Bertalanffy, 1957; Nelder, 1961; Brown et al., 1976; Blasco et al., 2003; Bahreini Behzadi et al., 2014; Rządkowski et al., 2015).

Table 3. Model expressions and parameters of growth functions
Çizelge 3. Büyüme fonksiyonlarının model ifadeleri ve parametreleri

Model	Equation	IPT	IPW
Brody	$Y_t = A(1 - b\exp(-kt))$	---	---
Gompertz	$Y_t = A\exp(-b\exp(-kt))$	$\ln(b)/k$	A/e
Von Bertalanffy	$Y_t = A(1 - b\exp(-kt))^3$	$(\ln 3b)/k$	8A/27
Logistic	$Y_t = A/(1 + b\exp(-kt))$	$-ln(1/b)/k$	A/2
Log-Logistic	$Y_t = A/(1 + b\exp(-kln(t)))$	$[(1+k)/b(k-1)]^{-1/k}$	A(k-1)/(2k)

Y_t: Plant length/weight, A: Asymptotic length/weight, b: Integration constant, k: Maturing index. IPT: Point of inflection time, IPW: Point of inflection weight

In model fit, both individual and average total data were analyzed. Since the analysis results of individual and averaged data were very close to each other, the analysis results of averaged data were evaluated in the study. In other words, the analysis was not made individually, but as a single analysis in the data set created by taking the average of 3 observations measured every week. These analyses were carried out as a general analysis for each feature. Analyzes were carried out with the SPSS 25.0 package program. Levenberg-Marquardt algorithm was chosen for model fitting.

To select the best model, coefficient of determination (R^2), number of iterations, Mean Square Error (MSE),

and Akaike information criterion (AIC) were performed (Echeverri et al., 2013; Tariq et al., 2013; Üçkardeş et al., 2013; Lupi et al., 2015; Yavuz et al., 2019). For each of these criteria, the optimum status was the highest level of the determination coefficient (pseudo R^2), the smallest number of the iterations needed, and the lowest value of the Akaike information criterion and Mean Square Error (Thomas et al., 2009; Yavuz et al., 2019). The Akaike information criterion was calculated as:

$$AIC = n * ln\left(\frac{SSE}{n}\right) + 2k \quad (1)$$

The R^2 and pseudo R^2 was calculated following:

$$R^2 = 1 - \frac{SSE}{SST} \quad (2)$$

$$pseudo\ R^2 = 1 - \frac{SSE}{SST_C} \quad (3)$$

The formula of MSE is as follows:

$$MSE = \frac{SSE}{n-k} \quad (4)$$

Where k is the number of parameters +1, SSE is the residuals sum of squares and n is the number of observations. SST is the total sum of squares, and $SST_{C\ is}$ the adjusted overall sum of squares.

RESULTS and DISCUSSION

The estimated parameters of the 11-week plant height measurements of the sorghum plant are presented in Table 4. All the estimated growth curve models fit well with the observed growth curves (R^2>0.99) in plant length (Table 4 and Figure 1).

Following analyses utilizing various non-linear growth functions, it was determined that the values estimated for A, which represented the mature length in each function (316.592-610.934 cm, Table 4) were reliable.

The values estimated for the k (maturing index) parameter are -0.072, 0.31, 0.234, 0.53, and 1.203 in Brody, Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively (Table 4). The point of inflection time for plant length is 4.184, 3.686, 5.037, and 5.886 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively. The point of inflection length is 128.656, 111.643, 158.296, and 266.443 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively (Table 4). The Brody model has no inflection point.

As seen in Table 4, the model with the best fit is the Gompertz model.

Table 4. Parameter coefficients and goodness of fit criteria for growth models (plant length).
Çizelge 4. Büyüme modelleri için parametre katsayıları ve uyum kriterleri (bitki uzunluğu).

Model	Brody	**Gompertz**	Von Bertalanffy	Logistic	Log-Logistic
A	610.934	349.722	376.794	316.592	581.198
b	0.979	2.683	0.625	8.494	13.468
k	-0.072	0.31	0.234	0.53	1.203
R^2	0.993	0.998	0.998	0.995	0.996
Pseudo R^2	0.9985	0.9996	0.9995	0.9989	0.9993
MSE	87.786	23.162	26.142	63.944	39.039
AIC	27.378	21.013	21.591	25.864	23.506
IPT	---	4.184	3.686	5.037	5.886
IPW	---	128.656	111.643	158.296	266.443

Models with the best goodness of fit are represented in bold

Table 5 provides the expected parameters for the sorghum plant's fresh weight measurements at 11 weeks. All of the calculated growth curve models (R2>0.99) in plant fresh weight fit the observed growth curves quite well (Figure 3). Several non-linear development functions were used in the analyses, and the values calculated for the A parameter, which in each function denotes the mature fresh weight, were found to be 311.557-1767.663 g (Table 5).

The values estimated for the k (maturing index) parameter are -0.001, 0.385, 0.288, 0.671, and 2.592 in Brody, Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively. The point of inflection time for plant fresh weight was 5.644, 5.257, 4.564, and 5.472 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively. The point of inflection weights were 573.18, 500.233, 701.489, and 542.847 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively (Table 5).

In the Brody model in Tables 4 and 5, the k parameter was found to be negative. This means that the weekly maturation rate of plant height and fresh weight is negative. However, in other models used, the weekly maturity rate is positive and is the expected result. Weekly maturity rates of the sorghum plant until its last developmental period are generally positive and there have been studies on this (Shi et al., 2013; Chrysostom et al., 2022).

The Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models for plant fresh weight's inflection point plots are shown in Figure 4.

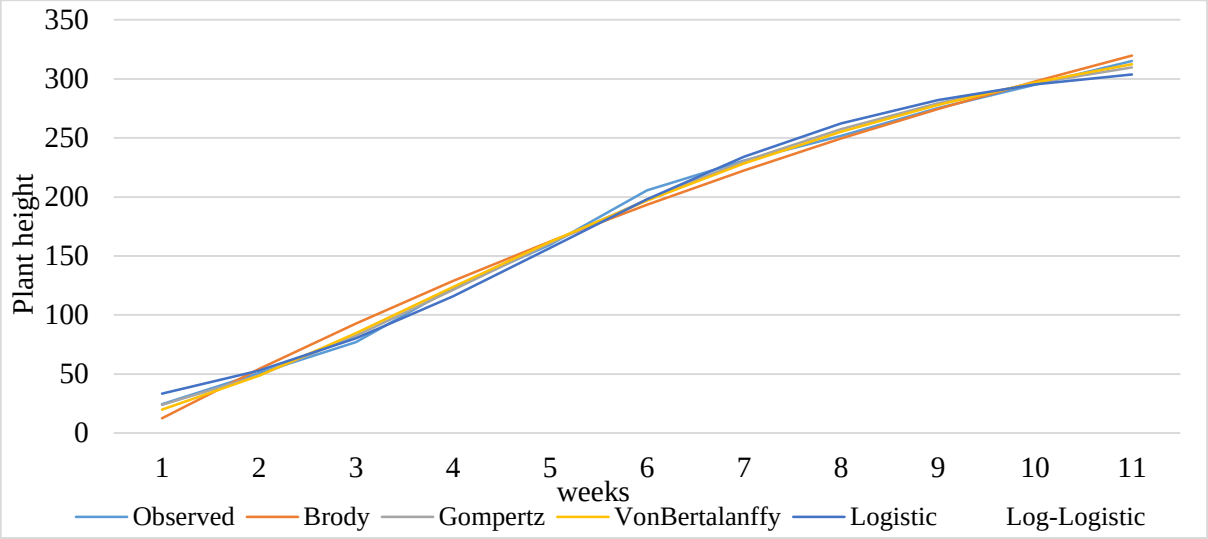


Figure 1. Growth curves of sorghum plant height (cm)
Şekil 1. Sorgum bitki boyunun büyüme eğrileri

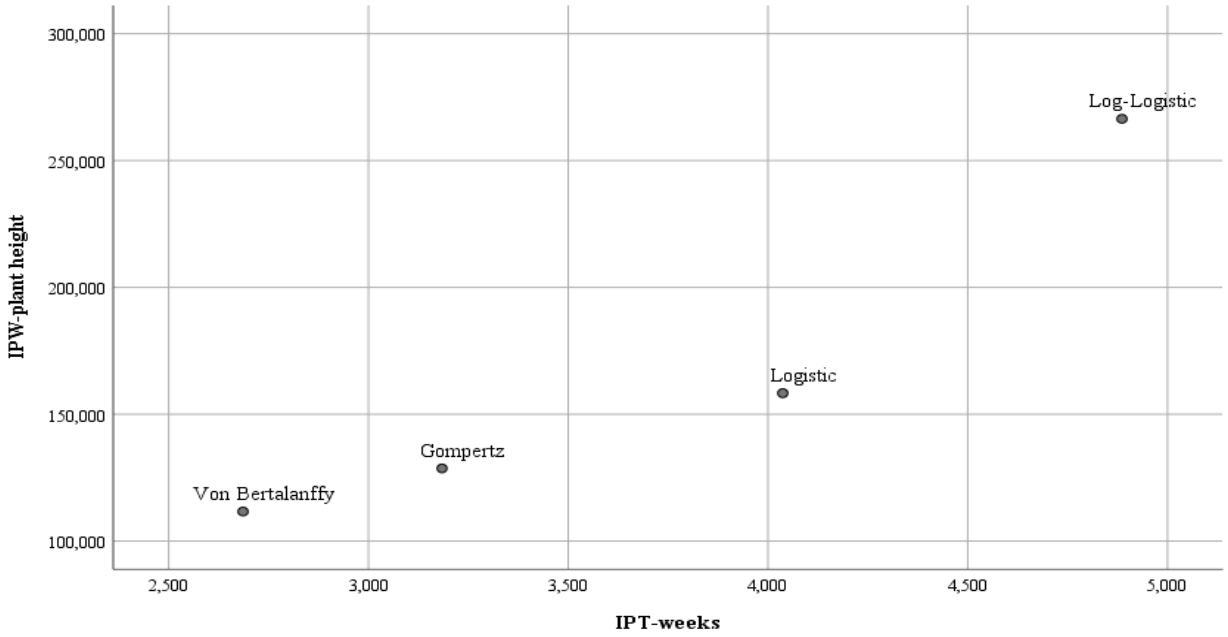


Figure 2. The point of inflection time for plant length
Şekil 2. Bitki uzunluğu için bükülme zamanı noktası grafiği

Table 5. Parameter coefficients and goodness of fit criteria for growth models (plant fresh weight).
Çizelge 5. Büyüme modelleri için parametre katsayıları ve uyum kriterleri (bitki yaş ağırlığı).

