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Kahramanmaraş Sütçü İmam Üniversitesi
Tarım ve Doğa Dergisi,
46100 – Kahramanmaraş/TÜRKİYE
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* Soyada göre sıralanmıştır.



Determination of *in vitro* Antioxidant, Anticholinergic, and Antiepileptic Activities of some Medicinal and Aromatic Plant Extracts

Bayram YURT¹, Rüya SAĞLAMTAŞ², Yeliz DEMİR³, Ebubekir İZOL⁴, Halit DİRİL⁵, Cüneyt ÇAĞLAYAN⁶

¹Department of Food Engineering, Faculty of Engineering and Architecture, Bingöl University, Bingöl, Türkiye, ²Vocational School of Health Services, Department of Medical Services and Technology, Agri Ibrahim Cecen University, Agri, Türkiye, ³Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan, Türkiye, ⁴Bee and Natural Products R&D and P&D Application and Research Center, Bingöl University, Bingöl, Türkiye, ⁵Medical Biochemistry Laboratory, Dursun Odabaş Medical Center, Van Yüzüncü Yıl University, Van, Türkiye, ⁶Department of Medical Biochemistry, Faculty of Medicine, Bilecik Şehy Edebalı University, Bilecik, Türkiye
¹<https://orcid.org/0000-0001-5447-1586>, ²<https://orcid.org/0000-0002-4400-2302>, ³<https://orcid.org/0000-0003-3216-1098>
⁴<https://orcid.org/0000-0003-0788-4999>, ⁵<https://orcid.org/0000-0003-4409-8268>, ⁶<https://orcid.org/0000-0001-5608-554X>
✉: eizol@bingol.edu.tr

ABSTRACT

Medicinal and aromatic plants such as *Crocus cancellatus*, and *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* have many different biological activities. While antioxidants are significant in preventing many diseases, inhibition of metabolic enzymes is also effective in preventing many diseases. In this study, antioxidant activities of water, ethanol, and dichloromethane extracts of four different medicinal and aromatic plant species were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and 2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS•+) radical scavenging and Cu²⁺, Fe³⁺, and Fe³⁺-2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) reducing assays. Enzyme inhibition studies were performed with metabolic enzymes acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase I and II isoenzymes. The ethanol extract of *A. nemorosa* showed the highest activity in DPPH and ABTS assays (IC₅₀: 17.36 µg mL⁻¹, IC₅₀: 7.02 µg mL⁻¹). In the Fe³⁺ reducing assay, the dichloromethane extract of *A. nemorosa* showed the highest activity (1.96±0.060 µg mL⁻¹). In the Cu²⁺ reducing assay, the dichloromethane extract of *J. oxycedrus* showed the highest activity (1.773±0.066 µg mL⁻¹). In the Fe³⁺-TPTZ reducing assay, the ethanol extract of *S. siberica* showed the highest activity (1.256±0.011 µg mL⁻¹). In the enzyme inhibition results, it was determined that all plants and all extracts inhibited the enzymes studied. As a result of this study, it was determined that these four medicinal and aromatic plants have high biological activities.

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Antioxidant and enzyme inhibition

Bazı Tıbbi ve Aromatik Bitki Ekstraktlarının *in vitro* Antioksidan, Antikolinerjik ve Antiepileptik Aktivitelerinin Belirlenmesi

ÖZET

Crocus cancellatus, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* gibi tıbbi ve aromatik bitkiler birçok farklı biyolojik aktiviteye sahiptir. Antioksidanlar birçok hastalığın önlenmesinde önemli rol oynarken, metabolik enzimlerin inhibisyonu da birçok hastalığın önlenmesinde etkilidir. Bu çalışmada, dört farklı tıbbi ve aromatik bitki türünün su, etanol ve diklorometan ekstraktlarının antioksidan aktiviteleri 1,1-difenil-2-pikrilhidrazil (DPPH•) ve 2,20-azino-bis-3-etilbenzthiazoline-6-sülfonik asit (ABTS•+) radikal giderme ve Cu²⁺, Fe³⁺ ve Fe³⁺-TPTZ indirgeme deneyleri ile belirlenmiştir. Enzim inhibisyon çalışmaları metabolik enzimler olan asetilkolinesteraz, bütirikolinesteraz, karbonik anhidraz I ve II izoenzimleri ile gerçekleştirilmiştir. *A. nemorosa*'nın etanol ekstresi DPPH ve ABTS deneylerinde en yüksek aktiviteyi göstermiştir (IC₅₀: 17.36 µg mL⁻¹, IC₅₀: 7.02 µg mL⁻¹). Fe³⁺ indirgeme deneyinde, *A. nemorosa*'nın

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Antioksidan ve enzim inhibisyonu

diklorometan ekstresi en yüksek aktiviteyi göstermiştir ($1.96 \pm 0.060 \mu\text{g mL}^{-1}$). Cu^{2+} indirgeme deneyinde, *J. oxycedrus*'un diklorometan ekstresi en yüksek aktiviteyi göstermiştir ($1.773 \pm 0.066 \mu\text{g mL}^{-1}$). Fe^{3+} -TPTZ indirgeme deneyinde, *S. siberica*'nın etanol ekstraktı en yüksek aktiviteyi göstermiştir ($1.256 \pm 0.011 \mu\text{g mL}^{-1}$). Enzim inhibisyon sonuçlarında, tüm bitkilerin çalışılan enzimleri inhibe ettiği belirlenmiştir. Bu çalışma sonucunda tıbbi ve aromatik bitkilerden olan bu dört bitkinin yüksek biyolojik aktiviteye sahip olduğu belirlenmiştir.

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INTRODUCTION

Medicinal and aromatic plants are used in many different fields and are the subject of scientific studies. Especially the biological activities they show with their phytochemical contents are very valuable. They act as pioneers in the treatment of many diseases and are the source of drug-active ingredients (Yılmaz et al., 2024; İzol et al., 2023; Yapıcı and İzol, 2023). In this study, the biological activities of four important medicinal and aromatic plants were investigated.

The *Crocus* has flowers in different colors (Ahouran et al., 2012). *Crocus* is a significant traditional medicinal herb (Abdullaev et al., 2003; Dimitra G et al., 2007; Fatehi et al., 2003). From Iran, Türkiye, and Jordan, the plant *Crocus cancellatus* is pretty common. Türkiye is a rich country regarding the *Crocus* species (Kandemir, 2010). *Crocus cancellatus* is called "Çiğdem," and grows on rocky slopes at an altitude of 50-2400 in the southeastern regions of Türkiye (Mammadov & Sahranc, 2003; Öntaş et al., 2020). This species' corms are available in local markets and consumed both cooked and raw (Ahouran et al., 2012).

The geographical range of *Scilla Siberica subsp. armena*, an Iranian-Turanian species, includes Georgia and Türkiye (Aydın et al., 2023). *Scilla Siberica subsp. armena*, (Grossh.) Mordak, known as "çamışkıran" in Türkiye (Guner et al., 2012). The bulbs of *S. siberica subsp. armena* are sold in Turkish markets and are used mainly as a garden herb (Aydın et al., 2023; Özdemir et al., 2016). This plant grows in rocky slopes at an altitude of 50-2400 in the southeastern regions of Türkiye (Özdemir & Yildirim 2016).

Juniperus oxycedrus subsp. oxycedrus is a variable species, particularly in the distribution range's western and central parts (Klimko et al., 2007). Folk medicine uses of *Juniperus* (Cupressaceae) species are widespread in developing nations (Orhan et al., 2012). It is growing on a variety of rocky sites from sea level up to 1600 m altitude (Orhan et al., 2011). In Türkiye, *J. oxycedrus subsp. oxycedrus* L. leaf decoction is used

to reduce blood sugar levels (Orhan et al., 2012).

Anthriscus, a member of the Apiaceae family and one of the fragrant herbs, is used therapeutically throughout the world in traditional medicine (Karakaya et al., 2019). *Anthriscus nemorosa* is called as 'gimigimi, peçek' in Türkiye. Fruits from the *A. nemorosa* plant have been used to treat inflammation, gastrointestinal disorders, and rheumatism (Bagci et al., 2016; Karakaya et al., 2019; Menemen, 2012). It grows in groves, rocky slopes, and watery meadows at an altitude of 500-3200 in all regions of Türkiye (Kiliç, 2017).

Alzheimer's disease (AD) is a progressive neurological condition marked by abnormal patient behavior and cognitive deficits (Güleç et al., 2022; Yaşar et al., 2021; Inci et al., 2023). Reactive oxygen species (ROS) have reportedly been linked to neuronal damage and cellular aging. Antioxidants may thereby slow the development of AD and prevent neuronal damage (Karageçili et al., 2023a; Demir et al., 2023; Osmaniye et al., 2022; Çelik et al., 2024). The ability of an antioxidant meal to suppress the main enzymes involved in the pathogenesis of AD, butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), is advantageous (İzol et al., 2021; Oztaskin et al., 2022; İzol, 2024; Bursal et al., 2021).

Carbonic anhydrases (CAs) are metalloenzymes that help a variety of biological systems produce bicarbonate and proton from carbon dioxide through a very straightforward hydration reaction (Karageçili et al., 2023b; Kaya et al., 2022). They control several pathological and physiological processes, including the transfer of CO_2 and bicarbonate ions between tissues involved in metabolism and the lungs, which helps keep the blood's pH and homeostasis in check (Ağgül et al., 2020; Buza et al., 2023; Yılmaz et al., 2023). Also, they are essential for the release of electrolytes from different tissues, bone resorption, and a few other biosynthetic processes like ureagenesis, lipogenesis, and gluconeogenesis (Bayindir et al., 2019; Çağlayan

& Gulcin 2018; Taslimi et al., 2017). The inhibition of CA isozymes may be responsible for several important physiological advantages against osteoporosis, epilepsy, hypertension, oedema, obesity, glaucoma, and cardiac hypertrophy (Ağgöl et al., 2020; Anil et al., 2022; Ozer et al., 2022).

Oxygen is an oxidizing agent that is highly reactive and non-metal and easily forms oxides, unlike other compounds. It exists in the atmosphere as a more stable biradical ($^3\text{O}_2$) and undergoes a gradual reduction process (Leyla & Gülçin, 2024; Gulcin, 2020). ROS are short-lived, active structures that contain oxygen atoms. Among them, singlet oxygen ($^1\text{O}_2$), superoxide anion radicals ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hypochlorite ions (ClO^-), and hydroperoxy radicals (HOO^{\cdot}) are the most abundant. These molecules are natural byproducts of the known methanolysis of oxygen and significantly affect the transmission of cell signals and homeostasis (Apak et al., 2022; Gulcin, 2020). These molecules have different half-lives. They are formed as radicals, molecules, and ions in various biological and chemical processes, including photosynthesis and the electron transport chain. ROS and free radicals are formed not only during metabolism but also due to the effects of various environmental sources such as exercise, exposure to chemicals, and sunlight (Durmaz et al., 2022; Kiziltas et al., 2021). Excessive levels of ROS in tissues and cells cause various disorders known as oxidative stress, including neurological and cardiovascular diseases, cancer, and lung diseases (Erdoğan et al., 2021; Polat Kose & Gulcin 2021). Antioxidants play a vital role in the human body and food systems, reducing ROS harmful effects and oxidative processes (Çakmakçı et al., 2015; Gulcin, 2020). Aerobic organisms have defense systems, including antioxidant compounds and enzymes to remove and repair damaged molecules. Cells are protected against oxidative stress by antioxidant enzyme networks (Davies, 1995; Gulcin, 2020).

The biological research done on plant extracts supports most species' traditional applications, but it falls short of fully supporting rational phytotherapy. Because there are so many species, physiotherapists continue looking for fresh sources of biologically active substances and assessing their pharmacological activity profiles, primarily based on *in vivo* and/or *in vitro* studies. All these studies must be performed in conjunction with a multicomponent pattern analysis of the extracts to evaluate the primary components. So, in the present study, tri extracts prepared from the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *sprengel* were investigated for their AChE, BChE, hCA I, hCA II, and antioxidant potential.

MATERIAL and METHOD

Chemicals

Butyrylthiocholine, acetylthiocholine, ethanol, dichloromethane, BHT, BHA, DPPH, ABTS, trolox, and α -tocopherol were commercially obtained from Sigma-Aldrich. The other chemicals were used as analytical grades.

Plant Material

In this research, four plants (*Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *Oxycedrus*, and *Anthriscus nemorosa* (M.bieb.) *sprengel*) were obtained from Bingöl region, Türkiye. The collected plants were identified in the Herbarium laboratory of Bingöl University, Department of Molecular Biology and Genetics, and added to the herbarium library.

Preparation of Extracts

Three different extracts from dried and ground plants were prepared separately. Extracts were prepared using dried herbs (2.5 g) and solvent (50 mL). The water extract was prepared using the boiling method, and the other extracts were prepared using the maceration method. Volatiles were extracted with a rotary evaporator and stored in the refrigerator until the study was carried out.

Enzyme Inhibition Assay

AChE and BChE enzyme inhibition studies were performed using Ellman's colorimetric method (Ellman et al., 1961). Based on this method, cholinesterases catalyze the breakdown reaction of ACh or BCh to thiocholine and acetate or butyrate. DTNB, which is used during inhibition studies, is formed as a result of the reaction with thiocholine, which is one of these degradation products, as a yellow compound, 5-thio-2-nitrobenzoic acid. An inhibition study was performed by measuring the color intensity of the colored compound formed at 412 nm.

The study purified both hCA I and II isoenzymes by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. Here, Sepharose-4B-L-Tyrosine-sulfanilamide is used as an affinity matrix for hCA isoenzymes. The activity of these isoenzymes is determined spectrophotometrically as in previous studies (Caglayan & Gulcin 2018; Gocer & Gulcin 2013). CA isoenzymes are considered to be the units in which they convert PNP from 348 nm PNA over 3 minutes at 25 °C (Verpoorte et al., 1967).

IC_{50} values were calculated by examining the three extracts (water WE, ethanol EE, and dichloromethane DME) prepared leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *sprengel* were investigated for their AChE, BChE, hCA I, hCA

II enzyme activities. For this purpose, enzyme activities at five different concentrations were measured spectrophotometrically for all extracts. The obtained data was drawn using the % activity [extract] graph. IC₅₀ values were calculated using the graph.

Antioxidant Activities Assays

DPPH solution was prepared daily and kept in a glass bottle in the dark (4°C). Plant extracts (1.5 mL) were dissolved in ethanol and transferred to fresh 500 µL of DPPH · solution (0.1 M). These mixtures were mixed vigorously and incubated in the dark for 30 minutes. Then, their absorbance was recorded spectrophotometrically at 517 nm (Aras et al., 2016; Köksal et al., 2009). ABTS⁺ was obtained by reacting ABTS (7.0 mM) with K₂S₂O₈ (2.5 mM). ABTS⁺ scavenging ability of extracts prepared using different solvents was determined according to the previously described spectroscopic method (Erdoğan et al., 2021). Fe³⁺-reducing effects of plant extracts were done in accordance with Oyaizu's method (Oyaizu, 1986). Cu²⁺-reducing effects of plant extracts were measured according to a minor modification of Apak et al. (2006) method (Bursal et al., 2019). The last reduction method we used, the FRAP method, is based on the complicated degradation of Fe³⁺-TPTZ. The increased absorbance of Fe²⁺-TPTZ was measured spectrometrically at 593 nm as described in previous studies (Gulcin et al., 2019; Polat Kose et al., 2020).

Çizelge 1. *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) *spreng* yapraklarının su, etanol ve diklorometan ekstraktlarının AChE ve BChE için IC₅₀ değerleri

Table 1. IC₅₀ values of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *spreng* for AChE and BChE

Samples	IC ₅₀ (mg mL ⁻¹)			
	BChE	r ²	AChE	r ²
<i>C. cancellatus</i> DME	63.58	0.9633	18.05	0.9957
<i>C. cancellatus</i> EE	74.52	0.9852	64.77	0.9544
<i>C. cancellatus</i> WE	51.72	0.9911	83.49	0.9815
<i>S. Siberica</i> subsp. <i>armena</i> DME	54.14	0.9392	97.61	0.9679
<i>S. Siberica</i> subsp. <i>armena</i> EE	41.5	0.9314	60.26	0.9839
<i>S. Siberica</i> subsp. <i>armena</i> WE	40.53	0.9601	91.18	0.9282
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> DME	66.63	0.9715	62.43	0.9950
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> EE	72.19	0.9943	60.79	0.9884
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> WE	31.5	0.9315	72.95	0.9819
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> DME	35.72	0.9649	17.46	0.9900
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> EE	15.4	0.9888	7.71	0.9139
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> WE	47.47	0.9719	38.72	0.9303

The studied plant extract demonstrated concentration-dependent inhibition of AChE, with activity ranging from 15.40 mg mL⁻¹ to 74.52 mg mL⁻¹. The AChE-inhibitory capacity of studied plant extracts is in the following order: *A. nemorosa* (M.bieb.) *Spreng*. EE (IC₅₀, 15.40 mg mL⁻¹, r²: 0.9888) > *J. oxycedrus* subsp.

Statistical Analyses

Statistical analyses were performed with SPSS, and p-values less than 0.05 were considered statistically significant at a 95% confidence interval. P values for differences were obtained as a result of a two-way (2x2) ANOVA analysis. Post-hoc Tukey test was used for pairwise comparisons.

RESULTS and DISCUSSION

Enzyme Inhibition Studies

Memory loss and other cognitive impairments are the earliest symptoms of AD, which are thought to be linked to acetylcholine (ACh) depletion, inflammation, and oxidative stress. Hence, consumption of antioxidant-rich vegetables can halt the onset of AD and neurodegeneration. Inhibiting AChE and BChE can be significant because it is a cutting-edge therapeutic strategy for treating neurodegenerative diseases. In the current study, the activities of the three extracts (water WE, ethanol EE, and dichloromethane DME) were prepared from *C. cancellatus* and *S. Siberica* Subsp. *armena*, *J. oxycedrus* subsp. *oxycedrus* and *A. nemorosa* were investigated against AChE and BChE. All extracts inhibited BChE and AChE in a dose-dependent manner. IC₅₀ values, which represent the inhibition effect of the tested extracts, were determined and are shown in Table 1.

oxycedrus WE (IC₅₀, 31.50 mg mL⁻¹, r²: 0.9315) > *A. nemorosa* (M.bieb.) *Spreng*. DME (IC₅₀, 35.72 mg mL⁻¹, r²: 0.9649) > *S. Siberica* Subsp. *armena* WE (IC₅₀, 40.53 mg mL⁻¹, r²: 0.9601) > *S. Siberica* Subsp. *armena* EE (IC₅₀, 41.50 mg mL⁻¹, r²: 0.9314) > *A. nemorosa* (M.bieb.) *Spreng* WE (IC₅₀, 47.47 mg mL⁻¹, r²: 0.9719)

> *C. cancellatus* WE (IC₅₀, 51.72 mg mL⁻¹, r²: 0.9911) > *S. Siberica* Subsp. *armena* DME (IC₅₀, 54.14 mg mL⁻¹, r²: 0.9392) > *C. cancellatus* DME (IC₅₀, 63.58 mg mL⁻¹, r²: 0.9633) > *J. oxycedrus* subsp. *oxycedrus* DME (IC₅₀, 66.63 mg mL⁻¹, r²: 0.9715) > *J. oxycedrus* subsp. *oxycedrus* EE (IC₅₀, 72.19 mg mL⁻¹, r²: 0.9943) > *C. cancellatus* EE (IC₅₀, 74.52 mg mL⁻¹, r²: 0.9852).

The concentration-dependent AChE inhibition effect of the WE of *C. cancellatus*, *S. Siberica* Subsp. *Armena*, and *J. oxycedrus* subsp. *oxycedrus* leaves were shown, to be higher than that of EE and DME. On the other hand, the AChE inhibition effect of the EE *A. nemorosa* (M.bieb.) *Spreng.* was shown, to be higher than that of WE and DME. The EE and WE AChE inhibition effects of *S. Siberica* Subsp. *Armena* plants were almost close to each other.

The studied plant extract demonstrated concentration-dependent inhibition of BChE, with activity ranging from 7.71 mg mL⁻¹ to 97.61 mg mL⁻¹. The BChE-inhibitory capacity of studied plant extracts is in the following order: *A. nemorosa* (M.bieb.) *Spreng.* EE (IC₅₀, 7.71 mg mL⁻¹, r²: 0.9139) > *A. nemorosa* (M.bieb.) *Spreng.* DME (IC₅₀, 17.46 mg mL⁻¹, r²: 0.9900) > *C. cancellatus* DME (IC₅₀, 18.05 mg mL⁻¹, r²: 0.9957) > *A. nemorosa* (M.bieb.) *Spreng.* WE (IC₅₀, 38.72 mg mL⁻¹, r²: 0.9303) > *S. Siberica* Subsp. *armena* EE (IC₅₀, 60.26 mg mL⁻¹, r²: 0.9839) = *J. oxycedrus* subsp. *oxycedrus* EE (IC₅₀, 60.79 mg mL⁻¹, r²: 0.9884) > *J. oxycedrus* subsp. *oxycedrus* DME (IC₅₀, 62.43 mg mL⁻¹, r²: 0.9950) > *C. cancellatus* EE (IC₅₀, 64.77 mg mL⁻¹, r²: 0.9544) > *J. oxycedrus* subsp. *oxycedrus* WE (IC₅₀, 72.95 mg mL⁻¹, r²: 0.9819) > *C. cancellatus* WE (IC₅₀, 83.49 mg mL⁻¹, r²: 0.9815) > *S. Siberica* Subsp. *armena* WE (IC₅₀, 91.18 mg mL⁻¹, r²: 0.9282) > *S. Siberica* Subsp. *armena* DME (IC₅₀, 97.61 mg mL⁻¹, r²: 0.9679). The concentration-dependent BChE inhibition effect of the EE of studied all plant leaves was shown, to be higher than that of WE and DME. When the results of this study were compared, *C. cancellatus* DME inhibited BChE enzyme 3.52 times more than AChE enzyme and *A. nemorosa* (M.bieb.) *Spreng.* DME inhibited 2.04 more. *A. nemorosa* (M.bieb.) *Spreng.* EE showed the best inhibition effect on the activity of both AChE and BChE enzymes. *J. oxycedrus* subsp. *oxycedrus* DME inhibited the two cholinesterase enzymes studied at close values.

As this is the first study to examine four plants using AChE and BChE enzymes, the data provided here cannot be compared to the literature currently in use. Studies on other species of these plants for AChE and BChE enzymes are available in the literature. For instance, Menghini et al. (2018) studied the effect of *C. sativus* L. Stigmas extract on AChE and BChE enzymes. This plant inhibited AChE and BChE enzymes with 2.51 ± 0.18 for AChE and 3.44 ± 0.13 galantamine equivalents g⁻¹ extract for BChE. Linardaki et al. (2017) investigated the neurotoxic

effects of aflatoxin B1 and the preventive effects of *C. sativus*. They tested the activity of AChE and BChE in the liver, cerebellum, and whole brain. They showed that pretreatment of aflatoxin B1-exposed mice with *C. sativus* infusion resulted in even lower activity in brain, cerebellar and liver AChE, while higher activity in brain BChE enzyme compared to aflatoxin B1-exposed mice. *A. nemorosa* essential oil was tested by Bagci et al. (2016) on rats given scopolamine to see how it affected their memory functions, anxiety levels, and depressive-like behaviours. Öztürk et al. (2011) looked at AChE and BChE enzyme inhibition effects by preparing acetone methanol and hexane extract of *J. oxycedrus* subsp. *oxycedrus* plant. It was found to be the hexane extract of this plant, having 81.40% inhibition at 200 mg mL⁻¹ against AChE. Hexane extract of this plant showed 95.75% inhibition against BChE.

One of the most popular and effective mechanisms for regulating pH in all biological systems is the CAs. (Aktaş et al., 2022). These enzymes are involved in numerous other biochemical and physiological processes; therefore, they are not just pH regulators. In clinical practice or as pharmacological tools, most CA inhibitors and activators are synthetic derivatives developed over time through conventional drug design campaigns from synthetic lead molecules (Hamide et al., 2022; Zengin et al., 2023). Yet, research into several natural items' CA inhibitory effects has also begun over the past ten years. This has resulted in substantial advancements in the field (Tugrak et al., 2021). All extracts inhibited hCA I and hCA II in a dose-dependent manner. IC₅₀ values, which represent the inhibition effect of the tested extracts, were determined and are shown in Table 2.

In this study, the plant extract demonstrated concentration-dependent inhibition of hCA I, with activity ranging from 14.59 mg mL⁻¹ to 68.61 mg mL⁻¹. The hCA I inhibitory capacity of studied plant extracts in the following order: *C. cancellatus* DME (IC₅₀, 14.59 mg mL⁻¹, r²: 0.9752) > *J. oxycedrus* subsp. *oxycedrus* DME (IC₅₀, 22.79 mg mL⁻¹, r²: 0.9671) > *A. nemorosa* (M.bieb.) *Spreng.* EE (IC₅₀, 26.55 mg mL⁻¹, r²: 0.9304) = *C. cancellatus* EE (IC₅₀, 26.55 mg mL⁻¹, r²: 0.9808) > *A. nemorosa* (M.bieb.) *Spreng.* WE (IC₅₀, 27.50 mg mL⁻¹, r²: 0.9698) > *S. Siberica* subsp. *armena* DME (IC₅₀, 34.85 mg mL⁻¹, r²: 0.9543) > *S. Siberica* subsp. *armena* WE (IC₅₀, 36.09 mg mL⁻¹, r²: 0.9420) > *S. Siberica* subsp. *armena* EE (IC₅₀, 41.75 mg mL⁻¹, r²: 0.9343) > *J. oxycedrus* subsp. *oxycedrus* EE (IC₅₀, 52.90 mg mL⁻¹, r²: 0.9247) > *A. nemorosa* (M.bieb.) *Spreng.* DME (IC₅₀, 53.31 mg mL⁻¹, r²: 0.9443) > *J. oxycedrus* subsp. *oxycedrus* WE (IC₅₀, 61.87 mg mL⁻¹, r²: 0.9244) > *C. cancellatus* WE (IC₅₀, 68.61 mg mL⁻¹, r²: 0.9609). The concentration-dependent hCA I inhibition effect of the DME of *C. cancellatus*, *S. Siberica* subsp. *armena* and *J. oxycedrus* subsp. *oxycedrus* leaves were shown, to be

higher than that of WE and EE. On the other hand, the order of inhibition in the leaves of *A. nemorosa* (M.bieb.) spreng. Is in the form of EE > WE > DME. DME extract of *C. cancellatus* inhibited hCA I enzyme 4.7 times more than WE. *C. cancellatus* and *A. nemorosa* EE inhibited the hCA I enzyme at the same rate. DME extract *J. oxycedrus* subsp. *oxycedrus* inhibited hCA I enzyme 2.71 times more than WE.

In the current study, the studied plant extract demonstrated concentration-dependent inhibition of hCA II, with activity ranging from 8.14 mg mL⁻¹ to 48.80 mg mL⁻¹. The HCA II inhibitory effect of studied plant extracts in the following order: *S. Siberica* Subsp. *armena* WE (IC₅₀, 8.14 mg mL⁻¹, r²: 0.9557) > *S.*

Siberica Subsp. *armena* EE (IC₅₀, 12.63 mg mL⁻¹, r²: 0.9215) > *C. cancellatus* WE (IC₅₀, 14.23 mg mL⁻¹, r²: 0.9468) > *S. Siberica* Subsp. *armena* DME (IC₅₀, 14.53 mg mL⁻¹, r²: 0.9716) > *J. oxycedrus* subsp. *oxycedrus* EE (IC₅₀, 14.78 mg mL⁻¹, r²: 0.9631) > *C. cancellatus* EE (IC₅₀, 18.53 mg mL⁻¹, r²: 0.9936) > *J. oxycedrus* subsp. *oxycedrus* DME (IC₅₀, 19.41 mg mL⁻¹, r²: 0.9708) > *A. nemorosa* (M.bieb.) Spreng. EE (IC₅₀, 27.18 mg mL⁻¹, r²: 0.9341) > *A. nemorosa* (M.bieb.) Spreng. WE (IC₅₀, 30.26 mg mL⁻¹, r²: 0.9859) > *C. cancellatus* DME (IC₅₀, 30.80 mg mL⁻¹, r²: 0.9346) > *A. nemorosa* (M.bieb.) Spreng. DME (IC₅₀, 35.18 mg mL⁻¹, r²: 0.9887) > *J. oxycedrus* subsp. *oxycedrus* WE (IC₅₀, 48.80 mg mL⁻¹, r²: 0.9139).

Çizelge 2. *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) spreng yapraklarının su, etanol ve diklorometan ekstraktlarının hCA I ve hCA II için IC₅₀ değerleri

Table 2. IC₅₀ values of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) spreng for hCA I and hCA II

Samples	IC ₅₀ (mg mL ⁻¹)			
	hCA I	r ²	hCA II	r ²
<i>C. cancellatus</i> DME	14.59	0.9752	30.8	0.9346
<i>C. cancellatus</i> EE	26.55	0.9808	18.53	0.9936
<i>C. cancellatus</i> WE	68.61	0.9609	14.23	0.9468
<i>S. Siberica</i> subsp. <i>armena</i> DME	34.85	0.9543	14.53	0.9716
<i>S. Siberica</i> subsp. <i>armena</i> EE	41.75	0.9343	12.63	0.9215
<i>S. Siberica</i> subsp. <i>armena</i> WE	36.09	0.9420	8.14	0.9557
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> DME	22.79	0.9671	19.41	0.9708
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> EE	52.90	0.9247	14.78	0.9631
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> WE	61.87	0.9244	48.80	0.9139
<i>A. nemorosa</i> (M.bieb.) spreng DME	53.31	0.9443	35.18	0.9887
<i>A. nemorosa</i> (M.bieb.) spreng EE	26.55	0.9304	27.18	0.9341
<i>A. nemorosa</i> (M.bieb.) spreng WE	27.5	0.9698	30.26	0.9859

Some studies in the literature include the effects of the plants studied in this study on the activities of other enzymes. Loizzo et al. (2016) studied *C. cancellatus* subsp. *damascenus* extract inhibited α-glycosidase and α-amylase. The IC₅₀ values of 68.6 for α-glycosidase and 57.1 μg/mL for α-amylase were determined. Another study examined the α-glycosidase and α-amylase inhibitory effect of *Scilla siberica* subsp. *armena* corm, flower, and leaf methanolic extracts. The flower extract displayed no inhibition against α-amylase as well as α-glycosidase inhibitory effect with an IC₅₀ value of 5239 μg mL⁻¹. Blue pollen, leaf, and Corm extracts showed no inhibition against α-glycosidase and α-amylase enzymes (Aydn et al., 2023).

Antioxidant Results

In this section, water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.)

Sprengel was prepared, and studies to determine the antioxidant capacities of these extracts were included. The ABTS•⁺ and DPPH• methods were used to determine how antioxidant plant extracts work and measure their ability to eliminate free radicals. In addition, the reduction capacity of copper ions (Cu²⁺) to copper ions (Cu⁺), the reduction capacity of ferric ions (Fe³⁺) to iron ions (Fe²⁺), and the reduction capacity of Fe³⁺-TPTZ by the FRAP method were determined by different methods. Comparisons were made with synthetic and standard antioxidants such as BHA, BHT, α-tocopherol, and the α-tocopherol analogue Trolox.

Determination of Radical Scavenging Effects

The ABTS•⁺ and DPPH• scavenging procedures are remarkable due to their rapidity, simplicity, sensitivity, and reproducibility (Aras et al 2016; Gulcin 2020). The DPPH• method is based on the DPPH• scavenging percentage of antioxidants in the plant extract. DPPH• has a dark blue colour and is a long-

lived nitrogen radical species capable of dimerization (Gulcin, 2020). This method was first reported as a decolourization assay by Blois (1958). Today, DPPH• is generally known as a reagent used to determine antioxidants' free radical scavenging activity. This molecule shows maximum absorbance at 517 nm (Bursal et al., 2020; Gulcin, 2020; Türkan et al., 2020). The difference between control values and different concentrations (10-30 µg mL⁻¹) of plant extracts was found to be statistically significant (p < 0.01). The IC₅₀ values of the extracts were between 19.86 and 38.93 µg mL⁻¹, and the values of the water extracts were higher than the others. The IC₅₀ values of the standards were calculated as 7.3 (r²: 0.9733) for Trolox and 8.35 (r²: 0.9823) for α-tocopherol (Table 3, Figure 1). The DPPH radical scavenging capacities of the studied plant extracts are in the following order: *A. nemorosa* (M.bieb.) *Spreng EE* (IC₅₀:17.36, r²:0.9513) > *S. Siberica* Subsp. *armena* DME (IC₅₀:19.86, r²:0.9555) > *A. nemorosa* (M.bieb.) *Spreng DME* (IC₅₀:20.2, r²:0.9053) > *J. oxycedrus* subsp. *Oxycedrus* DME (IC₅₀:27.18, r²:0.9247) > *S. Siberica* Subsp. *armena* EE (IC₅₀:28.17, r²:0.9107) > *S. Siberica* Subsp. *armena* WE (IC₅₀:29.12, r²:0.9549) > *C. cancellatus* EE (IC₅₀:30.13, r²:0.9417) > *J. oxycedrus* subsp. *Oxycedrus* EE (IC₅₀:32.54, r²:0.9420) > *C. cancellatus* DME (IC₅₀:33.31, r²:0.9552) ~ *C. cancellatus* WE (IC₅₀:33.97, r²:0.9631) > *A. nemorosa* (M.bieb.) *spreng WE* (IC₅₀:34.48, r²:0.9366) > *J. oxycedrus* subsp. *Oxycedrus*

WE (IC₅₀:38.93, r²:0.9556). The current study determined that extracts obtained from *C. cancellatus* had relatively higher IC₅₀ values than other plants.

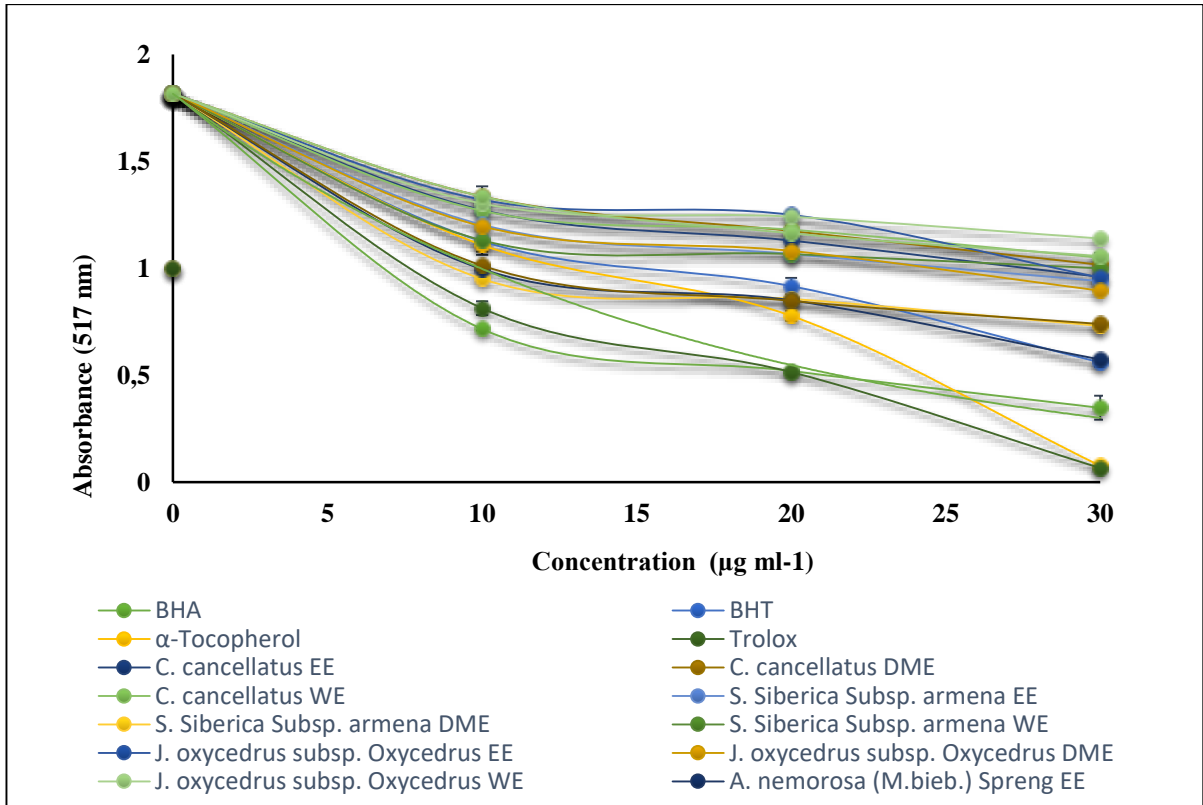
ABTS radical scavenging method is one of the different radical scavenging methods used to measure the antioxidant activities of extracts, pure substances, and food products (Gulcin, 2020). It can be easily applied as a spectrophotometric analysis method. It facilitates its use for routine screening and detection. ABTS•+ is generally obtained by the oxidation of ABTS with K₂S₂O₈ (Gülçin, 2012). The ABTS•+ radical can react rapidly with antioxidants and is easily used to determine the antioxidant effects of various food products and plant extracts, where it is effective over a wide pH range (Gulcin, 2020; Güven et al., 2023). For the ABTS radical scavenging method, control values and different concentrations of plant extracts (10-30 µg mL⁻¹) were studied, and the difference between the plant extracts was found to be statistically significant (p < 0.01). The IC₅₀ values of the extracts were between 7.02-84.51 µg mL⁻¹, and the values of the water extracts were higher than the other extracts in this method, as in the DPPH radical scavenging method. The IC₅₀ values of the standards were calculated as 7.06 (r²: 0.9420) for Trolox, 9.62 (r²: 0.9683) for α-tocopherol, 5.2 (r²: 0.9869) for BHA, and 9.68 (r²: 0.9465) for BHT (Table 3, Figure 2).

Çizelge 3. *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) *spreng* yapraklarının su, etanol ve diklorometan ekstraktlarının DPPH•, ABTS•+ süpürme aktiviteleri ve standart antioksidanlar için IC₅₀ (µg mL⁻¹) değerleri.

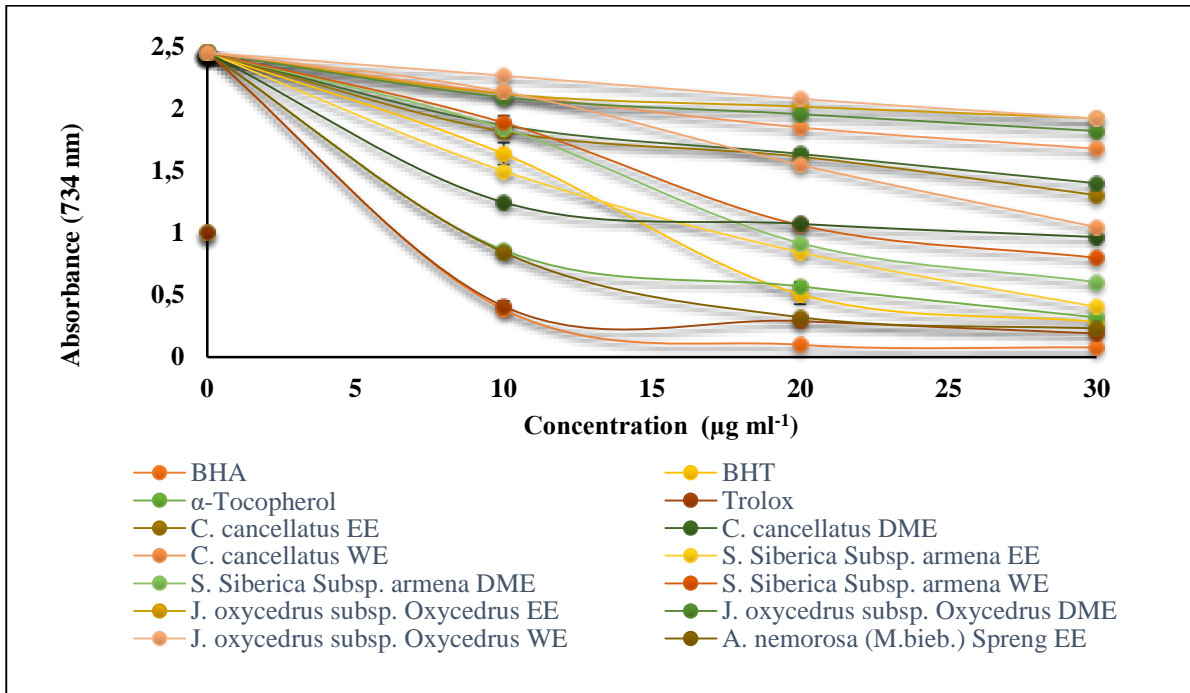
Table 3. IC₅₀ (µg mL⁻¹) values for DPPH•, ABTS•+ scavenging activities of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *spreng* and of standard antioxidants.

Antioxidants and Samples	DPPH• Scavenging		ABTS•+ Scavenging	
	IC ₅₀	r ²	IC ₅₀	r ²
BHA	11.57	0.9517	5.2	0.9869
BHT	18.05	0.9804	9.68	0.9645
Trolox	7.3	0.9733	9.62	0.9683
α-Tocopherol	8.35	0.9823	7.06	0.9420
<i>C. cancellatus</i> EE	30.13	0.9417	31.94	0.9707
<i>C. cancellatus</i> DME	33.31	0.9552	35.18	0.9710
<i>C. cancellatus</i> WE	33.97	0.9631	52.5	0.9939
<i>S. Siberica</i> subsp. <i>armena</i> EE	28.17	0.9107	12.07	0.9942
<i>S. Siberica</i> subsp. <i>armena</i> DME	19.86	0.9555	15	0.9650
<i>S. Siberica</i> subsp. <i>armena</i> WE	29.12	0.9549	18.33	0.9742
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> EE	32.54	0.9420	77	0.9170
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> DME	27.18	0.9247	64.17	0.9530
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> WE	38.93	0.9556	84.51	0.9997
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> EE	17.36	0.9513	7.02	0.9898
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> DME	20.2	0.9053	18.93	0.9671
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> WE	34.48	0.9366	26.86	0.9543

The results show significant differences (p < 0.05) in post-hoc comparisons between different groups.



Şekil 1. Ekstraktların radikal giderici etkileri (DPPH giderici etkileri)
 Figure 1. Radical scavenging effects of extracts (DPPH scavenging effects)



Şekil 2. Ekstraktların radikal giderici etkileri (ABTS giderici etkileri)
 Figure 2. Radical scavenging effects of extracts (ABTS scavenging effects)

The ABTS radical scavenging capacities of the studied plant extracts are in the following order: *A. nemorosa* (M.bieb.) *spreng*

EE (IC₅₀:7.02, r²:0.9898) > *S. Siberica* subsp. *armena* EE (IC₅₀:12.07, r²:0.9942) > *S. Siberica* subsp. *armena*

DME (IC₅₀:15.0, r²:0.9650) > *S. Siberica* subsp. *armena* WE (IC₅₀:18.33, r²:0.9742) ~ *A. nemorosa* (M.bieb.) *spreng* DME (IC₅₀:18.93, r²:0.9671) > *A. nemorosa* (M.bieb.) *spreng* WE (IC₅₀:26.86, r²:0.9543) > *C. cancellatus* EE (IC₅₀:31.94, r²:0.9707) > *C. cancellatus* DME (IC₅₀:35.18, r²:0.9710) > *C. cancellatus* WE

(IC_{50} :52.5, r^2 :0.9939)> *J. oxycedrus* subsp. *oxycedrus* DME (IC_{50} :64.17, r^2 :0.9530)> *J. oxycedrus* subsp. *oxycedrus* EE (IC_{50} :77, r^2 :0.9170)> *J. oxycedrus* subsp. *oxycedrus* WE (IC_{50} :84.51, r^2 :0.9997). The current study determined that extracts obtained from *J. oxycedrus* subsp. *Oxycedrus* had higher IC_{50} values than extracts from other plants. In the ABTS radical scavenging method, as in the DPPH method, it was determined that the extracts of the *A. nemorosa* (M.bieb.) *spring* plant had the lowest IC_{50} value.

A previous study reported that the root CH_2Cl_2 fraction of *Anthriscus nemorosa*, root essential oil, and the main compound α -pinene found in the root essential oil have antioxidant capacity (Karakaya et al., 2019). Another study on the branches and fruits of different *Juniperus* species reported that different extracts had DPPH and ABTS radical scavenging properties, and their total antioxidant capacity was relatively high (Gök et al., 2021). Taviano et al. (2011) examined the antioxidant potential of water and methanol extracts of branches of *Juniperus* species (*J. oxycedrus* subsp. *macrocarpa*, *J. communis* var. *communis*, *J. drupacea*, *J. communis* var. *saxatilis* and *J. oxycedrus* subsp. *oxycedrus*). They reported that *J. oxycedrus* subsp. Extracts had high DPPH scavenging activity.

Determination of Reducing Capacities

The reducing activity of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *spring* was evaluated by measuring their ability to reduce Fe^{3+} to Fe^{2+} . Compounds with functional groups such as -OH, -SH, and -COOH, essential electron donor groups found in plant extracts, are greatly important in reducing capacity. Fe^{3+} reduction abilities prepared with different solvents were determined. Plant extracts made with various solvents have been shown to have beneficial reducing effects by lowering ferric ions when categorized using standard criteria like BHT, Trolox, BHA, and α -Tocopherol. As seen in Table 4 and Figure 3, the Fe^{3+} reducing the ability of the extracts at 30 μ g/mL concentration showed absorbance in the range of 0.863-1.960 at 700 nm. Compared to standard antioxidants, the results obtained from this test showed that *A. nemorosa* (M.bieb.) *spring* DME, *A. nemorosa* (M.bieb.) *spring* EE, and *J. oxycedrus* subsp. *oxycedrus* DME, *S. Siberica* subsp. *armena* EE, *A. nemorosa* (M.bieb.) *spreng* WE, *J. oxycedrus* subsp. *oxycedrus* EE was found to have a very effective Fe^{3+} -reducing ability, and other extracts were found to have a close to moderate effect on α -Tocopherol and Trolox values.

The copper ions (Cu^{2+}) reducing capacity (CUPRAC) method was first developed and used by Apak's working group (Apak et al., 2006), and this CUPRAC reagent is stable and easily accessible compared to

chromogenic radical reagents. The method has been applied to various matrices containing both hydrophilic and lipophilic antioxidants, and positive results have been obtained. The method is based on the reduction of Cu^{2+} to Cu^+ or neocuproine (2,9-dimethyl-1,10-phenanthroline) via polyphenols in the aqueous ethanolic medium (Gulcin, 2020). In this method, the copper-reducing capacities of water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *spring* were determined to be between 1.02 and 1.773 absorbances at 30 μ g mL⁻¹ concentration. CUPRAC of the examined plant extracts and standard antioxidants are as follows: BHA (λ_{450} : 2.459 \pm 0.027, r^2 :0.9904)>BHT (λ_{450} : 1.975 \pm 0.091, r^2 :0.9422)> *J. oxycedrus* subsp. *oxycedrus* DME (λ_{450} : 1.773 \pm 0.066, r^2 :0.9084)> *S. Siberica* subsp. *armena* DME (λ_{450} : 1.74 \pm 0.003, r^2 :0.9354)> *J. oxycedrus* subsp. *oxycedrus* EE (λ_{450} : 1.598 \pm 0.095, r^2 :0.9221)> *C. cancellatus* DME (λ_{450} : 1.481 \pm 0.016, r^2 :0.9188) > *A. nemorosa* (M.bieb.) *spreng* EE (λ_{450} :1.436 \pm 0.032, r^2 :0.9457)> *A. nemorosa* (M.bieb.) *spreng* DME (λ_{450} :1.31 \pm 0.036, r^2 :0.9706)> *S. siberica* subsp. *armena* EE (λ_{450} :1.303 \pm 0.055, r^2 :0.9962)> *A. nemorosa* (M.bieb.) *spreng* WE (λ_{450} :1.133 \pm 0.028, r^2 :0.9543)> *S. siberica* subsp. *armena* WE (λ_{450} :1.204 \pm 0.020, r^2 :0.9739)> *C. cancellatus* EE (λ_{450} :1.195 \pm 0.015, r^2 :0.9245) > *J. oxycedrus* subsp. *oxycedrus* WE (λ_{450} :1.037 \pm 0.003, r^2 :0.9653)~ *C. cancellatus* WE (λ_{450} :1.020 \pm 0.03, r^2 :0.9479) > α -Tocopherol (λ_{450} :1.014 \pm 0.054, r^2 :0.9287)> Trolox(λ_{450} :0.987 \pm 0.007, r^2 :0.9663). When the results were examined, it was determined that plant extracts had better results than standard antioxidants, α -Tocopherol and Trolox.

Ferric reducing antioxidant power (FRAP assay) is known as a method based on measuring the reduction of ferric ions (Fe^{3+})-ligand complex by antioxidants in an acidic environment to intense, blue-coloured iron ions (Fe^{2+}) complex (Gulcin, 2020). This method was first used to analyze plasma assays and then began to be used in various places, including various biological fluids, plant extracts, foods, and beverages (Elmastas et al., 2006; Gülçin, 2012). In this method, Ferric reduced antioxidant power capacities (FRAP) of water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *Sprengel* was determined to be between 0.529 and 1.256 absorbances at 30 μ g mL⁻¹ concentration (Table 4, Figure 5). FRAP of the examined plant extracts and standard antioxidants are as follows: BHA (λ_{593} : 1.635 \pm 0.038, r^2 :0.9227) > Trolox (λ_{593} :1.443 \pm 0.020, r^2 :0.9603) ~ BHT (λ_{593} :1.441 \pm 0.006, r^2 :0.9202) > α -Tocopherol (λ_{593} :1.380 \pm 0.072, r^2 :0.9784)> *S. Siberica* subsp. *armena* EE (λ_{593} :1.256 \pm 0.011, r^2 :0.9554)> *J. oxycedrus*

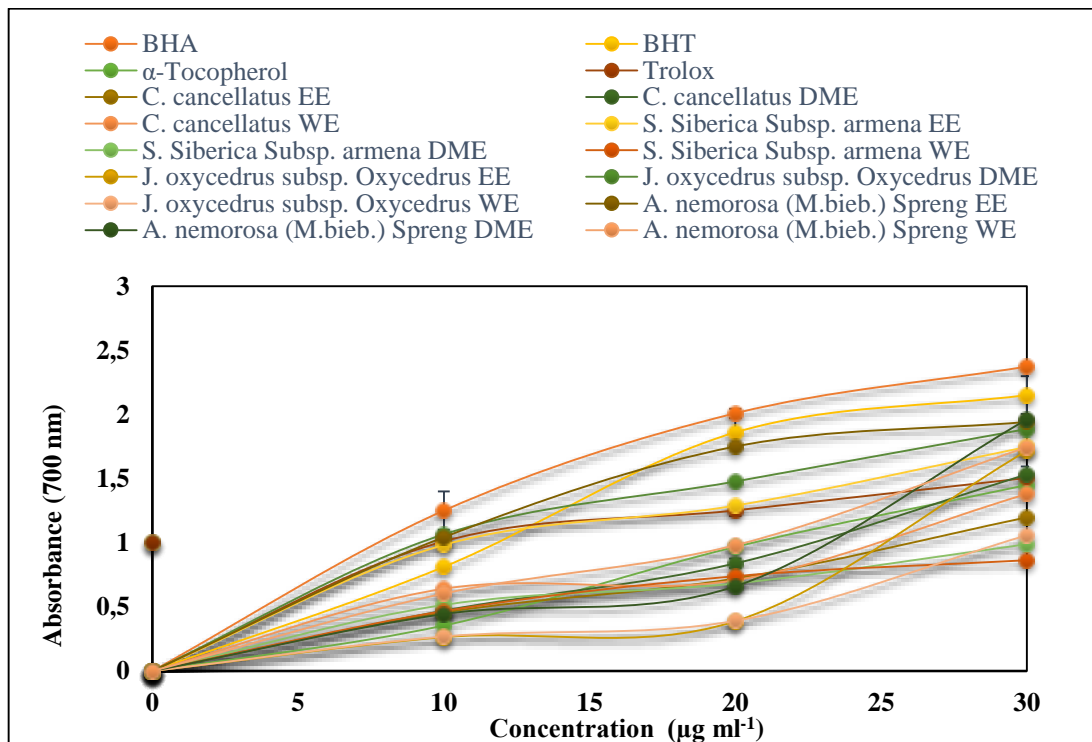
subsp. *oxycedrus* DME (λ_{593} :1.217±0.030, r^2 :0.9623) > *A. nemorosa* (M.bieb.) *spreng* DME (λ_{593} :1.185±0.012, r^2 :0.9884) > *A. nemorosa* (M.bieb.) *spreng* EE (λ_{593} :1.163±0.015, r^2 :0.9912) > *S. siberica* subsp. *armena* DME (λ_{593} :1.040±0.014, r^2 :0.9059) > *J. oxycedrus* subsp. *oxycedrus* EE (λ_{593} :0.956±0.041, r^2 :0.9533) > *S. Siberica* Subsp. *armena* WE

Çizelge 4. *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) *spreng* yapraklarının su, etanol ve diklorometan ekstraktlarının ve standart antioksidanların (30 µg mL⁻¹) Fe³⁺, Cu²⁺ indirgeme ve FRAP aktiviteleri

Table 4. Fe³⁺, Cu²⁺-reducing, and FRAP activities of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) spring and of standard antioxidants (30 µg mL⁻¹)

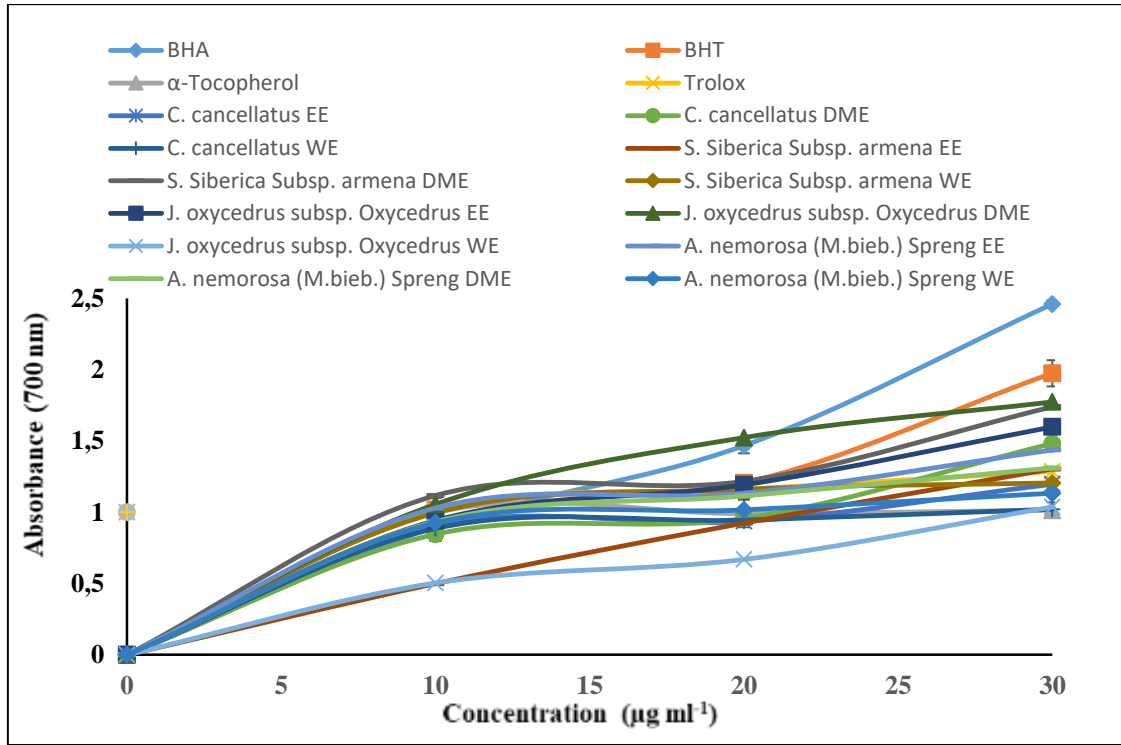
Antioxidants and Samples	Fe ³⁺ reducing		Cu ²⁺ reducing		Fe ³⁺ -TPTZ reducing	
	λ_{700}	r ²	λ_{450}	r ²	λ_{593}	r ²
BHA	2.372±0.020	0.9403	2.459±0.027	0.9904	1.635±0.038	0.9227
BHT	2.151±0.147	0.96	1.975±0.091	0.9422	1.441±0.006	0.9202
Trolox	1.449±0.047	0.9907	1.014±0.054	0.9287	1.380±0.072	0.9784
α -Tocopherol	1.504±0.016	0.9766	0.987±0.007	0.9663	1.443±0.020	0.9603
<i>C. cancellatus</i> EE	1.197±0.017	0.9891	1.195±0.015	0.9245	0.622±0.005	0.9333
<i>C. cancellatus</i> DME	1.532±0.060	0.9829	1.481±0.016	0.9188	0.564±0.022	0.9607
<i>C. cancellatus</i> WE	1.379±0.046	0.927	1.02±0.03	0.9479	0.529±0.018	0.9567
<i>S. Siberica</i> subsp. <i>armena</i> EE	1.747±0.039	0.9371	1.303±0.055	0.9962	1.256±0.011	0.9554
<i>S. Siberica</i> subsp. <i>armena</i> DME	0.988±0.017	0.9556	1.74±0.003	0.9354	1.040±0.014	0.9059
<i>S. Siberica</i> subsp. <i>armena</i> WE	0.863±0.015	0.9329	1.204±0.020	0.9739	0.915±0.005	0.9525
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> EE	1.716±0.028	0.9488	1.598±0.095	0.9221	0.956±0.041	0.9533
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> DME	1.886±0.015	0.9347	1.773±0.066	0.9084	1.217±0.030	0.9623
<i>J. oxycedrus</i> subsp. <i>oxycedrus</i> WE	1.058±0.040	0.9605	1.037±0.003	0.9653	0.854±0.033	0.9693
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> EE	1.94±0.062	0.9211	1.436±0.032	0.9457	1.163±0.015	0.9912
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> DME	1.96±0.060	0.9596	1.31±0.036	0.9706	1.185±0.012	0.9884
<i>A. nemorosa</i> (M.bieb.) <i>spreng</i> WE	1.744±0.040	0.9842	1.133±0.028	0.9543	0.867±0.028	0.95

The results show significant differences (p<0.05) in post-hoc comparisons between different groups.

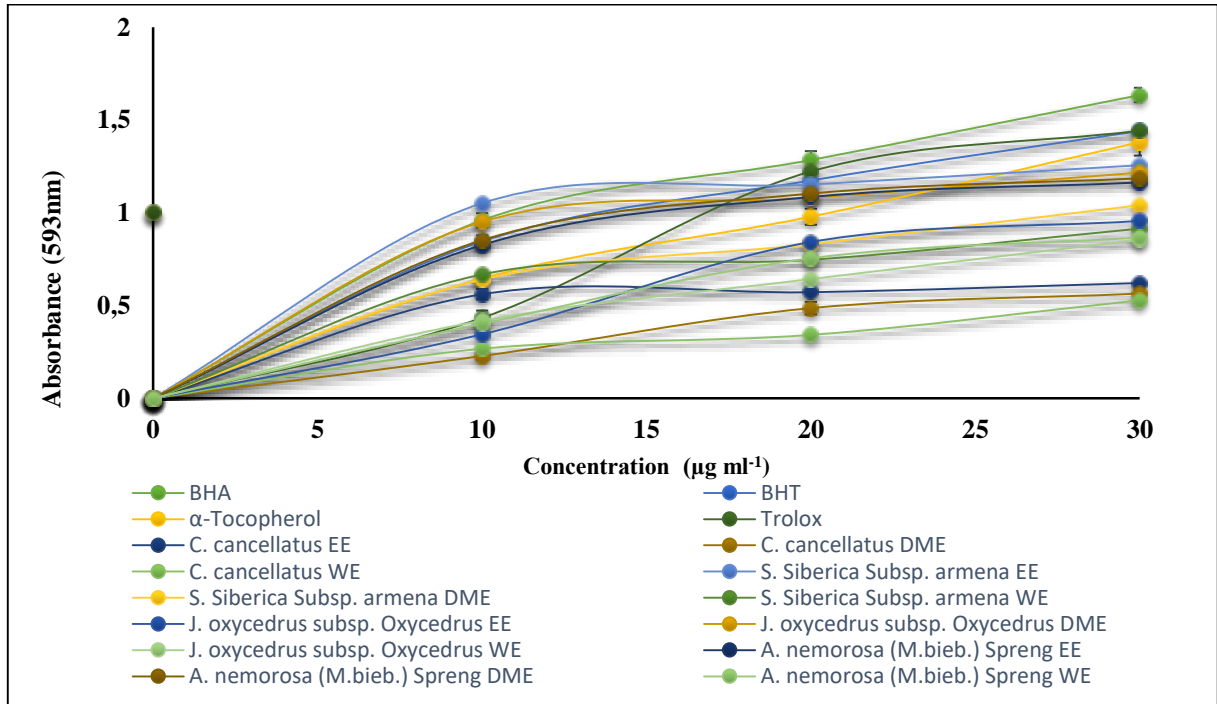


Şekil 3. Ekstraktların antioksidan aktiviteleri (Fe³⁺ indirgeme aktivitesi)

Figure 3. Antioxidant activities of extracts (Fe³⁺ reducing activity)



Şekil 4. Ekstraktların antioksidan aktiviteleri (Cu²⁺ indirgeme aktivitesi)
Figure 4. Antioxidant activities of extracts (Cu²⁺ reducing activity)



Şekil 5. Ekstraktların antioksidan aktiviteleri (Fe³⁺-TPTZ indirgeyici)
Figure 5. Antioxidant activities of extracts (Fe³⁺-TPTZ reducing)

(λ_{593} :0.915±0.005, r^2 :0.9525)> *A. nemorosa* (M.bieb.) spreng WE (λ_{593} :0.867±0.028, r^2 :0.9500)> *J. oxycedrus* subsp. *oxycedrus* WE (λ_{593} :0.854±0.033, r^2 :0.9693)> *C. cancellatus* EE (λ_{593} :0.622±0.005, r^2 :0.9333)> *C. cancellatus* DME (λ_{593} :0.564±0.022, r^2 :0.9607)> *C. cancellatus* WE (λ_{593} :0.529±0.018, r^2 :0.95).

CONCLUSION

This study was designed to reveal the health benefits of plants. In the study, four plants (*Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) spreng) used in different ways in our country and

many regions were used, and water, ethanol, and dichloromethane extracts of these plants were prepared. It was decided that the phenolics and flavonoids in the ethanol and dichloromethane extracts were what made them so good at reducing and scavenging radicals. It was also observed that the prepared extracts exhibited significant biological effects on critical metabolic enzymes, and in general, DME and EE fractions had significant inhibitory effects on enzyme activity. However, further research is needed to identify the phenolic active constituents that are among the main drivers of antioxidant activity and to evaluate their mechanisms of action *in vivo*.

Author's Contributions

Bayram Yurt, investigation, methodology **Rüya Sağlamtaş**, methodology, data curation, formal analysis, writing – original draft. **Yeliz Demir**, investigation, formal analysis, writing - review & editing, supervision. **Cuneyt Caglayan**, investigation, writing - review & editing, supervision. **Halit Diril**, methodology, writing – original draft. **Ebubekir İzol**, investigation, writing - review & editing, supervision. All authors have read and agreed to the published version of the manuscript.

Statement of Conflict of Interest

The authors declare no conflict of interest.

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Investigation of Flower, Leaf, and Stem Parts of Three *Gundelia* Species Growth in the Eastern Anatolia Region of Türkiye from a Biochemical Perspective

Fatih YILMAZ¹, İsmail TÜRKOĞLU², Görkem KIRMIZIKAYA ÖZMEN³, Ebru YÜCE BABACAN⁴
Ökkeş YILMAZ⁵

¹ Fırat University, Faculty of Education, Department of Mathematics and Science Education, 23169 Elazığ, Türkiye, ² Fırat University, Faculty of Education, Department of Mathematics and Science Education, 23169 Elazığ, Türkiye, ³ Fırat University, Faculty of Science, Department of Biology, 23169 Elazığ, Türkiye, ⁴ Munzur University, Munzur University, Pertek Sakine Young Vocational School, Department of Medical Services and Techniques, 62500 Tunceli, Türkiye, ⁵ Fırat University, Faculty of Science, Department of Biology, 23169 Elazığ, Türkiye
¹<https://orcid.org/0000-0002-5804-9240>, ²<https://orcid.org/0000-0001-7454-7605>, ³<https://orcid.org/0000-0001-8516-4933>
⁴<https://orcid.org/0000-0003-3128-3317>, ⁵<https://orcid.org/0000-0002-8276-4498>

✉: gkirmizikaya@firat.edu.tr

ABSTRACT

13 species of *Gundelia* species plants are endemic in Türkiye and are frequently used by the public for both nutrition and medicinal objectives. In this study, three parts (flower, leaf, and stem) of three species of *Gundelia* (*G. dersim*, *G. glabra*, and *G. munzurenensis*) were biochemically investigated. DPPH and ABTS analyses for antioxidant activity were performed on methanol extracts of plant parts. As a result of both analyses in parallel, the flower part of *G. munzurenensis* had the highest %Inhibition value (DPPH:91.85±0.78; ABTS:97.38±0) (P<0.001). In total phenolic (29.09 mg GAE/g) and total flavonoid (5.59 mg CE/g) measurements, the highest amount was found statistically quite significant (P<0.001) in the leaf part of *G. munzurenensis*. Gallic, vanillic, ferulic, rosmarinic, and cinnamic acids, which are phenolic acids and of the flavonoids, rutin, catechin, naringin, and naringenin, were determined in all plant parts. Vitamin D3, alpha-tocopherol, ergosterol, stigmasterol, and beta-sterol were detected in parts of all plant species. While palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids as the main fatty acids were detected in all parts of the three *Gundelia* species, lauric (12:0), tridecanoic (13:0), myristic (14:0), pentadecanoic (15:0), cis-10-pentadecanoic (15:1), heptadecanoic (17:0), cis-10-heptadecanoic (17:1), and eicosanoid (21:0) acids were also identified at different concentrations. Considering the biochemical properties of *Gundelia* species, it shows that they can be consumed as food and also used pharmacologically.

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Vitamins
Phenolic acids

Türkiye'nin Doğu Anadolu Bölgesinde Yetişen Üç *Gundelia* Türünün Çiçek, Yaprak ve Gövde Kısımlarının Biyokimyasal Açından İncelenmesi

ÖZET

Türkiye'de yayılış gösteren *Gundelia* L. cinsine ait 13 takson endemik olup halk tarafından hem beslenme hem de tıbbi amaçlarla sıklıkla kullanılmaktadır. Bu çalışmada *Gundelia*'ya ait üç türe (*G. dersim*, *G. glabra* ve *G. munzurenensis*) ait üç kısım (çiçek, yaprak ve gövde) biyokimyasal olarak incelenmiştir. Antioksidan aktiviteye yönelik DPPH ve ABTS analizleri bitki parçalarının metanol ekstraktlarında yapıldı. Her iki analizin paralel olarak yapılması sonucunda *G. munzurenensis*'in çiçek kısmı en yüksek % İnhibisyon değerine sahip olmuştur (DPPH:91.85±0.78; ABTS:97.38±0) (P<0.001). Toplam fenolik (29.09 mg GAE/g) ve toplam flavonoid (5.59 mg CE/g) ölçümlerinde en yüksek miktar *G. munzurenensis*'in yaprak kısmında istatistiksel olarak oldukça anlamlı (P<0.001) bulunmuştur. Fenolik asitlerden gallik, vanilic, ferulic, rosmarinik ve sinamik asitler ile flavonoidlerden rutin, kateşin, naringin ve naringenin tüm bitki

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Anahtar Kelimeler

Gundelia
Antioksidan aktivite
Yağ asitleri
Vitaminer
Fenolik asitler

kısımlarında tespit edilmiştir. Tüm bitki türlerinin bazı kısımlarında D3 vitamini, alfa-tokoferol, ergosterol, stigmasterol ve beta-sterol tespit edildi. Başlıca yağ asitleri palmitik (16:0), palmitoleik (16:1), stearik (18:0), oleik (18:1), linoleik (18:2) ve linolenik (18:3) yağ asitleridir. laurik (12:0), tridekanoik (13:0), miristik (14:0), pentadekanoik (15:0), cis-10-pentadekanoik (15:1), üç *Gundelia* türünün tüm kısımlarında tespit edildi. heptadekanoik (17:0), cis-10-heptadekanoik (17:1) ve henikosanoik (21:0) asitler de farklı konsantrasyonlarda tanımlandı. *Gundelia* türlerinin biyokimyasal özellikleri dikkate alındığında gıda olarak tüketilebildiği gibi farmakolojik olarak da kullanılabilceğini göstermektedir.

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INTRODUCTION

The genus *Gundelia* belongs to the *Asteraceae* family and is called "kenger" in Anatolia. With systematic studies, it has been found that the genus *Gundelia* is exemplified by 17 taxa in Türkiye and 13 of them are reported to be endemic (Ertas et al., 2021). *Gundelia* species are widely consumed by the public. It is also used for medical objectives for the treatment of many ailments such as liver, diabetes, heart, respiratory, and stomach pain (Ertas et al., 2021). *G. dersim* and *G. munzurensis* species discovered by Vitek et al. (2017) and *G. glabra* Mill species showed similar dispersion in the Tunceli province of Türkiye (Mikail et al., 2021). Comparative biochemical studies of these three species are important because the public frequently uses them for food and medical purposes and because they are similar. Besides, it was stated that species in ecologically different environments are important in point of biochemical studies (Mikail et al., 2021).

According to the literature review, *Gundelia tournefortii* L. was the most studied in detail among the *Gundelia* species (Coruh et al., 2007; Farhang et al., 2016; Hagi et al., 2011; Hajizadeh-Sharafabad et al., 2016; Khanzadeh et al., 2012; Matthäus and Özcan, 2011). At the same time, there were several studies on *G. dersim*, *G. glabra*, and *G. munzurensis* species (de la Luz Cádiz-Gurrea et al., 2020; Ertas et al., 2021; Mikail et al., 2021). However, in the present study, antioxidant properties, phenolic, and flavonoid contents, fatty acids, ADEK vitamins, and sterol profiles in methanol extracts of these three species were investigated separately. Therefore, this study comparatively examined the biochemical properties of the flower, leaf, and stem parts of these three types of plants (*G. dersim*, *G. glabra*, and *G. munzurensis*).

MATERIALS and METHODS

Plant materials and extraction

Among the plant materials, *G. munzurensis* (Figure 2) species were collected from Ovacık district (coordinates 39° 21' 30.0024" N and 39° 12' 57.9960" E) of Tunceli province, *G. dersim* and *G. glabra* (Figure 2) species were collected from Nizamiye district (coordinates 39° 10' 51.9960" N and 39° 49' 43.9968" E) of Tunceli province (Figure 1) in May 2021 (at the flowering period) by fieldwork. Species identification of the plants (Figure 2) was made by Prof. Dr. İsmail TÜRKÖĞLÜ and Prof. Dr. Ebru YÜCE BABACAN. Population homogeneity was considered when plants were collected. Plants are divided into flower, leaf, and stem parts as shown in Figure 2.

The methanolic extract was used to identify the antioxidant properties and phenolic and flavonoid contents homogenized three plants' flower, leaf, and stem parts were homogenized separately with 85% methanol at a rate of 1:5 (w/v). Afterwards, the homogenates were centrifuged at 10000 rpm for 10 min (4°C) and the analysis was carried out by using the methanolic supernatant.

To determine the fatty acids and lipophilic molecule contents of the plants, each plant part was homogenized with HIP (n-hexane/isopropyl alcohol (3/2, v/v)) at a rate of 1:10 (w/v). After centrifugation at 10000 rpm for 10 min, the supernatants were analyzed.

Antioxidant activities

DPPH (Free radical extinguishing activity)

Free radical extinguishing activity (DPPH), was performed per the method specified by Brand-Williams et al. (1995). 25 mg/L α , α -Diphenyl- β -picrylhydrazyl (DPPH), as a free radical was dissolved in methanol. 4

ml of DPPH solution will be added to the glass test tubes, respectively, and then 50, 100, and 200 µL of all plant extracts were added and mixed by vortex, then incubated for 30 min at room temperature and in the dark. At the end of the incubation, mixture absorbances were read at 517 nm opposite a blank in the spectrophotometer. Decreased absorbance, the left

behind amount of DPPH was determined as free radical extinguishing activity and the results were calculated according to the onlooking formula:

$$\% \text{Inhibition} = \left[\frac{\text{Control}_{\text{ABS}} - \text{Sample}_{\text{ABS}}}{\text{Control}_{\text{ABS}}} \right] \times 100$$

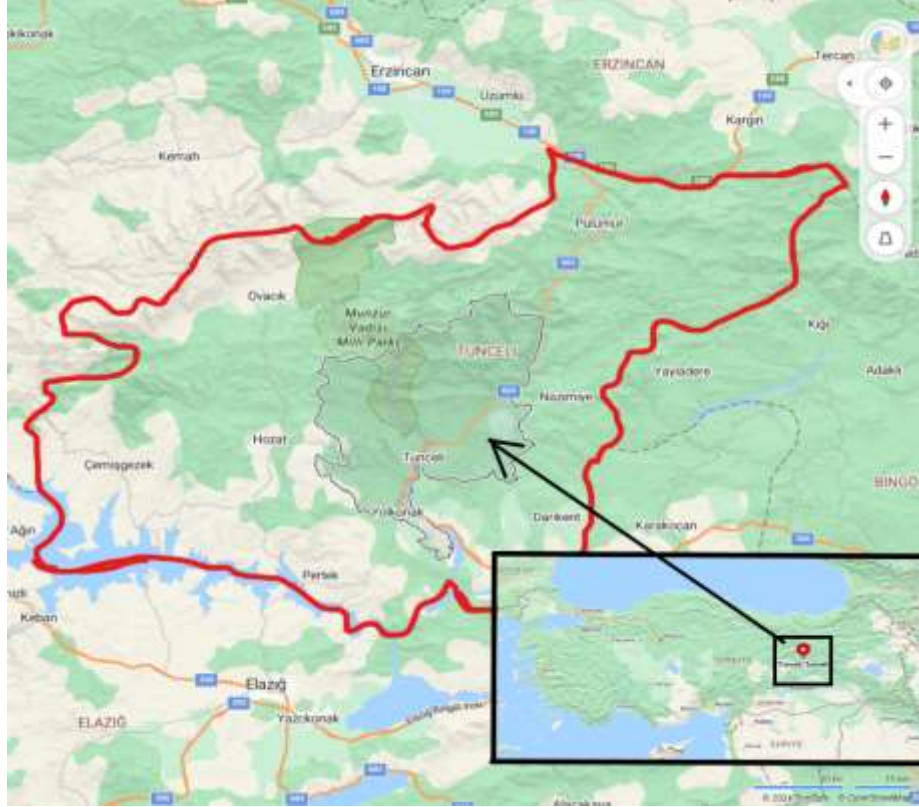


Figure 1. Tunceli province is the region where the *Gundelia* species used in the study are distributed (Bing Map, 2024)
Şekil 1. Tunceli ili, çalışmada kullanılan *Gundelia* türlerinin yayılış gösterdiği bölge (Bing Haritası, 2024)



Figure 2. *Gundelia dersim*, *Gundelia glabra*, and *Gundelia munzurenensis* species and parts
Şekil 2. *Gundelia dersim*, *Gundelia glabra* ve *Gundelia munzurenensis* türleri ve kısımları

ABTS (ABTS• radical elimination activity)

ABTS• radical elimination activity of plant samples Re et al. (1999) and Pellegrini et al. (2003) was performed according to these methods. After adding 2.45 mM potassium persulfate ($K_2S_2O_8$) to the final

concentration of 7 mM 2,2-azinobis (3-ethylbenzothiazolin-6-sulfonic acid) (ABTS), the solution was retained in the dark at room temperature for 12-16 h. ABTS• radical cation solution formed because of the oxidation of potassium persulfate with

ABTS was brought to an absorbance density of 0.70 at a wavelength of 734 nm with ethanol. After this process, 50, 100, and 200 µL of plant extract was added to 2 ml of ABTS• radical cation and retained in the dark for 15 min. The absorbance was then evaluated at a wavelength of 734 nm. The number of ABTS• radicals eliminated by the extracts was calculated using the onlooking formula:

$$\% \text{Inhibition} = [(Control_{ABTS} - Sample_{ABTS}) / Control_{ABTS}] \times 100$$

Phenolic components

The amount of total phenolic compounds

The amount of total phenolic compounds in plant extracts was measured by Singleton et al. (1999) method. Gallic acid was employed as standard. Briefly, 100 µL of plant extract was taken into a glass test tube and 0.5 mL of Folin-Ciocalteu reagent was suffixed to it. After 3 min, 3 mL of 2% Na₂CO₃ solution was suffixed to the samples and retained for 2 h with continuous mixing. At the end of this duration, the numbers of absorbances of the samples at 760 nm wavelength were read in the spectrophotometer, and results were calculated as gallic acid equivalent/g.

Determination of phenolic compounds by HPLC

Chromatographic analyses of phenolic acids were performed using a Prominence I LC-2030C3D plus compact HPLC system with some modifications. Chromatographic separations were realized on an Agilent Zorbax Eclipse XDB-C18 column and 4.6 mm x 150 mm, 3.5-µm particle size. The column was kept constant at 30±1°C during analysis. DAD signals for analytes were defined according to their spectrums acquired from the Lab Solutions LC/GC 5.91 Software. Suitable wavelengths for analysis were chosen between 254 nm and 333. Mobile phase A was 10 mM phosphoric acid and mobile phase B was methanol at an inflow ratio of 1 mL/min. The mobile phases flow program was as follows: 0-15 min (0-60% B), 15-20 min (60-80% B), 20-22 min (80-100% B), 22-27 min (100% B), and 27-32 min (0% B). The amount of samples injected into the system was 20 µL. Chromatographic peaks of the samples were compared with the spectra of standard references and sample quantity was determined (Dragovic-Uzelac et al., 2005; Gundogdu, 2013).

Flavonoid components

The number of total flavonoid compounds

The total amount of flavonoid substance was measured per the method applied by Kim et al. (2003). First, 0.3 mL of 5% sodium nitrite (NaNO₂) was added to 50 µL of plant extracts, and after 5 min 0.3 mL of 10% aluminum chloride (AlCl₃) was added. Thereafter, 2 mL of 1 M sodium hydroxide (NaOH) was added, and 2.4 mL of pure water was added and mixed by vortex.

The absorbance values of the samples at 510 nm wavelength were read in the spectrophotometer and the last results were calculated as catechin equivalent/g.

Determination of flavonoid components by HPLC

For chromatographic analysis of flavonoids, a 5 µm inner diameter PREVAIL C18 (15x4.6 mm) reverse-phase column was used. A mixture of methanol/water/acetonitrile (46/46/8, v/v/v) having 1% acetic acid was employed as the mobile phase (Zu et al., 2006). This mobile phase was filtered through a 0.45 µm diameter membrane filter and then deaerated in the ultrasonication device before use. These flavonoids were measured by DAD following RHPLC separation using a wavelength of 265 nm for camphor, 254 nm for rutin, myricetin, morin, and quercetin, 280 nm for catechin and naringin, and myricetin, morin and quercetin, 306 nm for resveratrol. Adjusted at 1.0 mL/min and the injection value was 10 µL. The chromatographic peaks of the analysis were approved by comparison of reaction times and UV spectra of standard references. Quantification was performed by peak coupling employing the standard method and all chromatographic procedures were performed at 25°C.

Determination of ADEK vitamins and sterols by HPLC

To analyze fatty acids and lipophilic molecules, the supernatants obtained with hexane/isopropyl alcohol were taken into 5 mL test tubes, mixed by adding 5 mL of 10% KOH and retained at 85°C for 15 min. The tubes were brought to room temperature, pure water was suffixed and mixed. Unsaponifiable lipophilic molecules were extracted by adding 5 mL of hexane. Thereafter the hexane phase was evaporated using nitrogen gas. The remaining residue was dissolved in 1.0 mL (60% + 40%, v/v) acetonitrile/methanol solution and taken into autosampler vials. Analysis was accomplished with the Shimadzu brand HPLC device. An acetonitrile/methanol (60%+40%, v/v) solution was employed as the mobile phase for analysis and the mobile phase flow ratio was determined as 1 mL/min. DAD-UV detector was employed for vitamin A, D, E, and K analysis. A Nucleodur LC 18 (15x4.6 cm, 5 µm; MN USA) column was employed as the column, the detection wavelength of 326 nm for vit A and 202 nm for vit E, D, K, and phytosterols were employed (Katsanidis and Addis, 1999; Lopez-Cervantes et al., 2006).

Determination of fatty acid profile by GC

Isolation and methylation of fatty acids: Fatty acids were obtained by adding 10 mL of hexane/isopropanol solution to the plant parts. Then, 5 mL of the hexane phase of the samples was taken into test tubes and 5 mL of 2% methanolic sulfuric acid was added. This mixture was mixed with vortex and left at 55°C for 12 h, and then brought to room temperature, 5 ml of 5%

NaCl solution was added and mixed with vortex. After the fatty acid methyl esters were received with 5 mL of n-hexane and treated with 5 mL of 2% potassium bicarbonate (KHCO₃) solution, the n-hexane phase was evaporated with nitrogen flow. Fatty acid methyl ester residues were dissolved in 1 mL of chloroform and received into autosampler vials.

Gas Chromatographic Analysis of Fatty Acids Methyl Esters: Fatty acids methyl esters were analyzed by SHIMADZU GC 17 gas chromatography and for this analysis, the SPTM-2380 capillary GC arm (LxI.D. 30 mx0.25 mm, df 0.20 µm) (Supelco, Sigma, USA) was employed. During the analysis, the column temperature was retained at 120–220°C, the injection temperature was 240°C, the detector temperature was 280°C, and the column temperature program was calibrated from 120°C to 220°C. Nitrogen gas was employed as bearer gas. First, by injecting mixtures of standard fatty acids methyl esters, the detention times of every fatty acid were determined, and then the programming was made and the analysis of fatty acids methyl esters of plant samples was performed (Christie, 1989).

Statistical Analysis

Statistical analysis of the obtained results was accomplished using the SPSS package program. ANOVA and LSD tests were applied to define the differences between the groups. The statistical significance limit was adopted as P<0.05. Data are given as mean ± SEM.

RESULTS

Antioxidant activities

DPPH (Free radical quenching activity)

DPPH results of flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species were measured at three different concentrations. DPPH results are given in Table 1 as %Inhibition. The 200 µL concentration of the flower part of *G. munzurenensis* (91.85±0.78) had the highest %Inhibition DPPH, followed by the 200 µL concentration of the stem part of *G. munzurenensis* (90.22±0.47). The lowest %Inhibition value was the 50 µL concentration of the stem part of *G. dersim* (4.47±0.28). When the concentration-dependent %Inhibition values of the flower, leaf, and stem parts of the three species were compared, the GdF50, GdF100, GdL50, GdL200, GdS50, GdS100, GgF200, GgS50, GgS100, and GmF200 groups were statistically more significant than all other groups (P<0.001). At the same time, the difference between GgF100&GmF50 and GgS200&GmS50 groups was statistically significant (P<0.05). Also, the difference between GmL100&GmL200 groups was statistically significant (P<0.01). Contrary to these results, the differences

between GdF200&GdS200, GdL100&GgF50, GgL50&GmF100, GgL100&GmL100&GmS200, GgL200&GmL200, GmL50&GmS100 groups were statistically insignificant (P>0.05).

ABTS (ABTS• radical elimination activity)

The percentage Inhibition ABTS values of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species at three different concentrations are shown in Table 1. According to the findings, the highest %Inhibition ABTS value was measured in the groups of 200 µL concentration (97.38±0.00) of the flower part of *G. munzurenensis*, 100 µL (97.27±0.11) and 200 µL concentration (97.09±0.06) of the stem part of *G. munzurenensis*, respectively. The 50 µL concentration of the stem part of *G. dersim* (12.20±0.25) had the lowest %Inhibition value. When all groups were compared, the difference between the GdF50, GdF200, GdS50, GdS200, GgF100, and GgS50 groups and the other groups was statistically significant (P<0.001). In addition, the difference between GdL100&GmF50, GgS200&GmF100&GmL200, GmF200&GgL50, GmF200&GmL100, GmL100&GmS100&GmS200 groups was statistically significant (P<0.01). On the other hand, the difference between GdL100&GgS100, GdL200&GmS50, and GgL50&GmS200 groups was statistically significant (P<0.05).

Phenolic components

The total number of phenolic components

The total phenolic contents of flowers, leaves and stems of *G. dersim*, *G. glabra*, and *G. munzurenensis* species were calculated as mg gallic acid equivalent/g and are shown in Figure 3. According to the findings, the group with the highest total phenolic content was the leaf part of *G. munzurenensis* (29.09 mg/g), followed by the leaf part of *G. glabra* (20.03 mg GAE/g). The least amount of phenolic was measured in the flower part of *G. dersim* (1.89 mg GAE/g) and the stem part of *G. dersim* (2.10 mg GAE/g). The difference between the flower part of *G. dersim* and the stem part of *G. dersim* was statistically insignificant (P>0.05). On the other hand, the differences between all other groups were statistically significant (P<0.001).

Determination of phenolic compounds

Gallic, vanillic, ferulic, rosmarinic, cinnamic, and caffeic acids from phenolic acids were determined in the methanolic extracts of flowers, leaves, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species by HPLC device. The results are given in Table 2 as µg/g. Gallic and ferulic acids were determined in high amounts in the leaf parts of *G. munzurenensis* and *G. glabra*. The highest amount of vanillic acid was measured in the stem part of *G. dersim*, rosmarinic acid in the leaf part of *G. glabra*, and cinnamic acid in

the leaf part of *G. munzurenensis*. Caffeic acid was determined only in the stem part of *G. dersim* (29.50 µg/g). In other words, except for caffeic acid, other

phenolic acids were determined almost in three parts of all plant species.

Table 1 ABTS and DPPH activities of methanolic extracts from *G. dersim*, *G. glabra*, and *G. munzurenensis* species
 Çizelge 1. *G. dersim*, *G. glabra* ve *G. munzurenensis* türlerinden elde edilen metanolik ekstraktların ABTS ve DPPH aktiviteleri

Plant	Plant parts	Abbreviation	Concentration (µL)	ABTS (% Inhibition)	DPPH (% Inhibition)
<i>G. dersim</i>	Flower	GdF	50	15.81±0.41***	6.77±0.40***
			100	28.93±1.04	12.35±0.13***
			200	44.88±0.73***	21.82±0.61
	Leaf	GdL	50	24.95±0.32	17.71±0.20***
			100	49.91±0.63**	33.11±0.28
			200	72.39±1.72*	58.65±0.68***
	Stem	GdS	50	12.20±0.25***	4.47±0.28***
			100	23.90±0.78	9.21±0.15***
			200	39.53±0.63***	21.60±0.20
<i>G. glabra</i>	Flower	GgF	50	28.71±1.45	33.38±0.84
			100	55.88±1.37***	46.61±0.13*
			200	89.73±0.68	77.25±0.67***
	Leaf	GgL	50	95.12±0.66	83.27±0.35
			100	96.14±0.38	89.60±0.08
			200	91.07±1.74	87.61±0.20
	Stem	GgS	50	34.13±1.05***	25.37±0.41***
			100	52.02±0.29	37.18±0.87***
			200	86.16±0.52	61.44±0.85*
<i>G. munzurenensis</i>	Flower	GmF	50	52.57±1.63	47.54±0.74*
			100	83.42±1.27**	83.31±0.55
			200	97.38±0.00	91.85±0.78
	Leaf	GmL	50	96.28±0.61	75.12±0.73
			100	94.72±0.23	89.55±0.55
			200	85.68±1.81	87.96±0.15
	Stem	GmS	50	70.35±1.30*	60.42±0.53*
			100	97.27±0.11	74.59±0.88
			200	97.09±0.06	90.22±0.47

***:P<0.001; **:P<0.01; *:P<0.005

Flavonoid components

1. The number of total flavonoid components

Total flavonoid amounts of flowers, leaves, and stems of *G. dersim*, *G. glabra*, and *G. munzurenensis* species were calculated as mg catechin equivalent/g and are shown in Figure 3. According to the findings, the group with the highest total flavonoid content was the leaf part of *G. munzurenensis* (5.59 mg CE/g), followed by the leaf part of *G. glabra* (4.45 mg CE/g). The least number of flavonoids was measured in the stem part of *G. dersim* (0.19 mg CE/g). The difference between the flower part of *G. dersim* and the stem part of *G. glabra* (0.43 mg CE/g and 0.40 mg CE/g, respectively) was statistically insignificant (P>0.05). In addition, the

differences between the flower part of *G. dersim* and the stem part of *G. dersim*, the stem part of *G. dersim* and the stem part of *G. glabra*, and the flower part of *G. glabra* and the flower part of *G. munzurenensis* were found to be statistically very significant (P<0.01).

2. Determination of flavonoid compounds

The flavonoids rutin, myricetin, catechin, naringin, naringenin, resveratrol, morin, quercetin, and kaempferol were determined by HPLC device in methanolic extracts of flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species. The results are shown in Table 2 as µg/g.

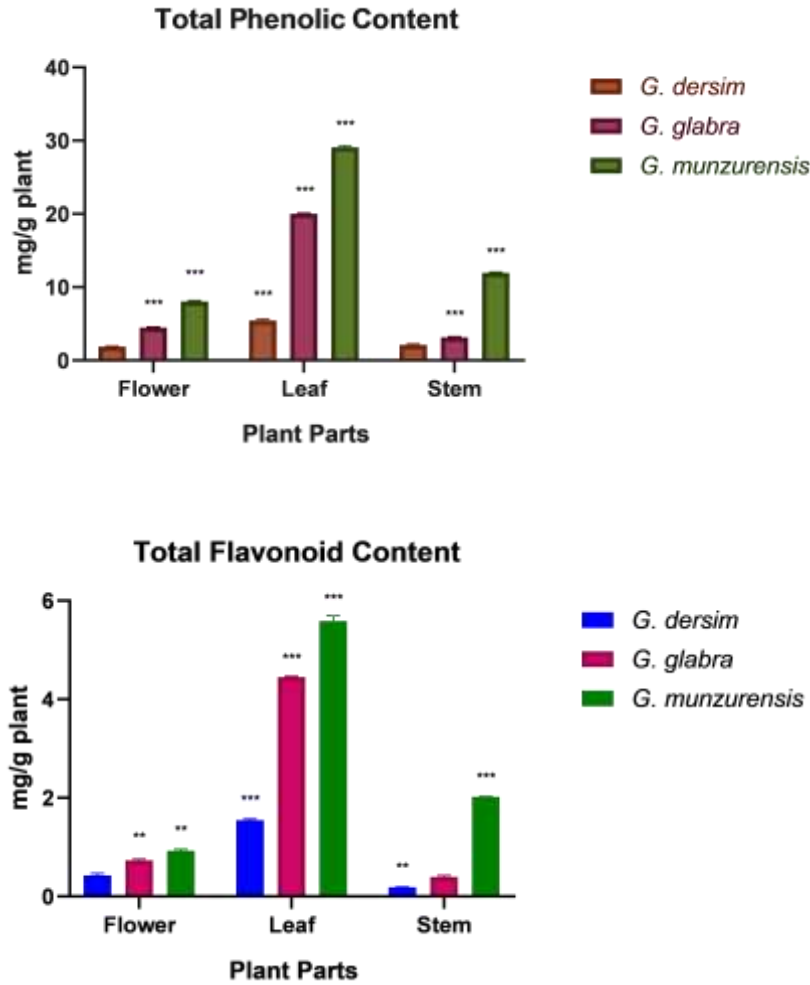


Figure 3. Total phenolic and flavonoid numbers of methanolic extracts obtained from *G. dersim*, *G. glabra*, and *G. munzurenensis* species (***: $P<0.001$; **: $P<0.01$; *: $P<0.005$)

Şekil 3. *G. dersim*, *G. glabra* ve *G. munzurenensis* türlerinden elde edilen metanolik ekstraktların toplam fenolik ve flavonoid sayıları (***: $P<0.001$; **: $P<0.01$; *: $P<0.005$)

Table 2. Phenolic acid and flavonoid contents of *G. dersim*, *G. glabra*, and *G. munzurenensis* species

Çizelge 2. *G. dersim*, *G. glabra* ve *G. munzurenensis* türlerinin fenolik asit ve flavonoid içerikleri

	<i>G. dersim</i>			<i>G. glabra</i>			<i>G. munzurenensis</i>		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Phenolic acids ($\mu\text{g/g}$)									
Galic acid	236.00	362.00	177.50	476.50	1072.50	251.00	789.00	1413.00	361.00
Vanillic acid	70.00	104.00	115.00	42.50	45.00	28.50	72.00	47.50	22.50
Ferulic acid	17.50	21.50	10.50	40.00	2201.00	128.50	28.50	1694.00	93.50
Rosmaniric acid	3.50	10.00	3.00	6.50	23.50	11.00	5.50	9.00	8.50
Hcinnamic acid	4.50	2.50	2.00	3.00	2.00	2.50	1.00	13.50	2.00
Caffeic acid	ND	ND	29.50	ND	ND	ND	ND	ND	ND
Flavonoids ($\mu\text{g/g}$)									
Rutin	6.25	156.50	16.75	139.00	1195.50	163.25	128.75	2619.75	1440.25
Myricetin	13.00	38.00	7.25	148.25	2630.00	ND	50.00	1930.75	319.25
Catechin	374.00	579.00	439.50	646.50	1109.25	397.75	74.00	84.00	409.00
Naringenin	4.25	118.25	8.25	115.25	1084.75	126.75	114.75	2148.50	1187.00
Naringenin	153.50	1.00	0.50	148.50	10.50	1.50	2.00	4.50	1.50
Resveratrol	1.00	6.00	ND	10.75	246.25	21.75	6.25	182.75	29.00
Morin	ND	3.50	0.50	1.00	4.75	ND	ND	7.50	ND
Quercetin	ND	1.00	0.50	5.00	47.50	1.00	1.00	24.75	3.75
Kaempferol	ND	ND	0.50	90.50	858.25	54.25	7.50	110.00	8.50

ND: Not Detected

Rutin, myricetin, and naringin were observed in high amounts in the leaf parts of *G. munzurenensis* and *G. glabra*. In addition, naringin was also high in the leaf part of *G. munzurenensis*. The highest amount of catechin, resveratrol, and kaempferol was determined in the leaf part of *G. glabra*. Naringenin, on the other hand, was quite high in the flower parts of *G. dersim* and *G. glabra*. Myricetin was not observed in the stem of *G. glabra*, resveratrol was not observed in the stem of *G. dersim*, and quercetin was not observed in the lower part of *G. dersim*. Rutin, catechin, naringin, and naringenin have been identified in greater or lesser amounts in three parts of all plant species.

Determination of ADEK vitamins and sterols

Vitamins K and D, tocopherol (stigma and alpha), sterol (ergo, stigma, beta), retinol, and retinol acetate were determined by HPLC device in the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species. The results are given in Table 3

as µg/g. According to the findings, D3, alpha-tocopherol, ergosterol, stigmasterol and beta-sterol were detected in more or fewer amounts in three different parts of three types of plants. While vitamin D3 was most abundant in the leaf part of *G. glabra* (57.00 µg/g), it was also high in the leaf part of *G. glabra* (46.75 µg/g). Ergo-sterol was found in very high amounts in the leaves of *G. munzurenensis* (1141.25 µg/g). Similarly, stigma-sterol was quite high in the leaves of *G. dersim* (3143.75 µg/g) but very low in the leaves of *G. glabra* (13.75 µg/g). Beta-sterol was detected in the highest amount in the stem part of *G. munzurenensis* (608.75 µg/g). Stigmatocopherol was not observed in the flower and stem parts of *G. glabra* yet was high in the leaf part (110.25 µg/g). Alpha-tocopherol was highest in the leaf part of *G. munzurenensis* (167.11 µg/g). Retinol was detected in all plant parts except the stem part of *G. munzurenensis*, and it was more abundant in the leaves of *G. glabra* (15.75 µg/g) than in the others.

Table 3. ADEK vitamin and sterol content of *G. Dersim*, *G. glabra* and *G. munzurenensis* species (µg/g)
Çizelge 3. *G. Dersim*, *G. glabra* ve *G. munzurenensis* türlerinin ADEK vitamini ve sterol içeriği (µg/g)

	<i>G. dersim</i>			<i>G. glabra</i>			<i>G. munzurenensis</i>		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Vitamin K2	6.00	65.50	ND	ND	ND	ND	4.00	ND	2.50
δ-Tocopherol	4.75	13.00	2.25	ND	110.25	ND	2.00	101.50	7.25
Vitamin D2	25.25	21.00	19.00	ND	ND	ND	8.50	19.00	5.50
Vitamin D3	8.50	57.00	4.25	5.50	46.75	10.75	14.25	9.50	30.25
α-Tocopherol	8.25	32.75	1.00	6.25	92.00	1.50	3.50	167.11	35.34
Ergosterol	31.25	186.00	44.75	104.75	475.25	40.50	371.50	1141.25	30.75
Stigmasterol	282.75	3143.75	237.50	315.75	13.75	348.00	40.50	73.25	317.50
β-Sitosterol	95.00	95.50	87.75	164.75	187.25	173.00	174.75	338.50	608.75
Retinol	1.50	1.25	0.50	2.50	15.75	1.00	0.50	0.50	ND
Retinol acetate	8.50	57.50	2.50	19.00	1.75	ND	1.50	0.50	0.50
Vitamin K1	ND	141.25	ND	4.50	301.5	3.25	55.00	28.00	19.25

ND: Not Detected

Determination of fatty acid profile

The fatty acid profiles of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* species defined by the GC device are shown in Table 4. Results are given as per cent concentration. These six essential fatty acids, called palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic fatty acid essential fatty acids, are present in different amounts in all parts of three types of plants. Palmitic acid was mostly detected in the leaves of *G. dersim* (33.753%) and was also in high concentration in the leaves of *G. glabra*, and *G. munzurenensis* (27.078, 30.228%, respectively). Palmitoleic acid, a monounsaturated fatty acid, was

observed mostly in the stem part of *G. dersim* and least in the flower part of *G. munzurenensis*. Stearic acid was determined mostly in the stem part of *G. munzurenensis*. Oleic acid was mostly observed in the stem part of *G. glabra*, and linoleic acid was observed most in the flower part of *G. munzurenensis*. Both fatty acids had the lowest concentration in the leaves of *G. munzurenensis*. In contrast, the leaf part of *G. munzurenensis* had the highest concentration of linolenic acid. In addition, high concentrations of linolenic acid were observed in the leaves of all three plant species. Apart from these fundamental fatty acids, lauric acid, tridecanoic, myristic acid, pentadecanoic, cis-10-pentadecanoic,

heptadecanoic, cis-10-heptadecanoic and eicosanoid acids were observed in various proportions in different

parts of three plant species and the data are given in Table 4.