Model	Brody	Gompertz	**Von Bertalanffy**	Logistic	Log-Logistic
A	311.557	1558.06	1688.287	1402.98	1767.663
b	1	5.976	1.136	35.294	109.523
k	-0.001	0.385	0.288	0.671	2.592
R^2	0.974	0.994	0.995	0.987	0.993
Pseudo R^2	0.9903	0.9977	0.9980	0.9952	0.9979
MSE	8992.44	2197.14	1871.141	4481.04	1940.435
AIC	49.493	42.760	41.993	46.165	42.167
IPT	---	5.644	5.257	4.564	5.472
IPW	---	573.18	500.233	701.489	542.847

Models with the best goodness of fit are represented in bold

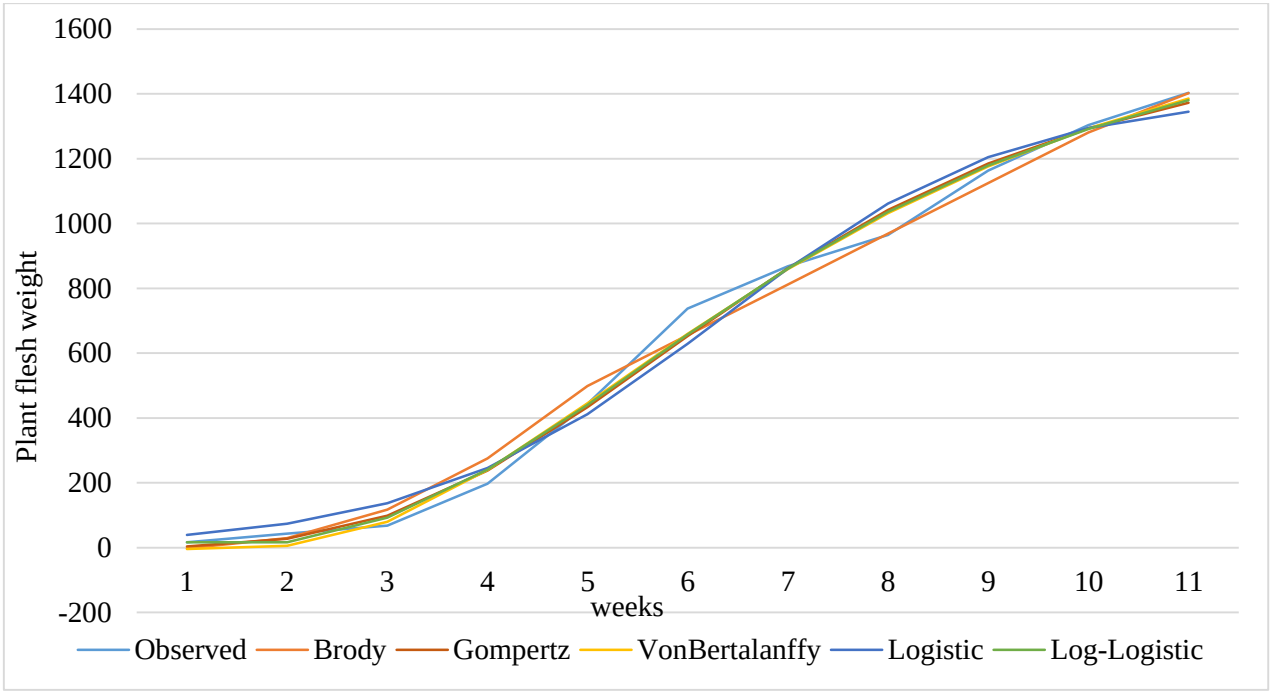


Figure 3. Growth curves of sorghum plant fresh weight
Şekil 3. Sorgum bitkisi yaş ağırlığının büyüme eğrileri