Table 4. Fatty acid contents of *G. dersim*, *G. glabra* and *G. munzurenensis* species (%)
Çizelge 4. *G. dersim*, *G. glabra* ve *G. munzurenensis* türlerinin yağ asidi içerikleri (%)

Fatty acids	<i>G. dersim</i>			<i>G. glabra</i>			<i>G. munzurenensis</i>		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Lauric acid (12:0)	0.298	ND	ND	0.272	ND	ND	ND	ND	ND
Tridecanoic acid (13:0)	ND	0.452	ND	ND	ND	ND	ND	0.550	ND
Myristic acid (14:0)	0.921	1.026	0.921	3.663	0.847	0.755	ND	1.173	ND
Pentadecanoic acid (15:0)	ND	ND	ND	0.316	ND	ND	ND	ND	ND
Cis-10-pentadecanoic acid (15:1)	ND	1.443	ND	ND	1.590	ND	ND	1.353	ND
Palmitic acid (16:0)	26.172	33.753	22.095	17.605	27.078	17.282	11.039	30.228	17.212
Palmitoleic acid (16:1)	2.442	2.368	3.541	1.492	1.202	1.663	0.385	1.254	0.875
Heptadecanoic acid (17:0)	ND	ND	ND	0.202	ND	ND	ND	0.351	ND
Cis-10-heptadecanoic acid (17:1)	1.366	ND	1.110	0.545	ND	ND	0.247	0.888	ND
Stearic acid (18:0)	4.039	3.675	3.867	5.432	3.613	3.532	3.742	2.301	7.898
Oleic acid (18:1)	24.128	5.429	28.190	21.610	16.292	35.067	16.321	5.018	29.567
Linoleic acid (18:2)	29.053	11.346	31.420	39.170	19.014	33.654	65.514	9.052	41.132
Linolenic acid (18:3)	11.582	39.584	8.856	9.694	30.364	8.047	2.753	47.831	3.316
Henicosanoic acid (21:0)	ND	0.923	ND	ND	ND	ND	ND	ND	ND
ΣSaturated	31.43	39.829	26.883	27.49	31.538	21.569	14.781	34.603	25.11
ΣUnsaturated	68.571	60.17	73.117	72.511	68.462	78.431	85.22	65.396	74.89
Σ MUFA	27.936	9.24	32.841	23.647	19.084	36.73	16.953	8.513	30.442
Σ PUFA	40.635	50.93	40.276	48.864	49.378	41.701	68.267	56.883	44.448

ND: Not Detected; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

DISCUSSION

Extracts of flowers, leaves and stems of *Gundelia* species were obtained using methanol for better extraction of antioxidants and other endogenous compounds (Sultana et al., 2007). Antioxidant activities in methanolic extracts of plants were determined by DPPH and ABTS analysis. ABTS shows cation radical and DPPH a free radical scavenging activity. According to the findings, when *G. dersim*, *G. glabra*, and *G. munzurenensis* species were compared in general, *G. munzurenensis* showed the highest DPPH activity first. *G. glabra* took the second order and *G. dersim* took the third order. %Inhibition ABTS activity results showed parallelism with DPPH activity results. When the flower, leaf, and stem parts of all three species were examined separately, the highest DPPH and ABTS activities were observed in the leaf, stem, and flower parts of *G. munzurenensis*. In the study of Ertas et al. (2021), disseminule ethanol extracts of seventeen *Gundelia* species were obtained and their

DPPH and ABTS activities were examined. According to the results of the study, the highest DPPH and ABTS activity was observed in *G. colemerikensis*. In addition, similar to the results of this study, both DPPH and ABTS activities were found to be from high to low: *G. munzurenensis*, *G. glabra*, and *G. dersim*. In another study examining the DPPH and ABTS activities of *G. dersim* and *G. glabra* extracts obtained by different extraction methods, it was stated that the infusion extract of *G. dersim* showed higher radical scavenging activity compared to the infusion extract of *G. glabra* (de la Luz Cádiz-Gurrea et al., 2020). Antioxidants are agents that inhibit radicals that damage macromolecules. Phenolic acids, polyphenols, and flavonoids found in plants are critical in terms of having antioxidant properties (Losso et al., 2007). There are reports that the phenolic components of plant extracts are related to their antioxidant activities (Aruoma et al., 2003; Škerget et al., 2005). When evaluated from this viewpoint, the total phenolic amounts and antioxidant activity values are consistent

with the findings of this study. In a study by Ertas et al. (2021), they found the total phenolic amount as $Gm > Gg > Gd$ and the total flavonoid amount as $Gd > Gm > Gg$ in ethanol extracts of plants. Although these results and total phenolic amounts in the current study were similar, total flavonoid amounts differed. This may be due to the difference in the solvent used to obtain the extract and the region where the plants are grown.

HPLC method is widely used for the identification of phenolic compounds in plant materials. It has also been stated that the extraction of these phenolic compounds is affected by their chemical structures, extraction method, particle sizes of the samples, storage time, and conditions (Naczka and Shahidi, 2004). Even though Ertas et al. (2021) detected rosmarinic acid and naringenin in *G. dersim*, *G. glabra*, and *G. munzurenensis* species, they could not detect kaempferol. In the present study, kaempferol was found in each part of all other plants except the flower and leaf parts of *G. dersim*. Ferulic acid and myricetin have been reported in *G. glabra*, whereas rosmarinic acid, quercetin, and kaempferol have been reported in *G. glabra* and *G. dersim* species (de la Luz Cádiz-Gurrea et al., 2020). In the findings obtained, ferulic and rosmarinic acids were determined in the highest amount in the leaf part of *G. glabra* species (Table 2). In addition, hydroxycinnamic acid derivatives were detected in *G. tournefortii* L. (Haghi et al., 2011), *G. glabra* and *G. dersim* species (de la Luz Cádiz-Gurrea et al., 2020). *G. tournefortii* has been reported to be affluent in phenolic compounds and flavonoids (Asadi-Samani et al., 2013; Haghi et al., 2011; Nakatani et al., 2000). Besides, the high antioxidant properties of *G. tournefortii* have been associated with the flavonoids gallic acid and quercetin (Coruh et al., 2007). In line with this information, the results of the present study show similarities with the literature.

The hexane extracts of three different parts of *Gundelia* species were analyzed for vitamins by HPLC and for fatty acid by GC device analysis. In a study, the amount of beta-sterol in *Gundelia* species was found to be $Gg > Gd > Gm$ from high to low (Ertas et al., 2021). In the current study, these results were found to be $Gm > Gg > Gd$. It has been reported that the biochemical analysis of plants may be affected depending on the ecological conditions in which the plant grows (stress, infection, photosynthesis rate, etc.) (Mikail et al., 2021). Matthäus and Özcan (2011) stated in their study that *G. tournefortii* L. has predominantly β -sitosterol in the total sterol content, followed by stigma-sterol. Additionally, α -tocopherol was reported as the major tocopherol in the same study. In the current study results, the predominant sterol was stigmasterol.

Ertas et al. (2021) reported that oleic, linoleic, palmitic, and stearic acids are the primary fatty acids in *Gundelia* species. The same researchers stated that the Σ Unsaturated/ Σ Saturated ratio of *Gundelia* species is >1 , and the plants are rich in unsaturated fatty acids. In parallel with these results, Σ the Unsaturated/ Σ Saturated ratios of three different parts of three plants were found to be >1 in the current study (Table 4). In a study investigating the fatty acid composition of *G. tournefortii* L. flower buds, linoleic acid was found the most, while oleic, palmitic, stearic, arachidic, and linolenic acids were identified (Matthäus and Özcan, 2011). In different studies on *G. tournefortii* L., linoleic, linolenic, and oleic acids were specified as the main fatty acids (Asghari et al., 2015; Khanzadeh et al., 2012; Mahmood et al., 2014). In a study investigating the fatty acid content of *G. rosea*, oleic and linoleic fatty acids were found as the main fatty acids (Dalar et al., 2019). Compared to the literature, the present study results show similarities in fatty acid compositions, even though the percentage values are different. It can be considered that the reason for these differences may be due to the various collection times, collection places, and ecological conditions of the plants.

CONCLUSION

Separate biochemical analyses of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurenensis* plants were performed and compared with each other. Considering the results of the study, *G. munzurenensis* showed the highest antioxidant activity and *G. dersim* showed the lowest activity. Total phenolic and total flavonoid amounts attributed to high antioxidant activity also supported these results. *Gundelia* species contained high levels of sterols and tocopherol, vitamin K, vitamin D, and small amounts of retinol. In terms of fatty acid content, the ratio of unsaturated fatty acids in these three plants was also observed to be high.

Considering the natural antioxidant properties of *Gundelia* species and their vitamin and fatty acid compositions, it shows that they can be consumed as food and used pharmacologically due to their health benefits and easy accessibility.

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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.

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Assessing the Efficacy of Moringa, Neem, and Tulsi in Remediation of Sewage Water: A Comparative Study.

Fatima Zehra KHAN¹, Zeenia AVARI²

¹Department of Life Sciences, Jai Hind College, Churchgate, Mumbai, Maharashtra, India 400020. ²Department of Life Sciences, Jai Hind College, Churchgate, Mumbai, Maharashtra, India 400020

¹<https://orcid.org/0009-0003-3070-6709>, ²<https://orcid.org/0009-0000-1429-6728>,

✉: fatimazehra.khan2735@gmail.com

ABSTRACT

The increase in a population's production and consumption habits causes an exponential rise in household waste, resulting in a lack of clean drinking water which leads to the main cause of water pollution. A cost-effective method is to use herbs as they are abundant in secondary metabolites. The purpose of this study was to understand how indigenous herbs can be utilized for treating sewage water, that can be used by citizens to get access to clean drinking water. A low-cost method was created to investigate the efficacy of herbs such as *Moringa oleifera* (Moringa), *Azadirachta indica* (Neem), and *Ocimum sanctum* (Tulsi), in the treatment of sewage water. Four combinations of herbs were selected, and the results were compared with municipal-treated water from a wastewater treatment facility. The samples were tested for parameters such as Estimation of Copper, pH, Chemical Oxygen Demand (COD), and Most Probable Number (Coliform). Coliform levels and copper levels in the herb-treated water were almost one-fourth the level as compared to sewage water. In both parameters, statistical significance was obtained. Statistical significance was considered at $p < 0.034$. The herb-treated samples showed a reduction in the COD and an increase in pH towards neutrality, as compared to the sewage water. In all combinations tested, the herbs were successful in improving the quality of water when compared to the sewage water as well as the municipal treated water. Hence, it can be concluded that herbs are a good natural resource that can be used for the treatment of sewage water, as they are easily available, and the method is sustainable.

Microbiology

Research Article

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INTRODUCTION

For human survival, water is a crucial natural resource (Ugwu et al., 2017) With the increase in population, industrialization, and economic growth freshwater consumption has increased and that has resulted in the mismanagement of natural resources. Rapid urbanization, increased farming activity, pesticide use, soil degradation, high population density, and improper waste management are only a few examples of the factors influencing freshwater sources' quality. Water scarcity stems from poor quality water – a UNESCO perspective (Villaseñor-Basulto et al., 2018). According to the Central Pollution Control Board (2020), and the Ministry of Home Affairs (2011), India produces 60 m³/p/a of wastewater and about 63% of India's wastewater is not treated before it is mingled with freshwater resources respectively. Untreated

water contains higher levels of carbon, nitrogen, and phosphorus, which contaminate water supplies by causing eutrophication and a decrease in dissolved oxygen. In addition to endangered aquatic species, this disturbs the ecosystem's balance. Recovering the nutrients from wastewater not only lowers emissions and protects aquatic life, but also strengthens the nation's economy and sense of independence (Gowd et al., 2023).

Wastewater treatment is the process of eliminating impurities from domestic and industrial sewage. It consists of impurities that are physical, chemical, and biological. Wastewater treatment's primary objectives are to reduce pollutants and provide a disposable effluent without harming the environment (DR et al., 2021). To overcome these issues, naturally available plant-based materials have been used to treat the

sewage water. The process of coagulation is crucial to the treatment of wastewater. Its use includes cleaning turbidity out of wastewater. It's a long-standing tradition where tiny pieces of drumsticks or drumstick powder were added to polluted water, after which the dirt particles would settle, an easy method to clear turbidity. Francis Kweku Amagloh and Amos Benang conducted an experiment that demonstrated the bio-coagulant effect of *Moringa oleifera* seeds (Amagloh & Benang, 2009). Physical-chemical processes like coagulation and flocculation, for example, have been found to reduce pollution and produce clean water for reuse (Delelegn et al., 2018).

The bio-coagulant used in this study, *Moringa oleifera* seeds, is the most crucial component in water purification. They have been used to treat wastewater because it is safe for humans and has no apparent disadvantages (Mehdinejad & Bina, 2018) (Alo et al., 2012). Bio-coagulants have the advantages of being affordable, easy to use, eco-friendly, and environmentally sustainable for developing nations. Compared to chemical coagulants, in which, the alkalinity of wastewater can be greater (Desta & Bote, 2021).

Another natural herb that helps in purifying water is *Ocimum sanctum* (Tulsi). It is one of the holiest and most revered of the numerous medicinal and health-giving herbs, giving it the title of "The Queen of Herbs", "The Legendary", and the "Incomparable One" of India (Maharjan, 2019). The herbal plant *Ocimum sanctum* has antibacterial properties against a variety of microbes in addition to possessing anticancer, anti-diabetic, and anti-ulcer properties (Sivaraja Bannari et al., 2012). It has been demonstrated that the Tulsi plant can prevent the toxic effects of industrial pollutants like copper sulfate, and other heavy metals too (Rana et al., 2022).

Neem is an important low-cost adsorbent that can help in wastewater treatment to a great extent due to its exceptional properties. Heavy metal ion removal from wastewater using neem leaf powder has shown excellent effectiveness (Hassan et al., 2018). Neem is also used as a natural adsorbent in the removal of copper ions from an aqueous solution (Al Moharbi et al., 2020). Parts of the neem plant exhibit an antibacterial function by inhibiting microbial growth and/or the capacity for cell wall breakdown (Alzohairy, 2016). When wastewater is dumped into rivers, fields, and other water bodies without being treated or just partially treated, it poses a major threat to public health and hygiene (Vikas et al., 2014). These properties prove that Neem, along with a combination of other herbs, can be an important component in adapting a simple technique to help purify untreated water.

MATERIAL and METHODS

Sampling Method

Five hundred ml of municipal treated water sample was collected from the Ghatkopar Wastewater treatment facility, managed by the Municipal Corporation of Greater Mumbai (MCGM), Mumbai, India. One liter of sewage water was collected from the same plant from the pre-treated water collection. Both samples were collected in plastic bottles and were stored at 4°C to prevent decomposition and ageing if any.

For the treatment process, both the fresh and dried forms of moringa seeds were used. The fresh *Moringa oleifera* seeds were extracted from drumsticks bought from the local market, and the dried seeds were bought from an agriculture-horticulture-based establishment. Fresh leaves of both Neem and Tulsi were used from the local market and plants grown locally respectively. The rest of the lab equipment, including glassware and chemicals, was provided by the Department of Life Sciences, Jai Hind College, Mumbai. The four combinations of herbs used for the treatment process were:

- a. Moringa + Tulsi
- b. Moringa + Neem
- c. Tulsi + Neem
- d. Moringa + Tulsi + Neem

The sewage water, the municipal treated water, and the four herb treatment combinations were divided into sets to be tested for different parameters such as Estimation of Copper, pH, Chemical Oxygen Demand, and Most Probable Number (Coliform). The entire setup was repeated twice.

Laboratory analyzes

Extraction Process

Method 1

The experiment was performed by two different methods. In the first set, 4g each of fresh Moringa seeds, Neem, and Tulsi leaves were macerated and added to 20 ml of untreated sewage water samples. The samples were incubated at room temperature for 5 days. The conical flasks were stirred every day.

Method 2

In the second set, mature dried Moringa seeds were used. The outer hard coat of Moringa seeds was removed manually, and ground using an electric mixer. 4g each of Neem and Tulsi leaves were ground similarly. The herbs were added to 20ml of the untreated sewage water sample, the flasks were shaken vigorously for 5 minutes and were incubated at room temperature for 2 days.

Estimation of Copper

Copper is an essential inorganic element for living organisms since it activates certain enzymes concerned with the oxidation process, particularly in plants. Copper in excess is, however, harmful to aquatic life. Pollution is through excess of fungicide, insecticide, and discharge of wastes from metallurgical and ceramic industries. The reaction of sodium diethyl thiocarbamate reagent with copper gives copper salt of diethyl dithiocarbamate, which is golden brown. The formation of this compound is one of the most sensitive methods for the estimation of copper and is unaffected by pH between the range of 5.7 and 9.2. Citric acid acts as a scavenger and chelates any other metal ion present. Liquor ammonia helps in maintaining the pH levels. The copper ions in the solution form a straw-coloured complex with carbamate which is extracted in the organic phase using isoamyl acetate or chloroform. Following separation, the samples' optical densities were measured with a colourimeter. (Bureau of Indian Standards, 1974) The amount of copper present was then determined using the following formula:

$$\text{Amount of copper} = \frac{\text{O.D of Sample}}{\text{O.D of Sample}} * \frac{\text{Concentration of standart}}{\text{Volume of sample}} * 1000$$

pH testing

The samples' pH was measured using pH strips, and the result was reported according to the visible change in colour.

Chemical Oxygen Demand (COD)

The Chemical Oxygen Demand (COD) is a measurement of the amount of organic matter in a water sample that is capable of being oxidized by a potent chemical oxidant. As a gauge of the organic and inorganic components present in water bodies as well as in municipal and industrial wastes' susceptibility to oxidation, COD is a frequently used method (Yao et al., 2014). The amounts of organic contaminants in wastewater are often estimated using the COD metric. Hence the parameter was used to estimate the organic contaminants present in the water sample treated.

The estimation of COD was done by titration, where the sample is checked for the amount of iodine liberated, by titrating it against sodium thiosulphate using starch as an indicator. The amount of Iodine liberated is the amount of Oxygen reduced by Potassium iodide (Aini & Juwitaningtyas, 2022). The COD levels were estimated using the following formula:

Blank(A) = Burette reading for blank

Flask (B) = Burette reading for sample water (treated and non-treated water sample) (A-B) = Amount of KMnO_4 required for oxidation

$$\text{COD} = \frac{(A-B) \text{ ml} * N/80 * 8 * 1000 \text{ ml}}{\text{Amount of sample water}}$$

Most Probable Number (Coliform)

Total Most Probable Number (coliform) testing is one of the simplest methods for determining whether a water source is contaminated with bacteria. Total coliform counts provide a broad indication of a water's sanitation condition. The test was performed to understand and study the number of coliforms present per 100 ml of sample. MacConkey's broth of double and single strength was used. After incubating for 24 hours the sample was then analyzed and the results were noted using MacConkey's index (Ukpong & Udechukwu, 2015).

Statistical Analysis

All statistical analysis was performed in Microsoft Office Excel 2019 (version 2403). Data was analyzed using One-way ANOVA for the Estimation of Copper and Most Probable Number (Coliform). Post-hoc tests (Tukey's HSD) were performed using Real Statistics Resource Pack (XRealStats). The level of significance was set at 0.034. Statistical significance was considered at $p < 0.034$.

RESULTS

Extraction Process:

Extraction obtained by first methodology

The outcomes of the 5-day incubation process for all of the water samples altered in colour, and fungal development was noticed, but no explicit changes in the water, like coagulation or a reduction in turbidity were seen. As a result, the water was discarded without additional testing or inspection.

Extraction obtained from the second methodology

The results obtained using the mature Moringa seeds and the ground leaves were significantly different from those of the first approach. The sample showed obvious flocculation and coagulation. Hence, the samples were used for additional analysis of the estimation of heavy metals like copper, pH, Most Probable Number (Coliform), and COD.

Estimation of Copper

Results of testing the water for heavy metals i.e. copper, found in the sewage water, municipal treated sewage water, and the different combinations of herb-treated sewage water have been plotted as a bar graph (Fig. 1), to help with better comprehension of the various treatments and how they differed from one another. Error Bars exhibiting the Standard deviation are displayed. According to the data, there is a statistical difference in the reduction in the cooper

levels when we compare the sewage water to the municipal treated sewage water and the different herb combinations used to treat the water. Statistical differences were observed (denoted as *). No

significant difference was seen among the other groups. Statistical significance was considered at $p < 0.034$.

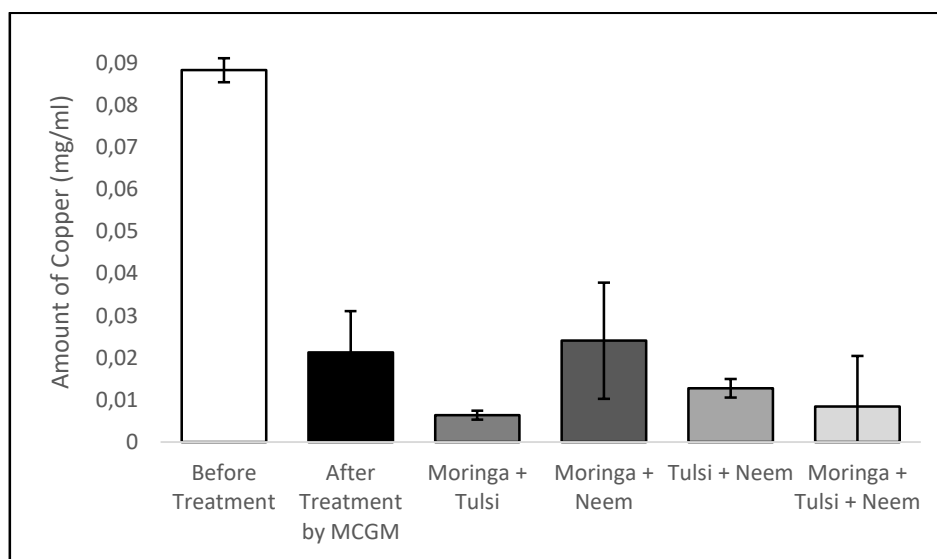


Figure 1. Bar graph displaying the amount of copper levels in sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. The amount of copper in all combinations of herb-treated sewage water, and the municipal-treated sewage water showed a significant decrease when compared to the sewage water (denoted as □). No significant difference was seen among other groups when compared to each other.

pH Testing:

In many chemical, medicinal, and environmental monitoring activities, the determination of pH values is crucial (Kumar Dewangan et al., 2023). pH is a measurement of the proportion of free hydrogen and hydroxyl ions in water. Water with more free hydroxyl ions is basic, and water with more free hydrogen ions is acidic. Since chemicals in the water can modify pH, it is a crucial sign of a chemical change in the water. Hence testing of pH is necessary to understand the

potential of Hydrogen ions in a sample.

The pH of the sample after the municipal treatment, and herb treatment was found to be approximately in the range of 6.0 to 8.0, indicating that it was neither too acidic nor too basic, and the results were plotted as a bar graph (Fig. 2). Additionally, when compared to the sewage water, the pH is on the lower side, indicating it is more acidic. Error Bars exhibiting the Standard deviation are displayed.

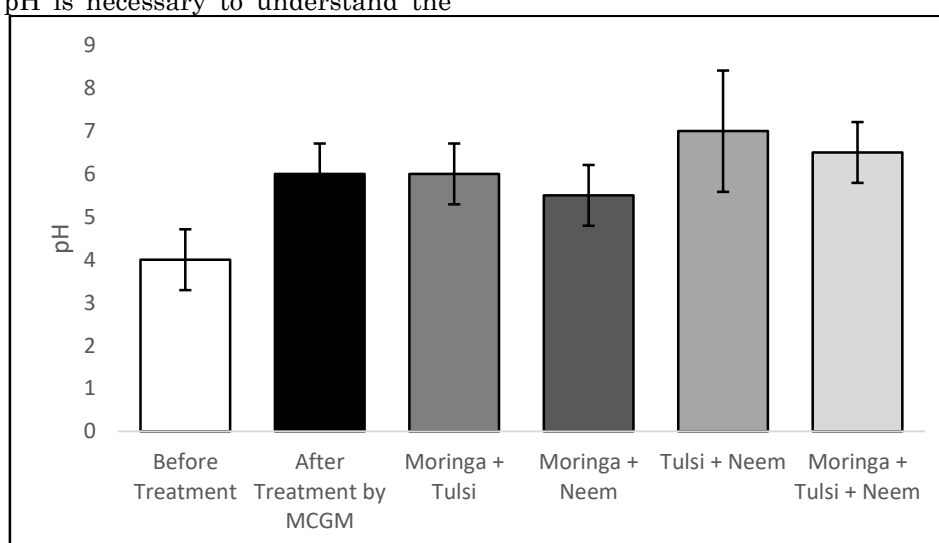


Figure 2. Bar graph displaying the pH range in sewage water, municipal treated water and the combinations of herbs used to treat sewage water. No significant difference was seen among other groups when compared to each other.

Most Probable Number (Coliform):

Compared to the sewage water, the Coliform levels in the municipal treated water decreased considerably. However, the sewage water treated with herbs had an even lower coliform level. The results when plotted in a bar graph (Fig. 3) give us a better understanding and

comparison of the result. Error Bars exhibiting the Standard deviation are displayed. It shows that the herb-treated water is statistically significant when compared to coliform levels in the sewage water (denoted as □). No significant difference was seen among the other groups. Statistical significance was considered at $p < 0.034$.

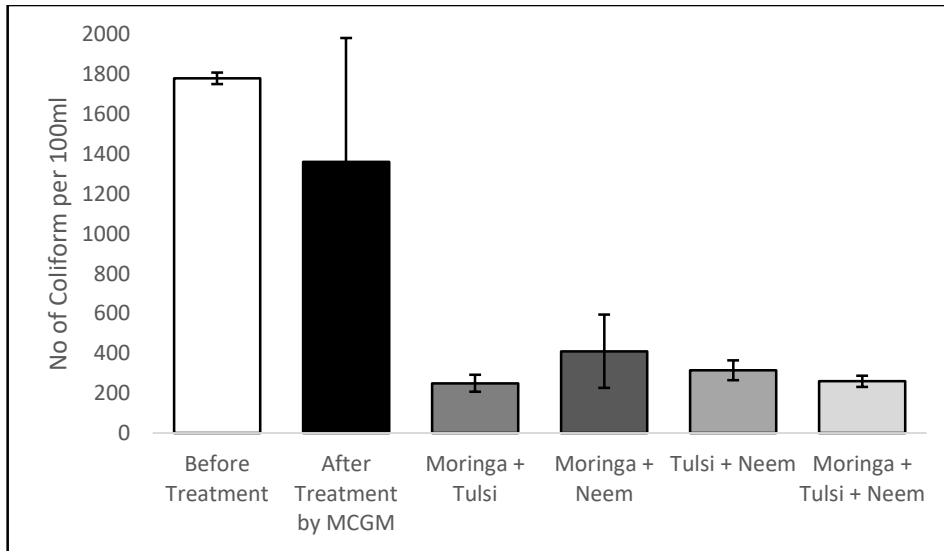


Figure 3. Bar graph displaying the MPN (Coliform) levels of sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. The coliform levels in herb-treated sewage water showed a significant decrease when compared to the sewage water (denoted as □). No significant difference was seen among other groups when compared to each other.

Chemical Oxygen Demand:

The results obtained after titration represent the Chemical Oxygen Demand (COD) levels in mg/ml. The results obtained showed that the herb-treated water has considerably less COD compared to the municipal-

treated water. The results have been represented in a bar graph (Fig. 4). Error Bars exhibiting the Standard error are displayed. The graph shows there is a comparative difference between the combinations of herb-treated water with municipal plant-treated water and sewage water.

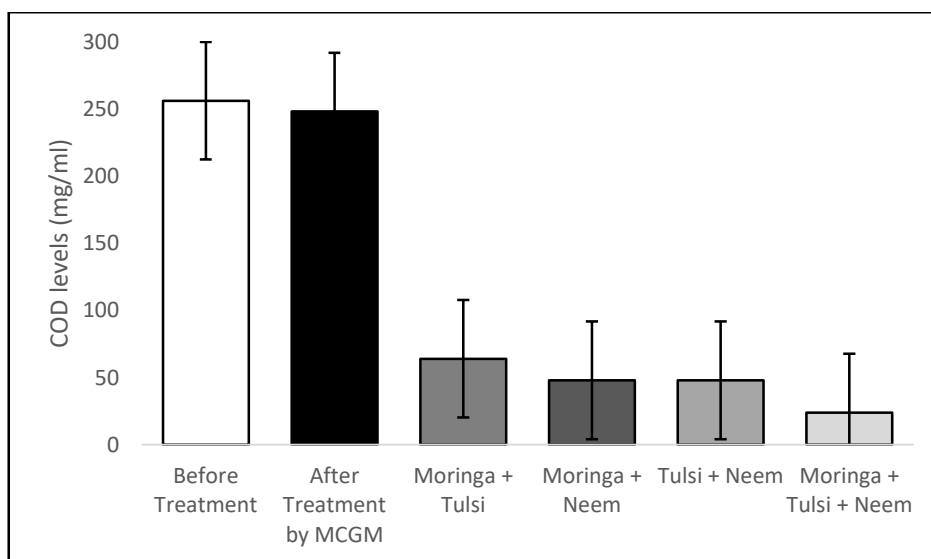


Figure 4. Bar graph displaying the COD levels difference in sewage water, municipal treated water, and the combinations of herbs used to treat sewage water. No significant difference was seen among other groups when compared to each other.

DISCUSSION

The aim of this study was to understand the effectiveness of natural herbs in treating sewage wastewater and compare the same with water treated at a municipal plant. This study found that *Moringa oleifera* seeds, a natural coagulant, and *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) are effective in purifying water. This was proved by examining the efficacy of these herbs on the Estimation of Copper, pH, Chemical Oxygen Demand levels, and Most Probable Number (Coliform) levels of the water, comparing them with water treated at a municipal treatment plant as well as the raw sewage water.

Copper is an essential inorganic element for living organisms, however, copper in excess is harmful to aquatic life as well as human life. Exposure to high levels of copper can lead to diarrhoea, hormonal imbalance, fatigue, nausea, and kidney problems. The Chemical Oxygen Demand measures the amount of oxygen required to oxidize organic and inorganic components in water. High levels of COD lead to reduced amounts of dissolved oxygen (required by aquatic life) which may lead to anaerobic conditions, causing the death of aquatic organisms. Coliform bacteria are commonly found in the digestive tract and are excreted in the faeces. They are often found in sewage and wastewater. A high coliform count in water indicates water contamination. The pH of water samples is an important regulatory test to confirm the portability of the water sample. Water with a pH of less than 6 is likely to be contaminated with pollutants making it unsafe to drink.

The results of this study showed that the herb-treated water had higher efficacy compared to municipal-treated water, with significantly decreased coliform and copper levels, reduced COD levels, and brought the pH levels close to neutrality. The study suggests that these natural herbs could be a cost-effective and efficient solution for treating sewage wastewater.

The costs of a treatment plant setup are high, and the amount of sewage generated in developing nations daily is also high. Herbs can be utilized to purify sewage water since they are more cost-effective and abundant in countries such as India, contributing to developing a cleaner environment. Although the experiment only utilized a small amount of water, the amount of wastewater that is disposed of every day is enormous, making it difficult to translate these results on an industrial scale. Hence, this method can be adopted for better efficiency when used in smaller units like groups of houses, colonies, municipal wards, etc. Further research needs to be done to find out whether this water can be recycled and used for agricultural and industrial purposes on a large scale.

CONCLUSION and RECOMMENDATIONS

With the increase in population, industrialization, and economic growth freshwater consumption has increased and that has resulted in mismanagement of the natural resources. According to the researchers, an exponential rise in household waste has been caused by improvements in the population's production and consumption habits. The main cause of water pollution is sewage, which is handled improperly and pollutes freshwater sources, specifically in metropolitan cities. Nearly 62% of wastewater in urban India remains untreated or partially treated, which further gets disposed of in natural water bodies. The usual treatment methods include the activated sludge process, oxidation ponds, aerated lagoons, and trickling filters. These methods need space, heavy equipment, and time, which drives up the cost of the treatment. The expense of the treatment process can be decreased by using natural coagulants. The indigenous medical system uses plants, which are abundant in secondary metabolites, to treat a variety of illnesses. This study investigated the properties of *Moringa oleifera*, *Ocimum sanctum* (Tulsi), and *Azadirachta indica* (Neem) in the treatment of wastewater. The results obtained from these herbs-treated water are compared with water treated at the treatment plants. It has been concluded that herbs are a good natural resource that can be used for the treatment of sewage water, which is cost-effective and easily available and a sustainable method of treating water.

This study conducted to test the effects of herbs on sewage water and municipal treated water shows promising results, however, it is a pilot study. To be introduced to the public on a larger scale in housing colonies and small-scale industries, different concentrations of the herb samples can be used to increase effectiveness. Replication of data is imperative and increasing the number of sets will help with advanced statistical analysis thereby increasing the validity of the positive results.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest Statement

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

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Network Pharmacology and Molecular Docking Perspectives into Lignans for Alzheimer's Disease Treatment

Seda ŞİRİN¹, Serap NİĞDELİOĞLU DOLANBAY²

^{1,2}Gazi University, Faculty of Science, Department of Biology, 06500, Teknikokullar, Ankara, Türkiye

¹<https://orcid.org/0000-0003-2636-725X>, ²<https://orcid.org/0000-0002-1238-0894>

✉: sdasirin@hotmail.com

ABSTRACT

Alzheimer's Disease (AD) is a debilitating neurodegenerative condition with limited treatment options. Lignans, a class of naturally occurring polyphenols found in various plants, have been shown to have the potential to modulate pathways associated with AD pathology. In this study, we used network pharmacology and molecular docking to investigate the therapeutic potential of lignans against AD by targeting specific proteins involved in disease progression. Our established interaction network includes key proteins such as EGFR, HSP90AA1, BCL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1. Molecular docking studies have revealed how lignans interact with these proteins and highlighted their potential to influence AD through mechanisms such as inflammation modulation, apoptosis regulation, and signal transduction pathways. The results suggest that lignans have significant binding abilities to these targets, potentially inhibiting their activity and thus alleviating AD symptoms by reducing amyloid-beta accumulation and tau phosphorylation. These findings support the viability of lignans as a basis for the development of new AD therapies and call for further *in vivo* studies to confirm their efficacy and safety. This integrated approach underscores the value of combining network pharmacology and molecular docking in the search for new therapeutic agents against complex diseases such as AD.

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Alzheimer Hastalığı Tedavisinde Lignanlara Yönelik Ağ Farmakolojisi ve Moleküler Yerleştirme Perspektifleri

ÖZET

Alzheimer hastalığı (AH), sınırlı tedavi seçeneklerine sahip, zayıflatıcı nörodejeneratif bir durumdur. Çeşitli bitkilerde bulunan doğal olarak oluşan bir polifenol sınıfı olan lignanların, AH patolojisiyle ilişkili yolları modüle etme potansiyeline sahip olduğu gösterilmiştir. Bu çalışmada, hastalığın ilerlemesinde rol oynayan spesifik proteinleri hedefleyerek lignanların AH'ye karşı terapötik potansiyelini araştırmak için ağ farmakolojisi ve moleküler yerleştirme kullanılmıştır. Kurulan etkileşim ağıımız EGFR, HSP90AA1, BCL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2 ve PIK3R1 gibi önemli proteinleri içermektedir. Moleküler yerleştirme çalışmaları, lignanların bu proteinlerle nasıl etkileşime girdiğini ortaya çıkarmış ve inflamasyon modülasyonu, apoptoz düzenlemesi ve sinyal iletim yolları gibi mekanizmalar yoluyla AH'yi etkileme potansiyellerini vurgulamıştır. Sonuçlar, lignanların bu hedeflere önemli bağlanma yeteneklerine sahip olduğunu, potansiyel olarak aktivitelerini inhibe ettiğini ve dolayısıyla amiloid-beta birikimini ve tau fosforilasyonunu azaltarak AH semptomlarını hafiflettiğini göstermektedir. Bu bulgular, yeni AH tedavilerinin geliştirilmesi için bir temel olarak lignanların yaşayabilirliğini desteklemekte ve bunların etkinliğini ve güvenliğini doğrulamak için daha fazla *in vivo* çalışma yapılması çağrısında bulunmaktadır. Bu entegre yaklaşım,

Moleküler Biyoloji

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 27.05.2024

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Anahtar Kelimeler

Alzheimer hastalığı

Lignan

Moleküler yerleştirme

Ağ farmakolojisi

AH gibi karmaşık hastalıklara karşı yeni terapötik ajanların araştırılmasında ağ farmakolojisini ve moleküler yerleştirmeyi birleştirmenin değerini vurgulamaktadır.

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INTRODUCTION

Alzheimer's disease (AD) is distinguished by several neuropathological changes, primarily extracellular amyloid aggregates (plaques), intraneuronal inclusions of phosphorylated tau (tangles), and neuronal and synaptic degeneration, which are accompanied by tissue reactions to astrogliosis and microglial activation that precede neuronal network disruptions in the symptomatic phase of the AD (Gobom et al., 2024). AD now affects 50 million people, with forecasts increasing to 152 million by 2050 (Dissanayaka et al., 2024; Oliveira Silva et al., 2024).

Currently, drugs licensed for AD therapy mostly provide symptomatic relief, and their effects are frequently poor. The FDA has authorized early-stage Alzheimer's drugs, such as cholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists, which only give short-term symptom relief and do not prevent disease progression (Arjmandi-Rad et al., 2024). In recent years, as the research area has expanded, amyloid-related treatment has emerged as a key trend in future clinical trials of novel medications. Aducanumab and lecanemab, amyloid-antibodies that can prevent or reverse AD, have received FDA approval. Nevertheless, this novel therapy against amyloid deposition is flawed by therapy management methods, expensive drug monitoring, and the need for professional tools and imaging studies (Park et al., 2024). Therefore, there is an urgent need to investigate AD pathogenesis and develop novel therapeutic agents to prevent AD's occurrence or delay its course. Hence, exploring the pathophysiological basis of AD and developing novel therapeutics to eradicate or at least slow AD progression is of utmost importance (Qin et al., 2024).

The chemical structure of plants contains secondary metabolites or bioactive compounds including phenols, terpenoids, alkaloids, anthocyanins, chlorogenic acids, flavonoids, tannins, glycosidic replacements, and lignans (Cedillo-Cortezano et al., 2024). Lignans are well-known for their antioxidant, anticarcinogenic, antimutagenic, and anti-estrogenic effects that benefit human health. They are synthesized through the shikimic acid pathway and composed of dimerized phenylpropanoid units. Their structure is characterized by an aromatic moiety carrying different oxidation levels and substitution patterns. The two

carbon atoms (8 and 8'), located at the center of the side chain of the phenylpropanoid unit with a C6C3 configuration, are dimerized to form the structure of lignans (Nawfetriyas et al., 2024).

Combining mathematics, bioinformatics, and many other fields, network pharmacology assists us in understanding the vast integrative and systematic properties of natural AD drugs obtained as a result of processing relevant plants. Research on molecular processes and the establishment of a drug ingredient target network are key processes of network pharmacology in helping study the AD therapy carried out with natural compounds based on plants through the lens of a systemic and wholesome approach. (Zhi et al., 2024).

Subsequently, lignans' anti-AD characteristics in AD were determined through a network pharmacology technique, which also provided a foundation for future experimental investigations and therapeutic applications. The combination of integrated network pharmacology and bioinformatics research revealed that the anti-AD pharmacological activities of lignans might be mainly attributed to blocking signaling pathways, hence slowing down the course of AD. These findings imply that lignans may be able to target the proposed therapeutic targets for the treatment of AD.

MATERIAL and METHOD

Determination of possible targets of lignans and AD

PubChem provided the canonical SMILES of the 8 lignans (enterodiol, enterolactone, etoposide, lariciresinol, matairesinol, pinoresinol, podophyllotoxin, and secoisolariciresinol) employed in this study. Using the canonical smiles of 8 lignans, their possible targets were obtained from SwissTargetPrediction (Table 1). Possible targets associated with AD were obtained from DisGeNET. Venny was used to link lignans with possible targets associated with AD (Trivedi et al., 2024; Xiaoying et al., 2023).

SwissADME

SwissADME was used to determine the physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry associated with lignans (Daina et al., 2014, 2017; Daina & Michelin, 2016).

Table 1. Canonical SMILES list of the 8 lignans

Çizelge 1. 8 lignanın kanonik SMILES listesi

Lignan name	Canonical SMILES	PubChem compound ID
Enterodiol	<chem>C1=CC(=CC(=C1)O)CC(CO)C(CC2=CC(=CC=C2)O)CO</chem>	115089
Enterolactone	<chem>C1C(C(C(=O)O1)CC2=CC(=CC=C2)O)CC3=CC(=CC=C3)O</chem>	10685477
Etoposide	<chem>CC1OCC2C(O1)C(C(C(O2)OC3C4COC(=O)C4C(C5=CC6=C(C=C35)O)CO6)C7=CC(=C(C=C7)OC)O)OC)O</chem>	36462
Lariciresinol	<chem>COC1=C(C=CC(=C1)CC2COC(C2CO)C3=CC(=C(C=C3)O)OC)O</chem>	332427
Matairesinol	<chem>COC1=C(C=CC(=C1)CC2COC(=O)C2CC3=CC(=C(C=C3)O)OC)O</chem>	119205
Pinoresinol	<chem>COC1=C(C=CC(=C1)C2C3COC(C3CO2)C4=CC(=C(C=C4)O)OC)O</chem>	73399
Podophyllotoxin	<chem>COC1=CC(=CC(=C1OC)OC)C2C3C(COC3=O)C(C4=CC5=C(C=C24)O)CO5)O</chem>	10607
Secoisolariciresinol	<chem>COC1=C(C=CC(=C1)CC(CO)C(CC2=CC(=C(C=C2)O)OC)CO)O</chem>	65373

Protein-Protein Interactions (PPI) Network Analysis

PPI networks are an important tool for understanding the complex interactions of biological processes and cellular functions. In our study, the STRING database was used to determine the interactions of relevant proteins. The STRING database is a large source of biological data integrating known and predicted PPI. The data were analyzed to determine the network structures of the identified proteins and the key nodes (hub proteins) in these networks. Then, the PPI network was visualized using Cytoscape software, and topological features were evaluated. This analysis allows us to better understand the biological functions of proteins and their roles in interaction networks (Szkłarczyk et al., 2023, 2019, 2016, 2015, 2010; Franceschini et al., 2016, 2012; Jensen et al., 2009; von Mering et al., 2003, 2005, 2007; Snel et al., 2000).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

GO enrichment analysis is used to determine whether a particular set of genes is significantly enriched in biological processes, molecular functions, and cellular components. In our study, the ShinyGo tool was used to determine the functional annotations of differentially expressed genes and their roles in biological processes. This tool evaluates the associations of gene sets with GO terms and identifies statistically significant enriched GO terms. The results obtained help us understand the biological functions of genes and their participation in processes (Bindea et al., 2009; Huang et al., 2009).

KEGG pathway enrichment analysis is used to determine the relationship of a given gene set to known biological pathways. In this study, the KEGG database was used to determine which biological pathways differentially expressed genes are associated with. Analysis was performed using the ShinyGo tool. These tools map gene sets to KEGG pathways and identify statistically significant enriched pathways. The resulting data enable us to understand which metabolic or signal transduction pathways genes are

involved in and how these pathways change in disease (Ge et al., 2020; Huang et al., 2009; Xie et al., 2011).

Molecular Docking CB-Dock2

CB-Dock2 is an improved version of the CB-Dock2 server for protein-ligand blind docking, integrating cavity detection, docking, and homologous template docking. Given the three-dimensional (3D) structure of a ligand and a target protein, it predicts their binding sites and affinities (Liu et al., 2022a; Xiaoying et al., 2023; Yang et al., 2022).

The 3D structure of the target protein was obtained from the Protein Data Bank (PDB) (Table 2). The specific PDB ID for the target protein was identified and downloaded. The protein structure was cleaned by removing any water molecules, ligands, or other heteroatoms that could interfere with the docking process. This was done using molecular visualization software such as PyMOL. Hydrogen atoms were added to the protein structure to ensure proper geometry and charge distribution. This step is crucial for accurate docking predictions.

Table 2. PDB code list of the proteins

Çizelge 2. Proteinlerin PDB kod listesi

Protein name	PDB codes
BCL2	1G5M
EGFR	5WB7
ERBB2	3PP0
ESR1	1XP1
HSP90AA1	81GI
HSP90AB1	1UYM
IL6	1ALU
JUN	1JUN
PIK3CA	7R9V
PIK3R1	5XGI

The 3D structures of the lignans were either obtained from chemical databases like PubChem or ChemSpider or drawn using molecular editing software such as ChemDraw. The structures of the lignans were optimized using quantum chemistry

methods or force field-based energy minimization to achieve a stable conformation. This was done using software such as Gaussian. The optimized lignan structures were converted to the SDF format, which is required for docking studies using CB-Dock2. This conversion was performed using AutoDockTools.

The prepared protein and ligand structures were uploaded to the CB-Dock2 server. CB-Dock2 automatically detected potential binding cavities on the protein surface. This is a crucial step for blind docking, as it identifies the regions where the ligand is most likely to bind. The docking process was initiated, where the ligand was docked into the identified cavities. CB-Dock2 used a combination of docking algorithms and scoring functions to predict the binding affinities and orientations of the ligand within the cavities.

The docking results were scored and ranked based on the predicted binding affinities. The top-ranked poses were selected for further analysis. The binding poses of the ligands were visualized using molecular visualization software to assess the interactions between the ligand and the protein. Key interactions, such as hydrogen bonds, hydrophobic interactions, and pi-pi stacking, were identified and analyzed.

RESULT and DISCUSSION

According to the oral bioavailability radar, the colored zone is the ideal physicochemical space for oral bioavailability when the following characteristics are taken into account: lipophilicity (XLOGP3 between -0.7 and +5.0), size (MW between 150 and 500 g/mol), polarity (TPSA between 20 and 130 Å²), solubility (log S not higher than 6), saturation (the carbon fraction in sp³ hybridization should not be less than 0.25), and flexibility (no more than 9 rotatable bonds) (Ibrahim et al., 2020; Mishra and Dahima, 2019). The lignans (enterodiol, enterolactone, etoposide, lariciresinol, matairesinol, pinoresinol, podophyllotoxin, and secoisolariciresinol) oral bioavailability radar is displayed in Figure 1. All other lignans are within the oral bioavailability radar range, with the exception of etoposide. Abd El-Razek et al. (2024) reported that colchicine and epimagnolin are found in the advised range. Compounds 1-4 were determined to have acceptable values when generated from dibenzylbutyrolactone lignans from *Hydrocotyle bonariensis* parameters related to the oral bioavailability radar, as reported by Souza et al. (2021). Compound 5, on the other hand, violated the saturation criterion and thus was not recommended. Depending on their structural features, lignans may have different acceptance ranges on the oral bioavailability radar.

The BOILED-Egg model was used for simultaneous prediction of blood-brain barrier (BBB) penetration and human gastrointestinal absorption (HIA) of

lignans and provides insight into their permeation characteristics (Majahan et al., 2024). The BOILED-Egg graphical interface also visually provides information on polarity (TPSA) and lipophilicity (WLOGP). This graph visually displays PGP (p-glycoprotein) responses and thus more clearly delineates the bioavailability of molecules. Membrane-bound PGP, a transporter that leads to substrate (PGP⁺) efflux, reduces intracellular concentrations and lowers molecular bioavailability (Nag et al., 2022). Figure 2 shows lignans' BOILED-Egg plot. Enterolactone (molecule 2) and pinoresinol (molecule 6) are the two molecules with positive BBB penetration and HIA properties as well as positive PGP effects. Enterodiol (molecule 1), lariciresinol (molecule 4), matairesinol (molecule 5), pinoresinol (molecule 6), podophyllotoxin (molecule 7), and secoisolariciresinol (molecule 8) are the molecules with a negative BBB penetration property and a positive HIA property when they are under the effect of the PGP. Etoposide (molecule 3) exerts a positive PGP effect on the molecule and displays a negative HIA property and a negative BBB penetration property. Chopade et al. (2021) reported that phyllanthin and hypophyllanthin originated from *Phyllanthus amarus* and passed through the BBB *in silico* BOILED egg models.

Majahan et al. (2024) reported favorable penetration HIA and BBB penetration properties for enterolactone, favoring the potential of the drug candidate in both bioavailability radar and BOILED Egg model. The lignans' structural characteristics may be influential in their HIA and BBB penetration in the BOILED Egg model.

Using canonical smiles of 8 lignans (enterodiol, enterolactone, etoposide, lariciresinol, matairesinol, pinoresinol, podophyllotoxin, and secoisolariciresinol), 201 possible targets were obtained from SwissTargetPrediction. 3173 possible AD-related targets were obtained from DisGeNET. Venny was used to link lignans to possible targets associated with AD. 224 possible common targets were identified (Figure 3 and Table 3).

Network pharmacology goes beyond the traditional single-drug-single-target paradigm by providing a holistic view of the interactions of ligands, targets, and diseases, enabling the development of multitarget therapies. Integrating systems biology, this approach enables the analysis of biological networks and pathways, thereby helping to elucidate the mechanisms of action of biologically active compounds and their effects on disease pathways (Hopkins, 2007; Li et al., 2011).

The PPI network was created by connecting 224 possible common targets with STRING. The number of nodes was determined as 228, the number of edges was 1165, the average node degree was 10.2 and the average local clustering coefficient was 0.462 (Fi. 4).

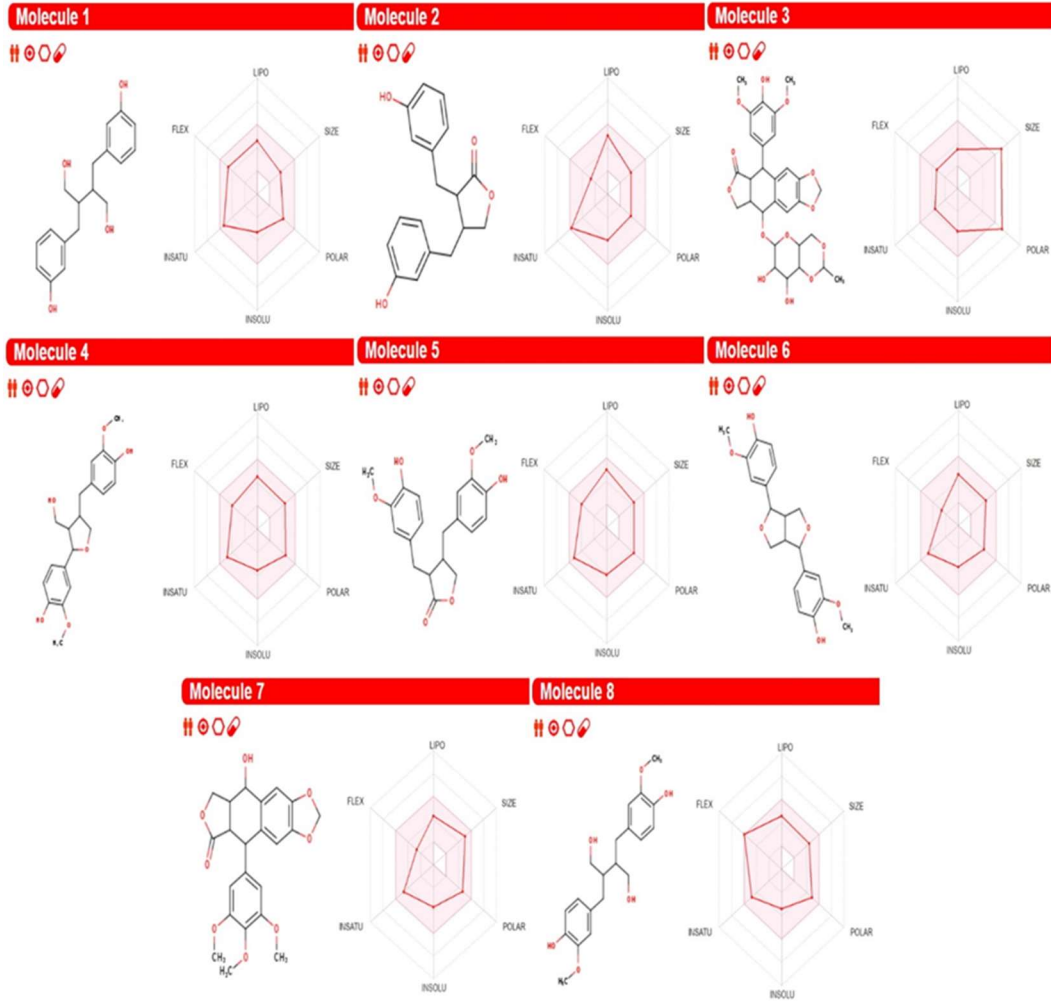


Figure 1. Oral bioavailability radar of lignans (enterodiol (molecule 1), enterolactone (molecule 2), etoposide (molecule 3), lariciresinol (molecule 4), matairesinol (molecule 5), pinoresinol (molecule 6), podophyllotoxin (molecule 7), and secoisolariciresinol (molecule 8))

Şekil 1. Lignanların (enterodiol (molekül 1), enterolakton (molekül 2), etoposid (molekül 3), larisiresinol (molekül 4), matairesinol (molekül 5), pinoresinol (molekül 6), podofilotoksin (molekül 7) ve sekoizolarisiresinol (molekül 8) oral biyoyararlanım radarı

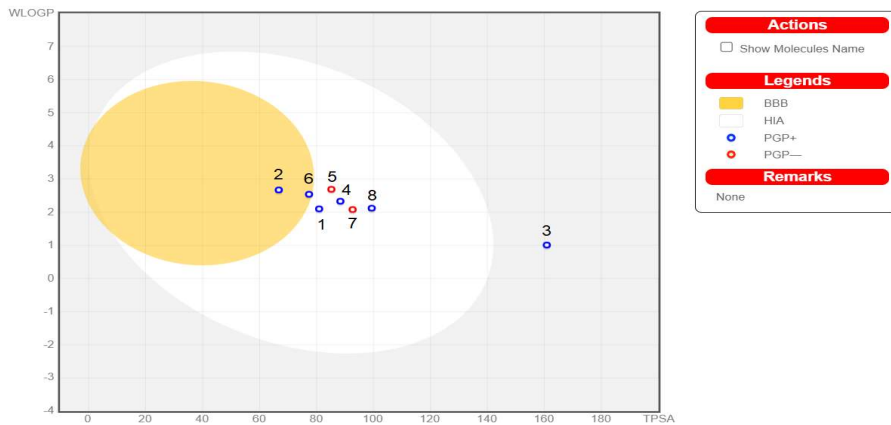


Figure 2. BOILED-Egg plot of lignans (enterodiol (molecule 1), enterolactone (molecule 2), etoposide (molecule 3), lariciresinol (molecule 4), matairesinol (molecule 5), pinoresinol (molecule 6), podophyllotoxin (molecule 7), and secoisolariciresinol (molecule 8))

Şekil 2. Lignanların (enterodiol (molekül 1), enterolakton (molekül 2), etoposid (molekül 3), larisiresinol (molekül 4), matairesinol (molekül 5), pinoresinol (molekül 6), podofilotoksin (molekül 7) ve sekoizolarisiresinol (molekül 8) haşlanmış yumurta grafiği

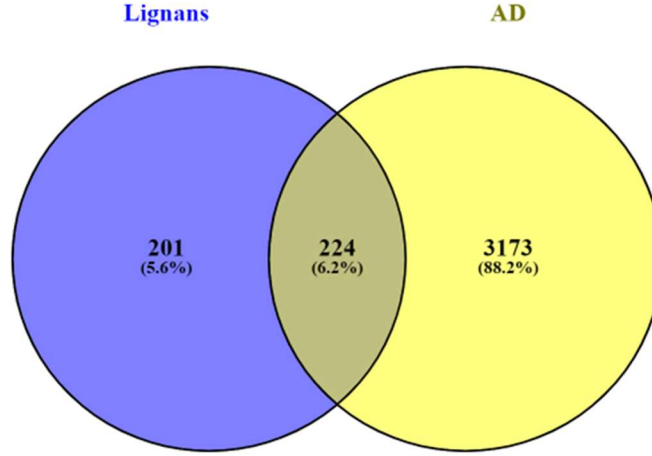


Figure 3. Venn diagram of lignans and possible targets of AD
Şekil 3. Lignanların ve AH'nin olası hedeflerinin Venn diyagramı

Table 3. 224 common targets in "Lignans" and "AD"
Çizelge 3. "Lignanlar" ve "AH'nin 224 ortak hedefi

KDR	MMP9	NOX4	MTOR	CDC25B	ALOX5AP
ESR2	MMP1	JUN	PIK3CA	ACE	QPCT
POLB	TYK2	FLT4	HCAR2	PIK3CB	GSK3A
ESR1	MAPK1	PITRM1	GBA	HCK	ABCC9
ALOX5	MMP8	ABL1	PIK3CD	PIK3CG	MTNR1A
NR3C1	PTGS2	MAP2	PNP	MAPK3	EDNRA
AR	ROCK2	MTNR1B	BCHE	EIF2AK3	GRM5
STS	TYR	DRD1	P2RX7	AKT2	PDE2A
TTR	SLC22A2	BCL2	EGFR	CDK4	PDE10A
ESRRA	NR1I3	ALB	LCK	SERPINE1	TAOK2
GPER1	SYK	FTO	PTGER3	CLK1	FLT1
SHBG	MAP3K7	SGK1	CASP3	DNM1	PDGFRB
WEE1	CAPN1	RAF1	GAPDH	HTR7	INSR
ADCY10	BACE1	MAPK8	MMP14	PNMT	IKKBK
ALOX15	HTR2C	PLK1	OGA	PRKACA	MAP2K3
ALOX12	RET	NOS1	SIRT2	MET	PRKAA2
CYP19A1	CHRM1	FGFR1	MME	PDK1	CAMK4
IGF1R	CHRM3	NOS2	TACR2	ABCG2	CHEK2
HSD17B1	ROCK1	ERBB2	NUDT1	PPARG	DAPK1
SLC6A4	RELA	ANPEP	MCL1	YES1	CAMKK2
CDK5	MAPK14	DYRK1A	MAPK9	EPHB2	INSRR
ADRA2A	HSP90AA1	PLA2G7	HIF1A	LYN	HMGCR
HTR1A	HSP90AB1	CYP2C9	CFTR	EPHA4	ACVRL1
CHEK1	CHRM2	CYP2C19	DPP4	BTK	XIAP
PGR	PARP1	SLC5A2	LNPEP	TYRO3	
IL6	BRD4	CYP3A4	ERN1	EPHA1	
ADAM17	NTRK1	ADORA1	FFAR1	OPRK1	
GLUL	MMP3	MMP13	HDAC6	BMP1	
GPBAR1	CREBBP	MMP2	HDAC2	PCNA	
SLC6A2	ADA	SLC29A1	PRSS3	KMO	
SLC6A3	OPRM1	IRAK4	CSF1R	TDP1	
JAK2	MAP2K1	ST6GAL1	F9	ABCB1	
LRRK2	NR1H3	PDE5A	TLR4	NR1H2	
GSK3B	PPARD	AGTR1	CXCR2	HDAC4	
CNR1	F2	HK1	CXCR1	MAP3K12	
CNR2	TGFBR1	P2RX3	PAK3	PIK3C3	
HDAC1	NQO2	CA2	PAK1	TDP2	
CDK1	ADORA2A	HSPA5	DHFR	MAPKAPK2	
MAP2K2	MMP7	F3	SLC2A1	NR3C2	
MAPKAPK5	HSD11B1	HSPA8	SOAT1	CTSD	

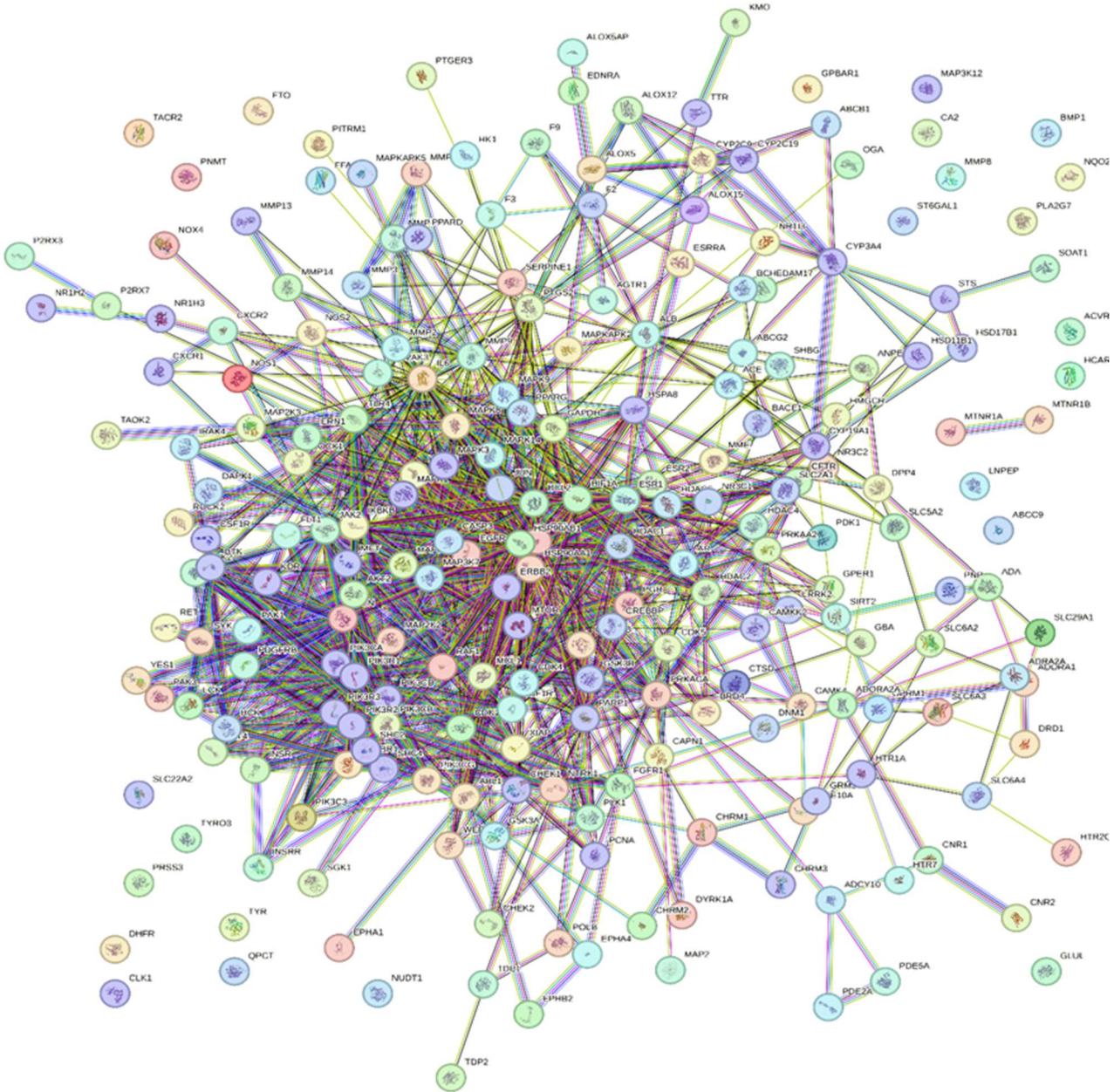


Figure 4. The PPI network

The different node colors show the different levels of interactions whereas the edge colors show their known, predicted, and other interactions. Color code for edges interpretation: neighborhood (green), gene fusion (red), cooccurrence (blue), coexpression (dark), experiments (pink), databases (sky blue), text mining (kelly), and homology (purple).

Şekil 4. PPI ağı

Farklı düğüm renkleri farklı etkileşim seviyelerini gösterirken kenar renkleri bilinen, tahmin edilen ve diğer etkileşimleri göstermektedir. Kenarların yorumlanması için renk kodu: komşuluk (yeşil), gen füzyonu (kırmızı), birlikte oluşum (mavi), birlikte ifade (koyu), deneyler (pembe), veritabanları (gök mavisi), metin madenciliği (kelly) ve homoloji (mor).

These data show that the network is highly dense and tightly connected, indicating that interactions between proteins are strong and reflect potentially important biological functions. The average node degree of 10.2 indicates that each protein interacts with approximately 10 other proteins on average, increasing the complexity and biological significance of the network. The average local clustering coefficient is 0.462, indicating that there is a high level of

interaction in subsets of the network and these interactions may play critical roles in biological processes. Such dense interaction networks mean that certain proteins play central roles and that these proteins may be potential therapeutic targets. The top 10 targets (EGFR, HSP90AA1, BCL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1), ranked according to their node degrees, have been determined (Table 4). PPI networks of the first 10

targets ranked according to their node degrees are drawn. It was determined that there were 43 interactions between 10 targets (Figure 5). These interactions help us understand the effects of these proteins on biological processes and disease mechanisms. Growth factor receptors such as EGFR and ERBB2 promote neuronal development and survival, whereas chaperone proteins like HSP90AA1 and HSP90AB1 can minimize the toxicity of amyloid beta and tau proteins by controlling protein folding (Ahsan et al., 2012; Dent et al., 2021). BCL2, an apoptosis regulator, and signal transduction modulators JUN, PIK3CA, and PIK3R1 play critical roles in nerve cell survival and death, providing neuroprotection (Behl et al., 1993; Jimenez et al., 2011). On the other hand, molecules like IL6 and ESR1 may contribute to AD through inflammatory responses and hormonal interactions (Boada et al., 2012; Liu et al., 2022b; Miron et al., 2018). While each of these genes contributes to the complicated etiology of AD in various ways, a thorough knowledge of these relationships may pave the way for the creation of new disease management and treatment options.

Table 4. Node and node degree of targets

Çizelge 4. Hedeflerin düğüm ve düğüm derecesi

#Node	Node_degree
EGFR	58
HSP90AA1	58
BCL2	45
HSP90AB1	44
IL6	41
JUN	41
ESR1	39
PIK3CA	39
ERBB2	38
PIK3R1	38

Although it is well known that the protein known as the epidermal development factor receptor (EGFR) is involved in cell development, differentiation, and survival, recent studies have indicated that EGFR may also have a role in AD. By stimulating tyrosine kinase signalling pathways that support brain cell growth and survival, EGFR may contribute to cellular dysfunction and neuronal death in AD (Jayaswamy et al., 2023). Moreover, it is postulated that EGFR signalling triggers neuroinflammatory processes by stimulating brain-resident immune cells called microglia and astrocytes (Qu et al., 2025). The production of amyloid beta peptides and aberrant tau protein phosphorylation, which are the main pathogenic features of AD, may also be impacted by these activities (Rajmoran and Reddy, 2017). Neuronal loss can result from either excessive or insufficient EGFR activation (Tavassoly et al., 2020).

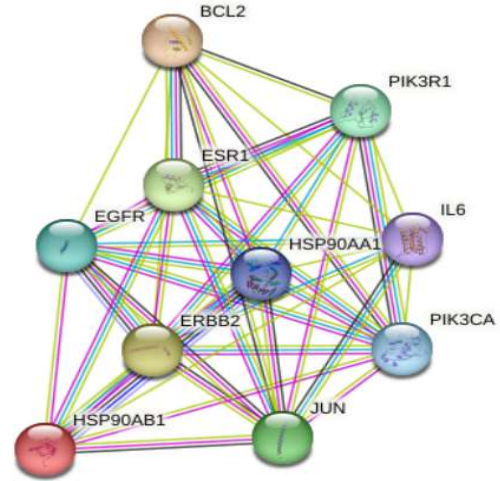


Figure 5. PPI network of top 10 targets

The different node colors show the different levels of interactions whereas the edge colors show their known, predicted, and other interactions. Color code for edges interpretation: neighborhood (green), gene fusion (red), cooccurrence (blue), coexpression (dark), experiments (pink), databases (sky blue), text mining (kelly), and homology (purple).

Şekil 5. İlk 10 hedefin PPI ağı

Farklı düğüm renkleri farklı etkileşim seviyelerini gösterirken kenar renkleri bilinen, tahmin edilen ve diğer etkileşimleri göstermektedir. Kenarların yorumlanması için renk kodu: komşuluk (yeşil), gen füzyonu (kırmızı), birlikte oluşum (mavi), birlikte ifade (koyu), deneyler (pembe), veritabanları (gök mavisi), metin madenciliği (kelly) ve homoloji (mor).

Intracellular heat shock protein 90 (HSP90) is involved in the folding, defense, and operation of proteins (Hoter et al., 2018). Heat shock protein 90 alpha family class A member 1 (HSP90AA1) and heat shock protein 90 alpha family class B member 1 (HSP90AB1) are its two isoforms. According to reports, HSP90AA1 and HSP90AB1 may have a significant impact on the accumulation of proteins and neuroprotective processes, which may influence the aetiology of AD (Gonzalez-Rodriguez et al., 2021). Heat shock protein 90 is significant because it inhibits the improper folding of tau and amyloid beta and their accumulation in the central nervous system, which lessens the harmful intracellular consequences of these proteins in neurodegenerative diseases (Bohush et al., 2019). By reducing the development of amyloid plaque and hyperphosphorylating tau, HSP90AA1 inhibition may be able to prevent neural damage and cell death. Additionally, this chaperone protein may benefit AD by promoting stress resistance of neuronal cells (Astillero-Lopez et al., 2024).

B-cell lymphoma 2 (BCL2), a protein that provides protection against cellular death and increases cell longevity, mainly functions by inhibition of apoptosis (Alam et al., 2021). It exerts cellular protective effects against neurodegeneration and thus has a central role

in AD (Shacka & Roth, 2005). AD is essentially characterized by apoptotic neuronal cell death through oxidative stress and mitochondrial malfunction (Eckert et al., 2003). Signals for cellular death can be effectively inhibited by BCL2 through the preservation of an intact mitochondrial membrane and the prevention of cytochrome c release (Scorrano & Korsmeyer, 2003). Hence, BCL2 can halt neuron loss in AD (Zhu et al., 2004).

Interleukin 6 (IL6) is a cytokine with pro-inflammatory properties and takes part in the regulation of immune response. It has been suggested that IL6 plays a role in the neuroinflammatory aspect of AD (Lyra e Silva, 2021). Accumulation of amyloid beta peptides and neurofibrillary tangles are responsible for the activation of immune cells, namely astrocytes, and microglia, in AD (Webers et al., 2020). According to Weisman et al. (2006), these deposits cause an increase in IL6 release, which fortifies the immunological response. According to Rubio-Perez et al. (2012), increased IL6 can cause neuronal injury and dysfunction, which would hasten the onset and course of the disease. On the other hand, IL6 may also have neuroprotective properties, such as promoting the survival and repair of neurons (Kummer et al., 2021).

The AP-1 transcription factor complex, which regulates cell growth and differentiation, is primarily composed of Jun proteins (Liebermann et al., 1998). The pathophysiological connection between the Jun proteins and AD is believed to be represented by neuroinflammation and neuronal responses to stress (Salminen et al., 2009). In reacting to oxidative stress or various undesirable stimuli, neurons produce proteins called Jun proteins, which control the expression of genes that aid cells in adapting and surviving (Maise and Chong, 2004). According to Yarza et al. (2016), there is evidence that Jun proteins contribute to AD-related neuronal damage and cell death. According to Wu et al. (2020), the neuroinflammatory response is defined by Jun activation, which leads to an increase in the generation of inflammatory cytokines as well as other mediators. This, in turn, damages neurons and speeds up the onset of disease.

Estrogen receptor 1 (ESR1), an important protein that is responsible for estrogen's cellular effects (Sundermann et al., 2010), has a pivotal role in AD, due to its and estrogen's neuroprotective properties (Lan et al., 2015). Estrogen favorably improves brain function and cognitive health by upregulating the expression of proteins that support neuronal development and survival (Russell et al., 2019). Decreased estrogen levels during the postmenopausal stage may be associated with AD in women (Pike, 2017). Facilitating ESR1-mediated estrogen signaling may improve neuronal endurance and synaptic plasticity in addition to avoiding amyloid-beta damage (Sato et al., 2023).

Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) and phosphoinositide-3-kinase catalytic subunit alpha (PIK3CA) are the two primary genes involved in the phosphatidylinositol 3-kinase (PI3K) pathway (Zhou et al. 2012). Essential biological processes like growth, proliferation, survival, and metabolism are carried out by this pathway (Martini et al. 2014). The PI3K/Akt signalling pathway is essential for brain cell survival and functioning during Alzheimer's disease (Razani et al., 2021). According to Munkley et al. (2015), PIK3R1 serves as the regulatory subunit and PIK3CA as the catalytic subunit. For the signalling pathway to be activated and controlled, they must work in concert. AD-related neuronal loss and damage may be caused by an impaired PI3K/Akt pathway (Hoxhaj and Mannig, 2020). A compromised PI3K/Akt pathway may have a negative impact on energy expenditure, synaptic plasticity, and cellular stress responses (Parihar and Brewer, 2010). These factors are closely linked to nerve cell loss and dysfunction in the aetiology of AD (Kumar and Bansal, 2022). Additionally, the route might trigger neuroprotective chemicals to shield cells from the damaging impacts of tau and amyloid-beta proteins (Fakhri et al., 2021).

The epidermal growth factor receptor ERBB2 receptor tyrosine kinase 2 (ERBB2), commonly referred to as HER2, controls cell survival, proliferation, and differentiation (Eccles, 2011). There is debate regarding its involvement in AD, and studies are being conducted to clarify its function in neurodegenerative illnesses (Ou et al., 2021). The effects of ERBB2 on the cell life cycle and neuronal signalling may be the cause of AD (Wang et al., 2017). According to Ledonne et al. (2018), ERBB2's action on synaptic plasticity and neuronal transmission is directly associated with both memory and learning impairments that are hallmarks of AD. Furthermore, it has been proposed that ERBB2 signalling influences neuroprotective processes, making neurons more vulnerable to the harmful effects of neurotoxic peptides, such as amyloid-beta. Nevertheless, overactivation of ERBB2 can cause dysfunction and set off detrimental processes in brain cells, as several cancer types show (Atoki et al., 2023). Gene and protein functions are examined in detail using advanced bioinformatics techniques such as GO analysis and KEGG pathway. GO analysis makes it easy to fully characterize the roles of target genes and proteins in biological processes, their molecular functions, and their location in cellular components. In this way, it is understood how the relevant biological processes are affected and which molecular functions come into play. KEGG pathway analysis shows the connections between various proteins in metabolic or signal transduction pathways and how these activities change in disease state. This method provides detailed information about complex biological networks and interactions so that the molecular mechanisms of

diseases can be better understood. These analyses are critical for understanding how targeted molecules affect disease processes and discovering potential therapeutic targets. Consequently, GO and KEGG analyses contribute to the creation of more effective and focused therapeutic strategies, thus playing an important role in combating diseases (Chen et al., 2017; Gene Ontology Consortium, 2017; Xing et al., 2016). The GO biological processes of the top 10 targets were identified (Figure 6). There are numerous distinct biochemical pathways that play a major role in the etiopathogenesis of AD. According to Lee et al. (2017), the ability of positive regulation of peptidyl-serine phosphorylation to hyperphosphorylate tau protein

indicates that it is the most dominant activity in AD. Furthermore, changes in global protein phosphorylation may impact neuronal function and cellular signalling networks, which may accelerate the course of the illness (Oliveira et al., 2017). Since neuronal loss is one of the main characteristics of AD, apoptotic process regulation and controlled death of cells are strongly related to the illness (Gong and Iqbal, 2008). Furthermore, as noted by Hernandez et al. (2009), intracellular signaling and its regulation play a critical role in neurodegenerative illnesses like AD. It will be crucial to carefully look at the roles that each of these processes plays in AD to develop successful treatment strategies and a deeper understanding of the disease.

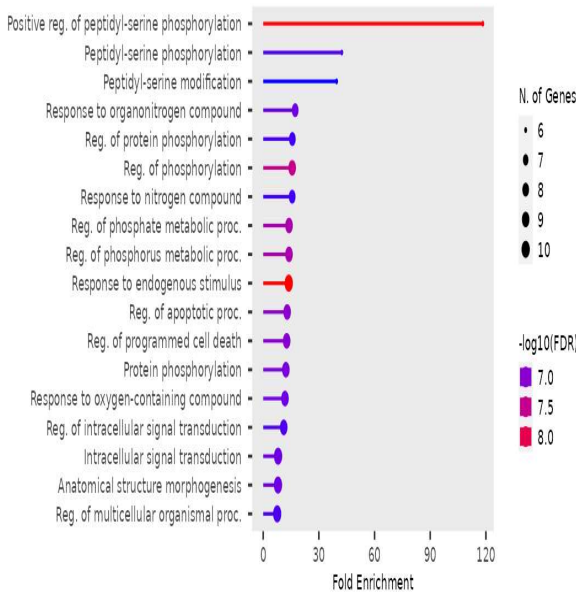
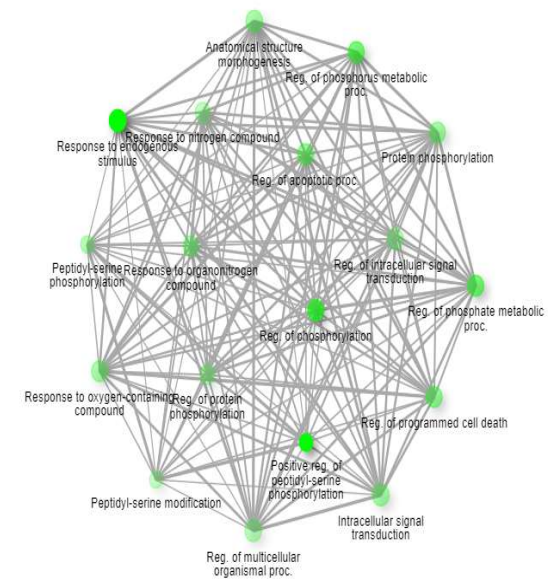


Figure 6. GO biological processes of top 10 targets
 Şekil 6. İlk 10 hedefin GO biyolojik süreçleri

GO cellular components of the top 10 targets were identified (Figure 7). Cellular components related to AD can be identified by considering the illness's fundamental features and the cellular processes it impacts. The modulation of neuronal signaling pathways and cell development is very critical in AD. In this setting, the phosphatidylinositol 3-kinase complex (particularly class 1 and class 1A) performs key roles in neural signaling and cell survival processes, making it intimately associated with AD (Fraser et al., 2008). Furthermore, because dendritic growth cones and axonal growth cones play critical roles in controlling neuronal development and connections, this condition may be linked to neuronal network disturbance (Weinkove et al., 2008). The myelin sheath is another critical cellular component that influences the speed and efficiency of neuronal transmission and can be impaired in AD (Fraser et al., 2008). To have a grip on AD and develop treatment alternatives, every one of these elements might be a key focus.



The top 10 targets' GO molecular functions were determined (Figure 8). The effect of AD on neuronal function and the roles of protein changes can be used to determine molecular activities associated with AD. In this regard, the regulation of nitric oxide synthase is essential for both inflammatory and neural processes, and it may also help prevent neurodegeneration in AD patients (Bredt et al., 1992). Reversal of protein phosphorylation and regulation of cellular signalling processes need two essential chemical reactions, namely protein phosphatase binding and phosphatase binding, which may be operational in the development of AD (Sontag & Sontag, 2014). Kinase and enzyme regulatory activities mediate signal transduction processes and preserve cellular homeostasis, the two processes that may potentially alter AD course (Austin and Katusic, 2016). Additionally, protein breakdown and cell longevity are related to ubiquitin-protein ligase and ubiquitin-like protein ligase binding, which may be involved in the regulation of protein aggregation and

cellular stress in AD (Prete et al., 2016). The pathophysiology of AD can be better explained and

potential new targets for treatment can be identified if these molecular effects are more deeply scrutinized.

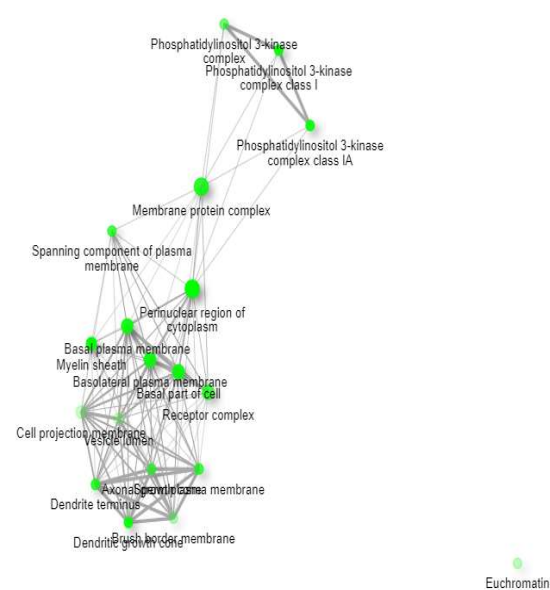
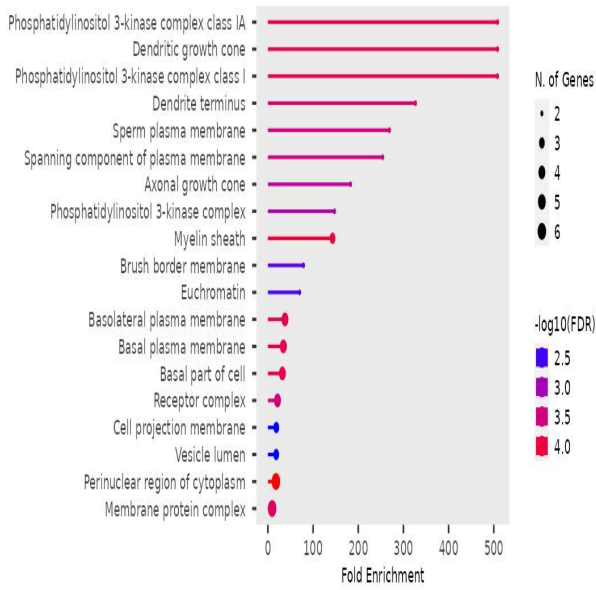


Figure 7. GO cellular components of top 10 targets
 Şekil 7. İlk 10 hedefin GO hücresel bileşenleri

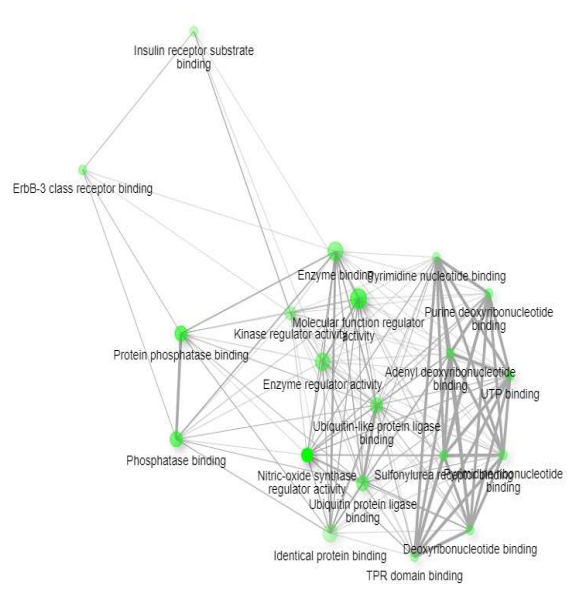
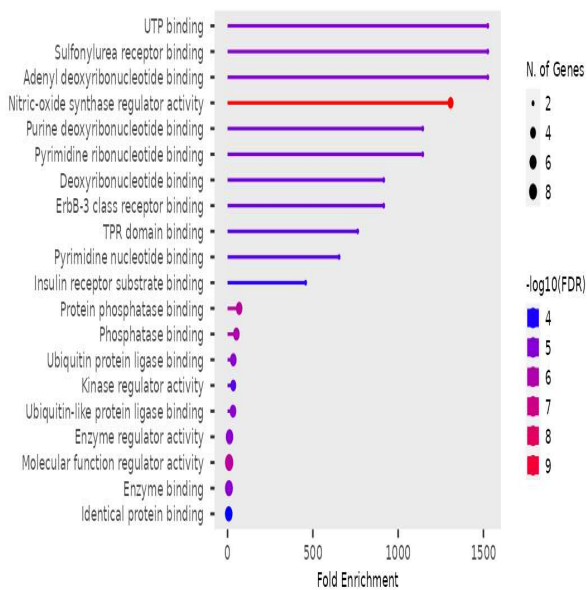


Figure 8. GO molecular functions of top 10 targets
 Şekil 8. İlk 10 hedefin GO moleküler fonksiyonları

KEGG pathways of the top 10 targets were determined (Figure 9). KEGG pathways related to AD are expected to reveal the basic characteristics of AD and the molecular interactions involved. According to Li et al. (2003), the ErbB signalling pathway is associated with the growth and survivability of neurons and may help to maintain the connections and functioning of neurons in AD. When activated in the event of neuronal stress and damage, the HIF-1 signalling pathway governs hypoxia-related cellular responses and may be crucial

in AD (Abdul and Butterfield, 2007). According to Jimenez et al. (2011), the PI3K-Akt signalling system regulates cell survival, proliferation, and metabolism, making it a potential target for halting the progression of AD. A thorough examination of these pathways may lead to novel therapy approaches as they are crucial for understanding how AD governs damage to neurons and how that damage influences the course of the disease.

The CB-Dock2 server provides the AutoDock vina-

based molecular docking technique and the curvature-based cavity identification approach to CB-Dock2, an enhanced version of the protein-ligand blind docking programme (URL1). Binding positions are evaluated using the binding energies expressed in kcal/mol when using the CBDOCK2 vina technique (Thangavel et al., 2021). Vina's empirical scoring method is based on a scoring function (Ugurlu et al., 2024). Greater affinity for binding is shown by a higher negative vina score (Quiroga and Villarreal, 2016). Table 5 displays the molecular docking data for the target proteins (EGFR, HSP90AA1, BL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1) and lignans (enterodiol, enterolactone, etoposide, lariciresinol, matairesinol, pinoresinol, podophyllotoxin, and

secoisolariciresinol). The target proteins have the following order of binding affinity for lignans: ERBB2 > PIK3CA > HSP90AB1 > PIK3R1 > EGFR > JUN > ESR1 > IL6 > HSP90AA1 > BCL2, in that order. Etoposide > enterolactone > pinoresinol > matairesinol > enterodiol > podophyllotoxin > lariciresinol > secoisolariciresinol is the order from highest to lowest in which the lignans attach to their particular target proteins. The molecular docking experiments revealed a considerable binding affinity between the target proteins (EGFR, HSP90AA1, BL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1) and the lignans, which indicated the potential of the proteins to function.

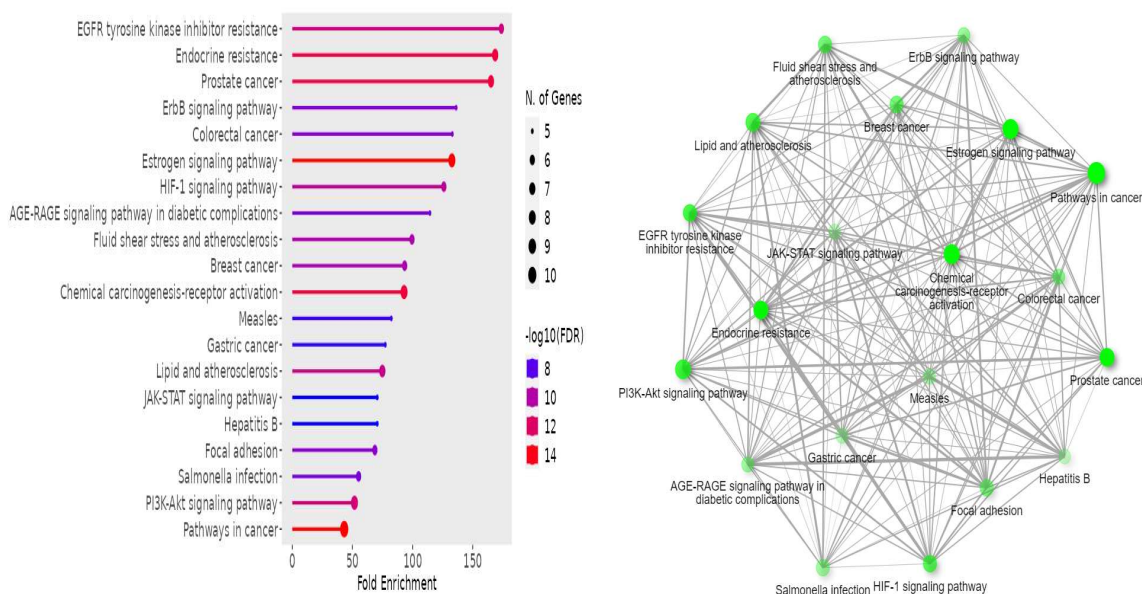


Figure 9. KEGG pathways of targets
Şekil 9. Hedeflerin KEGG yolları

The contact residues of the main lignans (enterolactone, etoposide, podophyllotoxin, and pinoresitol) to the target proteins (EGFR, HSP90AA1, BL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1) by the respective vina scores are shown on Table 6. The contact residues and bindings between the ligands and target proteins were found to have

contact amino acids and bond structures. The teal dotted lines show the reactions between hydrogen bonds. Aguilar-Carrillo et al. (2024) state that electrostatic interactions are displayed as yellow dotted lines, whereas hydrophobic interactions are depicted by grey dotted links.

Table 5. Molecular docking results (vina score) of lignans and target proteins

Çizelge 5. Lignanların ve hedef proteinlerin moleküler kenetlenme sonuçları (vina skoru)

Lignan	Target Protein									
	EGFR	HSP90 AA1	BCL2	HSP90 AB1	IL6	JUN	ESR1	PIK3CA	ERBB2	PIK3R1
Enterodiol	-8.1	-6.1	-6.2	-8.4	-7.0	-8.9	-8.0	-7.9	-8.7	-8.0
Enterolactone	-9.5	-6.9	-7.1	-9.4	-6.9	-7.8	-10.0	-8.5	-9.8	-7.8
Etoposide	-8.2	-7.7	-7.8	-8.7	-7.1	-9.8	-7.8	-9.5	-9.0	-10.8
Lariciresinol	-8.1	-6.3	-6.1	-8.4	-6.8	-7.2	-6.8	-8.5	-8.8	-7.8
Matairesinol	-8.3	-6.9	-6.0	-8.6	-6.8	-7.2	-8.7	-9.5	-9.0	-7.8
Pinoresinol	-8.5	-6.6	-6.8	-8.1	-6.1	-8.1	-7.8	-9.6	-9.5	-8.1
Podophyllotoxin	-7.7	-6.6	-6.4	-8.2	-7.3	-7.4	-6.7	-8.6	-8.1	-8.8
Secoisolariciresinol	-7.6	-6.4	-6.1	-7.8	-6.3	-7.0	-7.5	-7.2	-8.8	-7.4

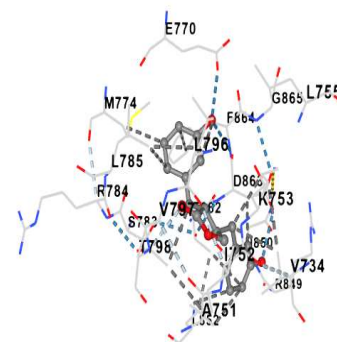
Table 6. Molecular docking results (contact residues) of lignans and target proteins

Çizelge 6. Lignanların ve hedef proteinlerin moleküler kenetlenme sonuçları (temas kalıntıları)

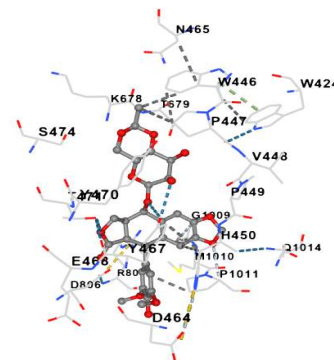
Lignan	Target Protein	Contact Residues	
Enterolactone	EGFR	Chain A: PHE723 VAL726 ALA743 ILE744 LYS745 LEU747 ARG748 GLU749 ALA750 THR751 SER752 PRO753 LYS754 ALA755 ASN756 GLU758 ILE759 LEU760 GLU762 ALA763 MET766 VAL769 CYS775 ARG776 LEU777 ILE780 LEU782 THR783 SER784 THR785 VAL786 LEU788 ILE789 MET790 THR854 ASP855 PHE856 GLY857 LEU858 LEU861 LEU862	
Etoposide	HSP90AA1	Chain A: ASN161 PRO162 ASN163 GLU164 GLY165 ALA166 THR167 THR189 GLU192 ASP195 LYS204 GLU207 GLU251 LYS252 ALA253 LYS254 GLU255 SER256 TRP257 MET259 LYS262 GLU263 GLU266 GLN267 ILE270 VAL271 LYS274 MET285 VAL288 ILE289 GLN290 TYR291 GLY292 LYS293 VAL295 SER296 TRP297 GLU299 MET300 GLU301 PHE320 LEU323 TYR327 LYS335 GLU338 CYS339 LYS342	
Etoposide	BCL2	Chain A: PHE23 CYS24 SER25 GLY26 ILE27 GLN28 ARG40 TYR41 LYS44 GLU45 GLU47 GLN48 ARG51 ARG55 SER58 GLN59 VAL100 SER103 GLU104 LEU106 SER107 ARG108 GLY109	
Enterolactone	HSP90AB1	Chain A: ASN413 MET414 PHE415 ARG417 LEU418 GLU421 Chain B: GLU370 ARG373 ILE400 GLN403 LEU404 GLU407 TYR411 Chain C: SER258 TRP259 MET261 ASN262 SER263 LYS266 SER298 TRP299 LEU300 GLU301 TYR302 GLU303 SER304 SER305 PHE306	

Podophyllotoxin	IL6	Chain A: GLU42 THR43 LYS46 SER47 ARG104 PHE105 GLU106 SER107 SER108 GLN156 ASP160 THR163	
Etoposide	JUN	Chain N: ILE535 ARG537 LYS538 ASN539 ARG541 GLN571 ARG572 HIS575 GLU576 LEU577 LYS664 ARG665 LYS666 ARG667 GLN669 Chain J: LYS268 ARG269 ARG271 ASN272 LYS283	
Enterolactone	ESR1	Chain A: MET343 LEU346 THR347 ASN348 LEU349 ALA350 ASP351 GLU353 TRP383 LEU384 LEU387 MET388 LEU391 ARG394 PHE404 MET421 ILE424 PHE425 LEU428 LYS520 GLY521 MET522 HIS524 LEU525 CYS530	
Pinoresitol	PIK3CA	Chain A: ARG154 ARG162 TYR165 VAL166 TYR167 PRO168 PRO169 ASN170 LYS253 ASP258 GLU259 TYR260 MET288 SER292 LEU293 GLN296 LEU297 PRO298 ASP300 GLN661 ARG662 PHE666 CYS695 GLY696 MET697 TYR698 HIS701 GLN749 GLY750 PHE751 LEU752 ASN756 PRO757 ALA758 GLN760 LEU761 GLY762 ASN763 PRO786	

Chain B: LEU726 GLY727 SER728
 VAL734 ALA751 ILE752 LYS753
 LEU755 GLU770 MET774 SER783
 ARG784 LEU785 LEU796 VAL797
 THR798 GLN799 LEU800 MET801
 PRO802 TYR803 GLY804 CYS805
 ASP808 ARG849 ASN850 LEU852
 THR862 ASP863 PHE864 GLY865
 PHE1004



Chain A: GLY364 TRP424 TRP446
 PRO447 VAL448 PRO449 HIS450
 LEU452 ASP454 LEU455 LEU456
 ASN457 PRO458 ILE459 ASN465
 CYS604 ASN605 LYS678 THR679
 ASP806 ARG808 LEU1006 GLY1009
 MET1010 PRO1011 GLN1014 SER1015
 PHE1016 ASP1017
 Chain B: ASP464 TYR467 GLU468
 TYR470 THR471 SER474



It has been observed that gefitinib ligand, while binding to the EGFR protein, interacts with residues ASP800, VAL726, LYS745, MET790, LEU788, ILE744, LEU844, ALA743, GLN791, LEU792, MET793, LEU718, PRO794, GLY796, SER719 (URL2; Yoskikawa et al., 2013). Erlotinib has been observed to interact with residues ASP831, VAL702, LYS721, THR830, LEU764, ILE720, ILE765, ALA719, THR766, GLN767, LEU820, LEU694, LEU768, MET769, PHE771, PRO770, GLY772, CYS773, ASP776 (URL3; Park et al., 2012). Lapatinib has been observed to interact with residues LEU792, MET1002, GLY796, CYS797, GLY719, ASP800, LEU799, ARG803, ARG841, PO481, LEU718, VAL726, LEU858, THR854, MET766, PHE856, ASP855, LEU777, ARG776, THR790, CYS775, LYS745, LEU788, ILE744, ALA743, GLN791, ILE789, MET793, LEU844 (URL4; Wood et al., 2004). Bilobol has been observed to interact with residues ASN86, ARG84, GLU60, ALA62, VAL36, VAL37, LEU38, GLY39, TYR251, GLY264, ALA265, THR249, THR266, PRO248, GLU221, SER222, ASP223, CYS236, CYS224, THR235, ALA234, LEU225, VAL226, CYS227, ARG231 (Adabi et al., 2023). 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9h-purine derivatives have been observed to interact with residues MET793, THR854, LEU718, LEU844, MET766, VAL726, ALA743, LYS745, MET790 (İbrahim et al., 2020). In our study, enterolactone and gefitinib, erlotinib, lapatinib, bilobol, and 2,9-disubstituted 8-phenylthio/phenylsulfinyl-9h-purine derivatives were found to interact with similar residues. Enterolactone was found to be more similar in interaction with gefitib

and lapatinib.

It has been observed that 17-AAG ligand, while binding to the HSP90AA1 protein, interacts with residues ASN161, PRO162, ASN163, GLU164, GLY165, ALA166, THR167, GLU192, ASP195, LYS204, GLU207, TRP257, MET259, LYS262, GLU263, GLN267, ILE270, VAL271, LYS274, MET285, VAL288, ILE289, GLN290, TYR291, GLY292, LYS293, VAL295, SER296, TRP297, GLU299, MET300, GLU301, PHE320, LEU323, TYR327, LYS335, GLU338, CYS339, LYS342 (URL5; Stebbins et al., 1997). Geldamycin has been observed to interact with residues ASN161, PRO162, ASN163, GLU164, GLY165, ALA166, THR167, GLU192, ASP195, LYS204, GLU207, TRP257, MET259, LYS262, GLU263, GLN267, ILE270, VAL271, LYS274, MET285, VAL288, ILE289, GLN290, TYR291, GLY292, LYS293, VAL295, SER296, TRP297, GLU299, MET300, GLU301, PHE320, LEU323, TYR327, LYS335, GLU338, CYS339, LYS342 (URL6; Stebbins et al., 1997). Radicicol has been observed to interact with residues ASN161, PRO162, ASN163, GLU164, GLY165, ALA166, THR167, GLU192, ASP195, LYS204, GLU207, TRP257, MET259, LYS262, GLU263, GLN267, ILE270, VAL271, LYS274, MET285, VAL288, ILE289, GLN290, TYR291, GLY292, LYS293, VAL295, SER296, TRP297, GLU299, MET300, GLU301, PHE320, LEU323, TYR327, LYS335, GLU338, CYS339, LYS342 (URL7; Roe et al., 1999). In our study, etoposide and 17-AAG, geldamycin, and radicicol were found to interact with similar residues.

It has been observed that ABT-737 ligand, while binding to BCL-2 protein, interacts with residues PHE23, ARG55, TYR41, ARG108 (URL8; Murray et al., 2019). Venetoclax (ABT-199) has been observed to interact with residues PHE23, TYR41, ARG55, GLU45, CYS24 (URL9; Birkinshaw et al., 2019). S55746 has been observed to interact with residues PHE23, ARG55, TYR41, GLU47 (URL10; Casara et al., 2018). Novel 9-(alkylthio)-Acenaphtho[1,2-e]-1,2,4-triazine derivatives has been observed to interact with residues GLU13, MET16, LYS17, HIS20, ALA32, ASP35, VAL36, GLU38, ASN39, THR41, ASP10, GLY46, GLU50, ASP35 (Mohammadi et al., 2014). In our study, etoposide and ABT-737, venetoclax (ABT-199), S55746, and novel 9-(alkylthio)-Acenaphtho[1,2-e]-1,2,4-triazine derivatives were found to interact with similar residues.

It has been observed that the 17-AAG ligand, while binding to the HSP90AB1 protein, interacts with residues GLU370, ARG373, ILE400, GLN403, LEU404, GLU407, TYR411 (URL5; Stebbins et al., 1997). Geldamycin has been observed to interact with residues ASN413, MET414, PHE415, ARG417, LEU418, GLU421 (URL6; Stebbins et al., 1997). Radicicol has been observed to interact with residues SER258, TRP259, MET261, ASN262, SER263, LYS266, SER298, TRP299, LEU300, GLU301, TYR302, GLU303, SER304, SER305, PHE306 (URL7; Roe et al., 1999). Chelerythrine has been observed to interact with residues TRP162, VAL150, ASP93, TYR139, PHE138, LEU107, MET98 (Sharma and Kumar, 2023). In our study, enterolactone and 17-AAG, geldamycin, radicicol, and chelerythrine were found to interact with similar residues.