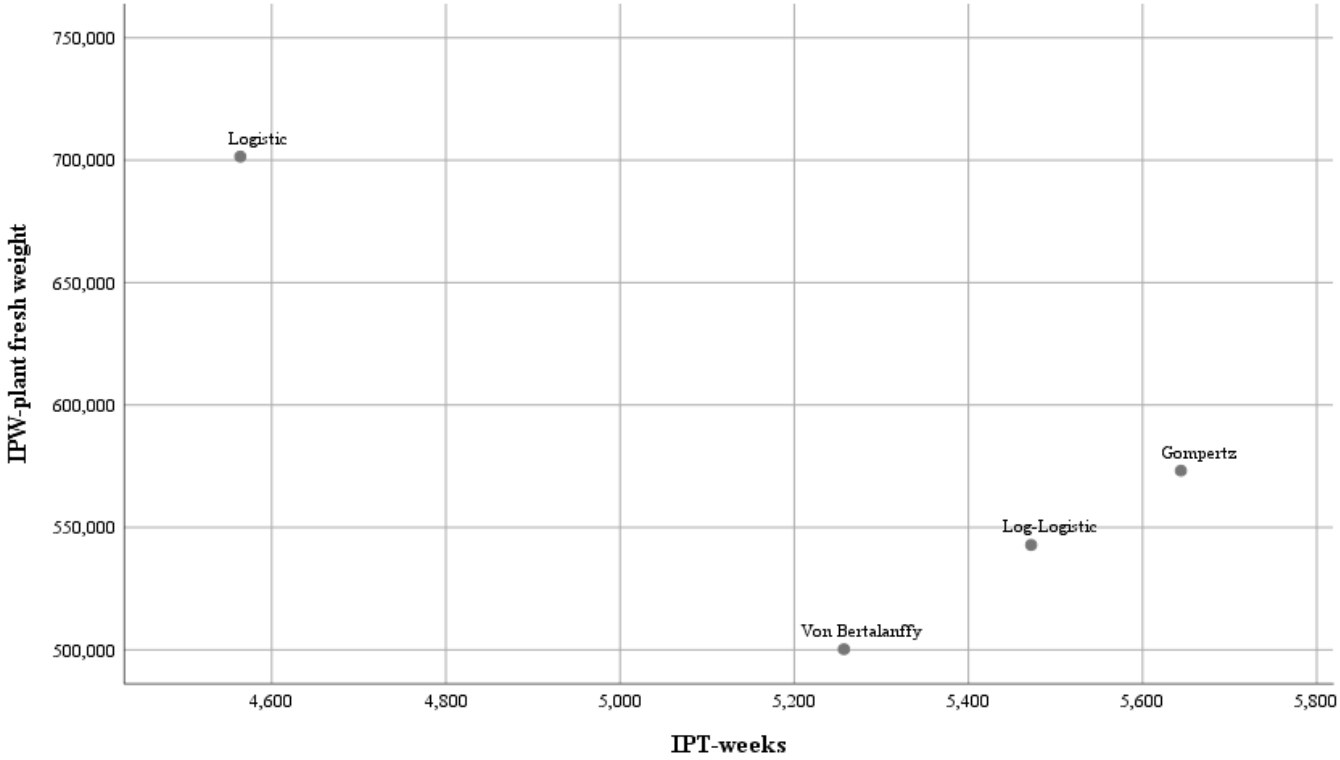


Figure 4. The point of inflection time for plant fresh weight
Şekil 4. Bitki yaş ağırlığı için bükülme zamanı noktası grafiği

Table 6 provides the expected parameters for the sorghum plant's 11-week plant dry weight measurements. In terms of plant dry weight, the estimated growth curves from models suit the observed growth curves well (R2>0.99) (Figure 5).

The analyses were carried out using several non-linear growth functions, and the values estimated for the A parameter, which represents the asymptotic dry weight in each function, were established (393.345-752.232 g, Table 6).

The values estimated for the k (maturing index) parameter are 0.001, 0.365, 0.205, 0.722, and 3.507 in Brody, Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively. The point of inflection time (week) for plant fresh weight is 7.109, 7.353, 5.708, and 7.137 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively. The point of inflection weight is 206.974, 222.884, 234.886, and 208.863 in Gompertz, Von Berttalanffy, Logistic, and Log-Logistic models, respectively (Table 6).

Table 6. Parameter coefficients and goodness of fit criteria for growth models (plant dry weight).
Çizelge 6. Büyüme modelleri için parametre katsayıları ve uyum kriterleri (bitki kuru ağırlığı).

Model	Brody	Gompertz	Von Bertalanffy	Logistic	**Log-Logistic**
A	393.345	562.613	752.232	469.771	584.35
b	1	9.299	1.226	110.847	1042.831
k	0.001	0.365	0.205	0.722	3.507
R^2	0.937	0.998	0.996	0.998	0.998
Pseudo R^2	0.9709	0.9993	0.9984	0.9992	0.9993
MSE	2202.82	53.532	124.629	55.26	51.007
AIC	42.773	25.015	29.052	25.167	24.784
IPT	---	7.109	7.353	5.708	7.137
IPW	---	206.974	222.884	234.886	208.863