It has been observed that the olokizumab ligand, while binding to the IL6 protein, interacts with residues GLU42, THR43, LYS46, ARG104, PHE105, GLU106, SER107, ASP160 (URL11; Shaw et al., 2014). Procyanidin has been observed to interact with residues GLU42, THR43, LYS46, SER47, ARG104, PHE105, GLU106, SER107, ASP160 (Zeng et al., 2023). In our study, podophyllotoxin, olokizumab, and procyanidin were found to interact with similar residues.

It has been observed that the 4-OHT ligand binds to the ESR1 protein and interacts with the MET343, LEU346, THR347, LEU349, ALA350, ASP351, GLU353, TRP383, LEU384, LEU387, MET388, LEU391, ARG394, PHE404, MET421, ILE424, PHE425, LEU428, LYS520, GLY521, MET522, HIS524, LEU525, CYS530 (URL12; Maximov et al., 2018). Chalcone derivatives (HNS10) have been observed to interact with residue LEU346, THR347, LEU349, ALA350, GLU353, LEU387, MET388, LEU391, ARG394, MET421, LEU525 (Muctaridi et al., 2017). Benzophenone imine inhibitors have been observed to interact with residues LEU346, THR347,

LEU349, ALA350, GLU353, TRP383, LEU384, LEU387, MET388, LEU391, ARG394, PHE404, MET421, ILE424, GLY521, HIS524, LEU525 (Shtaiwi et al., 2019). In our study, it was determined that enterolactone and 4-OHT, chalcone derivatives, and benzophenone imine inhibitors interact with similar residues.

It has been observed that the covalent inhibitor 19 ligand binds to the PIK3CA protein and interacts with the CYS862 residue (URL13; Borsari et al., 2022). Fragments 12 and 15 were observed to interact with residue GLU542 (URL14; Miller et al., 2017). Alpelisib (BYL719) has been observed to interact with residues ARG154, TYR165, TYR167, PRO169, ASP300, GLU542, ASP544 (Pattar et al., 2020). In our study, it was determined that pinoreisitol and covalent inhibitor 19, fragments 12 and 15, and alpelisib (BYL719) interact with similar residues.

It has been observed that doxazosin ligand, while binding to the ERBB2 protein, interacts with residues LEU726, GLY727, SER728, VAL734, ASP863, PHE864, MET774, SER783, LEU785, LEU796, THR798, MET801, TYR803, GLY804, CYS805, LEU852, THR862 (URL15). Gefitinib has been observed to interact with residues LEU726, VAL734, CYS805, LEU852, THR862, ASP863, and PHE864 (URL15). Lapatinib has been observed to interact with residues LEU726, ALA751, LYS753, GLU770, MET774, SER783, LEU785, LEU796, THR798, GLN799, LEU800, MET801, TYR803, GLY804, CYS805, LEU852, THR862, ASP863, PHE864, GLY865 (URL15). Tyrosine kinase inhibitors from *Panax biinnatifidus* and *Panax pseudoginseng* have been observed to interact with residues LYS753, ALA751, MET774, LEU852, THR798, LEU800, MET801, GLY804, CYS805, ASP808, LYS724, LEU726, VAL734, GLY729, ALA730, ASP863 (Paul et al., 2021). In our study, enterolactone and doxazosin, gefitinib, lapatinib, and tyrosine kinase inhibitors from *Panax biinnatifidus* and *Panax pseudoginseng* were found to interact with similar residues.

It has been observed that PI-103 ligand interacts with HIS450, LEU455, and ASP454 residues while binding to the PIK3R1 protein (URL16). Alpelisib has been observed to interact with residues GLY364, TRP424, and ASP806 (URL17). Wortmannin has been observed to interact with residues ASN605, CYS604, and GLY1009 (URL18). In our study, etoposide and P-103, alpelisib, and wortmannin were found to interact with similar residues.

The interaction residues between ligands and their target proteins were observed to significantly overlap with those detected for enterolactone, etoposide, podophyllotoxin, and pinoreisitol.

These consistent interactions suggest that the binding affinities and mechanisms of action of these ligands

may be comparable, thus common pathways or mechanisms can be investigated for therapeutic purposes.

Studies in the literature showing the relationship of enterolactone with EGFR, HSP90AB1, ESR1, and ERB2 are associated with cancer and hepatic fibrosis. There is no study showing its relationship with AD. However, Reddy et al. (2020), enterolactone is an inhibitor of acetylcholinesterase and butyrylcholinesterase and therefore provides neuroprotection against AD. Hoang et al. (2024) suggested that diets rich in matairesinol could inhibit the effect of A β ₁₋₄₂, since enterolactone is a matairesinol-derived metabolite produced through intestinal microbiota activity.

Studies in the literature showing the relationship of etoposide with HSP90AA1, BCL2, JUN, and PIK3R1 are associated with cancer. There is no study showing its relationship with AD. However, Lu et al. (2002) showed that although etoposide is neurotoxic, it also activates a cell survival pathway involving AMPA receptor-mediated activation of p42/p44 MAP kinases. They stated that agents that selectively inhibit cell survival or death pathways triggered by DNA damage may be useful in cancer and neurodegenerative diseases.

Studies in the literature show the relationship between podophyllotoxin and IL6 is associated with cancer. There is no study showing its relationship with AD. However, Xu et al. (2022), the extract from the Juniperus plant exhibited significant anti-butyrylcholinesterase activity, which was positively correlated with high levels of podophyllotoxin and deoxypodophyllotoxin.

Studies in the literature showing the relationship of pinosresitol with PIK3CA are associated with cancer. There is no study showing its relationship with AD. However, Lei et al. (2021) found that pinosresitol could alleviate neuroinflammation, neuronal apoptosis, and oxidative stress through the TLR4/NF- κ B and Nrf2/HO-1 pathways and ameliorate A β ₁₋₄₂-induced memory dysfunction in mice.

CONCLUSION

Our study explores the potential of lignans, natural polyphenols, that can be used in the treatment of AD. Using network pharmacology and molecular docking techniques, we examined how lignans interact with various key proteins associated with AD pathology. These proteins include EGFR, HSP90AA1, BCL2, HSP90AB1, IL6, JUN, ESR1, PIK3CA, ERBB2, and PIK3R1. Our findings suggest that interactions of lignans with these proteins may alleviate AD symptoms by modulating inflammation, regulating apoptosis, and affecting signal transduction pathways. Additionally, these interactions may reduce amyloid-

beta accumulation and tau phosphorylation, thus slowing disease progression. These results support the use of lignans as potential therapeutic agents in the treatment of AD and highlight the need for further *in vivo* studies. This integrated approach highlights the importance of developing new strategies in the treatment of complex diseases such as AD.

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None.

Contribution of Authors

SŞ and SND: Designed, performed, analyzed, writing, review and editing.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Committee Permission

An ethics committee permission is not required for the article.

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Maize Pests and Their Natural Enemies in the North-West of Türkiye

Abdurrahman Sami KOCA¹, Gülay KAÇAR²

^{1,2}Bolu Abant İzzet Baysal Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü, Bolu, Türkiye

¹<https://orcid.org/0000-0002-7657-5615>, ²<https://orcid.org/0000-0001-9800-8286>

✉: a.samikoca@yahoo.com.tr

ABSTRACT

Maize is one of the most significant cereal crops in the world, and insect pests cause the highest economic loss. The objective of this study was to assess the level of insect pests and their natural enemies during 2020 and 2022 in the maize fields of Düzce and Sakarya, Türkiye. We performed weekly surveys from the vegetative growth stage of maize to harvest from April through November. A hundred plants were selected with regular and irregular samplings from each field. In addition, light traps and pheromone traps were placed in regularly sampled fields. To detect overwintering larvae, the stalks left in the field after the harvest were cut just above the soil. In the maize fields, a total of 13 pest species from six families in four orders, along with 19 natural enemies from eight families in five orders, were identified. *Ostrinia nubilalis* Hbn. (Lepidoptera: Crambidae) was found as the primary pest, followed by *Helicoverpa armigera* Hbn. and *Mythimna unipuncta* Haw. (Lepidoptera: Noctuidae). *Meteorus pendulus* Müller (Hymenoptera: Braconidae) was a new record for the East Marmara and Western Black Sea regions and a new host record in Türkiye. Larval parasitoids of *M. unipuncta*, *Nemoraea pellucida* Meigen, and *Pales pavidus* Meigen (Diptera: Tachinidae) represent new host records for Türkiye. Among the predators, *Orius minutus* L. (Hemiptera: Anthocoridae) and coccinellids showed an especially notable common in the maize fields of both provinces.

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ÖZET

Mısır, dünyada tarımı yapılan en önemli tahıllardan birisidir ve zararlı böcek türleri yüksek ekonomik kayıplara neden olmaktadır. Bu çalışma 2020 ve 2022 yılları arasında Düzce ve Sakarya mısır alanlarında bulunan zararlı böcek türleri ve doğal düşmanlarını belirlemek amacıyla yürütülmüştür. Örneklemeler Nisan ayı ile Kasım ayı arasında mısırın vejetatif büyüme evresinden hasata kadar haftalık olarak gerçekleştirilmiştir. Örneklemelerin yapıldığı her bir tarladan toplam 100 bitki incelenmiştir. Ayrıca örnekleme yapılan tarlalara ışık tuzakları ve feromon tuzakları yerleştirilmiştir. Kışlayan larvaları tespit etmek için hasattan sonra tarlada kalan saplar laboratuvara getirilerek incelenmiştir. Bu çalışmada, mısır alanlarında dört takıma bağlı altı familyadan 13 zararlı böcek tür, beş takımdan sekiz familyaya bağlı 19 doğal düşman türü belirlenmiştir. *Ostrinia nubilalis* Hbn. (Lepidoptera: Crambidae), *Helicoverpa armigera* Hbn. ve *Mythimna unipuncta* Haw. (Lepidoptera: Noctuidae) sırasıyla başlıca zararlılar olarak tespit edilmiştir. *Meteorus pendulus* Müller (Hymenoptera: Braconidae), Doğu Marmara ve Batı Karadeniz Bölgeleri için yeni kayıt olmasının yanı sıra Türkiye için yeni konukçu kaydı niteliğindedir. *Mythimna unipuncta*'nın larva parazitoidleri olan *Nemoraea pellucida* Meigen ve *Pales pavidus* Meigen (Diptera: Tachinidae), Türkiye için yeni konukçu kaydı niteliğindedir. Predatör türlerden *Orius minutus* L. (Hemiptera: Anthocoridae) ve coccinellid türleri her iki ilde örnekleme yapılan mısır alanlarının birçoğunda yaygın olarak tespit edilmiştir.

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INTRODUCTION

Maize (*Zea mays* L.) is the most widely produced crop, with 1 billion tons worldwide. It has a high genetic diversity and can be grown in various climates, up to 3.800 meters above sea level and from the equator to around 50° north and 42° south latitudes (Ortega, 1987). In Türkiye, maize is the most widely grown crop after wheat and barley and is either sown as the main or the second crop by most farmers, with an approximate annual production of 6.750.000 tons (TUIK, 2021). Düzce and Sakarya provinces produce 332.000 tons of maize, representing 20% of the total production of Türkiye (TUIK, 2021). First-crop maize planting usually occurs between April and May, second-crop maize is in June and July, and harvesting is held from September to October in Düzce and Sakarya provinces. At the global level, maize is used 65-70% in animal feed, while around 20% is utilized in the production of sugar, starch, and oil, and the remaining is in the food industry (Özcan, 2009).

Animal pests, weeds, and pathogens threaten maize yield and quality (Oerke, 2006). Insect pests, especially lepidopteran species are the most critical factors limiting maize production. These pests attack any part of the plant, like foliage, tassel, ear, stem, or grain, and can infest at any stage of maize growth and storage, often causing severe damage (Ortega, 1987). Insect pests, particularly lepidopteran stem borers, severely reduce maize yields (Tonğa & Bayram, 2021). Currently, the most significant pests of maize in Türkiye are the European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Crambidae) and the Mediterranean corn borer, *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), which are the primary pests (Sertkaya et al., 2014). These corn borers cause economic loss from 2 to 4 million hectares of maize in Europe (Brookes, 2009). In Türkiye, other lepidopteran pests from the Noctuidae family include cotton leafworm (*Spodoptera littoralis* Boisd.), cutworms (*Agrotis* spp.), corn earworm (*Helicoverpa armigera* Hbn.), beet armyworm (*Spodoptera exigua* Hbn.), armyworms (*Mythimna* spp.), and the family Crambidae include spotted stem borer (*Chilo partellus* Swinhoe), which are significant cause of crop loss (Sertkaya et al., 2014; Akmeşe et al., 2017). Other pests of regional importance include wireworms such as *Agriotes* spp., *Cardiophorus cyanipennis* Mulsant & Wachanru, and *Melanotus fuscipes* Gyll. (Coleoptera: Elateridae), *Gryllotalpa gryllotalpa* (Orthoptera: Gryllotalpidae), *Tanymecus dilaticollis* Gyll. (Coleoptera: Curculionidae), spider mites (*Tetranychus* spp.), aphids (*Rhopalosiphum* spp.),

Hemiptera: Aphididae), thrips (Thysanoptera), leafhoppers (Hemiptera: Cicadellidae), and planthoppers (Hemiptera: Delphacidae, Cixidae) (Mutlu et al., 2008; Yılmaz et al., 2009; Sertkaya et al., 2010; Akmeşe et al., 2017).

Globalization has facilitated the increase in trade, transportation, travel, and tourism, eliminating natural barriers between specific regions, countries, and even continents and allowing many insect species to enter new habitats (Lowe et al., 2000). Additionally, in recent years, the impact of climate change has led to the emergence of some new insect pests as a frequent problem in various regions of the world.

Agricultural production has expanded considerably in the last 50 years because of the availability of synthetic fertilizers and high-yielding cultivars. In addition, the widespread use of pesticides, which provided farmers with better pest control, significantly contributed to the so-called green revolution (Newsom, 1980; Eichers, 1981; Kogan, 1998; Meissle et al., 2010). However, the rising use of chemicals in agriculture negatively affects the health of humans and animals, pollutes water and soil, and has side effects on beneficial insects such as natural enemies, decomposers, and pollinators (Metcalf, 1986; Pimentel, 2005). Insect pests substantially reduce maize yields, but an approach other than chemical control is lacking (Van Huis, 1981). The principle of knowing insect pests has a significant outcome, which can recognize the natural enemies that prey on or parasitize them. The capacity to correctly identify pests and beneficial insects is equally as critical as recognizing damaging ones because it can be used to improve pest management success (Ortega, 1987).

The primary purpose of the current study is to report the presence of pests and their natural enemies in maize fields of Düzce and Sakarya provinces. This study attempts to establish the basis for developing an integrated pest management program by identifying the pests and natural enemies in maize fields.

MATERIAL and METHOD

The study was performed in the first and second-crop maize fields in Merkez, Çilimli, Gümüşova districts of Düzce, and Hendek, Akyazı, Erenler districts of Sakarya between 2020 and 2022 (Figure 1). Regular samplings were made in 12 maize fields in Düzce and Sakarya provinces in 2021 and 2022. In addition, insect species were collected through irregular samplings from 54 different fields between 2020 and 2022. Field surveys were done from April to October each year, and samplings were carried out from the

vegetative growth stage to harvest. A hundred maize plants were checked in each field, with 20 randomly selected plants on 5 sampling spots. Each plant was carefully inspected, from the aerial root to the tassel. Additionally, solar-powered light traps and *O. nubilalis* pheromone traps (Z-11-Tetradecenyl acetate, E-11-Tetradecenyl acetate, Trece Incorporated, USA) were placed in regularly sampled fields. After the harvest, stalks remaining in the field were cut just above the soil, with 50 samples collected for each field and brought to the laboratory for detection of overwintering larvae in the stalks. Insect specimens were put into a plastic container (10 × 10 cm), falcon

tubes, and Petri dishes, labeled, and protected via an ice box until transferred to the laboratory. The collected insect samples were incubated in a plant growth chamber (25±1 °C, 60±10 RH, 14 L:10 D photoperiod) with their natural (fresh maize leaves, stems, and cobs) and artificial diets, including toasted soybean flour, stabilized wheat germ, sugar, vitamins, and mineral salts (Southland Products Inc, USA). Samples were checked daily until adult pests or any possible natural enemies such as parasitoids or predators emerged. After the first adults emerged, the samples were sent to the expert taxonomist for identification.



Figure 1. Map showing the location of Düzce and Sakarya provinces in Türkiye
Şekil 1. Çalışmaların yürütüldüğü Düzce ve Sakarya illerinin haritası

The molecular method based on DNA sequencing of ribosomal internal transcribed spacer (ITS) region was employed for the species identification of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) specimens from *O. nubilalis* eggs. The extraction nucleic acids of samples, preserved in 95% ethyl alcohol, were isolated utilizing the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the instructions provided by the manufacturer. The PCR amplification to yield their ITS2 was conducted with the universal primer pair

(forward 5'-TGTGAACTGCAGGACACATG-3' and reverse 5'-GTCTTGCTGCTCTGAG-3') according to Stouthamer et al. (1999). The amplified fragments were sequenced by a commercial company (Macrogen Inc., Seoul, South Korea) with the same primers. The obtained DNA sequences were subjected to alignment using ClustalW, a method for multiple-sequence alignment (Thompson et al., 1994), and analyzed using the Basic Local Alignment Search Tool (BLASTn: <http://blast.ncbi.nlm.nih.gov/Blast>) at NCBI (National Center for Biotechnology Information:

<https://www.ncbi.nlm.nih.gov/>) against the GenBank nucleotide database to identify the closest presented reference sequences.

RESULTS and DISCUSSION

We detected 13 different insect pests from the orders Lepidoptera, Hemiptera, Coleoptera, and Orthoptera, and 19 different natural enemies from the orders Diptera, Hymenoptera, Hemiptera, Coleoptera, and Neuroptera in the maize fields of Düzce and Sakarya.

Table 1. Pest species identified in maize fields of Düzce and Sakarya

Tablo 1. Düzce ve Sakarya ili mısır alanlarında tespit edilen zararlı türler

Order	Family	Species
Lepidoptera	Noctuidae	<i>Helicoverpa armigera</i> (Hübner, 1808)
	Noctuidae	<i>Mythimna unipuncta</i> (Haworth, 1809)
	Noctuidae	<i>Mythimna loreyi</i> (Duponchel, 1827)
	Noctuidae	<i>Mythimna congrua</i> (Hübner, 1817)
	Noctuidae	<i>Agrotis ipsilon</i> (Hufnagel, 1766)
	Noctuidae	<i>Autographa gamma</i> (Linnaeus, 1758)
	Crambidae	<i>Ostrinia nubilalis</i> (Hübner, 1796)
	Crambidae	<i>Loxostege sticticalis</i> (Linnaeus, 1761)
	Erebidae	<i>Hyphantria cunea</i> (Drury, 1773)
Hemiptera	Aphididae	<i>Rhopalosiphum padi</i> (Linnaeus, 1758)
	Aphididae	<i>Anoecia corni</i> (Fabricius, 1775)
Coleoptera	Curculionidae	<i>Tanymecus dilaticollis</i> (Gyllenhal, 1834)
Orthoptera	Gryllotalpidae	<i>Gryllotalpa gryllotalpa</i> (Linnaeus, 1758)

The European corn borer, *O. nubilalis* is the most common species in first and second-crop maize in Düzce and Sakarya provinces. It was detected in almost all the survey and sampled fields. Larvae of *O. nubilalis* were found on leaves, cobs, and stalks of maize and adults in pheromone and light traps. The pest was determined from the end of May until the harvest. In addition, overwintering the last stage larvae were found in the remaining corn stalks in the field after harvest. Pupae emerge as moths in May and June during the spring season. Previous investigations into the biological behavior of *O. nubilalis*, conducted in the field and laboratory, revealed the presence of first-generation larvae, typically from June to July, in the maize whorls. The pest overwinters as mature larvae in corn stalks and other plant refuse, including weed stems. (Özdemir, 1981). The second-generation larvae emerge from late July to August, causing severe damage (Özdemir, 1981; Melan et al., 1996). In the Cukurova region, the first generation is effective in late May and early June. 2nd, 3rd, and 4th generations were observed in July, August, and September, respectively (Kornoşor & Kayapınar, 1988a). The young larvae feed on the top tassels and leaves on the fresh shoots. Then, the larvae enter the cob and stem to transform into the pupal stage, causing the cob shedding and stalk tunnels, as well as restrictions on the cob and grains development (Hudon et al., 1989; Kaçar et al., 2023a). It causes considerable yield losses

Maize Pests in Düzce and Sakarya Provinces and Their Status in Türkiye

The most common and high-density species were *O. nubilalis*, *H. armigera*, and *M. unipuncta*. *Loxostege sticticalis* L. (Lepidoptera: Crambidae) has also the highest density, although its occurrence was restricted to 2022. The other species were found in various locations in Düzce and Sakarya, and their population densities remained low (Table 1).

in Central and South Europe and North America (Dicke and Guthrie, 1988; Krattiger, 1997; Velasco et al., 2007). Similarly, *O. nubilalis* is recognized as one of the most severe pests of maize in various parts of Türkiye, such as the Black Sea, Aegean, Mediterranean, and Central Anatolia regions (Özdemir, 1981; Derin, 1992; Sade, 2003; Öztemiz et al., 2008). *Ostrinia nubilalis* is the primary pest of maize crops along with *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), causing a 30% yield loss in second-crop maize (Özdemir, 1981; Cerit et al., 2006). However, in the study areas, *S. nonagrioides* was not detected in pheromone traps or light traps.

The corn earworm, *Helicoverpa armigera* is one of the most common pests after *O. nubilalis* in the maize fields of Düzce and Sakarya provinces. The pest was observed at a very low density in the first-crop maize during the 2020 and 2021 seasons. However, the pest was notably population density in the first-crop maize of 2022 and second-crop maize of all years. They have been frequently seen on second-crop maize from the end of August to the beginning of October while on first-crop maize from the second half of July to mid-August. According to earlier studies, *H. armigera* was generally a high population of second-crop maize, while the low density was in first-crop maize (Gözüaçık & Mart, 2005; Gözüaçık, 2016). *Helicoverpa armigera* larvae were found on leaves, silks, cobs, and tassels of maize, and adults in light traps. The larvae principally

damage the corn ear, which begins to feed on the silk of the cob soon. In addition to entering the cob and causing direct injury to the kernels, they also open access for infection by pathogens. Besides, the larvae may occasionally feed on leaf whorls or tassels when silks and cobs have not yet developed. *Helicoverpa armigera* is a globally distributed and devastating pest of agricultural and horticultural areas, containing over 181 plant species from 45 families. (Srivastava et al., 2005). The pest is a problem in a variety of crops in Africa, Middle East, Asia (Russia, China, Pakistan, India, Thailand), Europe (Portugal, Spain, Italy, Greece, Türkiye), Australia, New Zealand (Hardwick, 1965; Mohyuddin, 1985; Zalucki et al., 1986; Fitt, 1989; Karim, 2000, Scott et al., 2006; Fefelova & Frolov, 2008; Gözüaçık, 2020; Burgio et al., 2020). Also, *H. armigera* is regarded as one of the most significant pests of maize in some parts of Türkiye, including Black Sea, Central Anatolia, East Anatolia, Southeast Anatolia, Mediterranean, and Aegean (Özdemir & Uzunali, 1981; Şimşek & Sezer, 1983; Ünlü et al., 1995; Gözüaçık, 2004; Tiftikci and Kornoşor, 2015; Barış et al., 2020; Gözüaçık, 2020; Koca & Kaçar, 2021a). Management of *H. armigera* is complicated due to its high mobility, ability to adapt to adversities and complete many generations in a year, and capability to create pesticide resistance (Ali et al., 2009).

The study identified three armyworm species in the maize fields of Düzce and Sakarya provinces, namely *M. unipuncta*, *M. loreyi*, and *M. congrua*. Among these, *M. unipuncta* exhibited the highest population density, while *M. congrua* and *M. loreyi* were found in lower numbers. *Mythimna congrua* is a new record for the North-west of Türkiye, including East Marmara, and Western Black Sea Region. *M. congrua* has previously only been found in Izmir (Tanyeri et al., 2010), and Hatay (Can et al., 2018) provinces of Türkiye. *M. unipuncta* was found at low density in the first and second-crop maize in 2020. However, it was particularly population density in the second-crop maize of 2021 and the first and second-crop maize of the 2022 season. The pest was rarely found in the second half of July but frequently determined throughout August in the first-crop maize. In the second crop, it was intensely observed from the end of August until mid-October. *Mythimna* spp. consume the leaf tissue of maize plants. Feeding is usually confined to leaf margins, but larvae may occasionally strip the plants of leaf tissue. Additionally, it has been determined that they also feed on the silk of the cob as the leaves begin to harden or dry at the end of the vegetation period of the second-crop maize. In Çukurova, the first adults of *M. loreyi* were identified in late May using Robinson light traps in maize plants (İkincisoym, 1993). Similarly, the first adults of *M. unipuncta* were observed in early April, and adult emergence continued until May (Kara, 1994).

Armyworms generally prefer to lay eggs and feed upon plants in the family Gramineae, including weedy grasses. Although the larva feeds primarily on grasses (oats, wheat, fall rye, maize, barley, and forage grasses), it can be a pest of some vegetables such as beans, cabbages, carrots, onions, peas, peppers, radishes, sweet potatoes, alfalfas, lettuces, celeries, onions, cucumbers, sugar beets, and watermelons (Cook et al., 2004; Capinera, 2018). Armyworms occur in many areas of the world, including North, Central, and South America, Southern Europe, Central Africa, and Western Asia (E1-Sherif et al., 1972; Singh et al., 1987; Cabello, 1989; Capinera, 2018). They have been subjected to intensive investigations due to their damage to maize plants in several areas of Türkiye, such as the Black Sea, Mediterranean, East Anatolia, Southeast Anatolia, and Mediterranean regions (Kornoşor, 1999; Sertkaya & Bayram, 2005; Gözüaçık et al., 2009; Ölmez et al., 2010; Tiftikci & Kornoşor, 2015; Gözüaçık, 2020; Kaçar & Koca, 2021a).

The silver Y moth, *A. gamma* was determined in Düzce and Sakarya provinces for all years. However, its population was at very low densities in the first and second-crop maize. It was found between July and September in all maize fields, regularly sampled. The adults were captured in light traps, and the larvae were found on the cob. The larvae cause damage to the corn ear and the cob silk. The silver Y moth is an economically significant polyphagous pest of vegetables and field crops throughout the Palearctic region, including Europe, Asia, and North Africa (Balachowsky, 1972). *A. gamma* is mainly found in open habitats such as grasslands and forest edges. Females lay their eggs primarily on low-growing herbaceous plants, and they can frequently be seen feeding while taking nectar from flowering plants during the day or early evening. Larvae of *A. gamma* can cause significant economic damage due to their wide range of various crops and vegetables, including peas, soybeans, sugar beets, cabbage, tomatoes, and industrial crops (Chumakov & Kuznetsova, 2021; Nagy et al., 2022).

Agrotis spp. is a polyphagous species on numerous cultivated and herbaceous plants. Adults of the pest were captured in light traps in Düzce province (Alacamescit and Matı/Central) in May 2021 and 2022. The adults were also captured in Aktarla (Akyazı) and Hasanbey (Erenler) in Sakarya in 2021 and 2022, respectively. On the one hand, larvae were first detected in a field in Sakarya province (Fatih/Akyazı) in the half of June 2021. Larvae of *A. ipsilon* were found in 4-8 leaves of the first-crop maize. The larvae cut through the ground stem of maize, causing the plant to wilt and die. Black cutworm, *A. ipsilon* is known to attack at least 49 cultivated plants and cause considerable economic losses in various crops, particularly industrial plants and vegetables, in

Türkiye and around the world (Odiyo, 1975; Potter, 1998; Hong & Williamson, 2004; Liu et al., 2015). According to Lodos (1980), *Agrotis* spp. is one of the six primary pests of maize in Türkiye. Moreover, young larvae consume fresh leaves or growing plants until they reach the fourth instar during their early stages. Mature larvae can cut many plants in a single night, resulting in extensive damage (Santos & Shields, 1998; Capinera, 2001). While young larvae exhibit activity throughout the day, mature larvae are predominantly active during the night, with peak activity observed between midnight and one hour before sunrise (Williamson & Potter, 1997).

The meadow moth, *L. sticticalis* was detected in Düzce and Sakarya provinces only in 2022. Furthermore, it has been reported that this pest has become an outbreak in many regions of Türkiye, including Kocaeli, İstanbul, Bolu, Düzce, and Sakarya (Kaçar et al., 2023b). The larvae of *L. sticticalis* did not cause any damage to the maize plant. Adults of *L. sticticalis* were caught in numbers ranging from 10 to 50 per light trap in different locations in each province. The first-generation adults flight from the last week of June to the first half of July and the second generation was seen from the beginning to mid-August. The females of *L. sticticalis* lay their eggs on the underside of the host leaves. The larvae feed on the leaves, shoots, buds, and flower leaves of the plants, which can completely consume the plants during the outbreak years (Anonymous, 2008; Kuznetsova & Chumakov, 2008; Kaçar et al., 2023b). *Loxostege sticticalis* is one of the most destructive migratory pests in Europe, Asia, and North America, causing significant economic damage nearly yearly (Qu et al., 1999). This species is a dangerous polyphagous pest that is capable of feeding on 200 species from 35 plant families, including cereals, vegetables, fiber crops, beans, and oilseeds, but has a special preference for certain host plants (Qu et al., 1999; Yin et al., 2004; 2005; Zhang et al., 2010). They are highly adaptable, capable of reproduction, and harmful. It can populate large areas by flying great distances within two to three days. (Shurovenkov & Alekhin, 1984; Frolov et al., 2000; Smirnova, 2000).

The fall webworm, *H. cunea*, was only found in the second-crop maize of Düzce and Sakarya. This pest was previously detected in Düzce and Sakarya provinces on hazelnuts, mulberries, and some forest trees, and it was reported that the pest produced two generations per year. (Kaçar et al., 2019; Kaçar et al., 2022). As the host of *H. cunea*, maize is not an apparent preference. At the end of the season, it prefers to feed on maize leaves and cob tassels, which are relatively fresher than the leaves of trees such as mulberry and hazelnut. The larval stages of the second generation of *H. cunea*, which were close to overwintering, passed to the overwintering areas after feeding on the maize plant. *Hyphantria cunea* is a

polyphagous quarantined and serious invasive defoliator pest which causes significant damage and economic losses due to its great fecundity and extensive host range (Yang et al., 2006; Zhang et al., 2016). *H. cunea* defoliates 175 different plant species from 108 genera within 49 plant families, including ornamental, fruit, and forest trees as well as crops like maize, cotton, cabbage, and others (Qu, 1987; Wang, 1995; Yang et al., 2006). *H. cunea* was first detected in Turkey in 1975 and has spread to the Marmara, Black Sea, and North Aegean regions of Türkiye. It causes significant damage, especially in hazelnut orchards of the central Black Sea region (İren, 1977; Anonymous, 2011; Sullivan, 2011; Kaçar et al., 2019; Kaçar et al., 2022).

The maize leaf weevil, *T. dilaticollis*, was determined in Alacamescit (Cilimli) of Düzce province in May 2020, as well as Aktarla and Catalköprü (Akyazı) Sakarya province. They were detected in the 2-4 leaf period of maize and consume the leaf margins like a crescent shape. Barbulescu et al. (2001) reported that *T. dilaticollis* adults are more dangerous during the early vegetation stages of maize, while damage to its larvae is not important. So, adults consume leaves and destroy apical meristems. Besides that, *T. dilaticollis* has one generation per year and overwinters as an adult in the soil (Paulian, 1972). *Tanymecus dilaticollis* is a significant pest of maize and damage in the seedling period in Europe, Anatolia, Iran, Russia, South-western Asia, and the Caucasus (Draganova et al., 2012; Alonso-Zarazaga et al., 2017; Davidian, 2019). This species is a common pest in maize fields in many regions of Türkiye (Gözüaçık, 2019). Lodos (1980) reported that *T. dilaticollis* is one of the six main pests of maize fields of Türkiye. Although *T. dilaticollis* is considered a polyphagous species, it prefers maize for optimal development for the larvae and is the most preferred food by adults (Bărbulescu & Voinescu, 1998).

The mole cricket, *G. gryllotalpa* was only determined in a maize field in Seyfeler/Akyazı (Sakarya province) in 2021. The nymph and adult of the pest cut through the ground stem of maize, causing the plant to die. *Gryllotalpa gryllotalpa* is a significant pest of many crops in various parts of the world, causing damage to seedlings, roots, and tubers. They are distributed throughout temperate and tropical regions such as Europe, Russia, Turkey, Central Asia, Iran, Afghanistan, Central and Southern Asia, North Africa, America, and Southern Ukraine (Klechkovskii, 1967). The pest damages many crops such as soybean, cowpea, maize, turf, vegetable, tobacco, sunflower, cotton, fruits, and some tree seedlings (Frank & Parkman, 1999; Bhamrah, 2007; Akmeşe et al., 2017; Javadzadeh et al., 2017). *Gryllotalpa gryllotalpa* feeding on the roots of plants causes plant stress and even death (Thompson, 2003). In addition, plant

pathogenic fungi and bacteria can enter a plant through damage caused by feeding the pest (Thompson & Brandenburg, 2005).

Aphids are frequently found on maize leaves and tassels in large and dense colonies. Aphid infestation results in deformed and chlorotic showing leaves. If severely damaged, the tassel can become infertile and negatively affect the production of seeds (Bosque-Perez, 1995). Aphids, also produce honeydew on which the molds grow. It may limit the pollination process of maize when tassels and silks become covered in honeydew. Also, if aphids feed more inside the whorl before tassel emergence, it may affect kernel growth or result in sterile ears (Stray et al., 1994). Besides, *R. padi* transmits maize dwarf mosaic virus (Ferro et al., 1980). Maize aphids were detected in the Hendek and

Akyazı districts of Sakarya and Merkez, Çilimli, and Gümüşova districts of Düzce. These species were primarily found in the first-crop maize, which varies from year to year but usually appears between mid-June and the end of August. Also, maize aphids were rarely detected in the second crop at the end of August.

Natural Enemies Detected in Maize Fields in Düzce and Sakarya Provinces

The study has revealed 19 beneficial species belonging to eight families from five different orders from the maize fields of Düzce and Sakarya. Nine parasitoid species were identified, including eight larval and egg parasitoids (Table 2).

Table 2. Parasitoids identified in maize fields of Düzce and Sakarya

Tablo 2. Düzce ve Sakarya ili mısır alanlarında tespit edilen parazitoitler

Family	Species	Host	Location
Diptera			
Tachinidae	<i>Voria ruralis</i> (Fallén, 1810)	<i>H. armigera</i> <i>A. gamma</i>	Merkez (Düzce) Matı (Sakarya) Hendek (Sakarya)
Tachinidae	<i>Lydella thompsoni</i> (Herting, 1959)	<i>O. nubilalis</i>	Merkez (Düzce) Çilimli (Düzce) Akyazı (Sakarya) Erenler (Sakarya) Hendek (Sakarya)
Tachinidae	<i>Peleteria iavana</i> (Wiedemann, 1819)	Unknown	Merkez (Düzce)
Tachinidae	<i>Nemoraea pellucida</i> (Meigen, 1824)	<i>M. unipuncta</i>	Merkez (Düzce) Hendek (Sakarya)
Tachinidae	<i>Pales pavid</i> a (Meigen, 1824)	<i>M. unipuncta</i>	Merkez (Düzce)
Tachinidae	<i>Peribaea tibialis</i> (Robineau-Desvoidy, 1851)	<i>M. unipuncta</i>	Merkez (Düzce)
Hymenoptera			
Braconidae	<i>Meteorus pendulus</i> (Müller, 1776)	<i>M. unipuncta</i>	Merkez (Düzce) Erenler (Sakarya)
Ichneumonidae	<i>Hyposoter didymator</i> (Thunberg, 1822)	<i>H. armigera</i>	Merkez (Düzce)
Trichogrammatidae	<i>Trichogramma brassicae</i> (Bezdenko, 1968)	<i>O. nubilalis</i>	Merkez (Düzce) Çilimli (Düzce) Akyazı (Sakarya) Erenler (Sakarya) Hendek (Sakarya)

The larval parasitoids, *Meteorus pendulus* (Müller, 1776) from the Braconidae (Hymenoptera), were collected in the Alacamescit (Düzce) and Hasanbey (Sakarya) locations on *Mythimna unipuncta* Haw. (Lepidoptera: Noctuidae) larvae. It has been noted as a new record for the East Marmara and Western Black Sea regions and a new host record in Türkiye. *Meteorus pendulus* was only recorded in Adana province, in the Mediterranean region of Türkiye (Yılmaz et al., 2010). It is a solitary endoparasitoid that emerges from the larval stage of various Lepidopteran families, including Noctuidae, Tortricidae, Geometridae, Lycaenidae, Lasiocampidae, and Lymantriidae (Yu et al., 2012). Another larval

parasitoid *H. didymator*, obtained from *H. armigera* larvae, is a solitary koinobiont endoparasitoid (Medina et al., 2007). This species is widely distributed in many regions, including Europe, Asia, the Middle East, North Africa, and Australia (Schneider et al., 2003). *Hyposoter didymator*, highlighted as an important parasitoid of *H. armigera*, has been documented to be an effective parasitoid under natural conditions on *Helicoverpa* and *Spodoptera* species, as well as economically significant harmful noctuids (Sertkaya et al., 2004; Mironidis & Savopoulou-Soultani, 2009).

The species *N. pellucida* and *P. pavid*a, larval parasitoids of *M. unipuncta*, represent new host records for both species in Türkiye. *Peribaea tibialis*, a

solitary parasitoid species collected from *M. unipuncta* larvae, was previously discovered in the Bartın province of Turkey (Atay, 2017). In general, Tachinidae species are generally known to parasitize the Macrolepidoptera and Microlepidoptera larvae and to be polyphagous (Tschorsnig & Herting, 1994). The parasitoid *L. thompsoni*, obtained from *O. nubilalis* larvae, was previously reported in the Western Blacksea region of Türkiye (Melan & Kara, 2004), and it was widely documented in many countries worldwide (Cagáncaron et al., 1999). Gözüaçık (2020) obtained this parasitoid from *O. nubilalis* larvae in maize fields in the Iğdır province, reporting a parasitism rate ranging from 21.2% to 39.7%. Another tachinid species identified in our study, *V. ruralis*, was obtained from *H. armigera* larvae. This species was previously reported on *H. armigera* and *A. gamma* larvae in Türkiye (Kara & Özdemir, 2000). *Voria ruralis* is generally known as a parasitoid commonly found in the Noctuidae larvae (Tschorsnig & Herting, 1994). Among the Tachinidae species, *L. thompsoni* is the most frequent in both provinces, and following *V. ruralis* and *N. pellucida* were listed as common tachinids.

Among the identified parasitoid species, only an egg parasitoid has been detected. The identification of *Trichogramma* species was carried out by assessing the size of their ITS2 PCR product. BLASTn queries based on the ITS2 products of *Trichogramma* specimens in the maize fields of Sakarya and Düzce

provinces showed that the sequences of isolates were 99–100% identical to those of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) isolates in the GenBank database. The resultant sequences were deposited in GenBank under the accession numbers OP597676 to OP597679. In previous studies conducted in the region, the same species has been identified (Koca et al., 2018; Gülser & Öztemiz, 2020). Egg parasitoids of the *Trichogramma* genus have been commonly used in inundative biological control against a variety of agricultural pests, including Lepidoptera, Diptera, Coleoptera, Hemiptera, Hymenoptera, and Neuroptera (Smith, 1996). In Türkiye, *Trichogramma* species are mass-reared, and these parasitoids are released, particularly in maize fields (Öztemiz et al., 2008). These parasitoids play a significant role in maintaining the ecosystem in some areas by controlling the population of lepidopteran pests without pesticides (Thomson et al., 2003). Earlier studies in the Black Sea region of Türkiye have shown that the natural occurrence of the egg parasitoids, *T. evanescens* and *T. brassicae*, can effectively suppress the populations of *O. nubilalis* (Özdemir, 1981; Melan et al., 1996; Kutuk, 2017). In addition, *T. evanescens* is released against *H. armigera*, reducing up to 80% of *H. armigera* larvae (Öztemiz et al., 2009). Therefore, *Trichogramma* species are a significant mortality or reducing factor in *O. nubilalis* and *H. armigera* populations and may play a crucial role in their (Öztemiz et al., 2009; Koca & Kaçar, 2021b).

Table 3. Predators identified in maize fields of Düzce and Sakarya
 Tablo 3. Düzce ve Sakarya ili mısır alanlarında tespit edilen predatörler

Family	Species	Location
Hemiptera		
Miridae	<i>Stenodema virens</i> (Linnaeus, 1767)	Çilimli (Düzce)
Anthocoridae	<i>Orius minutus</i> (Linnaeus, 1758)	Merkez, Çilimli (Düzce) Akyazı, Erenler, Hendek (Sakarya)
Coleoptera		
Coccinellidae	<i>Adalia decempunctata</i> (Linnaeus, 1758)	Çilimli, Merkez (Düzce) Erenler (Sakarya)
Coccinellidae	<i>Propylea quatuordecimpunctata</i> (Linnaeus, 1758)	Merkez (Düzce) Akyazı, Erenler (Sakarya)
Coccinellidae	<i>Harmonia axyridis</i> (Pallas, 1773)	Çilimli (Düzce) Erenler, Hendek (Sakarya)
Coccinellidae	<i>Coccinula quatuordecimpustulata</i> (Dobzhansky, 1925)	Merkez, Çilimli (Düzce) Akyazı, Hendek (Sakarya)
Coccinellidae	<i>Scymnus rubromaculatus</i> (Goeze, 1778)	Merkez, Çilimli (Düzce) Akyazı (Sakarya)
Coccinellidae	<i>Subcoccinella vigintiquatuorpunctata</i> (Linnaeus, 1758)	Merkez, Çilimli (Düzce) Akyazı, Erenler (Sakarya)
Coccinellidae	<i>Coccinella septempunctata</i> (Linnaeus, 1758)	Merkez, Çilimli (Düzce) Akyazı, Erenler, Hendek (Sakarya)
Neuroptera		
Chrysopidae	<i>Chrysoperla carnea</i> (Stephens, 1836)	Merkez, Çilimli (Düzce) Akyazı (Sakarya)

In addition, 10 predators were detected, with seven belonging to the Coccinellidae family and one each from the Miridae, Anthocoridae, and Chrysopidae families (Table 3). Six coccinellids were predators, and one species (*Subcoccinella vigintiquatuor punctata*) was phytophagous. Coccinellids play a crucial role in sustainable plant protection strategies, serving as fundamental members of biological control and famous predators in agroecosystems (Khan et al., 2007; Kaçar & Koca, 2020).

Among these taxa, *O. minutus* showed an especially notable presence in the maize fields of Düzce and Sakarya, practically ubiquitous across virtually all areas subject to sampling efforts. Similarly, coccinellids were also widely distributed in both provinces. *Stenodema virens* was detected only in the Matı localion in the first year of the study in Düzce province, whereas *C. carnea* emerged in relatively low population densities in different locations.

CONCLUSION

The study revealed nine Lepidopteran pests, two Hemipteran pests, and one Orthopteran and Coleopteran pest. Furthermore, the study has identified six parasitoid species belonging to the Tachinidae family, as well as one species from the Braconidae, Ichneumonidae, and Trichogrammatidae families. Besides, this research has also revealed six species from the Coccinellidae family and one species each from the Miridae, Anthocoridae, and Chrysopidae families as predator species. The primary pest was identified as the European corn borer, *O. nubilalis*, damaging maize cultivation in Düzce and Sakarya, followed by the species *H. armigera* and *M. unipuncta*. Over the past five years, problems with Lepidopteran pests, such as *O. nubilalis*, *H. armigera*, and *Mythimna* spp., have experienced a notable increase. This rise could be attributed to the expanding populations of pests, potentially influenced by warmer climatic conditions or the excessive application of pesticides.

Naturally occurring parasitoids and predators, which play an important role in biological control in the field, are frequently harmed by broad-spectrum insecticide applications. In recent years, there has been a rise in the prevalence of *H. cunea*, reaching epidemic levels in hazelnut orchards in Düzce. To manage the *H. cunea* population in these areas, a combination of registered and unregistered pesticides is frequently employed. Due to the interweaving of maize fields within hazelnut orchards, the regular application of pesticides against *H. cunea* could harm natural enemies and disrupt the biocontrol of *O. nubilalis* by *Trichogramma* species. Therefore, the chemicals used in the control of pests should be eco-friendly and not harm on natural enemies. In addition, a decrease in insecticide usage

will contribute to enhanced biological control. Parasitoids and predators can also be promoted with specific measures, including diverse crop patterns, and managing field margins such as flower strips and hedges to provide food and overwintering sites. Besides seasonal population fluctuations, natural enemies of pest species should be followed to develop integrated pest management programs as an alternative against chemical control. In addition, it would be beneficial to research to determine the effectiveness of natural enemies.

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

Conflicts of Interest

The authors declare no conflict of interest.

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Allelopathic Effects of Black Radish (*Raphanus sativus* L. var. *niger* J. Kern.) and Garden Cress (*Lepidium sativum* L.) Plants on Johnsongrass (*Sorghum halepense* (L.) Pers.) Plant in Tomato Cultivation

Muhammad ELSEKRAN¹, Tamer ÜSTÜNER²

^{1,2}Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection, Kahramanmaraş

¹<https://orcid.org/0000-0001-6672-7016>, ²<https://orcid.org/0000-0003-3584-4249>

✉: asdr.ag198024@gmail.com

ABSTRACT

This study aimed to investigate the effectiveness of black radish and garden cress as pre-plant in field trials and their aqueous extracts under greenhouse conditions to control johnsongrass. In the field experiment, black radish and garden cress were grown as a pre-plant, then mixed with the soil and used with and without mulch against johnsongrass. The effects of these applications on johnsongrass development and tomato yield and quality were evaluated. Different concentrations of pre-plant extracts (2, 5, and 10%) were also investigated on johnsongrass and tomato seedling growth in greenhouse conditions. The lowest johnsongrass density was recorded in black radish and garden cress with mulch (106.7 and 97.2 number m⁻²). Black radish and garden cress with mulch achieved johnsongrass control efficiency of 80.2 and 84.0% compared to those without mulch 56.8 and 58.2%. The effect of all treatments was positive in increasing the quantity and improving the quality of tomato production. The results of greenhouse experiments showed that high concentrations (10%) of black radish and garden cress extracts achieved high levels in preventing the germination and growth of johnsongrass. In these treatments, johnsongrass seeds and rhizomes germination rates were 17.5 and 7.5%, 20.0 and 12.5% respectively. GC-MS analysis showed that five isothiocyanates (ITCs) were in black radish, and their total percentages were 40.4%. In the garden cress, it was found that there is only one ITC with a percentage of 61.0%. Black radish and garden cress effectively inhibit the germination and growth of Johnsongrass due to their allelopathy effects. Sustainable management of johnsongrass can be achieved by introducing these plants into a crop rotation which may be an alternative or reduce the use of herbicides.

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Keywords

Black radish (*Raphanus sativus* L. var. *niger* J. Kern.)
Garden cress (*Lepidium sativum* L.)
Johnsongrass (*Sorghum halepense* (L.) Pers.)
Tomato
Allelopathy

Domates Yetiştiriciliğinde Siyah Turp (*Raphanus sativus* L. var. *niger* J. Kern.) ve Tere (*Lepidium sativum* L.) Bitkilerinin Geliç (*Sorghum halepense* (L.) Pers.) Bitkisi Üzerine Allelopatik Etkileri

ÖZET

Bu çalışma, geliş mücadelesinde tarla denemelerinde ön bitki ve sera koşullarında sulu özüt olarak siyah turp ve tere etkinliğini araştırmayı amaçlamıştır. Tarla denemesinde siyah turp ve tere ön bitki olarak tarlada yetiştirildikten sonra toprağa karıştırılarak geliç'e karşı malçlı ve malçsız olarak kullanılmıştır. Bu uygulamaların geliç'in gelişmesine ve domates verim ve kalitesine etkileri değerlendirilmiştir. Sera koşullarında ön bitki özütlerinin farklı konsantrasyonlarının (%2, 5 ve 10), geliş ve domates fide büyümesi üzerine etkileri de araştırılmıştır. En düşük geliş yoğunluğu malçlı siyah turp ve tere uygulamalarda kaydedilmiştir (106.7 ve 97.2 adet m⁻²). Malçlı siyah turp ve tere uygulamalarda geliç'in mücadele etkinliği %80.2 ve %84.0 olurken malçsız uygulamalarda %56.8 ve %58.2 kayıt edilmiştir. Tüm uygulamaların etkisi domates üretim miktar ve kalitesinin artırılmasında olumlu olarak değerlendirilmiştir. Sera deneme sonuçları, siyah turp ve tere özütleri yüksek konsantrasyonlarının (%10) geliç'in çimlenme ve büyümesini önlemede yüksek seviyede etkili

Herboloji

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 08.02.2024

Kabul Tarihi : 18.07.2024

Anahtar Kelimeler

Siyah turp (*Raphanus sativus* L. var. *niger* J. Kern.)
Tere (*Lepidium sativum* L.)
Geliç (*Sorghum halepense* (L.) Pers.)
Domates
Allelopati

olduğunu göstermiştir. Bu uygulamalarda geliş tohum ve rizomlarının çimlenme oranları sırasıyla %17.50 ve %7.50, %20.00 ve %12.50 olarak tespit edilmiştir. GC-MS analizine göre siyah turpta beş isotiyosiyanat (ITC) bileşen bulunduğu, toplam oranları ise %40.4 olarak tespit edilmiştir. Tere ise %61.0 oranında sadece bir ITC'nin bulunmuştur. Siyah turp ve tere, allelopati etkileri nedeniyle gelişin çimlenme ve büyümesini etkili bir şekilde engellemiştir. Sürdürülebilir geliş yönetimi, bu bitkilerin, herbisitlerin kullanımına alternatif olabilecek veya kullanımını azaltabilecek bir ürün rotasyonuna dahil edilmesiyle sağlanabilir.

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INTRODUCTION

Johnsongrass (*Sorghum halepense* (L.) Pers.) is a perennial weed native to the Mediterranean region, belongs to the Poaceae family, and reproduces by seeds and rhizomes (Warwick & Black, 1983; Davis, 1988). Johnsongrass causes yield loss of up to 88% in economically important crops (Peerzada et al., 2017). Chemical herbicides are widely used to reduce the damage caused by johnsongrass. Herbicides cause damage to the environment and human health. They persist for many years in the soil, and weeds are developing resistance to them. Therefore, it is necessary to find alternative and healthy control methods to achieve sustainable agriculture (Ustuner et al., 2020; Yazlik & Uremis, 2022).

Some plants contain allelochemicals produced through secondary metabolite reactions. These substances affect the germination and growth of other plants positively or negatively. Allelopathic negative effects can benefit weed control, while positive effects can be used as growth regulators. In addition, the synergistic effect between plants can be benefited by taking it into the appropriate agricultural rotation system (Bellostas et al., 2007; Jabran, 2017; Acar et al., 2019). Several studies showed the allelopathic potential of many plants of the cruciferous family and their efficiency in weed control (Uremis et al., 2009; Bangarwa et al., 2011; Jabran et al., 2015; Elsekran, 2022; Elsekran & Ustuner, 2024). It was reported that when the *Brassica napus* plant was incorporated into the soil, it inhibited weed biomass by 96% in subsequent potato production (Boydston & Hang, 1995). In the tomato (*Lycopersicon esculentum* L.) field, *B. rapa*, *B. juncea*, and *Sinapis alba* were used as cover crops, in combination with polyethylene mulch, to control johnsongrass. Brassicaceae cover crops achieved johnsongrass control of 46% two weeks after planting (Bangarwa & Norsworthy, 2014). Malik et al. (2008), reported that incorporating wild radish into the soil before the cultivation of corn significantly reduces the density of *Digitaria sanguinalis*. The allelopathic potential of six

species of cruciferous plants was evaluated in laboratory and field conditions. According to this study, all species inhibited the development of johnsongrass while black radish (*Raphanus sativus* L. var. *niger* J. Kern.) was the most efficient (Uremis et al., 2009). According to Qasem (1994), aqueous extracts of wild cress (*Lepidium draba*) leave inhibited wheat and barley seed germination rate (36% and 75%), coleoptile elongation (52% and 85%), and root length (63% and 94%). The aqueous extract of the wild cress plant increased the germination and root development of barley seeds at 1% concentration, and the germination rate decreased when the concentration was increased to 3% (Erez, 2009). Allelochemicals in garden cress (*Lepidium sativum* L.) seeds and seedlings were reported to be effective in reducing seedling growth in *Amaranthus caudatus* and lettuce (Iqbal & Fry, 2012).

The allelopathic effects of cruciferous plants are attributed to compounds called isothiocyanates (ITCs). These compounds, which are characterized by high bioactivity, do not exist in healthy plants but result from the hydrolysis of glucosinolates (GSLs) by the enzyme myrosinase (β -thioglucosidase). GSLs are secondary metabolites and do not have toxic effects (Rask et al., 2000; Jafariehyazdi & Javidfar, 2011). GSLs are divided into three classes namely aliphatic GSLs, aromatic GSLs, and indole GSLs based on the difference in the side chain of their structure. Therefore, the ITCs differ based on these GSL types and their effects on weeds (Graser et al., 2000; Norsworthy & Meehan, 2005).

This study aimed to determine the level of control of johnsongrass using black radish and garden cress as pre-plants, with and without black plastic mulch. Additionally, the study aimed to evaluate the allelopathic effects of these plants on biomarkers of johnsongrass growth and development under both field and greenhouse conditions. The effect of these treatments on the quantity and quality of the tomato crop was also estimated. The greenhouse experiments

aimed to study the effect of aqueous extracts of black radish and garden cress at different concentrations (2, 5, and 10%) on the germination and growth of johnsongrass seeds and rhizomes in pots.

MATERIALS and METHODS

Fields Experiments

Experiment site and climatic characteristics

The experiments were performed in the fields of the faculty of Agriculture at Kahramanmaraş Sütçü İmam University, Türkiye (37°35'37.3" N 36°48'53.0" E) in 2019 and 2020. The soil analysis of the experiment area showed that the texture of the soil was clay loam, pH neutral (7.04), slightly salty (0.30%), organic matter ratio of 3.32%, and potassium and phosphorus content moderate. According to the meteorological data of the field trial area, it was observed that the hottest months are July and August, and the coldest is January. In addition, the highest precipitation was in January, while the rains were absent or infrequent in June, July, August, and September (Table 1).

Experiment design and treatments

The experiment was set up in three replications (blocks) according to the randomized complete block design. In each block, there were 7 applications, weed-free control (Cnt 1), black radish (BR), garden cress (GC), black polyethylene mulch 100 microns thick and had UV additives (mulch), black radish + mulch (BR+M), garden cress + mulch (GC+M), and johnsongrass control (Cnt 2). The dimensions of the

experimental plots in the field experiment were 2 × 5 m. While a distance of 1 m was left between the plots, 1.5 m between the blocks, and 1.5 m as side effects areas around the experimental areas. All experiments were repeated twice throughout 2019 and 2020.

BR and GC were planted first as pre-plants, and then tomato seedlings were planted. The varieties used in this experiment were BT- Bur Siyah for BR Zeybek for GC, and F1 Ege pembesi for tomato. The seeds of these plants were obtained from the Teta-Tohumculuk-seed company in Türkiye. There was no need for infection with johnsongrass because the experiment area was a natural area for it (Figure 1).



Figure 1. Johnsongrass spread in the experiment area naturally

Şekil 1. Geliç bitkisinin deneme alanında doğal olarak yayılması

Table 1. Meteorological data of Kahramanmaraş province for 2019 and 2020*

Çizelge 1. Kahramanmaraş ili 2019 ve 2020 yılına ait meteorolojik verileri*

Months	Minimum temperature (°C)		Maximum temperature (°C)		Average relative humidity (%)		Total precipitation (mm)	
	2019	2020	2019	2020	2019	2020	2019	2020
January	-5.0	-4.2	16.1	14.1	84.5	82.3	265.8	105.8
February	-0.9	-3.2	19.5	18.7	83.0	78.1	111.6	75.2
March	-0.3	0.3	23.9	25.9	69.4	74.6	143.4	4.6
April	0.8	0.4	29.5	30.9	72.2	66.1	32.2	33.0
May	1.7	7.5	41.3	41.2	47.5	54.4	3.6	23.0
June	10.3	11.4	39.9	41.0	50.1	50.2	5.2	0.0
July	18.9	18.5	41.1	44.6	49.8	46.4	0.2	0.0
August	14.7	15.9	39.7	45.0	43.3	40.9	0.0	0.0
September	11.1	11.8	36.2	37.6	48.6	48.2	0.0	0.0

*Kahramanmaraş meteorology station

Before sowing seeds of BR and GC, the soil of the trial area was ploughed twice. NPK fertilizer, which is a mixture containing 15% nitrogen, 15% phosphorus, and 15% potassium at a rate of 500 kg ha⁻¹, was applied with half of the amount used during the sowing seeds of BR and GC, and the other half during the planting of tomato seedlings. BR and GC were planted on March 1, 2019, and February 1, 2020. Whole plant samples of both BR and GC were taken from 1 m² during the

flowering stage, the fresh weights (3.17 and 2.51 kg m⁻²) and the dry weights (0.78 and 0.57 kg m⁻²) of samples were determined, then ground and kept in the refrigerator at -4°C to use in greenhouse experiments and chemical analysis. In this stage whole plants were incorporated into the soil using a hoeing machine (30 cm depth), at the same time, mulch was applied. Tomato seedlings were planted on 10 June in the first year of the study and on 7 May in the second year. All

weeds were removed from Cnt 1 periodically, while only johnsongrass was left in Cnt 2.

Effect of treatments on biomarkers of johnsongrass growth

At the ripening stage of johnsongrass seeds, the density was calculated by determining the number of johnsongrass stems in 1 m² for each treatment separately. In the same way, the length of 30 stems was measured by a ruler of 2m in both 2019 and 2020.

In the last harvest phase of tomatoes, weed stems were cut from 1 m² area from each treatment, and fresh weight was calculated. The cut stems were dried at 25 ± 2°C in room conditions for 4 weeks then the dry weight was determined.

The rhizomes formed in the soil were collected by digging 1m² at a depth of 0.5 m per treatment, then they were cleaned from the soil and the fresh weight was determined (Figure 2). Rhizomes were dried under room conditions for a month and their dry weight was calculated.



Figure 2. Collecting johnsongrass plant rhizomes from the soil

Şekil 2. Geliç bitkisi rizomlarının topraktan toplanması

Control efficiency of Johnsongrass

Control efficiency refers to the rate of dry biomass reduction of johnsongrass both above-ground (stems) and in-soil parts (rhizomes) due to applied treatments. Control efficiency was calculated as a percentage of treatment reduction of biomass over Cnt 2 according to formula (1).

$$\text{Control efficiency} = [1 - (Wt/W0)] \times 100 \quad (1)$$

Where, Wt: dry weight of stems and rhizomes of johnsongrass in treatment; W0: dry weight of stems and rhizomes of johnsongrass in Cnt 2

Determine the effect of the treatments on the yield of tomato

To determine the average weight of tomato fruit grown under different treatments, the average weight of 10 fruits was calculated for each treatment separately for each harvest, and then the general average of all harvests was calculated.

The tomato yield was calculated from 1 m² for all harvesting operations for each treatment, and then the yield per hectare was calculated. Then the rate of increase in tomato yield over Cnt 2 (5016 kg ha⁻¹ in 2019 and 5975 kg ha⁻¹ in 2020) was calculated using formula (2).

$$\text{Tomato yield increase rate} = [(Yx/Y0) - 1] \times 100 \quad (2)$$

Where, Yx: yield of tomato in treatment, Y0: yield of tomato in Cnt 2.

The tomato yield loss rate caused by johnsongrass was also calculated under the effect of the treatments over Cnt 1 (47771 kg ha⁻¹ in 2019 and 64943 kg ha⁻¹ in 2020) using formula (3).

$$\text{Tomato yield loss rate} = [1 - (Yt/Yc)] \times 100 \quad (3)$$

Where, Yt: yield of tomato in treatment, Yc: yield of tomato in Cnt 1

To determine the difference in the quality of tomato fruits under the influence of the treatments, the fruits were analyzed in terms of nutritional content (glucose, fructose, protein, and potassium) and color characteristics (color depth). Tomato samples (2 kg) from the treatments were analyzed at Kahramanmaraş Sütçü Imam University Research and Application Development Center (USKIM).

Greenhouse Experiments

Experiment site and design

Greenhouse experiments were carried out in the greenhouse of Kahramanmaraş Sütçü Imam University Faculty of Agriculture in the years 2020-2021. The greenhouse was glass, with concrete floors, and a gable roof with dimensions of 16 × 8 m and a height of 5 m. The pots were placed on single-layer iron beds with 0.5 × 6.5 m dimensions, and 25 cm high from the floor, inside the greenhouse (Figure 3). Greenhouse climate conditions were recorded using a thermometer and hygrometer. The average maximum temperature was from 32°C to 34°C, the average minimum temperature was from 17°C to 18°C, and the average relative humidity was from 68% to 72%.

The greenhouse experiments were set up according to the completely randomized plot design with four replications twice in 2020 and 2021. BR and GC extracts were applied at 2, 5, and 10% concentrations against johnsongrass seeds and rhizomes.



Figure 3. Greenhouse experiment setting
Şekil 3. Sera deneme ortamı

Johnsongrass seeds and rhizomes used in the greenhouse experiments were collected from the field experiments after the last harvest of tomatoes. Johnsongrass seeds dormancy was broken by sandpaper, while rhizomes were cut 1 cm long and each part contained one bud (AL Sakran et al., 2020; Elsekran et al., 2023). Then 100 g of dried BR powder was weighed, and 1 liter of distilled water was added to prepare an extract with a concentration of 10%. Distilled water was added to obtain concentrations of 5 and 2%, and GC extracts were also prepared in the same way. The extracts were left at 25°C for 1 day and were manually agitated frequently, then filtered with filter papers of dimensions 50 × 50 cm twice. After preparing the extracts, 300 ml of each was added to pots containing 1:1:1 ratio of sand, soil, and peat in which one tomato seedling was planted, in addition to 20 seeds or 10 pieces of rhizome of johnsongrass. The experiment continued for forty days in greenhouse conditions.

Effect of extracts on the growth of johnsongrass and tomato seedlings

The germination rate of johnsongrass seeds and rhizomes was calculated at the end of the experiment according to formula (4).

Germination rate = (number of germinated seeds or rhizomes/total number of seeds or rhizomes) × 100 (4)

At the end of the experiment, johnsongrass stems were cut from the soil surface and their length was measured using a ruler.

These stems were dried under room conditions for 4 weeks and their dry weight was calculated.

Tomato plant height was measured with a plastic ruler (100 cm) 40 days after planting seedlings.

Determination of ITCs

To determine the ITCs content of BR and GC by GC-MS analysis, samples were prepared according to the method described by Vaughn and Berhow (2005); and

Elsekran et al. (2023). First, the samples were defatted using hexane, then dichloromethane and potassium phosphate were added, and placed in a shaker for 8 hours at 25 °C and 200 rpm. Then, NaCl and Na₂SO₄ were added and mixed. The mixtures were filtered by filter paper. Dichloromethane solutions were placed in ampoules and numbers were given according to the plant. Samples were analyzed in Ataturk University, Department of Chemistry by GC-MS.

Data Analysis

Data from field studies in 2019, and 2020 were subjected to multivariate analysis of variance (MANOVA) by IBM SPSS 26.0 program. Differences between values were grouped using the LSD test ($P \leq 0.05$). Greenhouse data in 2020, and 2021 were subjected to one-way analysis of variance (ANOVA). Differences between values were grouped using Duncan's test ($P \leq 0.05$). Data from field experiments in 2019 and 2020 and greenhouse in 2020 and 2021 were compared by t-test. There were no significant differences between the two years of study for greenhouse experiments, so the average results of the two years were analyzed.

RESULTS

Field Experiments

Effect of treatments on biomarkers of johnsongrass growth

There was no significant difference between the years of study for all treatments (Table 2). Johnsongrass density was very high in Cnt 2 parcels (426.7 and 448.8 number m⁻² in 2019 and 2020). The lowest johnsongrass density was recorded in GC+M and BR+M treatment (97.2, 106.7, and 101.7, 115.3 number m⁻² in 2019 and 2020 respectively). While the highest density was observed in mulch treatment in both years.

The shortest stem length of johnsongrass was observed in the GC+M treatment with 90.2 cm in 2019 and 92.9 cm in 2020. While the length of the stem was higher in the treatment of mulch. It was also found that there were no significant differences in terms of stem length in GC and BR treatments with and without mulch, as well as between the years of study. The average stem length of johnsongrass in the Cnt 2 was 142.7 and 144.5 cm in the two years of the study (Table 2).

The lowest fresh and dry stem weight was recorded in GC+M and BR+M treatments, followed by GC and BR. While the fresh weight in the GC+M treatment was 431.7 and 466.7 g m⁻², it was 3152.8 and 3239.2 g m⁻² in the Cnt 2. As for the dry weight, it was 92.2 and 96.8 g m⁻² in the GC+M treatment and 625.1 and 661.1 g m⁻² in the Cnt 2 in the two years of the study (Table 3).

Table 2. The density (number m⁻²) and stem length (cm) of johnsongrass
Çizelge 2. Geliş bitkisi yoğunluğu (adet m⁻²) ve sap uzunluğu (cm)

Treat.	Density (number m ⁻²)			Stem length (cm)		
	2019	2020	t Test	2019	2020	t Test
*BR	227.3bc**	233.6b	Sig.=0.638	93.87a	94.3a	Sig.=0.927
GC	209.1b	228.2b	Sig.=0.126	91.2a	93.6a	Sig.=0.679
BR+M	106.7a	115.3a	Sig.=0.482	93.7a	93.9a	Sig.=0.972
GC+M	97.2a	101.7a	Sig.=0.750	90.2a	92.9a	Sig.=0.548
Mulch	248.1c	251.7b	Sig.=0.314	105.9b	107.8b	Sig.=0.651
Cnt 2	426.7d	448.8c	Sig.=0.184	142.7c	144.5c	Sig.=0.672
LSD	25.6	27.0		8.1	10.9	

*BR: Black radish, GC: Garden cress, BR+M: Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch, Cnt 2: Johnsongrass control.

**Values followed by the same letter(s) in the same column do not differ significantly from each other ($p \leq 0.05$).

Table 3. The wet and dry weight of johnsongrass stems (g m⁻²)
Çizelge 3. Geliş bitkisi saplarının yaş ve kuru ağırlığı (g m⁻²)

Treat.	Stems wet weight (g m ⁻²)			Stems dry weight (g m ⁻²)		
	2019	2020	t Test	2019	2020	t Test
*BR	1027.8b**	1078.9b	Sig.=0.273	224.5b	229.9b	Sig.=0.773
GC	929.2b	1017.8b	Sig.=0.117	201.6b	218.7b	Sig.=0.349
BR+M	486.7a	549.2a	Sig.=0.154	102.8a	111.6a	Sig.=0.349
GC+M	431.7a	466.7a	Sig.=0.244	92.2a	96.8a	Sig.=0.440
Mulch	1312.4c	1355.2c	Sig.=0.454	263.0c	281.6c	Sig.=0.256
Cnt 2	3152.8d	3239.2d	Sig.=0.460	625.1d	661.1d	Sig.=0.070
LSD	124.7	123.1		29.4	29.0	

*BR: Black radish, GC: Garden cress, BR+M: Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch, Cnt 2: Johnsongrass control.

**Values followed by the same letter(s) in the same column do not differ significantly from each other ($p \leq 0.05$).

Johnsongrass produced large amounts of rhizomes that were 2502.2 and 2513.0 g m⁻² in the Cnt 2 in the two years of the study. The wet weight of rhizomes decreased significantly in GC+M and BR+M treatments and was 396.5, 490.8, and 438.5, 564.2 g m⁻² in the two years of study, respectively (Table 4).

Although the growth of johnsongrass was prevented in Cnt 1, this procedure did not exhaust all rhizomes in the soil, as 125.0 g m⁻² fresh weight of the rhizomes were collected in 2019 and 136.7 g m⁻² in 2020. The effect of mulch treatment on the dry weight of rhizomes was weak (576.2 and 576.6 g m⁻²) and did not differ significantly from Cnt 2.

The effects of GC and BR treatments with and without mulch on the dry weight of rhizomes were similar to their effects on the fresh weight, and no significant difference was observed between the two years of study (Table 4).

Control efficiency of Johnsongrass

The highest control efficiency of johnsongrass was

achieved by GC+M treatment which was 84.8% in 2019 and 84.0% in 2020. The lowest control efficiency was calculated in the mulch treatment, and it was 33.4 and 32.8% in the two years of the study. Figure (4) shows the efficiency of the applied treatments in johnsongrass control in the two years of the study and the significant differences between them.

The effect of the treatments on the yield of tomato

The highest weight of tomato fruits was obtained from Cnt 1 (164.6 and 166.2 g), and there was no significant difference between this treatment and GC+M and BR+M treatments in both years of the study. The fruit weight was statistically similar in all applied control methods. The weight of the fruit in Cnt 2 decreased significantly from the other treatments and the lowest weight of the fruits was recorded in it (76.5 and 80.8 g) in the two years of the study (Figure 5).

Johnsongrass caused losses at different rates to the tomato yield according to the applied treatments. The lowest rate of losses was achieved in the GC+M treatment (10.4 and 16.7%) in the two years of study.

The rate of loss in Cnt 2 was very high (89.3 and 90.1%), and the highest rate of loss was in the BR and mulch treatments. There were also significant differences between the two years of the study, and the rate of loss in production in 2020 was greater than in

2019 in all treatments (Table 5). The reason is that the tomato season in 2020 was longer than in 2019. That means the increase in the length of the season was in the interest of the weed, not the cultivated plant.

Table 4. The wet and dry weight of johnsongrass rhizomes ($g\ m^{-2}$)

Çizelge 4. Geliç bitkisi rizomlarının yaş ve kuru ağırlığı ($g\ m^{-2}$)

Treat.	Rhizomes wet weight ($g\ m^{-2}$)			Rhizomes dry weight ($g\ m^{-2}$)		
	2019	2020	t Test	2019	2020	t Test
*Cnt 1	125.0a**	136.7a	Sig.=0.421	30.7a	33.5a	Sig.=0.700
BR	1159.5c	1348.3c	Sig.=0.068	283.6c	321.1c	Sig.=0.326
GC	1049.5c	1286c	Sig.=0.053	257.6c	314.4c	Sig.=0.052
BR+M	490.8b	564.2b	Sig.=0.187	121.5b	140.6b	Sig.=0.391
GC+M	396.5b	438.5b	Sig.=0.196	96.1b	107.1b	Sig.=0.426
Mulch	2292.1d	2310d	Sig.=0.866	562.2d	576.6d	Sig.=0.802
Cnt 2	2502.2f	2513.0f	Sig.=0.923	613.7d	615.3d	Sig.=0.965
LSD	165.8	144.9		63.4	64.3	

*Cnt 1: weed-free control, BR: Black radish, GC: Garden cress, BR+M: Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch, Cnt 2: Johnsongrass control.

**Values followed by the same letter(s) in the same column do not differ significantly from each other ($p \leq 0.05$).

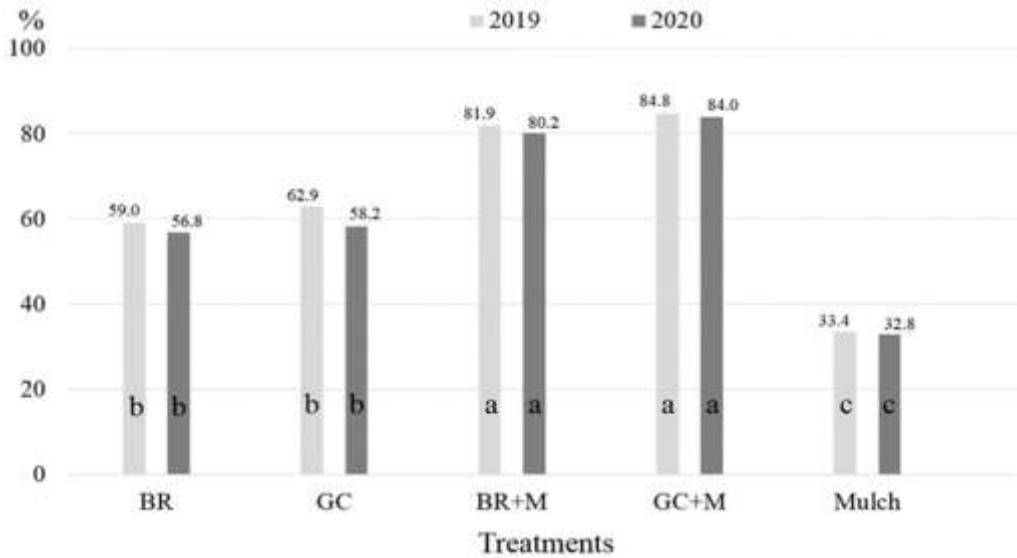


Figure 4. Control efficiency of johnsongrass (%)

Şekil 4. Geliç bitkisi mücadele etkinliği (%)

BR: Black radish, GC: Garden cress, BR+M: Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch.

LSD ($p \leq 0.05$) = 6.8 (2019) and 6.4 (2020). The same letter(s) do not differ significantly ($p \leq 0.05$).

The highest rate of increase in tomato yield was obtained in GC+M (737.2 and 741.7%) in the two years of study. The lowest rate of increase in yield was in the treatments of mulch, BR, and GC. With the exception of Cnt 1 and mulch, there was no significant difference between the two years of study in the rate of increase in yield (Table 5).

According to the analysis of the nutritional content and color of tomato fruits, the GC+M treatment was superior to the other treatments in characteristics of tomato fruits, followed by the BR+M treatment and

Cnt 1, while the quality of fruits in Cnt 2 was very low (Table 6).

Greenhouse Experiments

Germination of johnsongrass

GC extract at a concentration of 10% was more effective in reducing the germination rate of seeds (7.50%) than BR extract (17.50) while it was statistically similar against rhizomes (12.50 and 20.00%). With the exception of GC extract, which stimulated seed germination (87.50%), the low

concentration (2%) had no effect on the germination of seeds and rhizomes. There was an increase in the

negative effect on johnsongrass germination with increasing concentration of extracts (Figure 6).

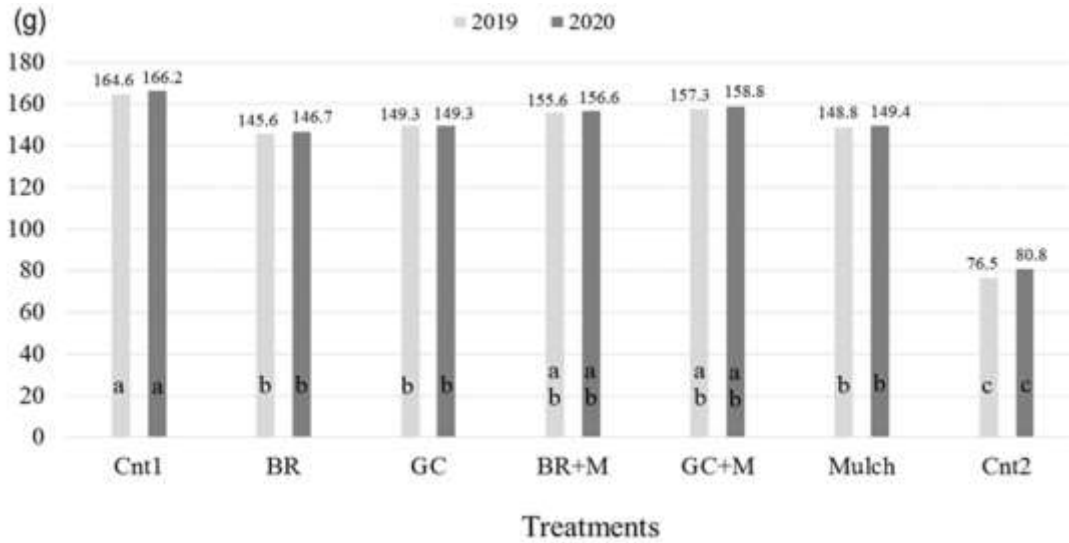


Figure 5. The average weight of tomato fruit (%)

Şekil 5. Domates meyvesinin ortalama ağırlığı (%)

Cnt 1: weed-free control, BR: Black radish, GC: Garden cress, BR+M: Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch, Cnt 2: Johnsongrass control.

LSD ($p \leq 0.05$) = 13.2 (2019) and 14.5 (2020). The same letter(s) do not differ significantly ($p \leq 0.05$).

Table 5. Tomato yield loss and yield increase (%)

Çizelge 5. Domateste verim kaybı ve verim artışı (%)

Treat.	Tomato yield loss (%)			Tomato yield increase (%)		
	2019	2020	t Test	2019	2020	t Test
*Cnt 1	-	-		834.5a	910.2a	Sig.=0.028
BR	40.3d	45.5d	Sig.=0.038	457.9d	450.5d	Sig.=0.667
GC	36.3c	43.7c	Sig.=0.005	495.3d	468.7d	Sig.=0.160
BR+M	19.2b	23.5b	Sig.=0.005	655.1c	672.8c	Sig.=0.081
GC+M	10.4a	16.7a	Sig.=0.001	737.2b	741.7b	Sig.=0.554
Mulch	36.7cd	44.6cd	Sig.=0.001	491.6d	459.6d	Sig.=0.017
Cnt 2	89.3f	90.1f	Sig.=0.196	-	-	
LSD	3.8	1.6		38.4	19.1	

*Cnt 1: weed-free control, BR: Black radish, GC: Garden cress, BR+M: BR+M Black radish + mulch, GC+M: garden cress + mulch, Mulch: Black polyethylene mulch, Cnt 2: Johnsongrass control.

Values followed by the same letter(s) in the same column do not differ significantly from each other ($p \leq 0.05$).

Table 6. Tomato fruits content of some nutrients and color depth

Çizelge 6. Domates meyvelerinin bazı besin içerikleri ve renk derinliği

Treat.	Glucose (%)	Fructose (%)	Protein (%)	Potassium (mg kg ⁻¹)	Color depth (%)		
					L*	a*	b*
Cnt 1	3.28	2.12	5.32	29.76	37.21	33.80	27.92
BR	2.61	1.90	4.71	28.81	38.71	33.03	27.40
GC	2.80	1.71	4.90	28.90	36.30	32.72	26.73
BR+M	3.35	2.11	5.21	29.15	36.90	33.20	27.51
GC+M	3.44	2.29	5.43	29.69	36.61	34.40	27.53
Mulch	2.68	1.90	5.10	28.51	37.11	33.23	28.10
Cnt 2	1.10	0.91	4.03	28.22	39.26	30.18	25.47

*=D65 was made with daylight and 10 degrees' perspective. The fruits' color was L (brightness; 100 white, 0 black), a (+ red; - green) and b (+ yellow; - blue) was measured on the cheek area (Kaymak et al., 2010).

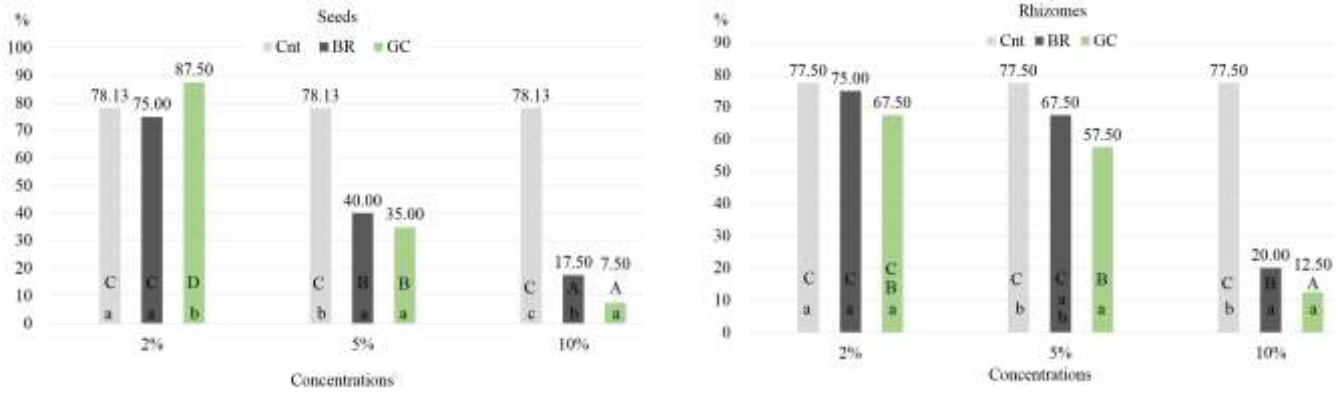


Figure 6. Effects of extracts on germination of johnsongrass seeds and rhizomes (%)

Şekil 6. Özütlelerin geliş bitkisi tohum ve rizomlarının çimlenme üzerine etkileri (%)

Cnt: Control, BR: Black radish, GC: Garden cress.

The same small letter(s) in the same concentration group and the same capital letter(s) at different concentrations of the same plant extract do not differ significantly according to Duncan's test ($p \leq 0.05$).

Stem length of Johnsongrass

Concentrations of 5 and 10% of the extracts of both plants significantly reduced the length of stems growing from seeds and rhizomes. While the

concentration of 2% was effective on the stem length of the rhizomes only. It was also found that GC was more effective in reducing the stem length of johnsongrass than BR (Figure 7).

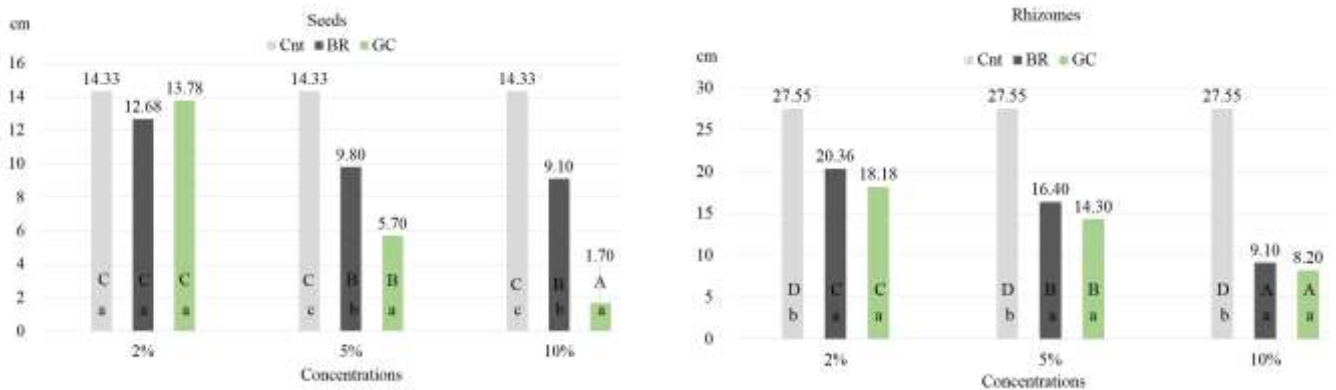


Figure 7. Effects of extracts on stem length of johnsongrass seeds and rhizomes (cm).

Şekil 7. Özütlelerin geliş tohum ve rizom sürgünleri uzunluğuna etkileri (cm)

Cnt: Control, BR: Black radish, GC: Garden cress.

The same small letter(s) in the same concentration group and the same capital letter(s) at different concentrations of the same plant extract do not differ significantly according to Duncan's test ($p \leq 0.05$).

Dry weight of Johnsongrass

GC and BR extracts at 10% concentration significantly reduced the dry weight of stems growing from seeds (0.01 and 0.11 g) compared to the control (0.97 g). Also, the dry weight of the stem of the rhizomes in these treatments was 0.13 and 0.18 g, compared to 2.07 g in the control. All concentrations of extracts of both plants (except for GC 2% on seeds) had a significant effect on reducing the dry weight of stems grown from both seeds and rhizomes (Figure 8).

Tomato seedling length

The growth of tomato seedlings in the greenhouse experiment was affected by different concentrations of GC extracts. The highest seedling length was obtained from GC extract at a concentration of 10% (33.8 cm)

and the shortest from BR extract at a concentration of 2% (23.9 cm). No negative effect of extracts was observed for all concentrations on the length of tomato seedlings. Figure (9) shows the effect of BR and GC extracts on the length of tomato seedlings.

Determination of ITCs

According to GC-MS analysis, 5 different ITCs were determined as a result of GSL components hydrolysis in BR. These components were tert-butyl-ITC, 2-propenyl-ITC, benzyl-ITC, 4-methylthio-3-butenyl-ITC, and 2-phenylethyl-ITC, while their ratios were determined as 4.7, 5.6, 6.6, 19.3, and 4.2%, respectively, and their total percentages were 40.4% (Figure 10).

Only one ITC compound was recorded in GC, which is

benzyl-ITC, and its percentage was 61%, as shown in Figure (11).

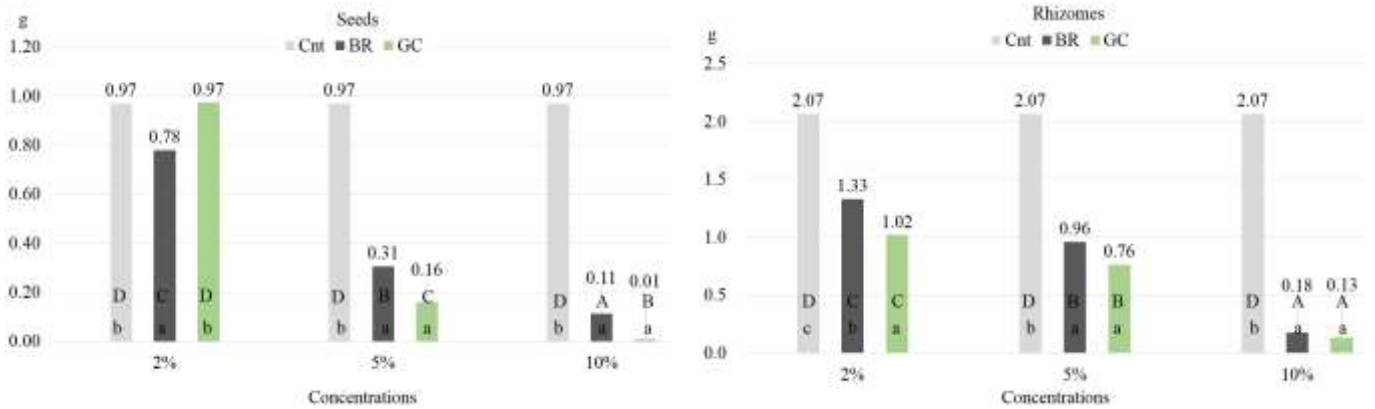


Figure 8. Effect of extracts on the dry weight of johnsongrass grown from seeds and rhizomes (g)

Şekil 8. Özütlerin tohum ve rizomlardan süren geliş kuru ağırlığına etkileri (g)

Cnt: Control, BR: Black radish, GC: Garden cress.

The same small letter(s) in the same concentration group and the same capital letter(s) at different concentrations of the same plant extract do not differ significantly according to Duncan's test ($p \leq 0.05$).

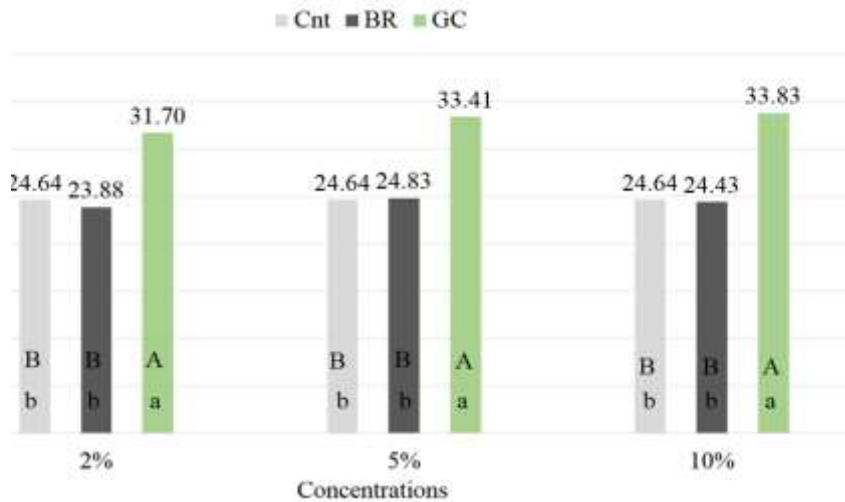


Figure 9. The effect of extracts on the length of tomato seedlings (cm)

Şekil 9. Özütlerin domates fide uzunluğuna etkileri (cm)

Cnt: Control, BR: Black radish, GC: Garden cress.

The same small letter(s) in the same concentration group and the same capital letter(s) at different concentrations of the same plant extract do not differ significantly according to Duncan's test ($p \leq 0.05$).

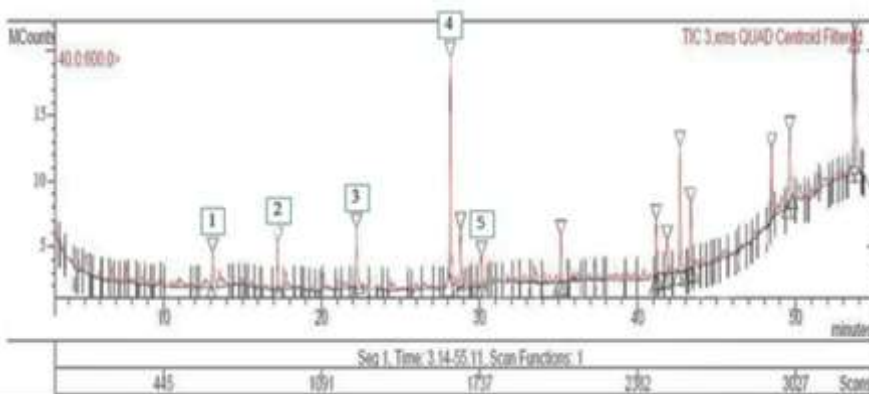


Figure 10. ITC components in black radish (%)

Şekil 10. Siyah turpta ITC bileşenleri (%)

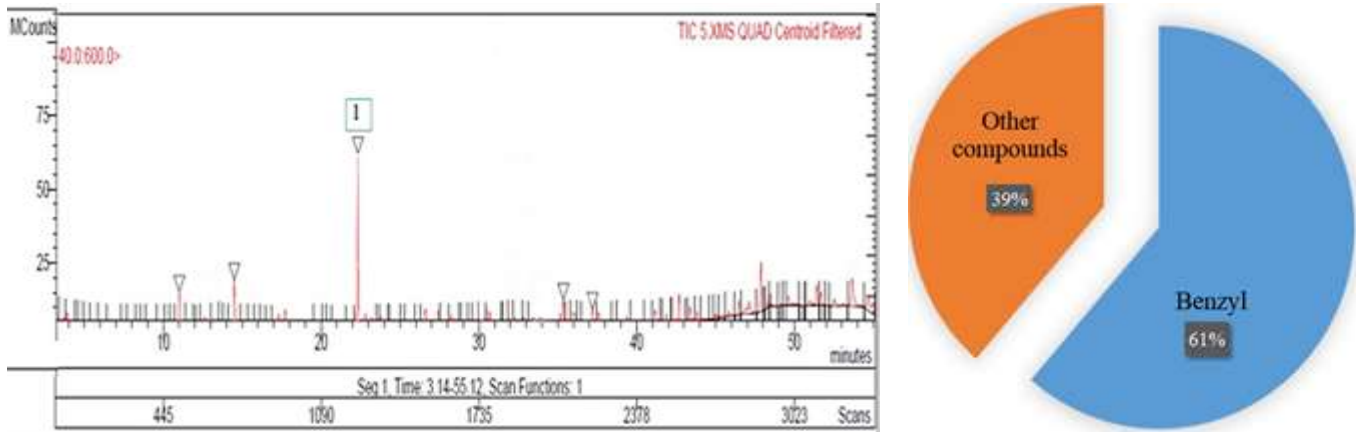


Figure 11. ITC components in garden cress (%)
Şekil 11. Terede ITC bileşenleri (%)

DISCUSSION

The treatments of BR+M and GC+M were the most effective in reducing the growth of johnsongrass stems, where the fresh biomass in these treatments was (466.7 and 549.2 g m⁻²) while it was 3239.2 g m⁻² in Cnt 2.

According to Bangarwa and Norsworthy (2014), cruciferous plants (*B. rapa*, *B. juncea*, and *S. alba*) + mulch decreased johnsongrass fresh weight by 34-46%. The reason for increasing the effectiveness of BR and GC compared to these plants may be due to the differences in their ITCs content. Many studies mentioned that the ITCs content of cruciferous plants varies according to variety, species, and growth conditions (Nakamura et al., 2008; Shah et al., 2016; Elsekran et al., 2023).

BR+M and GC+M treatments reduced fresh rhizome biomass with high efficiency (564.2 and 438.5 g m⁻²) compared to Cnt 2 (2513.0 g m⁻²). On the other hand, cruciferous plants contain GSLs, which are enzymatically hydrolyzed in a pH-neutral medium to ITCs (Uda et al., 1986). The results of the analysis showed that the soil in the study site is neutral in acidity (pH=7.04), which is an ideal medium for the decomposition of GSLs into ITCs.

Mulch treatment had no effect on rhizomes, although it reduced the biomass of stems. Johnsongrass was unable to penetrate the mulch used in this experiment. The growth space was limited to the holes designated for tomato seedlings, so the application of mulch reduced the biomass of johnsongrass above the soil surface. The rhizomes were observed in the form of a net on the surface of the soil under the mulch, growing densely, and this explains the lack of reduction in the biomass of the rhizomes compared to Cnt 2.

Covering BR and GC with mulch immediately after incorporating them into the soil increased the control efficiency of johnsongrass (80.2 and 84.0%) compared to them without mulch (56.8 and 58.2%), while the

efficacy of mulch alone was less (32.8%). The reason may be due to the fact that ITCs are short-lived compounds in the soil, and they volatilize from it quickly (van Ommen Kloeke et al., 2012; Price and Norsworthy 2013). The use of mulch leads to an increase in the life of these compounds in the soil, and thus an increase in the period of exposure of roots and rhizomes to them, thus the effect on their growth. The control efficiency of BR+M and GC+M treatments was relatively high and may be sufficient to be an effective alternative to herbicides in controlling johnsongrass. Ustuner et al. (2023) showed that chemical control using fluazifop-P-butyl (1.5 L ha⁻¹) achieved johnsongrass control by 69.8%, while according to Karkanis et al. (2022), this percentage was 90%.

According to the results of this research, the control methods that had the greatest impact on the yield and quality of tomatoes were the pre-plant with mulch treatments. Pre-plant with mulch treatments achieved the highest tomato plant fruit quality, sugar, protein percentage, and color characteristics. The highest increase in tomato yield was GC+M treatment and ranged between 737.2 and 741.7% in two years of study. This is due to the low density of johnsongrass in this treatment, as the tomato plants may have benefited from the organic materials and ITCs contained in the GC. Also, it was shown in greenhouse experiments that GC extracts have an effect that stimulates the growth of tomato seedlings.

The results of the greenhouse experiments were consistent with those obtained from the field experiments. BR and GC extracts at 10% concentration were effective in reducing the germination of seeds (17.5 and 7.5%) and rhizomes (20.0 and 12.5%) of johnsongrass, compared to the controls (78.13 and 77.5%).

Elsekran et al. (2023) showed that cruciferous plants such as garden rockets at 10% concentration reduce the germination of johnsongrass seeds by 100.0% and rhizomes by 83.9%. The difference is due to the fact

that Elsekran et al. (2023) carried out the experiment in closed petri dishes, which prevented the ITCs from volatilizing and thus increased the effect period on johnsongrass seeds and rhizomes. In addition, there is a difference between the species of cruciferous plants used in the two experiments.

Extracts of BR and GC at a concentration of 10% reduced the dry biomass of johnsongrass both grown from seeds and rhizomes in a greenhouse experiment. Also, the effect of the extracts at a concentration of 5% was significant in reducing the dry biomass. These results are similar to what Uremis et al. (2009) reached when using BR extracts at a concentration of 8% on johnsongrass rhizomes, where the rate of inhibition of rhizome germination was 45.5%.

Plant extracts at all concentrations did not have a negative effect on the growth of tomato seedlings, while the effect of GC extracts at all concentrations was stimulative. The reason is that the negative effect of allelopathic substances in cruciferous plants is greater on the seeds and this effect decreases on established plants. Bangarwa et al. (2012) showed that tomato seedlings were not negatively affected by the allelochemicals contained in cruciferous plants. Some research also indicated that allelochemicals can play as plant growth regulators that enhance the growth of cultivated plants (Bellostas et al., 2007; Jabran 2017).

According to the results of GC-MS analysis, five ITC compounds were detected in BR, and their total percentages were 40.4% of the GSLs hydrolysis products. It was also found that the dominant ITC was 4-methylthio-3-butenyl-ITC, which is an aliphatic compound with a percentage of 19.3%. Yi et al. (2015) reported that one of the most abundant GSL components in radish is glucoraphasatin, which hydrolyses into 4-methylthio-3-butenyl-ITC.

In GC, it was found that there is only one compound that belongs to the ITC-aromatic group, which is benzyl-ITC with a percentage of 61.0%. Radwan et al. (2007), and Sarikami and Yanmaz (2011) reported benzyl-GSL, the major GSL component of GC.

CONCLUSION

The results of these experiments showed that both black radish and garden cress have strong allelopathic potentials that inhibit the germination and growth of johnsongrass seeds and rhizomes very effectively, especially if they are used with black plastic mulch immediately after incorporating them into the soil. The density of johnsongrass in both black radish and garden cress with plastic black mulch treatments was 97.2, 106.7, and 101.7, 115.3 number m⁻² in 2019 and 2020 respectively, while it was 426.7 and 448.8 number m⁻² in 2019 and 2020 in the control plots respectively. Also, the control efficiency of johnsongrass was achieved by both black radish and garden cress with

plastic black mulch treatments of 84.8% and 81.9%, respectively. This process will reduce the bank of seeds, and the rhizomes of johnsongrass in agricultural lands, hence the reduction of its population gradually in the following years. Thus, sustainable management of johnsongrass can be achieved by introducing black radish and garden cress into a crop rotation which may be an alternative to or reduce the use of herbicides.

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Conflicts of Interest

Authors have declared no conflict of interest.

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The Effects of Boron-Containing Compounds against *Monilinia fructigena* Mycelium Growth

Ferah YILMAZ¹, Şaban KORDALI², İsmail ŞEN³, Gülsüm PALACIOĞLU⁴

Department of Plant Protection, Fethiye Faculty of Agriculture, Muğla Sıtkı Koçman University, Muğla, Türkiye, ²Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Türkiye

¹<https://orcid.org/0000-0003-0954-7478>, ²<https://orcid.org/0000-0001-5669-5831>, ³<https://orcid.org/0000-0001-5760-5535>,

⁴<https://orcid.org/0000-0002-3603-2413>

✉: gulsumpalacioglu@mu.edu.tr

ABSTRACT

Monilinia fructigena is the causative agent of brown rot in pome fruits, contributing to substantial economic losses, especially in storage facilities. The effects of boron-containing compounds have been considered as an environmentally friendly alternative to fungicides. The objective of the study was to determine suitable boron-containing compounds for inhibiting the mycelium growth of *M. fructigena*. Eight different compounds with pH adjusted to neutral (pH 7) and non-neutral were tested with concentrations of 0 (untreated control), 5, 10, 20, 40, 60, 80, and 100 mM *in vitro* conditions. The mycelium growth of the pathogen was totally inhibited with the application of 20 mM of potassium tetrafluoroborate and 10 mM of sodium tetrafluoroborate. The tested concentrations of ammonium pentaborate tetrahydrate, antidot-67, sodium metaborate, and sodium tetraborate decahydrate were not sufficiently effective in inhibiting the mycelium growth of *M. fructigena*, but the experiment of higher concentrations of them could be utility against the pathogen. The pH of boron-containing compounds was crucial in improving the efficacy of compounds, and the non-neutral compounds showed better results against inhibition of *M. fructigena* mycelium growth. The results showed that boron-containing compounds may be pathogen-specific and that the activity of these compounds is related to pH.