Models with the best goodness of fit are represented in bold

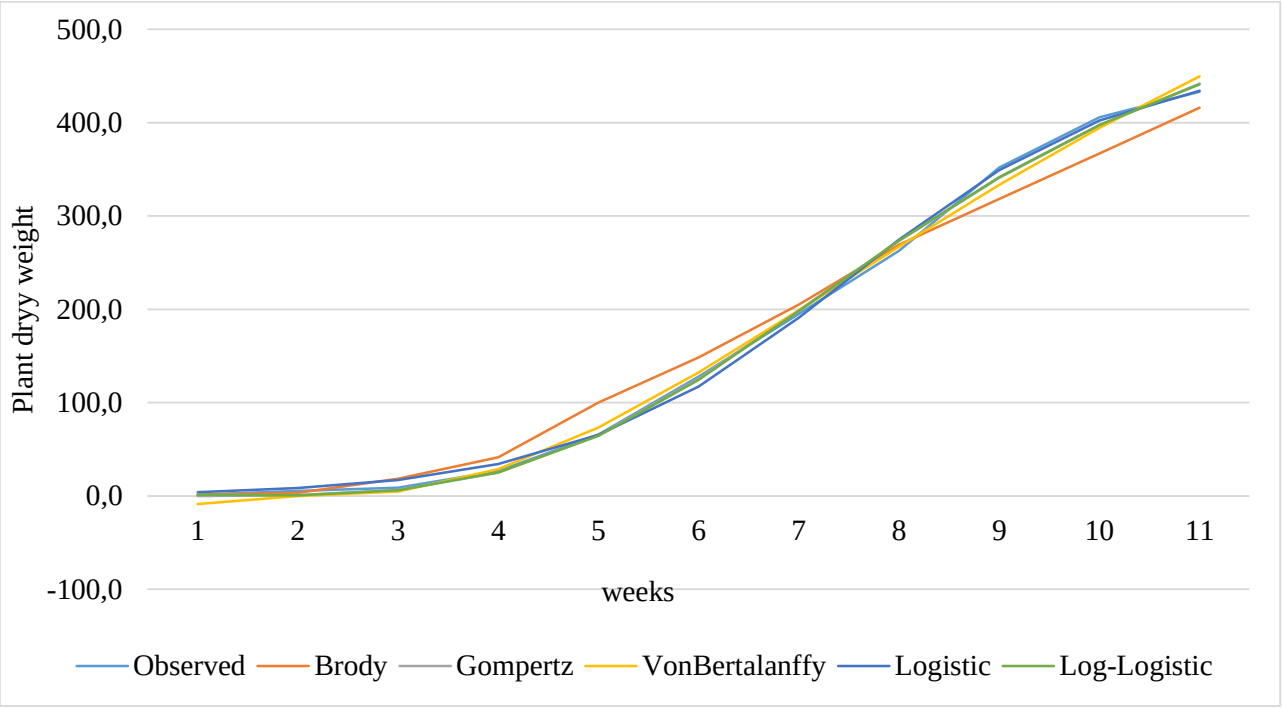


Figure 5. Growth curves of sorghum plant dry weight
Şekil 5. Sorgum bitkisi kuru ağırlığının büyüme eğrileri

Figure 6 displays the inflection point charts for the Gompertz, Von Bertalanffy, logistic, and log-logistic models for plant dry weight.

Tables 4, 5, and 6 explain the goodness of fit statistics obtained for the five models studied. It was observed that for plant length (Table 4), the Gompertz model provided the best fit. On the other hand, in what concerns fresh and dry weight, different models provided best fits for datasets of different features. Different growth curve models with different characteristics showed the best performance. It was observed that for plant fresh weight (Table 5), the Von Bertalanffy model provided the best fit. It was observed that for plant dry weight (Table 6), the Log-Logistic model provided the best fit. Thus, it was not possible to determine a model as being superior to the others.

Descriptive statistics regarding plant height, fresh weight, and dry weight are presented in Table 7.

In a study (Chrisostomo et al., 2022), while leaf diameter and length had a superior fit to the nonlinear Wood model, the biometric variables plant height and leaf width had a better fit to the linear model. Corn, pearl millet, and sorghum did not differ from one another in terms of the biometric variables (p=0.1863), and the model curves for corn and sorghum overlapped

for all variables. The Gompertz model provided the best fit for leaf growth. In this study, the Gompertz model was the most appropriate model for plant height. It seems to be compatible with the results of this study in that the best model is the same.

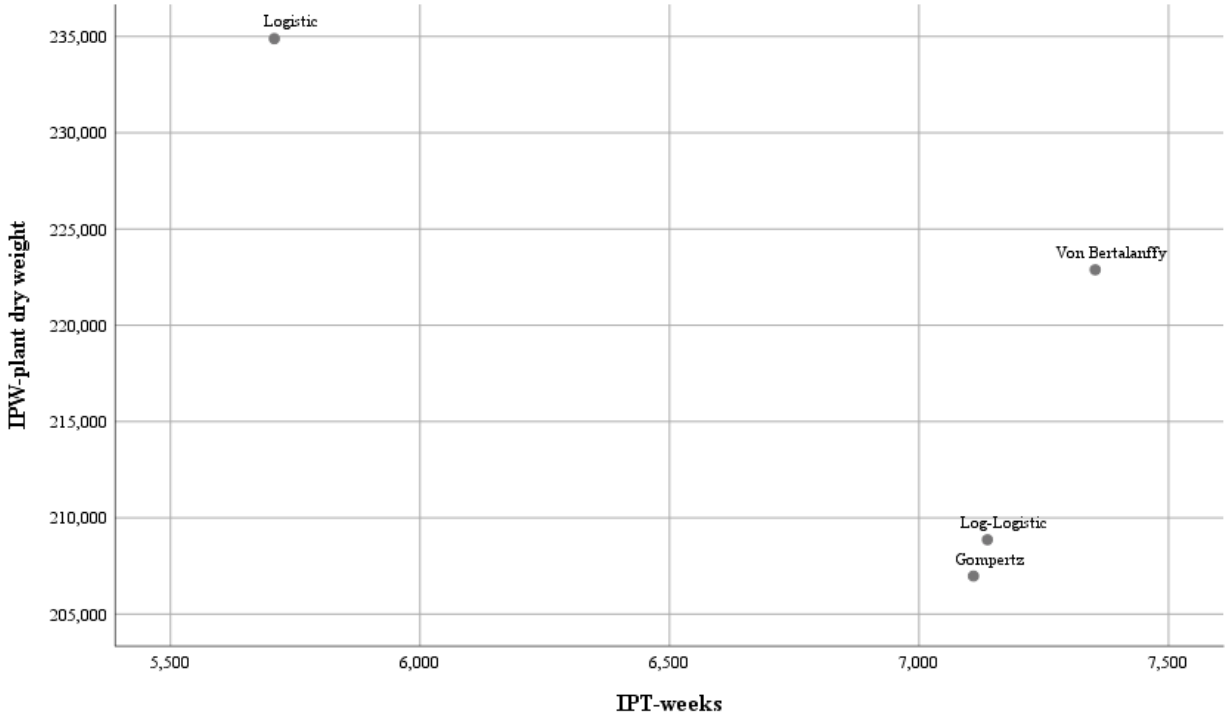


Figure 6. The point of inflection time for plant dry weight
Şekil 6. Bitki kuru ağırlığı için bükülme zamanı noktası grafiği