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ÖZET

Monilinia fructigena yumuşak çekirdekli meyvelerde kahverengi çürüklük etmenidir ve özellikle depolama tesislerinde önemli ekonomik kayıplara neden olmaktadır. Bor içeren bileşiklerin patojenlere karşı etkileri ise fungusitlere alternatif çevre dostu bir uygulama olarak değerlendirilmektedir. Bu çalışmanın amacı *M. fructigena*'nın misel gelişimini inhibe etmek için uygun bor içeren bileşiklerin belirlenmesidir. Bu bağlamda, nötr (pH 7) ve nötr olmayan pH ile ayarlanmış sekiz farklı bor bileşiğinin, sekiz farklı konsantrasyonun (0; işlenmemiş kontrol, 5, 10, 20, 40, 60, 80 ve 100 mM) patojene etkililikleri *in vitro* koşullarda araştırılmıştır. Uygulamalar arasında 20 mM potasyum tetraflorborat ve 10 mM sodyum tetraflorboratın fungusun miselyum gelişimini tamamen engellediği tespit edilmiştir. Amonyum pentaborat tetrahidrat, etidot-67, sodyum metaborat ve sodyum tetraborat dekahidratın test edilen konsantrasyonları etmenin miselyum gelişimini yeterince inhibe etmemiş, ancak daha yüksek konsantrasyonlarının patojene karşı daha yüksek etki gösterebilecekleri değerlendirilmiştir. Bor içeren bileşiklerin pH'sı, bileşiklerin etkinliğinin artırılmasında önemli bir kriter olmuş ve nötr olmayan bileşiklerin *M. fructigena*'nın miselyum gelişiminin inhibisyonunda daha iyi sonuçlar verdiği gözlenmiştir. Elde edilen sonuçlar, bor içeren bileşiklerin etmene spesifik olabileceğini ve bu bileşiklerin etkinliğinin pH ile ilişkili olduğunu göstermiştir.

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INTRODUCTION

Monilinia fructigena (Aderhold and Ruhland) Honey is one of the most important fungal pathogens which cause brown rot on pome fruits. The pathogen generally infects the fruits before and during storage, while blossom and branch infections in plants are rarely observed (Hrustić et al., 2012). Wounds or growth cracks inflicted by insects and birds cause increased disease occurrence and are known to cause economic losses of more than 1.5 million euros (Batra, 1991; Bautista-Baños, 2014). Brown rot pathogens primarily infect the fruits, and the disease can occur during storage, so reducing fruit injuries during harvest is of great importance for managing the disease (Van Leeuwen et al., 2000). However, fungicides are mostly used to control brown rot in orchards, but the usage of fungicides is not allowed by the European Union as well as Türkiye during the post-harvest and in storage facilities (Karabulut & Baykal, 2004; Hrustić et al., 2012; 2018). Alternative control methods are needed due to the formation of resistance to fungicides such as thiophanate-methyl and benzimidazoles, which are commonly used against the pathogen (Ivić et al., 2021). Eco-friendly disease control methods with UV-C and heat treatment, biocontrol using some yeasts, heated water immersion, thyme essential oils and boron-containing compounds are used by many researchers (Marquenie et al., 2002; Larena et al., 2005; Karabulut et al., 2010; Thomidis & Exadaktylou, 2010; Mari et al., 2012; Tanovic et al., 2015; Thomidis et al., 2017; Yildiz & Coskuntuna, 2019; Zhang et al., 2020; Ardiç et al., 2021).

Boron products such as boric acid and borax can be used as food preservatives against some types of bacteria and fungi (Estevez-Fregoso et al., 2021). It has been stated that boron application in fruits reduces cuticular microcracks and membrane permeability, and is protective against brown rot factors, especially in storage facilities (Xuan et al., 2000; Thomidis & Exadaktylou, 2010). Various boron-containing compounds, such as Borax, Power B, and the commercial product Nutrel B, have been reported to inhibit *Monilinia laxa* mycelium growth (Thomidis & Exadaktylou, 2010; Thomidis et al., 2017). Similarly, different researchers have reported the inhibitory effects of boric acid and potassium tetraborate on *Botrytis cinerea* (Omama & Karima, 2007; Qin et al., 2010). The number of identified boron-containing compounds is increasing because of improved natural identification and innovative synthesis procedures, and there is a need to determine the species-specific

effects of these compounds on fungi, to find environmentally friendly preservatives, and to select suitable boron-containing compounds and their appropriate concentrations as preservatives against specific fungal pathogens (Estevez-Fregoso et al., 2021).

The effectiveness of boron depends on concentration and pH (Woods 1994). Therefore, determining the optimum pH values of the compounds is very important in terms of increasing their effectiveness. Qin et al. (2010) stated that alkaline potassium tetraborate gave better results than the neutral solution in the control of *B. cinerea*. The pH of the boron compounds tested against *M. laxa* was determined as 7 by Thomidis & Exadaktylou (2010) and Thomidis et al. (2017). The aims of this study were (i) to determine the effects of several boron-containing compounds on *M. fructigena* mycelium growth, (ii) to find suitable concentrations of these compounds, and (iii) to evaluate the interactions between boron-containing compounds and pH with *M. fructigena* mycelium growth.

MATERIAL and METHODS

Isolation of *Monilinia fructigena*

Monilinia fructigena was isolated from an infected Granny Smith apple tree. The infected parts of the tree including fruits and blossoms were sampled and transferred to the laboratory. The surface of the collected samples was sterilized superficially to avoid the development of other microorganisms that might exist on the samples. The samples were washed with tap water and then sterilized with 0.5% sodium hypochlorite for 5 minutes. The sterilized samples were cleaned by rinsing sterile distilled water three times and dried on sterile filter paper. The infected tissues were cut into 2–5 mm pieces with sterile scalpel and inoculated into media of 2% w/w potato dextrose agar (PDA-Merck Millipore) which includes 0.5 g/L streptomycin sulfate powder (Sigma Aldrich) to avoid bacterial contamination. The Petri dishes were incubated at 25 °C for 12 hours for consecutive dark and light periods in a controlled climate chamber. After seven days, the cultures were sub-cultured to a fresh nutrient medium to obtain pure *M. fructigena* isolate. The morphological characters such as color, aerial mycelium, margin shape, etc. were determined under a light microscope for characterization of the isolate (Batra, 1991). The obtained culture of *M. fructigena* has been confirmed by Lane (2002) and Van Leeuwen et al. (2002). The morphological identification

was also confirmed with PCR assay as described by Côté et al. (2004). The *M. fructigena* culture was stocked at 4 °C until the experiments.

Experimental Design

Eight different boron-containing compounds given in Table 1 were used to determine the effects on *M. fructigena* mycelium growth. The final concentrations of tested boron-containing compounds were provided as 0 (untreated control), 5, 10, 20, 40, 60, 80, and 100 mM. Furthermore, two blocks were created to evaluate how the pH value influenced the efficacy of each tested compound. At the first, the pH of each growth media was measured by using electronic probes (Mettler Toledo S-210) and observed pH values were given in Table 1. In this experiment, the pH of tested media was not intervened and was considered as non-neutral media. In the second, the pH of growth media was adjusted to 7 (\pm 0.2 unit) by using hydrochloric acid

(1N) for alkaline media and potassium hydroxide (1N) or sodium hydroxide (1N) for acidic media and considered as neutral media. The neutral (pH adjusted as 7.0) and non-neutral growth media were used as a factor for each concentration of tested compounds. Agricultural chemicals called Luna® 450 (200 g/L fluopyram + 200 g/L tebuconazole; 25 ml/100 L water) and Signum WG (26.7% boscalid + 6.7% pyraclostrobin; 40 g/100 L water) which are known to control *M. fructigena* mycelium growth were also used as control at the doses recommended by Republic of Türkiye Ministry of Agriculture and Forestry. The solutions of each compound were prepared in sterile distilled water and added to 2% w/w potato dextrose agar (PDA). The media was poured into 9 cm Petri dishes and 8 mm diameter agar disks taken from actively growing *M. fructigena* cultures were inoculated and incubated at 25 °C. Six replicates were used in each experiment and repeated twice.

Table 1. pH values of Boron-containing compounds used in the study

Çizelge 1. Çalışmada kullanılan bor içerikli bileşiklerin pH değerleri

Compounds	Tested pH (H ₂ O)							
	0	5	10	20	40	60	80	100
Ammonium pentaborate tetrahydrate	5.5	6.2	6.3	6.5	6.6	6.8	6.8	6.8
Boron oxide	5.5	5.8	5.7	5.7	5.5	5.5	5.4	5.3
Etidot-67	5.5	7.1	7.2	7.2	7.3	7.3	7.4	7.5
Potassium metaborate	5.5	7.1	7.6	7.9	8.1	8.3	8.4	8.4
Potassium tetrafluoroborate	5.5	5.4	5.1	4.6	4.2	3.9	3.6	3.6
Sodium metaborate tetrahydrate	5.5	7.5	7.9	8.2	8.4	8.6	8.7	8.7
Sodium tetraborate decahydrate	5.5	7.2	7.3	7.4	7.6	7.7	7.8	7.9
Sodium tetrafluoroborate	5.5	4.9	4.9	4.7	3.9	3.6	3.5	3.5

After seven days of incubation, the colony diameter of each Petri dish was calculated to evaluate the effects of boron-containing compounds on *M. fructigena* mycelium growth. A digital caliper (MarCal 16 EWR IP 67) was used to measure the colony diameter taking two orthogonal measurements and the final colony diameter per Petri dish was calculated as the average of both measurements. The colony growth of treatments was calculated as the diameter of the final colony in Petri dishes minus the diameter of the preliminary agar disk (8 mm). The mycelial development inhibition rates (%) of boron compounds on the 7th day were calculated with the Abbott formula (Karman, 1971).

Statistical Analysis

R software environment (version 3.6.2, R Core Team 2017) was used to carry out the statistical analysis. Before analysis, the normality and homogeneity of data were checked by Shapiro-Wilk (shapiro.test

function) and Levene test (leveneTest function in “car” package). The datasets were transformed by Ordered Quantile Normalization (QRQ) to meet the assumptions of Analysis of Variances (ANOVAs) and some noises were added to datasets to break down ties by using the “jitter” function (Peterson & Cavanaugh, 2019). The datasets were back transformed to obtain means of data and to create the illustrations. The effects of concentrations of compounds and the pH (factors) on mycelium growth were analyzed by ANOVA. The posthoc analyses were conducted by Tukey Honest Significant Difference test (TukeyHSD function) when significant interaction between the factors was obtained and the “multcomp” package was used to compare between group means (Hothorn et al., 2008). The effect size statistics (partial eta square - η^2_p) for ANOVAs were calculated by using “etaSquared” function in the “lsr” packages (Navarro, 2013).

RESULTS

The effects of several boron-containing compounds on the mycelial development of *M. fructigena* isolate, whose morphological and molecular identification was completed, were tested in the study. The results showed that the boron-containing compounds had statistically different effects on mycelium development (Table 2, Figure 1). The most effective applications in preventing pathogen development were sodium tetrafluoroborate (STFB) and potassium tetrafluoroborate (PTFB), respectively. The different concentrations of STFB significantly affected *M. fructigena* mycelial growth ($F_{7, 181} = 36.46$, $p < 0.01$, $\eta^2_p =$

0.60), and the development was not observed when the concentration was higher than 10 mM (Figure 1h). Similar to STFB, mycelium development was greatly inhibited in applications of 20 mM ($F_{7, 184} = 68.80$, $p < 0.01$, $\eta^2_p = 0.73$) and above among different concentrations of PTFB. Although pH adjustment had no effects on the growth media ($F_{1, 184} = 0.98$, $p = 0.32$, $\eta^2_p = 0.005$), the interactions of these two parameters had significant effects ($F_{7, 184} = 3.57$; $p < 0.01$, $\eta^2_p = 0.12$). The non-neutral growth media showed better inhibitory effects on *M. fructigena* development compared to neutral growth media.

Table 2. Average values of *Monilinia fructigena* growth after seven days of incubation (mm)

Çizelge 2. Yedi gün inkübasyondan sonra *Monilinia fructigena* gelişiminin ortalama değerleri (mm)

Compounds		Concentrations (mM)							
		0	5	10	20	40	60	80	100
Ammonium pentaborate tetrahydrate (APBT)	Non-neutral	51.26±5.40	66.23±1.11	66.44±1.53	69.75±0.53	74.03±1.45	28.68±1.45	17.70±2.65	10.93±2.63
	Neutral	67.57±4.96	67.89±1.41	68.38±1.47	72.69±1.00	66.09±1.13	27.06±1.41	15.51±2.02	10.06±1.60
Boron oxide (BO)	Non-neutral	51.26±5.40	39.18±0.48	40.09±0.64	38.85±1.87	39.47±2.70	47.28±0.93	51.49±0.73	51.74±1.01
	Neutral	67.57±4.96	67.55±3.66	58.53±1.28	57.46±2.12	59.54±1.89	64.94±1.27	63.45±1.46	62.12±1.74
Etidot-67 (ETI)	Non-neutral	51.88±5.19	57.30±2.01	58.12±1.39	65.24±2.80	42.05±3.04	26.18±1.40	15.10±2.85	7.03±1.25
	Neutral	67.57±4.96	52.58±2.36	58.03±1.63	65.95±1.35	49.49±1.58	39.27±1.25	34.47±0.61	28.95±1.16
Potassium metaborate (PMB)	Non-neutral	51.26±5.40	57.40±2.14	57.38±3.70	56.05±3.45	57.24±1.60	63.64±2.23	65.66±2.96	65.92±1.40
	Neutral	67.57±4.96	64.11±2.97	61.17±3.65	60.72±4.31	58.09±2.56	58.88±3.05	59.81±4.07	60.43±2.89
Potassium tetrafluoroborate (PTFB)	Non-neutral	51.26±5.40	33.62±1.93	6.65±1.19	ng*	ng	ng	ng	ng
	Neutral	67.57±4.96	50.25±1.65	26.24±0.63	ng	ng	ng	ng	ng
Sodium metaborate tetrahydrate (SMT)	Non-neutral	51.26±5.40	51.22±1.97	46.16±3.31	46.86±5.67	40.93±3.62	35.94±1.72	23.43±3.28	15.86±1.83
	Neutral	67.57±4.96	49.77±3.29	45.97±2.82	45.44±2.39	38.31±3.13	36.69±4.43	35.14±2.09	34.30±4.03
Sodium tetraborate decahydrate (STBD)	Non-neutral	51.88±4.95	54.43±4.82	56.04±2.79	62.34±5.46	50.55±0.93	25.93±2.76	18.53±2.41	2.35±1.50
	Neutral	67.57±4.96	43.67±1.32	43.98±1.67	42.77±0.73	41.24±0.79	33.99±1.22	26.20±0.48	23.10±1.04
Sodium tetrafluoroborate (STFB)	Non-neutral	51.26±5.40	7.10±0.72	ng	ng	ng	ng	ng	ng
	Neutral	67.58±4.96	23.63±0.71	ng	ng	ng	ng	ng	ng

*ng: no grow

The different concentrations of ammonium pentaborate tetrahydrate (APBT) have controlled *M. fructigena* mycelial growth by varying degrees ($F_{7, 175} = 183.58$, $p < 0.001$, $\eta^2_p = 0.88$). Although pH adjustment of media did not show a significant impact ($F_{1, 175} = 0.09$, $p = 0.75$), the interaction of these two factors had significant effects on *M. fructigena* development ($F_{7, 175} = 27.54$, $p < 0.01$, $\eta^2_p = 0.54$). APBT with higher levels than 40 mM had a better controlling tendency and 100 mM concentration of APBT showed the best results to control with 10.06±1.60 mm and 10.93±2.63 mm mycelial growth for neutral and non-neutral growth media, respectively (Table 2, Figure 1a). Unfortunately, none of the concentrations of APBT tested in this study were able to fully inhibit *M.*

fructigena development. The concentrations of boron oxide (BO) ($F_{7, 165} = 35.11$, $p < 0.01$, $\eta^2_p = 0.59$) and the pH adjustment ($F_{1, 165} = 735.66$, $p < 0.01$, $\eta^2_p = 0.82$) had statistically significant effects, but the applications did not able to control *M. fructigena* mycelium growth (Figure 1b). The neutral growth media supported mycelium growth compared to non-neutral growth media and the pH factor had higher effects than the concentration of BO. The interactions of these two factors had significant effects on mycelium growth ($F_{7, 165} = 11.63$, $p < 0.01$, $\eta^2_p = 0.34$). *M. fructigena* development decreased significantly with increasing concentrations of epidote-67 application (disodium octaborate tetrahydrate – ETI) and the best result was observed when the concentration was 100 mM non-

neutral medium with 7.03 ± 1.25 mm mycelium growth (Table 2, Figure 1c). Although potassium metaborate (PMB) concentrations and pH changes have statistically different effects, it was observed that they

could not prevent fungal growth ($F_{7, 184} = 8.30$, $p < 0.01$, $\eta^2_p = 0.25$ for concentration, $F_{1, 184} = 17.20$, $p < 0.01$, $\eta^2_p = 0.09$ for pH adjustment) (Figure 1d).

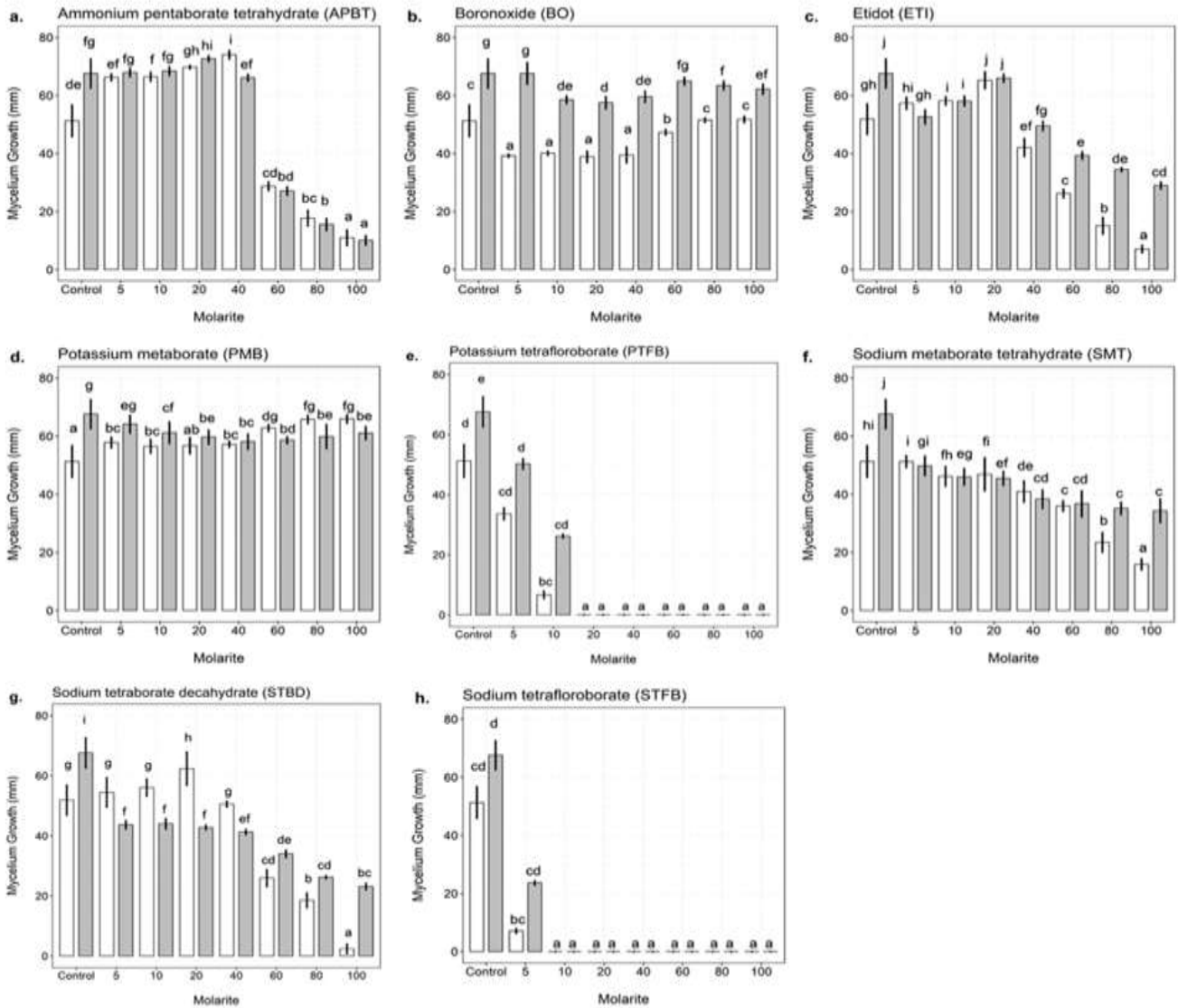


Figure 1. Effects of boron-containing compounds on the growth of *Monilinia fructigena* after seven days of incubation (a. Ammonium pentaborate tetrahydrate, b. Boron oxide, c. Etidot-67, d. Potassium metaborate, e. Potassium tetrafluoroborate, f. Sodium metaborate tetrahydrate, g. Sodium tetraborate decahydrate, h. Sodium tetrafluoroborate). *The white bars indicate non-neutral growth media while grey bars are neutral growth media)

Şekil 1. Yedi gün inkübasyondan sonra bor içerikli bileşiklerin *Monilinia fructigena* gelişimine etkileri (a. Ammonium pentaborate tetrahydrate, b. Boron oxide, c. Etidot-67, d. Potassium metaborate, e. Potassium tetrafluoroborate, f. Sodium metaborate tetrahydrate, g. Sodium tetraborate decahydrate, h. Sodium tetrafluoroborate). * Beyaz barlar nötr olmayan gelişme ortamını gösterirken gri barlar nötr gelişme ortamını göstermektedir)

The increasing concentration of sodium metaborate tetrahydrate (SMT) and sodium tetraborate decahydrate (STBD) substantially reduced *M. fructigena* mycelium growth by 15.86 ± 1.83 mm and 2.35 ± 1.50 mm, respectively, at 100 mM concentrations. Although pH adjustment of the growing medium in the

SMT application had significant, albeit low, effects on mycelium growth ($F_{1, 184} = 31.66$, $p < 0.01$, $\eta^2_p = 0.15$), pH adjustment in the STBD application did not have a significant effect on mycelium growth ($F_{1, 176} = 1$, $p = 0.31$, $\eta^2_p = 0.006$). However, the interaction between these factors significantly affected mycelium growth

for both treatments ($F_{7, 184} = 19.74$ $p < 0.01$, $\eta^2_p = 0.44$ for SMT; $F_{7, 176} = 89.19$, $p < 0.01$, $\eta^2_p = 0.79$ for STBD). The results showed that 100 mM SMT and STBD with non-neutral growth media can control *M. fructigena* development (Figure 1f, 1g). Additionally, Luna and Signum used as controls completely inhibited *M. fructigena* mycelium growth.

The highest inhibition values of boron-compounds in *M. fructigena* mycelial development were observed in STFB and PTFB applications similar to the effect of mycelial diameter values (Figure 2). Mycelial development was prevented 100% at concentrations of ≥ 10 mM of STFB and ≥ 20 mM of PTFB. Doses of 60 mM \geq APBT and STBD applications, gradually

increased the percentage of inhibition of mycelial development of the pathogen, regardless of pH. However, 100 mM concentration of STBD inhibited mycelial growth at a higher rate than neutral, with a 95.47% inhibition rate in non-neutral media. BO application inhibited mycelial growth by up to 24.20% and showed that inhibition rates varied between neutral and non-neutral media. While an increasing inhibition from 18.95% to 86.45% was recorded with the ETI application, this range varied from 9.95 to 69.06% with the SMT application. Pathogen development could not be significantly prevented in PMP application, but it was revealed that there was an adverse effect between media.

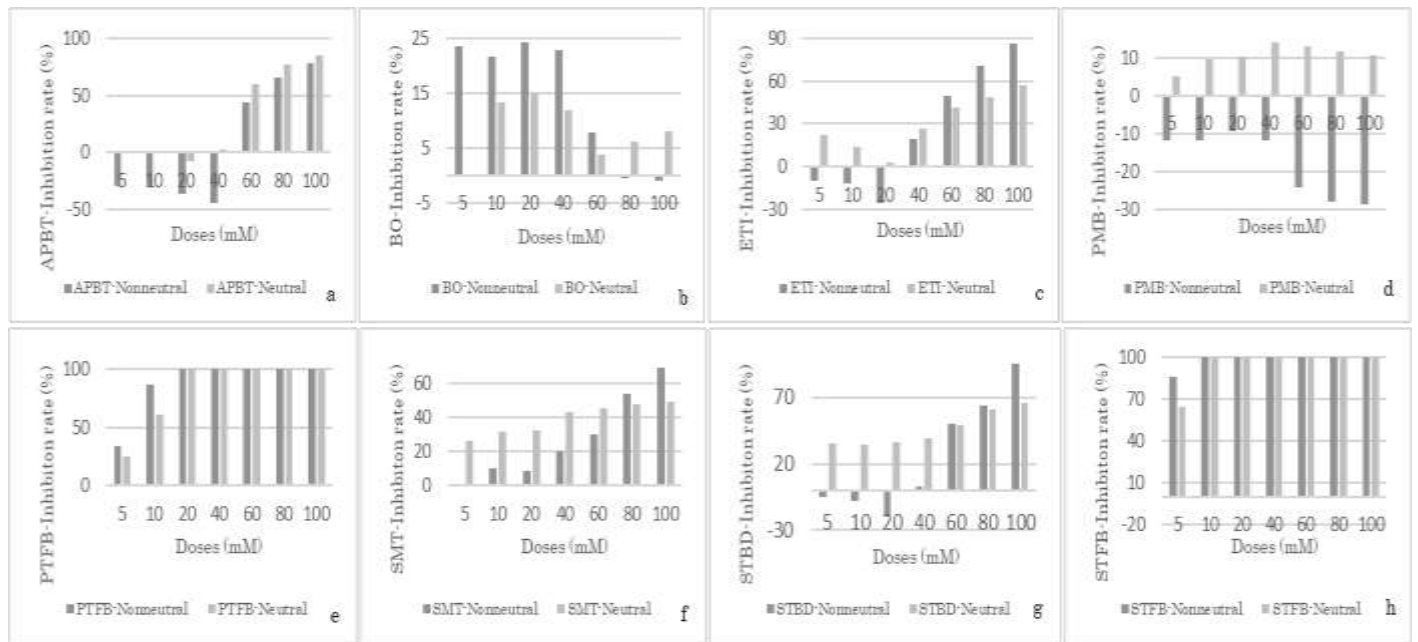


Figure 2. Inhibition rates (%) of boron-compounds on *Monilinia fructigena* mycelial growth (a; APBT, B; BO, c; ETI; d; PMB, e; PTFB, f; SMT, g; STBD, h; STFB).

Şekil 2. Borlu bileşiklerin *Monilinia fructigena* miselyal gelişimini engelleme oranları (%) (a; APBT, B; BO, c; ETI; d; PMB, e; PTFB, f; SMT, g; STBD, h; STFB).

DISCUSSION and CONCLUSION

Boron is widely used in many fields such as industry and medicine and is also one of the important micronutrients for plants (Woods, 1994; Bolt et al., 2017; Shireen et al., 2018). The effects of boron-containing compounds in fungi have been investigated in recent years and the attention to the application of boron has been increasing because of its environmental-friendly preservative effects against several plant pathogens (Gedük et al., 2020; Estevez-Fregoso et al., 2021; Gür et al., 2021). Finding pathogen-specific boron-containing compounds and determining their appropriate concentrations are important for the management of plant diseases. The aim of this study was to investigate various boron-containing compounds to control brown rot caused by *Monilinia fructigena*.

Sodium tetrafluoroborate (STFB) and PTFB were the most effective compounds in inhibiting pathogen growth among applications. It was observed that mycelial development was prevented completely after 10 mM STFB and 20 mM PTFB applications (Figure 1). Moreover, although the pH of these compounds did not have a significant effect on mycelium development, non-neutral media with lower concentrations showed better results than neutral media. Qin et al. (2010) showed that alkaline potassium tetraborate (pH 9.2) was more effective in controlling *B. cinera* than neutral compounds. *Monilinia fructigena* can optimally grow at pH 3.5 and it can also tolerate the alkaline growth medium at pH 9 (Holb, 2004; Hrustić et al., 2020). Holb (2004) hypothesized that *M. fructigena* decreased the pH of the substrate, thus the disease grows exponentially after acidity levels of fruits reached the

optimal degree. However, the pH of boron-containing compounds applied against brown rot agents generally adjusted at 7 (Thomidis & Exadaktylou, 2010). Even though the results did not show significant effects of the pH of STFB and PTFB on *M. fructigena* mycelium growth, the pH of other tested compounds was significant (Figure 1). The main acidification agent of brown rot caused by *M. fructicola* was gluconic acid and the accumulation of gluconic acid created an unstable cell membrane as well as increased disease susceptibility (De Cal et al., 2013). Boron application could improve the cell membrane integrity in pears and reduce the micro cracking on the fruit surface (Thomidis & Exadaktylou, 2010; Xuan et al., 2002). Tetrafluoroborate anions can be associated with antifungal effects on *M. fructigena* because it is likely that fluorine improves the antifungal effects of these compounds (Kirk 2006).

Tested concentrations of APBT, ETI, SMT, and STBD failed to completely inhibit mycelium growth, but it appears that higher concentrations of these compounds may be useful in controlling *M. fructigena* mycelial growth. In particular, the non-neutral growth media with these compounds (except APBT) showed better results in controlling pathogen development. The higher concentrations of these compounds are slightly alkaline to alkaline and the effectiveness of these compounds against *M. fructigena* mycelium growth is increasing with the high level of pH. Boronoxide could not inhibit mycelial growth, and interestingly, the higher concentrations of BO increased it. Similarly, the pH of PMB was observed as one of the highest levels, but it cannot inhibit mycelium growth. The concentration and pH of boron-containing compounds are crucial for selecting appropriate pathogen-specific preservatives, and it is thought that these compounds may be pathogen-specific. Therefore, the selection of suitable boron-containing compounds against specific pathogens is necessary to find an environmentally friendly alternative to fungicides.

In conclusion, this study showed that the boron-containing compounds with tetrafluoroborate anions could be candidates as an alternative to fungicides against *M. fructigena*. Likewise, APBT, ETI, SMT, and STBD could be useful to control *M. fructigena* mycelium growth with higher concentrations than 100 mM when the pH of these compounds was alkaline. Further studies are needed to investigate how much boron can be absorbed by fruits after application and the toxicological effects of this boron on human health.

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Researchers' Contribution Rate Statement

Boron applications and experiments were carried out by İ. Ş. and F. Y. Ş. K. provided funding and supervised the experiment. Identification of the isolate and first draft of the manuscript was prepared by G. P. All authors read previous versions of the manuscript and approved the final manuscript.

Conflicts of Interest Statement

No potential conflict of interest was reported by the authors.

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Curculionoidea (Insecta: Coleoptera) Species Detected on Some Weeds in Kahramanmaraş Province, Türkiye Part I

Zehra Sena GÖZÜBENLİ¹, Mahmut Murat ASLAN², Kevser SABANCI³

^{1,2,3}Kahramanmaraş Sütçü İmam University Faculty of Agriculture, Plant Protection Department, TR 46050, Kahramanmaraş, Türkiye.

¹<https://orcid.org/0000-0002-4949-9223>, ²<https://orcid.org/0000-0002-4586-1301>, ³<https://orcid.org/0000-0001-8012-0229>

✉: aslan@ksu.edu.tr

ABSTRACT

This study was conducted to determine the Curculionoidea species on weeds found in non-agricultural areas in Kahramanmaraş Province between 2021 and 2022, weekly from late March early April until the end of September after the weeds begin to germinate, and at fifteen-day intervals when the weeds start to dry towards the end of September. Studies were carried out. As a result of this study two genera belonging to the family Curculionidae, Lixinae Schoenherr, 1823 subfamily of the superfamily Curculionoidea, and fourteen species belonging to these genera. *Larinus cinerascens* Capiomont, 1874, one of the identified species, is a new record for the Curculionoidea fauna of Türkiye. A total of five species, including *Lixus algirus* L., *Lixus vilis* (Rossi, 1790), *Larinus cinerascens* Capiomont, 1874, *Larinus hedenborgi* Boheman, 1845, *Larinus turbinatus* Gyllenhal, 1835, are new records for Kahramanmaraş Province. In addition, twelve weed species were identified as new host plants for the identified Curculionoidea species.

Entomology

Research Article

Article History

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Anahtar Kelimeler

Curculionoidea

Curculionidae

Weed

Host Plant

Kahramanmaraş

Kahramanmaraş İlindeki Bazı Yabancı Otlar Üzerinde Saptanan Curculionoidea (Insecta: Coleoptera) Türleri Kısım I

ÖZET

Bu çalışma Kahramanmaraş İlinde 2021-2022 yılları arasında tarım dışı alanlarda bulunan yabancı otlar üzerindeki Curculionoidea türlerini belirlemek amacıyla yabancı otların çimlenmeye başlamasını takiben mart sonu-nisan ayı başlarından eylül ayının sonuna kadar haftalık olarak, eylül ayının sonuna doğru yabancı otların kurumaya başlamasıyla 15 günlük aralıklarla arazi çalışmaları yürütülmüştür. Yürütülen bu çalışma sonucunda Curculionoidea üst familyasının Curculionidae familyası, Lixinae Schoenherr, 1823 alt familyasına bağlı 2 cins ve bu cinslere ait 14 tür tespit edilmiştir. Tespit edilen türlerden *Larinus cinerascens* Capiomont, 1874 Türkiye Curculionoidea faunası için yeni kayıttır. *Lixus algirus* L., *Lixus vilis* (Rossi, 1790), *Larinus cinerascens* Capiomont, 1874, *Larinus hedenborgi* Boheman, 1845, *Larinus turbinatus* Gyllenhal, 1835 olmak üzere toplam 5 tür ise Kahramanmaraş İli için yeni kayıt niteliğindedir. Ayrıca belirlenen Curculionoidea türleri için 12 yabancı ot türü yeni konukçu bitki olarak belirlenmiştir.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 06.02.2024

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Keywords

Curculionoidea

Curculionidae

Yabancı Ot

Konukçu Bitki

Kahramanmaraş

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INTRODUCTION

One of the most important factors affecting crop production is weeds. Weeds survive in the same environment as crops and compete with them for water, nutrients, and light, negatively affecting the

quality of agricultural products, and also causing a loss of approximately \$ 7.6 billion worldwide (Pacanoski, 2007).

The order of Coleoptera is important among the insects used for biological weed control (Kısmalı & Madanlar,

1990). Within this order, the superfamily Curculionoidea has more weed hosts than other species (Oberprieler et al., 2007). Larvae mostly prefer the root collar and roots of plants as feeding habitat, and they feed on the root parts by forming galls (Volovnik, 2010) within the plant tissue (Trnka et al., 2015) or by moving freely in the soil. This group of insects is also important for biological control of weeds, depending on their specific nutritional characteristics (Stinson et al., 1994; Story et al., 2006; Gültekin et al., 2019).

Studies on the species of the superfamily Curculionoidea began in the 1700s, and many foreign and local researchers contributed by carrying out systematic, taxonomic, and faunistic studies. Some of them; Winkler (1924-1932), Emden (1944), Lodos (1960; 1971; 1972), Voss (1962), Altay et al. (1972), Lodos et al. (1978; 2003), Dieckmann (1980), Alonso-Zarazaga & Lyal (1999), Gültekin (2001), Marvaldi & Lanteri (2005), Pehlivan et al. (2005a; 2005b), Keskin (2005), Gültekin (2006c), Davidian & Gültekin (2006), Wanat (2007), Bolu & Legalov (2008), Erbey (2010), Uzun & Tezcan (2011), Avgın & Colonnelli (2011), Gültekin & Podlussany (2012), Aydın (2013), Talamelli (2014), Gürler (2014), Yılmaz (2015), Aydın & Hacet (2016), Özgen et al. (2016), Erdem (2016), Güven (2019), Kapucu (2019), Hacet & Colonnelli (2019), Erbey & Bolu (2021).

In this study, weeds are plants adapted to different climatic conditions and soil structures. They serve as intermediate hosts for many living organisms due to their ability to withstand difficult ecological conditions and create population diversity in the ecosystem. For this reason, two genera belonging to the subfamily Lixinae Schoenherr, 1823 of the superfamily Curculionoidea, the family Curculionidae, which has a very important place among the weeds found in non-agricultural areas of Kahramanmaraş Province, and fourteen species belonging to these genera were identified.

MATERIAL AND METHOD

This study was conducted on the weeds found in non-agricultural areas in Kahramanmaraş Province during 2021-2022.

Material

The primary material of this study consists of species belonging to the superfamily Curculionoidea found in Kahramanmaraş Province, Türkiye, and the weeds these species feed on. In the study, a trap, killing jar, culture containers, sample containers, polyethylene bags, suction tube, 70% ethyl alcohol, forceps, insect needle, petri dish, cotton, soft-tipped brush, tulle, rubber, and GPS device were used.

Method

Field and Laboratory Studies

The study was carried out in weekly intervals, starting from the end of March or early April and till the end of September, after the germination of the weeds in the non-agricultural areas of Kahramanmaraş Province, and at fifteen-day intervals when the weeds started to dry up towards the end of September. In the samples, all the weeds were examined because the species of the superfamily Curculionoidea were found in the roots, stems, leaves, and generative organs of the weeds. The phenological period of each weed in which the species was found, the part where the insect feeds and the locations of the weed species were determined by GPS.

Weeds belonging to the superfamily Curculionoidea were observed in the wild, with large species collected by hand, small species collected with a suction tube, and a trap used for collection. Some life stages of Curculionoidea species such as egg, larva, pupa, and adult from the collected samples were brought to the laboratory conditions and cultured with the plant whether they fed on. To maintain the humidity of the cultured samples, water was sprayed at regular intervals, and nutrients were replaced as necessary. Cultured weedy plant samples were checked at regular intervals, and egg, larva, pupa, and adult emergence dates were recorded.

Adult insect species samples collected during field works were killed with the help of the killing jars or directly brought to the laboratory in separate sample containers with their label information. The location where the samples were collected, latitude and longitude, was recorded using GPS. With this information, the samples were labeled and prepared for expert identification. Herbaria of weed species belonging to the superfamily Curculionoidea were also designed and made available for identification.

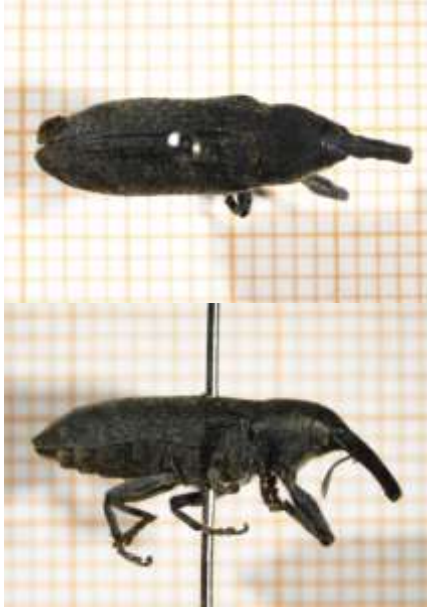
Dieckman (1977), Gültekin (2008a), and Erbey (2010) were used to determine the morphological characteristics of the species belonging to the superfamily Curculionoidea. Identifications of insect specimens belonging to the superfamily Curculionoidea. Associate. Prof. Dr. Mahmut ERBEY (Kırşehir Ahi Evran University, Faculty of Science and Letters, Department of Molecular Biology and Genetics) and identifications of weeds by Associate. Prof. Dr. Tamer ÜSTÜNER (Kahramanmaraş Sütçü İmam University Faculty of Agriculture, Department of Plant Protection) was done.

RESULT AND DISCUSSION

In this study conducted in Kahramanmaraş Province, two genera belonging to the family Curculionidae of the superfamily Curculionoidea and the Lixine subfamily and fourteen species belonging to these genera were identified. Among the species identified,

Larinus cinerascens Capiomont, 1874 was determined as a new record for the Türkiye fauna. A total of five species belonging to the genera *Lixus* Fabricius, 1801 and *Larinus* Dejean, 1821, including *Lixus algirus* L., *Lixus vilis* (Rossi, 1790), *Larinus cinerascens*

Capiomont, 1874, *Larinus hedenborgi* Boheman, 1845, *Larinus turbinatus* Gyllenhal, 1835, are new records for Kahramanmaraş Province. Furthermore, 12 weed species were identified as new host plants for the identified Curculionoidea species (Table 1).



Lixus algirus L.



Lixus circumcinctus Bohemann, 1836



Lixus cardui Olivier, 1808



Lixus elongatus Goeze, 1777





Lixus scolopax Bohemann, 1836



Larinus cinerascens Capiomont, 1874



Larinus latus (Herbst, 1874)



Lixus vilis (Rossi, 1790)



Larinus hedenborgi Boheman, 1845



Larinus minutus Gyllenhal



Larinus onopordi (Fabricius, 1787)



Larinus sturnus Schaller, 1873



Larinus turbinatus Gyllenhal, 1835



Larinus rudicollis Petri, 1907

Figure 1. Curculionoidea (Insecta: Coleoptera) species detected in the weeds in Kahramanmaraş Province
Şekil 1. Kahramanmaraş İli yabancı otlar üzerinde tespit edilen Curculionoidea (Insecta: Coleoptera) türleri

Table 1. Curculionoidea (Insecta: Coleoptera) species and their host plant detected in Kahramanmaraş Province
Çizelge 1. Kahramanmaraş İlinde tespit edilen Curculionoidea (Insecta: Coleoptera) türleri ve konukçu bitkileri

Species	Host Plant	References
<i>Lixus algerus</i>	<i>Cirsium arvense</i>	Pierre, 1901
	<i>Cirsium palustris</i>	Krauss, 1963
	<i>Echinops orientalis*</i>	Bardner, 1983
	<i>Euphorbia spinosa</i>	
	<i>Gundelia tournefortii*</i>	
	<i>Vicia faba</i>	
<i>Lixus cardui</i>	<i>Amygdalus communis</i>	Hofmann, 1954
	<i>Carduus nutans*</i>	Lodos et al., 1978; 2003
	<i>Carduus</i> sp.	Ter-Minasyan, 1978
	<i>Centaurea</i> sp.	Gültekin et al., 2000
	<i>Cirsium</i> sp.	Swirepik & Smyth, 2002

	<i>Cynara scolymus</i>	Huwer et al., 2004
	<i>Crataegus</i> sp.	Pehlivan et al., 2005a
	<i>Echinops orientalis</i> *	Kluge & Zachariades, 2006 Gültekin, 2007; 2008c
	<i>Hypericum</i> sp.	
	<i>Juglans regia</i>	Harizanova et al., 2010
	<i>Onopordum acanthium</i>	Gültekin & Perrin, 2011
	<i>Onopordum alexandrinum</i>	Shahriyari-Nejad et al., 2013
	<i>Onopordum bracteatum</i>	Bolu, 2016
	<i>Onopordum carduiforme</i>	Güven, 2019
	<i>Onopordum heteracanthum</i>	Alewi et al., 2019
	<i>Onopordum illyricum</i>	
	<i>Onopordum leptolepis</i>	
	<i>Onopordum palaestinum</i>	
	<i>Onopordum</i> sp.	
	<i>Planatus</i> sp.	
	<i>Prunus avium</i>	
	<i>Prunus domestica</i>	
	<i>Pyrus elaeagrifolia</i>	
	<i>Salix</i> sp.	
	<i>Solanum esculentum</i>	
	<i>Triticum</i> sp.	
	<i>Verbascum</i> sp.	
	<i>Vicia cracca</i>	
	<i>Vicia faba</i>	
<i>Lixus circumcinctus</i>	<i>Amygdalus communis</i>	Ter-Minasyan, 1978
	<i>Crambe orientalis</i>	Gültekin & Korotyev, 2011
	<i>Crambe</i> sp.	Bolu, 2016
		Korotyev et al., 2016
<i>Lixus elongatus</i>	<i>Beta</i> sp.	Hoffmann, 1954
	<i>Carduus acanthoides</i>	Balachowsky, 1963
	<i>Carduus nutans</i>	Ter-Minasyan, 1978
	<i>Carduus</i> sp.	Eber et al., 1999
	<i>Centaurea</i> sp.	Lodos et al., 2003
	<i>Cirsium lanceolatum</i>	
	<i>Cirsium</i> sp.	
	<i>Cretaeagus</i> sp.	
	<i>Onopordum</i> sp.	
	<i>Prunus armenica</i>	
	<i>Sinapis</i> sp.	
	<i>Sisymbrium</i> sp.*	
	<i>Tamarix</i> sp.	
	<i>Triticum</i> sp.	
	<i>Verbascum</i> sp.	
<i>Lixus scolopax</i>	<i>Centaurea solstitialis</i>	Lodos et al., 1978; 2003
	<i>Centaurea</i> sp.	Gültekin, 2007
	<i>Echinops orientalis</i> *	
	<i>Echinops sphaerocephalus</i>	
	<i>Medicago sativa</i>	
	<i>Onopordum</i> sp.	
	<i>Prunus amygdalus</i>	
	<i>Quercus</i> sp.	
<i>Lixus vilis</i>	<i>Carduus nutans</i> *	Stierlin, 1883
	<i>Centaurea</i> sp.	Dieckman, 1983
	<i>Erodium cicutarium</i>	Lodos et al., 2003
	<i>Erodium</i> sp.	Pehlivan et al., 2005a Heijerman, 2007
	<i>Fraxinus</i> sp.	Ghahari et al., 2009
	<i>Malus sylvestris</i> subsp. mitis	Stejskal & Trnka, 2013

	<i>Onopordum</i> sp.	Stejskal et al., 2014
	<i>Pistacia lentiscus</i>	Forbicioni et al., 2019
	<i>Salix</i> sp.	
	<i>Sisybrium</i> sp.*	
<i>Larinus cinerascens</i>	<i>Carthamus dentatus</i> * <i>Centaurea lugdunensis</i>	Alphonse, 1934
<i>Larinus hedenborgi</i>	<i>Echinops cephalotes</i> <i>Echinops</i> sp. <i>Echinops sphaerocephalus</i> <i>Echinops orientalis</i> *	Gültekin, 2006b; 2008a Skuhrovec et al., 2022
<i>Larinus latus</i>	<i>Acacia</i> sp. <i>Carduus nutans</i> <i>Carduus onopordioides</i> <i>Carduus</i> sp. <i>Carthamus lanatus</i> <i>Centaurea</i> sp. <i>Cirsium haussknechtii</i> <i>Cirsium</i> sp. <i>Cirsium vulgare</i> <i>Cynara cardunculus</i> <i>Cynara scolymus</i> <i>Cynara</i> sp. <i>Echinops sphaerocephalus</i> <i>Onopordum acanthium</i> <i>Onopordum bracteatum</i> <i>Onopordum candidum</i> <i>Onopordum carduchorum</i> <i>Onopordum</i> sp. <i>Onopordum tauricum</i> <i>Quercus</i> sp. <i>Silybum marianum</i> <i>Silybum</i> sp. <i>Verbascum</i> sp.	Hoffman, 1954 Zwölfer et al., 1971 Lodos et al., 1978; 2003 Karaat et al., 1986 Briese & Sheppard, 1992 Michalakis & Olivieri, 1992 Rosenthal et al., 1994 Briese et al., 1995 Briese, 2000 Gültekin et al., 2000 Abdel-Moniem, 2002 Briese et al., 2002 Gültekin, 2004 Pehlivan et al., 2005a Ottai & Abdel-Moniem, 2006 Gültekin, 2008c Yardibi & Tozlu, 2013 Abad et al., 2016 Bozdoğan & Uygur, 2019 Güven, 2019
<i>Larinus minutus</i>	<i>Astragalus</i> sp. <i>Carduus nutans</i> <i>Carduus</i> sp. <i>Centaurea diffusa</i> <i>Centaurea maculosa</i> <i>Centaurea solstitialis</i> * <i>Centaurea</i> sp. <i>Centaurea stoebe</i> <i>Centaurea stoebe</i> subsp. <i>micranthos</i> <i>Centaurea virgata</i> <i>Cirsium</i> sp. <i>Echium</i> sp. <i>Elaeagnus angustifolia</i> <i>Genista</i> sp. <i>Medicago sativa</i> <i>Onopordum</i> sp. <i>Quercus</i> sp. <i>Rosa</i> sp. <i>Rubus</i> sp. <i>Vincetoxicum</i> sp. <i>Vitis vinifera</i>	Groppe et al., 1990 Kashefi & Sobhian, 1998 Lodos et al., 2003 Seastedt et al., 2003 Lejeune et al., 2005 Smith & Mayer, 2005 Myers et al., 2009 Knochel et al., 2010 Bourchier & Crowe, 2011 Stephens & Myers, 2013 Bolu, 2016 Hoebeke & Spichiger, 2016 Güven, 2019
<i>Larinus onopordi</i>	<i>Carduus</i> sp. <i>Centaurea</i> sp. <i>Cirsium</i> sp.	Ter-Minasyan, 1967 Lodos et al., 1978; 2003 Pehlivan et al., 2005a

	<i>Echinops</i> sp.	Gültekin, 2006b
	<i>Echinops lalesarensis</i>	Mathesona et al., 2008
	<i>Echinops sphaerocephalus</i>	Gültekin, 2008c
	<i>Echinops pungens</i>	Shahriyari-Nejad et al., 2013
	<i>Onopordum</i> sp.	Özgen et al., 2016
	<i>Onopordum cynarocephalum</i>	Güven, 2019
	<i>Pinus</i> sp.	
	<i>Echinops orientalis</i> *	
<i>Larinus sturnus</i>	<i>Arctium</i> sp.	Zwölfer et al., 1971
	<i>Carduus crispus</i>	Zwölfer, 1974; 1979b
	<i>Carduus</i> sp.	Koch, 1992
	<i>Centaurea</i> sp.	Lodos et al., 2003
	<i>Cirsietum rivularis</i>	Ghahari et al., 2009
	<i>Onopordum</i> sp.	Skuhrovec & Gosik, 2011
	<i>Cirsium oleraceum</i>	
	<i>Cirsium</i> sp.	
	<i>Cirsium spinosissimum</i>	
	<i>Cirsium vulgare</i>	
	<i>Solanum melongena</i>	
	<i>Echinops orientalis</i> *	
<i>Larinus turbinatus</i>	<i>Arrhenatheretum elatioris</i>	Rabaud, 1913
	<i>Carduus</i> sp.	Zwölfer, 1975b
	<i>Carduus acanthoides</i>	Batra et al., 1981
	<i>Carduus crispus</i>	Zwölfer & Brandl, 1989
	<i>Carduus hamulosus</i>	Koch, 1992
	<i>Carduus nervosus</i>	Skuhrovec et al., 2008 Balalaikins et al., 2011
	<i>Carduus nutans</i>	
	<i>Carduus nutans</i> subsp. <i>nutans</i>	Gosik & Skuhrovec, 2011 Yegorenkova et al., 2011
	<i>Carduus thoermeri</i>	
	<i>Carduus uncinatus</i>	Delbol, 2012
	<i>Centaurea behen</i>	Ghahari & Arzanov, 2012 Gültekin, 2004
	<i>Centaurea solstitialis</i>	Bolu, 2016
	<i>Cirsium arvense</i>	Maciejowski & Petryszak, 2017
	<i>Cirsium eriophorum</i>	Dumont & Tonnancour, 2019
	<i>Cirsium heterophyllum</i>	Güven, 2019
	<i>Cirsium incanum</i>	
	<i>Cirsium oleraceum</i>	
	<i>Cirsium setosum</i>	
	<i>Cirsium</i> sp.	
	<i>Cirsium tuberosum</i>	
	<i>Cirsium ukrainicum</i>	
	<i>Cirsium vulgare</i>	
	<i>Onopordum acanthium</i>	
	<i>Picnoman acarna</i> *	
<i>Larinus rudicollis</i>	<i>Carduus</i> sp.	Lodos et al., 2003
	<i>Centaurea</i> sp.	Pehlivan et al., 2005a
	<i>Cirsium</i> sp.	Gültekin, 2008c
	<i>Cistus</i> sp.	Ghahari et al., 2010
	<i>Echinops orientalis</i> *	Bolu, 2016
	<i>Echinops pungens</i>	Korotyayev et al., 2016
	<i>Echinops</i> sp.	Szenası et al., 2019
	<i>Echinops sphaerocephalus</i>	
	<i>Lactuca scariola</i>	
	<i>Medicago sativa</i>	
	<i>Onopordum</i> sp.	
	<i>Pinus</i> sp.	
	<i>Sinapis</i> sp.	