Table 7. Descriptive statistics of plant height (cm), plant fresh weight (g) and plant dry weight (g) ($\bar{X} \mp s_{\bar{x}}$)
Çizelge 7. Bitki boyu (cm), bitki yaş ağırlığı (g) ve kuru ağırlığına (g) ait tanımlayıcı istatistikler ($\bar{X} \mp s_{\bar{x}}$)

Weeks	N	Plant height	Plant fresh weight	Plant dry weight
1	3	24.333∓0.882	16.000∓0.577	1.470∓0.057
2	3	50.667∓3.480	43.333∓3.333	5.300∓0.401
3	3	77.000∓2.309	68.333∓4.410	8.767∓0.601
4	3	123.000∓7.211	196.667∓34.921	26.733∓4.823
5	3	159.333∓11.566	443.333∓85.942	65.767∓11.919
6	3	205.667∓14.769	736.667∓13.017	128.000∓4.359
7	3	230.667∓17.285	868.000∓20.551	195.000∓8.660
8	3	251.667∓14.814	965.000∓29.297	262.667∓3.930
9	3	275.333∓6.360	1163.333∓86.667	351.900∓26.467
10	3	295.000∓5.000	1303.333∓44.190	405.633∓12.632
11	3	315.000∓2.887	1403.333∓20.276	433.333∓10.929

$\bar{X}$: Mean, $s_{\bar{x}}$: Standard error.

The comparison between the observed values and the predicted values by the logistic model was displayed for the dry weight of sweet sorghum (Shi et al., 2013). In the logistic model, parameter values were found to be A=213 (asymptotic dry weight), b=7.73, and k=0.1237. The R^2 of the model was found to be 0.940. The parameter coefficients representing the sorghum dry weight in this study and the best-fitting model differed.

In Italy, sorghum hybrids' average plant height was 222 and 181 cm to biomass hybrids and forage hybrids in 2005, respectively. The sorghum hybrid's average plant height was measured at 338 and 284 cm for biomass hybrids and forage hybrids in 2006, respectively. The plant height of biomass hybrids was higher than forage hybrids as observed in 2005 with an average value of 338 cm (Pannacci and Bartolini 2016). Plant height values were found to be different from the values in this study.

In a study conducted in Italy, the plant height of different varieties of sorghum plants was found to be between 199 and 344 cm (Habyarimana et al., 2004).

The highest plant height was obtained from the H 132 variety with 344 cm. This was followed by Mamhonne (295 cm), IS 21055 (291 cm), and Brown sweta (291 cm). When compared to the sorghum plant height in this study, H 132 sorghum was higher than the result of this study, while other varieties were lower.

CONCLUSION

In this study, 5 different growth curves were compared using the plant height, and plant fresh and dry weights from the day of emergence to the 11th week of the sorghum plant. In the Gompertz model that best describes the plant length, the asymptotic plant length (A) was estimated as 349.7 cm and the adult growth rate (k) was 0.31. Gompertz model $Y_t = 349.7\exp(-2.683\exp(-0.31t))$ was determined as.

In the Von Berttalanffy model that best describes the plant's fresh weight; the maximum fresh plant weight (A) was estimated at 1688.287 g and the adult growth rate (k) was 0.288. The time of the highest wet weight gain was 5.257 weeks. Von Berttalanffy model $Y_t = 1688.287(1 - 1.136\exp(-0.288t))^3$ was determined as.

In the Log-Logistic model that best describes the plant dry weight, the maximum dry weight of plants (A) was estimated as 584.35 g and the adult growth rate (k) was 3.507. Log-Logistic model $Y_t = 584.35/(1 + 1042.831\exp(-3.507ln(t)))$ was determined as.

Although the growth curves appear to be close to each other when looking at the figures, it has been determined that the model fits give different results. As a result, when the 11-week growth curves of the plant were examined, plant height, and fresh and dry weight characteristics were modeled differently. Brody's model showed the lowest model performance of all models. Gompertz, Von Bertalanffy, and Log-Logistic models were the models that best describe the growth for plant height, wet weight, and dry weight in sorghum plants, respectively. Defining growth is important in determining the most appropriate time for agricultural practices. Growth differences seen between plant characteristics require the use of different models in the adaptation of growth data. In choosing the model to be used in the fitting of growth curves, the structure of the data of the estimated parameters should be considered. With such growth functions, it may be possible to predict the plant height, fresh weight, and dry weight that plants can reach in the future.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

Abreha, K. B., Enyew, M. A., Carlsson, S., Vetukuri, R. R., Feyissa, T., Motlhaodi, T., Ng'uni, D., & Geleta, M. (2022). Sorghum in dryland: Morphological, physiological, and molecular responses of sorghum under drought stress. *Planta, 255*. [DOI: 10.1007/s00425-022-03808-0]

Alam, T., Suryanto, P., Susyanto, N., Kurniasih, B., Basunanda, P., Putra, E. T. S., Kastono, D., Respatite, D. W., Widyawan, M. H., Nurmansyah, N., Ansari, A., & Taryono, T. (2022). Performance of 45 non-linear models for determining the critical period of weed control and acceptable yield loss in soybean agroforestry systems. *Sustainability, 14*(13), 7636. [DOI: 10.3390/su14137636]

Almodares, A., Taheri, R., & Adeli, S. (2007). Inter-relationship between growth analysis and carbohydrate contents of sweet sorghum cultivars and lines. *Journal of Environmental Biology, 28*, 527-531.

Bahreini Behzadi, M. R., Aslaminejad, A. A., Sharifi, A. R., & Simianer, H. (2014). Comparison of mathematical models for describing the growth of Baluchi Sheep. *J. Agr. Sci. Tech., 14*, 57-68.

Bem, C. M., Filho, A. C., Facco, G., Schabarum, D. E., Silveira, D. L., Simões F. M., & Uliana, D. B. (2017). Growth models for morphological traits of sunn hemp. *Seminar: Ciênc. Agrár., 38*, 2933-2944.

Bem, C. M., Filho, A. C., Chaves, G. G., Kleinpaul, J. A., Pezzini, R. V., & Lavezo, A. (2018). Gompertz and logistic models for the productive traits of Sunn hemp. *J. Agric. Sci., 10*, 225-238.

Bertalanffy, L. (1957). Quantitative laws for metabolism and growth. *The Quarterly Review of Biology, 32*, 217-231.

Blasco, A., Piles, M., & Varona, L. (2003). A Bayesian analysis of the effect of selection for growth rate on growth curves in rabbits. *Genet. Sel. Evol., 35*, 21-41.

Brody, S. (1945). Bioenergetics and growth. *Rheinhold Pub. Corp. N.Y.*

Brown, J. E., Fitzhugh Jr, H. A., & Cartwright., T. C. (1976). A Comparison of nonlinear models for describing weight-age relationship in cattle. *J. Anim. Sci., 42*, 810-818.

Chrisostomo, P. H. B., Camilo, M. G., Baffa, D. F., Process, E. F., Gloria, L. S., Fernandes, A. M., & Oliveria, T. S. (2022). Biometric evaluation and nutrients of the corn, pearl millet, and sorghum crops. *Pesqulsa Agropecuaria Brasilleira, Brasília, 57*.

Echeverri, A. M. L., Bergmann, J. A. G., Toral, F. L. B., Osorio, J. P., Carmo, A. S., Mendonça, L. F., Moustacas, V. S., & Henry, M. (2013). Use of nonlinear models for describing scrotal circumference growth in Guzerat bulls raised under

grazing conditions. *Theriogenology, 79*(5), 751-759. [DOI: 10.1016/j.theriogenology.2012.11.021]

FAO. (2022). Food and Agriculture Organization of the United Nations: Home. Retrieved from http://apps.fas.usda.gov/faostat/en/#data/QCL

Faci, J. M. (1991). Efecto de la densidad de plantas sobre la producción de sorgo bajo un suministro variable de riego. *IX Jornadas Te´cnicas de Riego*, 37–45, Granada, Spain.

Gnansounou, E., Dauriat, A., & Wyman, C. E. (2005). Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. *Bioresource Technology, 96*, 985-1002. [DOI: 10.1016/j.biortech.2004.06.025]

Habyarimana, E., Laureti, D., Ninno, M. D., & Lorenzoni, C. (2004). Performances of biomass sorghum [Sorghum bicolor (L.) Moench] under different water regimes in the Mediterranean region. *Industrial Crops and Products, 20*, 23–28. [DOI: 10.1016/j.indcrop.2004.03.004]

IBM Corp. (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Impa, S. M., Perumal, R., Bean, S. R., Sunoj, V. S. J., & Jagadish, S. V. K. (2019). Water deficit and heat stress-induced alterations in grain physico-chemical characteristics and micronutrient composition in field-grown grain sorghum. *J. Cereal Sci., 86*, 124–131. [DOI: 10.1016/j.jcs.2019.02.004]

Jane, S. A., Fernandes, F. A., Silva, E. M., Muniz, J. A., Fernandes, T. J., & Pimentel, G. V. (2020). Adjusting the growth curve of sugarcane varieties using nonlinear models. *Ciência Rural, 50*.

Karizaki, A. R., Rezvantalab, N., & Gholamalipour Alamdari, E. (2022). Quantification of grain dry matter accumulation trends in barley cultivars. *Plant Physiology Reports, 1-12*. [DOI: 10.1007/s40502-022-00647-5]

Lacasa, J., Hefley, T. J., Otegui, M. E., & Ciampitti, I. A. (2021). A practical guide to estimating the light extinction coefficient with nonlinear models—a case study on maize. *Plant Methods, 17*(1), 1-11. [DOI: 10.1186/s13007-020-00720-2]

Liu, X., Woodward, J. E., Kelly, B., Lewis, K. L., Byrd, S. A., & Chen, Y. (2021). Effects of production practices on temporal disease progress of Verticillium wilt of cotton (Gossypium hirsutum L.) in the Texas High Plains, USA. *Crop Protection, 140*, 105429. [DOI: 10.1016/j.cropro.2020.105429]

Lupi, T. M., Nogales, S., León, J. M., Barba, C., & Delgado, J. V. (2015). Characterization of commercial and biological growth curves in the Segureóna sheep breed. *Animal, 9*(8), 1-8. [DOI: 10.1017/S1751731115000855]

Mastrorilli, M., Katerji, N., & Rana, G. (1999). Productivity and water use efficiency of sweet sorghum as affected by soil water deficit occurring at different vegetative growth stages. *European Journal of Agronomy, 11*(3-4), 207-215. [DOI: 10.1016/S1161-0301(99)00032-4]

Mathur, S., Umakanth, A. V., Tonapi, V. A., Sharma, R., & Sharma, M. K. (2017). Sweet sorghum as biofuel feedstock: recent advances and available resources. *Biotechnol Biofuels, 10*, 146. [DOI: 10.1186/s13068-017-0829-y]

McCann, T., Krause, D., & Sanguansri, P. (2015). Sorghum—new gluten-free ingredient and applications. *Food Aust, 67*(6), 24–26. [DOI: 10.1016/j.biortech.2004.06.025]

McLaren, J. S., Lakey, N., & Osborne, J. (2003). Sorghum as a bioresources platform for future renewable resources. Proceedings 57th Corn and Sorghum Research Conference. CD ROM. American Seed Trade Association, Alexandria, VA, USA.

Murungweni, C., Van Wijk, M. T., Smaling, E. M. A., & Giller, K. E. (2016). Climate-smart crop production in semi-arid areas through increased knowledge of varieties, environment, and management factors. *Nutrient Cycl. Agroecosyst, 105*, 183-197. [DOI: 10.1007/s10705-016-9802-0]

Nelder, J. A. (1961). The fitting of a generalization of the logistic curve. *Biometrics, 17*, 89-110. [DOI: 10.2307/2527682]

Pannacci, E., & Bartolini, S. (2016). Evaluation of sorghum hybrids for biomass production in central Italy. *Biomass and Bioenergy, 88*, 135-141. [DOI: 10.1016/j.biombioe.2016.03.014]

Rahemi-Karizaki, A., Khaliliaghdam, N., & Biabani, A. (2021). Predicting the time trend of dry matter accumulation and leaf area index of winter cereals under nitrogen limitation by non-linear models. *Plant Physiology Reports, 26*(3), 443-456. [DOI: 10.1007/s40502-021-00647-5]

Rządkowski, G., Rządkowski, W., & Wójcicki, P. (2015). On some connections between the Gompertz function and special numbers. *Journal of Nonlinear Mathematical Physics, 22*, 374–380. [DOI: 10.1080/14029251.2014.911347]

Sari, B. G., Lúcio, A. D. C., Santana, C. S., Olivoto, T., Diel, M. I., & Krysczun, D. K. (2019). Nonlinear growth models: An alternative to ANOVA in tomato trials evaluation. *European Journal of Agronomy, 104*, 21-36. [DOI: 10.1016/j.eja.2019.01.002]

Sari, B. G., Lúcio, A. D. C., Santana, C. S., & Savian, T. V. (2019). Describing tomato plant production using growth models. *Scientia Horticulturae, 246*, 146-154. [DOI: 10.1016/j.scienta.2018.11.035]

Shi, P. J., Men, X. Y., Sandhu, H. S., Chakraborty, A., Li, B. L., Ou-Yang, F., Sun, Y. C., & Ge, F. (2013). The "general" ontogenetic growth model is inapplicable to crop growth. *Ecological Modelling, 266*, 1-9. [DOI: 10.1016/j.ecolmodel.2013.06.013]

Sousa, I. F., Neto, J. E. K., Muniz, J. A., Guimarães, R. M., Savian, T. V., & Muniz, F. R. (2014). Fitting nonlinear autoregressive models to describe coffee

seed germination. *Cienc. Rural., 44*, 2016-2021. [DOI: 10.1590/0103-8478cr20130118]

Tariq, M. M., Iqbal, F., Eyduran, E., Bajwa, M. A., & Huma, Z. (2013). Comparison of non-linear functions to describe the growth in the Mengali sheep breed of Balochistan. *Pakistan Journal of Zoology, 45*, 661-665. [DOI: 10.17582/journal.pjz/2013.45.3.661.665]

Tesso, T. T., Claflin, L. E., & Tuinstra, M. R. (2005). Analysis of stalk rot resistance and genetic diversity among drought-tolerant sorghum genotypes. *Crop Sci., 45*, 645-652. [DOI: 10.2135/cropsci2004.0293]

Tyagi, R. C., Singh, D., & Hooda, I. S. (1998). Effect of plant population, irrigation, and nitrogen on yield and its attributes of spring maize (Zea mays). *Indian Journal of Agronomy, 43*(4), 672–676. [DOI: 10.1007/s10681-013-0885-1]

Thomas, M. S. C., Annaz, D., Ansari, D., Serif, G., Jarrold, C., & Karmiloff-Smith, A. (2009). Using developmental trajectories to understand developmental disorders. *Journal of Speech, Language, and Hearing Research, 52*(2), 336-358. [DOI: 10.1044/1092-4388(2009/07-0203)]

USDA-FAS. (2018). World Agricultural Production. Circular Series WAP10-18 [DOI: 10.1021/jf508703f]

Üçkardeş, F., Korkmaz, M., & Ocal, P. (2013). Comparison of models and estimation of missing parameters of some mathematical models related to in situ dry matter degradation. *Journal of Animal and Plant Sciences, 23*, 999–1007. [DOI: 10.11648/j.ajae.20130103.12]

Winsor, C. P. (1932). The Gompertz curve as a growth curve. *Proc. National Academy of Science, 18*(1), 1-8. [DOI: 10.1073/pnas.18.1.1]

Yavuz, E., Önem, A. B., Kaya, F., Çanga, D., & Şahin, M. (2019). Modeling of individual growth curves in Japanese quails. *Black Sea Journal of Engineering and Science, 2*(1), 11-15. [DOI: 10.32569/bsengineering.615738]