*New host plant

Superfamily: Curculionoidea

Family: Curculionidae

Subfamily: Lixinae Schoenherr, 1823

Tribus: Lixini Schoenherr, 1823

Genus: *Lixus* Fabricus, 1801

Species: *Lixus algirus* L.

Material examined: Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, May 11, 2022 (number of insect samples: 2), on *Gundelia tournefortii* L. (new host plant); Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, May 31, 2022 (number of insect samples: 2), on *Echinops orientalis* Trautvetter (new host plant).

Distribution in Türkiye: İçel, Kırşehir, Kocaeli, Samsun, Zonguldak (Lodos et al., 1978; Pehlivan et al., 2005a; Erbey, 2010; Yılmaz, 2015), Kahramanmaraş (new record).

Species: *Lixus cardui* Olivier, 1808

Material examined: Kahramanmaraş, Onikişubat, Üngüt Neighbourhood, N37°35'43,613/E36°50'34,120, August 5, 2021 (number of insect samples: 24); March 29, 2022 (number of insect samples: 12), on *Carduus nutans*; Kahramanmaraş, Pazarcık, Akçalar Village, N37°31'29,326/E37°26'14,770, May 11, 2022 (number of insect samples: 6), on *Carduus nutans*; Kahramanmaraş, Türkoğlu, Kızıleniş Village, N37°20'48,6/E36°46'40,7, April 8, 2022 (number of insect samples: 13), on *Carduus nutans*; Kahramanmaraş, Dulkadiroğlu, Hacımustafa Village, N37°28'29,776/E36°53'7,2773, April 25, 2022 (number of insect samples: 18); May 5, 2022 (number of insect samples: 6), on *Carduus nutans*; Kahramanmaraş, Dulkadiroğlu, Ayaklıcaoluk Village, N37°36'29,314/E37°2'38,830, August 7, 2021 (number of insect samples: 6); March 29, 2022 (number of insect samples: 3), on *Carduus nutans*; Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, May 31, 2022 (number of insect samples: 5), on *Echinops orientalis* (new host plant); Kahramanmaraş, Dulkadiroğlu, Sekamer, N37°35'28,975/E37°3'30,066, 28.04.2022 (number of insect samples: 6), on *Carduus nutans* (new host plant).

Distribution in Türkiye: Adana, Ankara, Antalya, Aydın, Bartın, Balıkesir, Bilecik, Burdur, Bursa, Çanakkale, Çankırı, Denizli, Diyarbakır, Edirne, Eskişehir, Gaziantep, Isparta, Iğdır, İçel, İzmir, Kahramanmaraş, Karaman, Karabük, Kastamonu, Kayseri, Kırklareli, Kırşehir, Kütahya, Konya, Manisa, Mersin, Muğla, Niğde, Osmaniye, Sakarya, Uşak, Yozgat (Lodos et al., 1978; 2003; Sert, 1995; Pehlivan et al., 2005a; Erbey, 2010 ; Yardibi & Tozlu, 2013; Gürler, 2014; Bolu, 2016; Güven, 2019).

Species: *Lixus circumcinctus* Bohemann, 1836

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 2, 2019 (number of insect samples: 3); May 15, 2019 (number of insect samples: 8); May 16, 2019 (number of insect samples: 3); May 23, 2019 (number of insect samples: 3); April 14, 2022 (number of insect samples: 3), on *Crambe orientalis*.

Distribution in Türkiye: Ankara, Diyarbakır, Kayseri, Kırşehir, Mardin (Sert, 1995; Sert & Çağatay, 1999; Pehlivan et al., 2005a; Bolu, 2016), Kahramanmaraş (new record).

Species: *Lixus elongatus* Goeze, 1777

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2019 (number of insect samples: 2), on *Sisybrium* sp. (new host plant); May 9, 2019 (number of insect samples: 13), on *Carduus nutans*.

Distribution in Türkiye: Adana, Antalya, Ankara, Aydın, Bilecik, Bursa, Denizli, Düzce, Gaziantep, Hatay, İçel, Kahramanmaraş, Karaman, Kayseri, Kırklareli, Kırşehir, Konya, Manisa, Nevşehir, Niğde, Uşak (Lodos et al., 1978; 2003; Altınayar, 1981; Sert, 1995).

Species: *Lixus scolopax* Bohemann, 1836

Examined material: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, December 11, 2017 (number of insect samples: 3), on *Echinops orientalis*; Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°39'31,17/E36°82'53,69, July 8, 2021 (number of insect samples: 2), on *Echinops orientalis* (new host plant).

Distribution in Türkiye: Adana, Antalya, Denizli, Edirne, Gaziantep, Kahramanmaraş, Mersin, Niğde, Osmaniye, Tekirdağ, Karabük (Lodos et al., 1978; 2003; Erbey, 2010; Yardibi & Tozlu, 2013; Bolu, 2016).

Species: *Lixus vilis* (Rossi, 1790)

Material examined: Kahramanmaraş, Onikişubat, Şehit Abdullah Çavuş Neighborhood, N37°34'55,825/E36°53'10,106, April 21, 2022 (number of insect samples: 2), on *Sisybium* sp. (new host plant); Kahramanmaraş, Pazarcık, Akçalar Village, N37°31'29,326/E37°26'14,1770, May 11, 2022 (number of insect samples: 2), on *Carduus nutans* (new host plant).

Distribution in Türkiye: Adana, Afyonkarahisar, Aksaray, Ankara, Aydın, Balıkesir, Bursa, Çanakkale, Edirne, Hatay, İzmir, Kastamonu, Kırklareli, Kütahya, Manisa, Mardin, Muğla, Niğde, Osmaniye (Lodos et al., 1978; 2003; Fremuth, 1983; Pehlivan et al., 2005; Erbey, 2010; Avgın & Colonnelli, 2011),

Kahramanmaraş (new record).

Genus: *Larinus* Dejean, 1821

Species: *Larinus cinerascens* Capiomont, 1874

Material examined: Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, July 8, 2021 (number of insect samples: 2); July 2, 2022 (number of insect samples: 3); June 6, 2022 (number of insect samples: 6), on *Carthamus dentatus*; Kahramanmaraş, Pazarcık, Akçalar Village, N37°31'29,326/E37°26'14,770, July 6, 2022 (number of insect samples: 6), on *Carthamus dentatus* (new host plant).

Distribution in Türkiye: A new record for Türkiye.

Species: *Larinus hedenborgi* Boheman, 1845

Material examined: Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, August 2, 2022 (number of insect samples: 7), on *Echinops orientalis* (new host plant).

Distribution in Türkiye: Adıyaman, Balıkesir, Gaziantep, Malatya, Siirt, Sivas, Şanlıurfa (Gültekin, 2008a; Gültekin & Podlussany, 2012), Kahramanmaraş (new record).

Species: *Larinus latus* (Herbst, 1874)

Material examined: Kahramanmaraş, Dulkadiroğlu, Hacımustafa Village, N37°28'29,776/E36°53'7,2773, April 25, 2022 (number of insect samples: 12), on *Carduus nutans*; Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 17, 2022 (number of insect samples: 3), on *Carduus nutans*.

Distribution in Türkiye: Adana, Adıyaman, Afyonkarahisar, Amasya, Ankara, Antalya, Aydın, Balıkesir, Bitlis, Burdur, Bursa, Çankırı, Çanakkale, Çorum, Denizli, Diyarbakır, Erzurum, Eskişehir, Hatay, Isparta, Iğdır, İçel, İzmir, Kahramanmaraş, Karaman, Karabük, Kayseri, Kırşehir, Kırklareli, Konya, Manisa, Mersin, Nevşehir, Niğde, Osmaniye, Siirt, Sivas, Yozgat (Lodos et al., 1978; 2003; Sert & Çağatay, 1994; Sert, 1995; Pehlivan et al., 2005a; Gültekin, 2008b; Erbey, 2010; Yardibi & Tozlu, 2013; Gürler, 2014; Yılmaz, 2015; Güven, 2019).

Species: *Larinus minutus* Gyllenhal

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 17, 2022 (number of insect samples: 2), on *Centaurea solstitialis*; Kahramanmaraş, Onikişubat, Bulutoğlu Village, N37°38'17,7/E37°46'02,9, June 25, 2022 (number of insect samples: 3), on *Centaurea solstitialis* (new host plant).

Distribution in Türkiye: Adana, Adıyaman, Ankara, Antalya, Balıkesir, Bitlis, Burdur, Çanakkale, Çorum, Diyarbakır, Edirne, Eskişehir, Gaziantep, Hakkari, Iğdır, İçel, İzmir, Karaman, Kahramanmaraş, Kayseri,

Kırşehir, Kırklareli, Kilis, Malatya, Mardin, Mersin, Muğla, Niğde, Osmaniye, Şanlıurfa, Yozgat (Lodos et al., 1978; 2003; Pehlivan et al., 2005a; Sert, 1995; Erbey, 2010; Gürler, 2014; Yılmaz, 2015; Bolu, 2016; Güven, 2019).

Species: *Larinus onopordi* (Fabricius, 1787)

Material examined: Kahramanmaraş, Türkoğlu, Kızılören Village, N37°20'48,6/E36°46'40,7, April 8, 2022 (number of insect samples: 8), on *Echinops orientalis*; Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'33,557/E36°50'16,226, March 29, 2022 (number of insect samples: 10); March 8, 2022 (number of insect samples: 11); May 24, 2022 (number of insect samples: 9); May 11, 2022 (number of insect samples: 7); May 31, 2022 (number of insect samples: 8); August 8, 2022 (number of insect samples: 3); August 11, 2022 (number of insect samples: 6), on *Echinops orientalis*; Kahramanmaraş, Pazarcık, Salmanıpak Village, N37°43'0,782/E37°21'5,700, May 26, 2022, on *Sorghum halapense*; Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 24, 2022 (number of insect samples: 8); April 27, 2022 (number of insect samples: 4), on *Echinops orientalis*; Kahramanmaraş, Dulkadiroğlu, Hacımustafa Village, N37°28'29,776/E36°53'7,2773, April 25, 2022 (number of insect samples: 11), on *Echinops orientalis*; Kahramanmaraş, Pazarcık, Sarerik Village, N37°20'50,503/E37°6'16,540, May 11, 2022 (number of insect samples: 12), on *Echinops orientalis* (new host plant).

Distribution in Türkiye: Adana, Adıyaman, Afyonkarahisar, Antalya, Artvin, Aydın, Balıkesir, Bingöl, Bitlis, Burdur, Bursa, Çanakkale, Diyarbakır, Edirne, Elazığ, Erzurum, Erzincan, Gaziantep, Hakkari, Hatay, Isparta, Iğdır, İçel, İzmir, Kahramanmaraş, Karaman, Karabük, Kars, Kırşehir, Kırklareli, Kilis, Malatya, Manisa, Mardin, Mersin, Nevşehir, Niğde, Osmaniye, Sivas, Şanlıurfa, Şırnak (Lodos et al., 1978; 2003; Pehlivan et al., 2005a; Gültekin, 2006c; Göktürk, 2009; Erbey, 2010; Yardibi & Tozlu, 2013; Bolu, 2016; Özgen et al., 2016; Güven, 2019; Sert & Özdemir, 2019).

Species: *Larinus sturnus* Schaller, 1873

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, July 18, 2018 (number of insect samples: 2), on *Echinops orientalis* (new host plant).

Distribution in Türkiye: Adana, Ankara, Artvin, Çankırı, Isparta, İçel, İzmir, Kars, Kastamonu, Kırşehir, Konya, Mersin, Niğde (Lodos et al., 1978; 2003; Sert & Çağatay, 1994; Sert, 1995; Pehlivan & al., 2005a; Erbey, 2010; Gürler, 2014; Yılmaz, 2015), Kahramanmaraş (new record).

Species: *Larinus turbinatus* Gyllenhal, 1835

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 2), on *Carduus nutans*; May 24, 2022 (number of insect samples: 2), on *Picnomon acarna*; Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°39'66,15/E36°84'84,06, July 8, 2021 (number of insect samples: 3), on *Picnomon acarna*; Kahramanmaraş, Pazarcık, Sarierik Village, N37°20'50,503/E37°6'16,540, on *Carduus acanthoides*; Kahramanmaraş, Dulkadiroglu, Hacımustafa Village, N37°28'29,776/E36°53'7,2773, April 25, 2022 (number of insect samples: 2), on *Carduus nutans*; Kahramanmaraş, Türkoğlu, Damobası Village, N37°39'92,03/E36°81'95,65, July 8, 2021 (number of insect samples: 2), on *Picnomon acarna* (new host plant).

Distribution in Türkiye: Afyonkarakisar, Ankara, Antalya, Artvin, Balıkesir, Bayburt, Bingöl, Bitlis, Burdur, Çankırı, Diyarbakır, Düzce, Hakkari, Iğdır, Karaman, Kastamonu, Kırşehir, Mardin, Mersin, Niğde (Lodos et al., 1978; Pehlivan et al., 2005a; Erbey, 2010; Gürler, 2014; Yılmaz, 2015), Kahramanmaraş (new record).

Species: *Larinus rudicollis* Petri, 1907

Material examined: Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, July 4, 2017 (number of insect samples: 2); July 6, 2017 (number of insect samples: 5); July 13, 2017 (number of insect samples: 3); September 12, 2017 (number of insect samples: 4); October 25, 2019 (number of insect samples: 7); December 11, 2017 (number of insect samples: 4), on *Echinops orientalis* (new host plant).

Distribution in Türkiye: Adana, Adıyaman, Antalya, Bayburt, Bingöl, Bitlis, Elazığ, Erzincan, Gaziantep, Hatay, İçel, Kahramanmaraş, Kilis, Malatya, Muş, Osmaniye, Van (Lodos et al., 2003; Pehlivan et al., 2005a; Gültekin, 2008c; Bolu, 2016; Korotyaev et al., 2016).

CONCLUSION

As a result of this study, It was found that the Curculionoidea fauna feeding on weeds in non-agricultural areas of Kahramanmaraş Province is quite rich. In addition to investigating the relationship between Curculionoidea and weeds, this study has provided new data that will form the basis for future biological control and other regional studies.

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Contribution of the Authors as Summary

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Determination of Biology and Prey Preference of the Predator Insect, *Xylocoris flavipes* (Reuter) (Heteroptera: Anthocoridae) Against Storage Pests

Gönül USTA GEBEŞ¹, Celalettin GÖZÜAÇIK²

¹University of Iğdır, Faculty of Agriculture, Department of Plant Protection, ²University of Iğdır, Faculty of Agriculture, Department of Plant Protection, Iğdır

¹<https://orcid.org/0000-0002-4961-4658>, ²<https://orcid.org/0000-0002-6543-7663>

✉: gnlusta@hotmail.com

ABSTRACT

This study was carried out to determine the biology of *Xylocoris flavipes* Reuter (Heteroptera: Anthocoridae), an important predator of stored product pests, and its prey preference on some warehouse pests. The study was conducted under laboratory conditions at 25±2°C temperature and 55±10% humidity. *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were used to determine the biology of *X. flavipes*. *E. kuehniella*, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), and *Trogoderma granarium* Everts (Coleoptera: Dermestidae) larvae were utilized to detect the prey preferences of *X. flavipes*. The mean development time (days) of five nymphs, female and male adults of *Xylocoris flavipes* on *E. kuehniella* eggs were as follows: 4.2±0.63; 3.1±0.57; 2.9±0.57; 3.0±0.00; 4.5±0.53; 104.2±17.01; 114.6±12.34, respectively. The average egg consumption for the aforementioned stages was: 5.0±1.76; 9.9±3.07; 9.1±6.01; 13.3±4.14; 22.5±4.79; 515.6±75.46; 286.2±24.39, respectively. Although mature male *X. flavipes* survived longer than females, females consumed more *E. kuehniella* eggs than males. The mean oviposition period of the females was 36.7±12.39 days, and the average laid egg number was 117.3±29.86. The consumed *E. kuehniella* eggs and larvae, *T. confusum*, and *T. granarium* larvae during the predator nymph period were on average 63.1±6.01; 49.6±2.76; 26.8±3.52; 12.8±4.29, respectively. The average development time (days) of the nymphs for the aforementioned consumption stages were 16.4±1.96; 15.4±0.52; 16.2±1.14; and 21.6±4.62, respectively. The consumed *E. kuehniella* eggs and larvae, *T. confusum*, and *T. granarium* larvae during the predator adult period were on average 570±231.32; 249.5±142.10; 218.1±110.57; 22.2±6.23, respectively. The average development time (days) of the adults for the aforementioned consumption stages were 87.5±30.92; 75.9±41.18; 89.3±43.55; and 24.5±7.18 days, respectively. *X. flavipes* preferred mostly *E. kuehniella* eggs among the preys during the nymph and adult stages, and least *T. granarium* larvae.

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Depo Zararlılarına Karşı Predatör Böcek, *Xylocoris flavipes* (Reuter) (Heteroptera: Anthocoridae)'in Biyolojisi ve Av Tercihinin Belirlenmesi

ÖZET

Bu çalışma, depolanmış ürün zararlılarının önemli bir predatörü olan *Xylocoris flavipes* Reuter (Heteroptera: Anthocoridae)'nin biyolojisi ve bazı depo zararlıları üzerindeki av tercihinin belirlemek amacıyla yürütülmüştür. Çalışma 25±2°C sıcaklık, %55±10 neme ayarlı laboratuvar koşullarında yapılmıştır. *Xylocoris flavipes*'in biyolojisinin belirlenmesinde *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) yumurtaları, av tercihinde ise *E. kuehniella*, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) ve *Trogoderma granarium* Everts (Coleoptera: Dermestidae) larvaları kullanılmıştır. Çalışma sonucunda, *X. flavipes*'in ergin dişi, erkek ve beş nimf döneminin *E. kuehniella* yumurtalarında ortalama gelişme süreleri sırasıyla

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104.2±17.01; 114.6±12.34; 4.2±0.63; 3.1±0.57; 2.9±0.57; 3.0±0.00; 4.5±0.53 gün, ortalama tükettikleri yumurta sayıları sırasıyla 515.6±75.46; 286.2±24.39; 5.0±1.76; 9.9±3.07; 9.1±6.01; 13.3±4.14; 22.5±4.79 adet bulunmuştur. Erkek *X. flavipes* bireyleri dişilerden daha uzun süre hayatta kalmıştır ve dişiler erkek bireylerden daha fazla *E. kuehniella* yumurtası tüketmişlerdir. Dişi bireyin ortalama ovipozisyon süresi 36.7±12.39 gün, bıraktığı ortalama yumurta sayısı ise 117.3±29.86 adet olarak belirlenmiştir. Nimf döneminde depo zararlılarından *E. kuehniella* yumurta, birinci ve ikinci dönem larvası, *T. confusum*, *T. granarium* birinci ve ikinci dönem larvalarından sırasıyla ortalama 63.1±6.01; 49.6±2.76; 26.8±3.52; 12.8±4.29 adet olarak ortalama sırasıyla 16.4±1.96; 15.4±0.52; 16.2±1.14; 21.6±4.62 günde; ergin döneminde ise 570±231.32; 249.5±142.10; 218.1±110.57; 22.2±6.23 adet şeklinde sırasıyla 87.5±30.92; 75.9±41.18; 89.3±43.55; 24.5±7.18 günde tüketmiştir. *Xylocoris flavipes* nimf ve ergin dönemlerinde avlarından en fazla *E. kuehniella* yumurtası, en az *T. granarium* 1. ve 2. dönem larvalarını tercih etmiştir.

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INTRODUCTION

Pests, rodents, and microorganisms cause about 10% quantitative and qualitative loss in stored products (Prevett, 1975). The geography and climate conditions of Türkiye are favorable for pest development. The pests are especially deleterious for stored products in the Southern parts of Türkiye (Ferizli & Emekçi, 2010). Pest residues as body parts and various secretions deteriorate crop quality. High pest contamination density in stored commodities accelerates processes such as molding, heat, and fermentation. Consumption of pest-containing commodities also leads to potential health risks. The general approaches to get rid of the pests are crop destruction or pesticide application. Pest management in stored products encounters many limitations including restrictions on many pesticides worldwide and progress of insecticide resistance in pest populations. Therefore, environment-friendly sustainable approaches are required (Bell et al., 1996). Many parasitoids and predators are used for biological control in Integrated Pest Management (IPM) systems (Schöller & Flinn, 2000; Schöller et al., 2006). Insect natural enemies could be a potential solution for the biological control of pests (Haines, 1984; Brower et al., 1995; Hokkanen et al., 1995; Zettler & Arthur, 2000). There are 22 predator and parasitoid species known in storage ecosystems. *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) is a predator of many stored crop pests. This bug is commercially produced and sold for biological control in crop storage in North America (Mason & Huber, 2001; Taro et al., 2008). The presence of predator mites has been detected in vegetable fields in our country (Kaymak et al., 2023).

Similar studies exist. However, the predator *X. flavipes* was not detected in warehouses and was not used. The potential of the biological agent *X. flavipes* as a predator of stored product pests was first evaluated by Jay et al. (1968). Ballal et al. (2013) noted that *X. flavipes* are an important predator of stored product pests. *X. flavipes* is a predator bug of many storage pests and has the potential for use in biological control programs. Because *X. flavipes* is able to regulate populations of stored-product pests, this study concentrates on the investigation and incorporation of this valuable predator as a biological control agent in Türkiye. This study was carried out to determine the biology of *X. flavipes*, an important predator of stored product pests, and its prey preference on some crop storage pests.

MATERIALS AND METHODS

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), Jacquelin du Val (Coleoptera: Tenebrionidae), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) crop storage pests and *X. flavipes* predator were cultured and produced in Iğdır University, Faculty of Agriculture, Department of Plant Protection, Entomology laboratory. In the study, 5 lt plastic containers, (9 × 1.5 cm) of petri dishes, 25 ml glass bottles with cork stoppers, filter paper, gauze, rubber, flour, wheat, bulgur, rice, incubator, soft tip brush, plastic tube, binocular stereo microscope were used. The study was conducted under laboratory conditions at 25±2°C temperature and 55±10% humidity.

Definition of *Xylocoris flavipes* Biology on *Ephestia kuehniella* Eggs

Same day eggs of *X. flavipes* were laid in petri dishes containing blue cardboard paper on the bottom. In the study, 10 petri dishes each with 10 predator eggs were used. Egg hatching was tracked, and *X. flavipes* first instar nymphs were released after egg hatching. The instars were fed with daily laid eggs of *E. kuehniella*.

20 eggs per petri dish adhered to the cardboard strips using water and a soft-tipped brush. Cardboard strips were checked every 24 hours, and the number of consumed eggs was recorded and replaced with new ones. This process continued until *X. flavipes* matured and died. Average egg consumption and days of life stages for *X. flavipes* 1st, 2nd, 3rd, 4th, and 5th instar nymph, as well as adults, were determined (Table 1).

Table 1. Average life period (in days) of nymphs and adults of *Xylocoris flavipes* and number of consumed *Ephestia kuehniella* eggs

Çizelge 1. *Xylocoris flavipes*'in nimf ve ergin dönemlerinin süresi ve bu dönemlerde tükettikleri *Ephestia kuehniella* yumurta sayıları

Biological Stages of <i>Xylocoris flavipes</i>	Average life cycle Days±SD (Min.-Max.)	Average consumed <i>Ephestia kuehniella</i> Eggs±SD (Min.-Max.)	P
1. Nymph	4.2±0.63 (3-5)	5.0±1.76 (2-7)	<0.001
2. Nymph	3.1±0.57 (2-4)	9.9±3.07 (5-15)	0.383
3. Nymph	2.9±0.57 (2-4)	9.1±6.01 (0-19)	<0.001
4. Nymph	3.0±0.00 (3-3)	13.3±4.14 (7-21)	<0.001
5. Nymph	4.5±0.53 (4-5)	22.5±4.79 (17-30)	<0.001
Total	17.7 (14-21)	59.8 (31-92)	
Female adult	104.2±17.01 (76-122)	515.6±75.46 (427-629)	<0.001
Male adult	114.6±12.34 (100-131)	286.2±24.39 (259-319)	<0.001

The abdomen of females has a longitudinal symmetrical elongated appearance on both sides and in the males, there is a left offset and ventral notch on the left side of the 8th and 9th segments. Adults of *X. flavipes* is dark brown to black with rostrum 3 and antennae 4 segmented. Female adults have long symmetrical abdomens from the laterals, and the abdomen of the male adult is tilted to the left (Fig. 1g, h)

Detection of *Xylocoris flavipes* Preoviposition, Oviposition, and Postoviposition Times on *Ephestia kuehniella* Eggs

X. flavipes adults were placed in pairs (1♀, 1♂) in one petri dish containing blue cardboard on the base and the experiment was conducted with 10 replications. The adults were fed with *E. kuehniella* eggs adhered to the cardboard strips using water and a soft-tipped brush. The period until the first egg lay of the female adult was recorded as preoviposition. The Petri dishes were checked every 24 hours, and the number of eggs laid by *X. flavipes* was recorded and placed in other petri dishes. This process continued until female adults stop laying eggs and this period was recorded as oviposition. The days between oviposition (stop laying eggs) and death of *X. flavipes* female was recorded as postoviposition.

Consumption of *Ephestia kuehniella* Eggs and Larvae, and Larvae of *Tribolium confusum* and *Trogoderma granarium* by Pre-mature and Adult Stages of *Xylocoris flavipes*

1st and 2nd instar nymph and one adult of *X. flavipes* were placed separately in a petri dish with filter paper on the bottom and the experiment was conducted with 10 replications. 20 eggs of *E. kuehniella* per petri dish were adhered to the cardboard strips using water and a soft-tipped brush. Every 24 hours, the number of consumed *E. kuehniella* eggs was recorded and new cardboard strips were added. This process continued until nymphs of *X. flavipes* matured and their adults died. Likewise, one nymph and one adult of *X. flavipes* were placed separately per 25 ml bottles with cork stoppers, and 10 bottles per nymph and adult were used. 20 first and second-stage larvae of *E. kuehniella* were added to each bottle. Consumed *E. kuehniella* larvae at intervals of five days were counted and replaced with new ones. This process continued until the predator nymphs matured and the adults died. Flour was added to the bottles to feed *E. kuehniella* larvae. The method applied for the consumption of *E. kuehniella* larvae was also applied for the consumption of *T. confusum* and *T. granarium* larvae. The production of *Tribolium confusum* pest was carried out by considering the work of Gökçe et al. (2021).

Statistical Analysis

Descriptive statistics were determined for the average egg consumption (number) and lifespan (days) of the nymph and adult periods. However, the width and length data of the nymph, adult, and egg periods; days of preoviposition, oviposition, and postoviposition and the number of eggs laid; Mean, standard error, and minimum-maximum values were calculated for the

larvae and eggs (number) consumed and the time (day) data consumed in the prey preferences of adults and nymphs. Shapiro and Levene tests were applied to the above-mentioned data and the normal distribution and constant variance assumptions of the data were examined. One-way analysis of Variance (ANOVA) followed by the Tukey test was applied to the data providing the hypotheses and pairwise group

comparisons were made. Since the data on the number of harmful larvae consumed by adults did not meet the assumptions of normal distribution and constant variance, it was subjected to the Kruskal-Wallis test, which is a non-parametric method, and then Dunn's test for pairwise group comparisons. The experiments were conducted with 10 replications.



Figure 1. Eggs of *Xylocoris flavipes* (a), 1st nymph (b), 2nd nymph (c), 3rd nymph (d), 4th nymph (e), 5th nymph (f), adult male (g) and adult female (h)

Şekil 1. *Xylocoris flavipes*'in yumurtası (a), 1. nimf (b), 2. nimf (c), 3. nimf (d), 4. nimf (e), 5. nimf (f), ergin erkek (g) ve ergin dişi (h)

RESULTS AND DISCUSSION

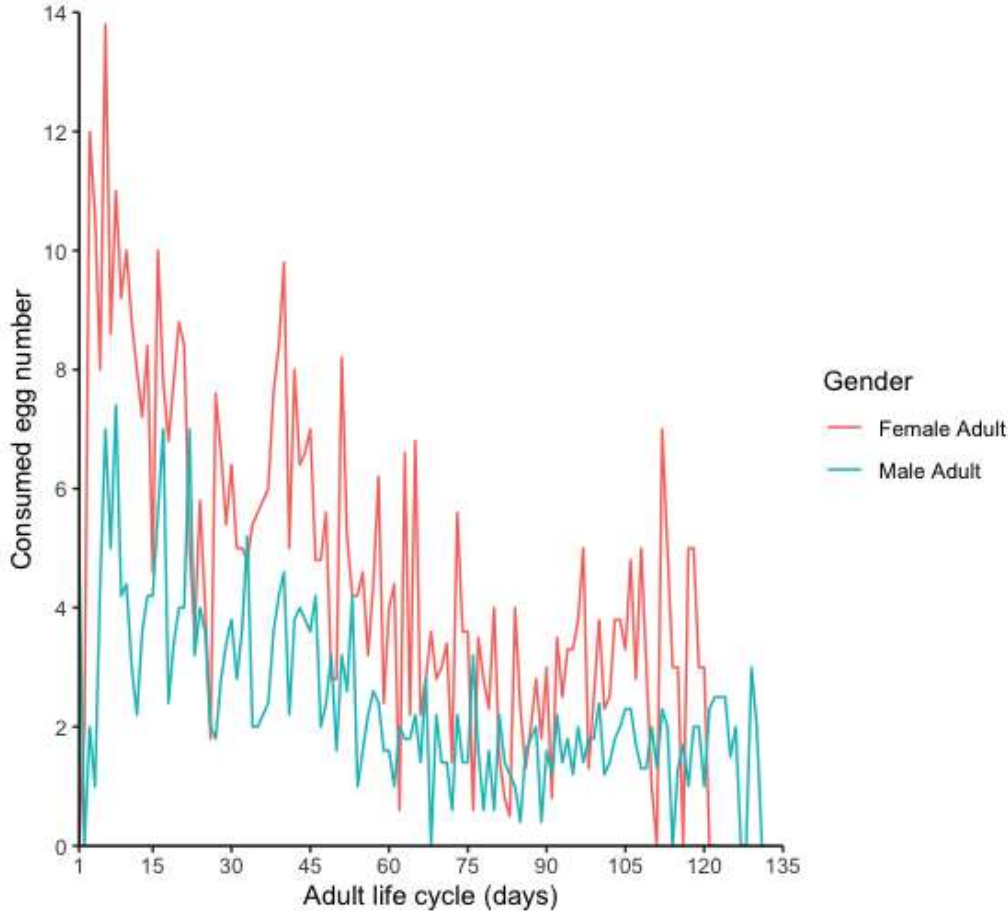
Definition of *Xylocoris flavipes* and Biology on *Ephestia kuehniella* Eggs

Eggs of *X. flavipes* is cylindrical (ellipsoid) with a white-yellowish transparent cover on one side, turning to orange when the nymph exits approaches, as well as with reddish eyes and scent glands (Fig. 1a). Egg hatching time was estimated average as 5.2 ± 0.42 days. 1st Stage nymphs are generally light orange with red eyes and visible scent glands with transparent antennae, rostrum, and legs (Fig. 1b). The development time of the 1st stage nymph was estimated to average 4.2 ± 0.63 days (Table 1). The 2nd

stage nymph has similar characteristics to the 1st stage nymph but with more prominent scent glands (Fig. 1c) and an average development time of 3.1 ± 0.57 days (Table 1). The 3rd stage nymph has a darker color than the 2nd stage nymph (Fig. 1d) with a development time of average 2.9 ± 0.57 days. The 4th stage nymph has a dark orange, brown color (Fig. 1e) with development time of average 3.0 ± 0.00 days (Table 1). The 5th stage nymph looks like the adult with light brown color (Fig 1f) and development time of average 4.5 ± 0.53 days (Table 1). The 5th stage nymph consumed the highest number of *E. kuehniella* eggs and the 1st stage nymph the lowest as illustrated in

Table 1. Figure 2 shows that the egg consumption of *X. flavipes* was highest in the 5th nymphal stage (peaking on day 4 with an average of 9 eggs) and lowest in the 1st nymphal stage. The life cycle for female adult was average 104.2 ± 17.01 days, and 114.6 ± 12.34 days for

the male (Table 1). The consumed *E. kuehniella* eggs for female adult was average 515.6 ± 75.46 eggs, and 286.2 ± 24.39 eggs for the male as seen in Table 1. It was determined that *X. flavipes* adult females consumed more *E. kuehniella* eggs than males (Figure 3).



Şekil 2. *Xylocoris flavipes*'in nimf dönemlerinin gelişme süreleri ve tükettikleri *Ephestia kuehniella* yumurta sayıları arasındaki ilişkiyi gösteren çizgi grafiği

Figure 2. Line graph showing the relationship between development time of *Xylocoris flavipes* nymph stages and consumed *Ephestia kuehniella* eggs.

The female adult's highest egg consumption was during day 6th with an average of 14 eggs, and the male adult's highest egg consumption was during day 8th with an average of 7 eggs. Arbogast et al. (1971) and Awadallah and Tawfik (1972a) reported that adults of *X. flavipes* are bright brown to dark in color and about 2 mm tall. Additionally, gender detection is most easily made from the abdomen. Lokanath (2012a) reported that the average development times of the *X. flavipes* nymph stages using *C. cephalonica* eggs under laboratory conditions were 3.2 ± 1.30 ; 2.8 ± 0.84 ; 2.8 ± 0.86 ; 3.2 ± 0.84 ; 3.6 ± 0.65 . These results are consistent with our findings. However, the life cycle was reported to be 22.4 ± 1.42 days for the female adult, and 24.4 ± 1.80 days for the male adult. These results are much lower than our findings (Table 1). The males lived longer than the females which was consistent with our results. Parajulee et al. (1994),

Rahman et al. (2009) determined in their research that females of the biological agent consumed more prey than male hunters. Russo (2004) reported 24.4 days and Awadallah and Tawfik (1972b) stated 21.7 days life period for the adults. These findings are lower than our results (Table 1).

Detection of *Xylocoris flavipes* Preoviposition, Oviposition and Postoviposition Times on *Ephestia kuehniella* Eggs

Xylocoris flavipes laid eggs randomly about 4.9 ± 1.37 days after mating (Table 2). The preoviposition, oviposition and post-oviposition times of the predator were examined on 10 female adults and the mean preoviposition time was on average 4.9 ± 1.37 days. The oviposition and post-oviposition times were about 36.7 ± 12.39 and 1.1 ± 0.99 days, respectively (Table 2).

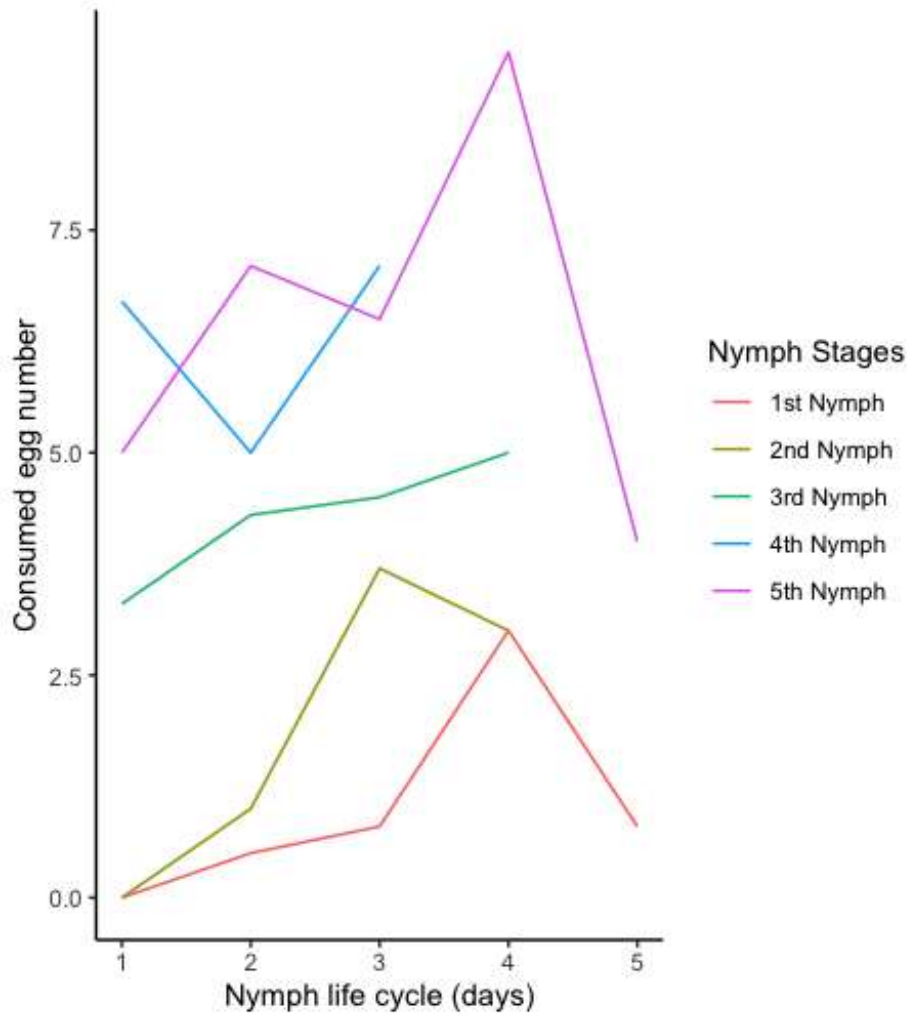


Figure 3. The relationship between development time of female and male adult of *Xylocoris flavipes* and number of *Ephestia kuehniella* eggs they consume

Şekil 3. *Xylocoris flavipes*'in ergin erkek ve dişi dönemlerinin gelişme süreleri ve tükettikleri *Ephestia kuehniella* yumurta sayıları arasındaki ilişki

Table 2. Average preoviposition, oviposition and postoviposition development times and laid eggs by *Xylocoris flavipes*

Çizelge 2. *Xylocoris flavipes*'in preovipozisyon, ovipozisyon, postovipozisyon dönemlerinin ortalama gelişme süreleri ve bırakılan ortalama yumurta sayısı

Stage	AverageDays±SD (Min.-Max.)	AverageEggs±SD (Min.-Max.)
Preoviposition	4.9±1.37 (3-8)	0.0±0.00 (0-0)
Oviposition	36.7±12.39 (20-62)	117.3±29.86 (73-175)
Postoviposition	1.1±0.99 (0-3)	0.0±0.00 (0-0)

The average number of eggs laid by the female adult during oviposition was 117.3±29.86 with minimum egg number of 73 and maximum 175 eggs per female (Table 2). It was found that there was a positive linear relationship ($r=0.85$) between the number of eggs laid by *X. flavipes* females and the consumption of *E. kuehniella* eggs, and as the number of eggs laid by predator females increased, the consumption of *E.*

kuehniella eggs also increased (Figure 4).

Lokanath (2012b) reported that under laboratory conditions using *Corcyra cephalonica* (Stainton) eggs, female adults of *X. flavipes* laid 24-32 eggs during the oviposition period lasting for 23-28 days. Rahman (2004) found that the average total highest egg numbers produced by female *X. flavipes* when fed with *Tribolium castaneum* (Herbst) and *T. confusum*

(Jacquelin du val) were 98.65 ± 1.81 , 97.05 ± 1.73 , respectively. Arbogast (1975) stated about 30 eggs for 29 days; Awadallah and Tawfik (1972c) found about 41.6 eggs for 17.5 days; Rahman (2007) reported on average 27.27 ± 2.52 eggs during oviposition.

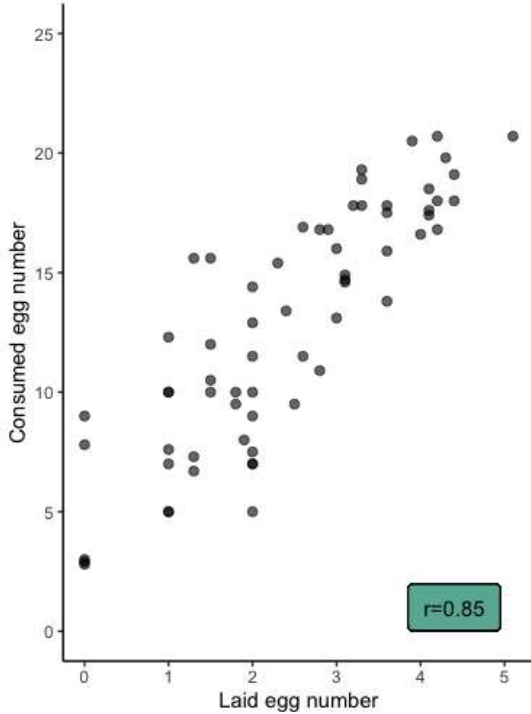


Figure 4. The relationship between *X. flavipes* female adult laid eggs and consumption of *E. kuehniella* eggs.

Şekil 4. *Xylocoris flavipes*'in ergin dışısının ovipozisyon döneminde tükettiği *Ephestia kuehniella* yumurta sayısı ile yumurtladığı yumurta sayısı arasındaki ilişki

Consumption of *Ephestia kuehniella* Eggs and Larvae, and Larvae of *Tribolium confusum* and *Trogoderma granarium* by Pre-mature and Adult Stages of *Xylocoris flavipes*

It was determined that *X. flavipes* nymphs consumed about 63.1 ± 6.01 *E. kuehniella* eggs, and the adults consumed 570 ± 231.32 eggs (Table 3). Mean development times for nymph and adults of *X. flavipes* during *E. kuehniella* egg consumption are 16.4 ± 1.96 ; 87.5 ± 30.92 , respectively. The average consumed *E. kuehniella* larvae was 49.6 ± 2.76 for nymphs, and 249.5 ± 142.10 for adults of *X. flavipes* with an average development time of 15.4 ± 0.52 days, 75.9 ± 41.18 days, respectively. The average consumed *T. confusum* larvae was 26.8 ± 3.52 for nymphs, and 218.1 ± 110.57 for adults of *X. flavipes* with an average development time of 16.2 ± 1.14 days, 89.3 ± 43.55 days, respectively. The average consumed *T. granarium* larvae was 12.8 ± 4.29 for nymphs, and 22.2 ± 6.23 for adults of *X. flavipes* with an average development time of 21.6 ± 4.62 days, 24.5 ± 7.18 days, respectively (Table 3). As shown in Figure 5, *X. flavipes* nymphs consumed the most *E. kuehniella* larvae and the least *T. granarium* larvae. Similarly, *X. flavipes* adults consumed most *E. kuehniella* larvae and the least *T. granarium* larvae (Figure 6).

X. flavipes nymph and adults preferred most *E. kuehniella* eggs and least *T. granarium* larvae. *E. kuehniella* eggs were consumed more because of their small size, and *T. granarium* larvae were preferred less because they were extremely hairy. LeCato and Collins (1976) looked at the maximum number of prey that a predator could consume in a lifetime. They used *T. castaneum* as the prey species and determined that *X. flavipes* could consume an average of 539 eggs, 34 larvae, or 14 pupae in a laboratory environment. Schöller and Prozell (2011) reported that the predator *X. flavipes* could suppress *T. confusum* in small amounts or a thin layer of flour.

Çizelge 3. *Xylocoris flavipes*'in nimf ve ergin dönemlerinin ortalama gün ve bu dönemlerde tükettikleri depo zararlıları ortalama av miktarları

Table 3. Average development times and pray consumption for nymph and adults of *Xylocoris flavipes*

Type of prey	Period of hunt	<i>Xylocoris flavipes</i> nymph		<i>Xylocoris flavipes</i> adult	
		Average Consumption \pm SD (Min.-Max. Piece)	Average Day \pm SD (Min.-Max.)	Average Consumption \pm SD (Min.-Max. Piece)	Average Day \pm SD (Min.-Max.)
<i>Ephestia kuehniella</i>	Egg	63.1 ± 6.01 (55-76)	16.4 ± 1.96 (14-20)	570 ± 231.32 (289-895)	87.5 ± 30.92 (55-135)
<i>Ephestia kuehniella</i>	Larvae	49.6 ± 2.76 (44-53)	15.4 ± 0.52 (15-16)	249.5 ± 142.10 (36-421)	75.9 ± 41.18 (10-126)
<i>Tribolium confusum</i>	Larvae	26.8 ± 3.52 (22-32)	16.2 ± 1.14 (15-18)	218.1 ± 110.57 (50-381)	89.3 ± 43.55 (30-145)
<i>Trogoderma granarium</i>	Larvae	12.8 ± 4.29 (8-20)	21.6 ± 4.62 (17-28)	22.2 ± 6.23 (15-31)	24.5 ± 7.18 (12-37)

The reason for the higher egg consumption compared to larvae could be the smaller size and immobility of the eggs. LeCato and Davis (1973) found that *X. flavipes* preferred certain pest species and stages to

others probably due to pest size and sclerotization stage. It was detected that prey size was the most important factor affecting early and late-stage larvae consumption of *T. castaneum*, *Oryzaephilus*

surinamensis Linnaeus, *P. interpunctella* Hübner and *Lasioderma serricornis* Fabricius. Early-stage larvae were more consumed than late stage. *L. serricornis*

larva is small but hairy and sticky. Consequently, the preference for small-sized prey was confirmed.

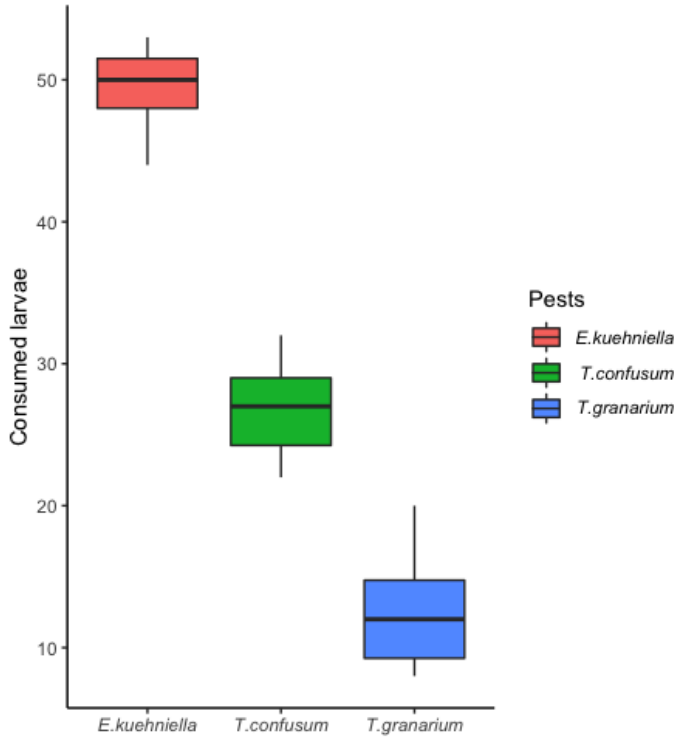


Figure 5. Boxplot showing the number of consumed larvae of *Ephestia kuehniella*, *Tribolium confusum* and *Trogoderma granarium* by *Xylocoris flavipes* nymphs.

Şekil 5. *Xylocoris flavipes* nimf döneminin kutu diyagramına göre tükettiği *Ephestia kuehniella*, *Tribolium confusum* and *Trogoderma granarium* larva sayısı

CONCLUSIONS

Insect damage is one of the biggest problems facing managers of stored grain ecosystems worldwide. In the study, it was seen that one of the biological agents that can reduce insect damage with biological control, which is one of the integrated control methods, is the predator *X. flavipes*. *X. flavipes* preferred the most *E. kuehniella* eggs and the least *T. granarium* larvae as prey in adult and nymph stages. *E. kuehniella* eggs were consumed more as prey because they were smaller, and *T. granarium* larvae were less preferred because they contained excessive hair. The mean number of *E. kuehniella* eggs (63.1 ± 6.01 ; 570 ± 231.32) consumed in *X. flavipes* nymph and adult stages found too much is higher than the number of larvae (49.6 ± 2.76 ; 249.5 ± 142.10). It is thought that the prey is smaller and immobile because the predator consumes more eggs than the larva. In this study, it was concluded that the *X. flavipes* predator would be successful in suppressing harmful insect populations of stored products. It also reveals that mass culture of *X. flavipes* can be established on *E. kuehniella* eggs in the

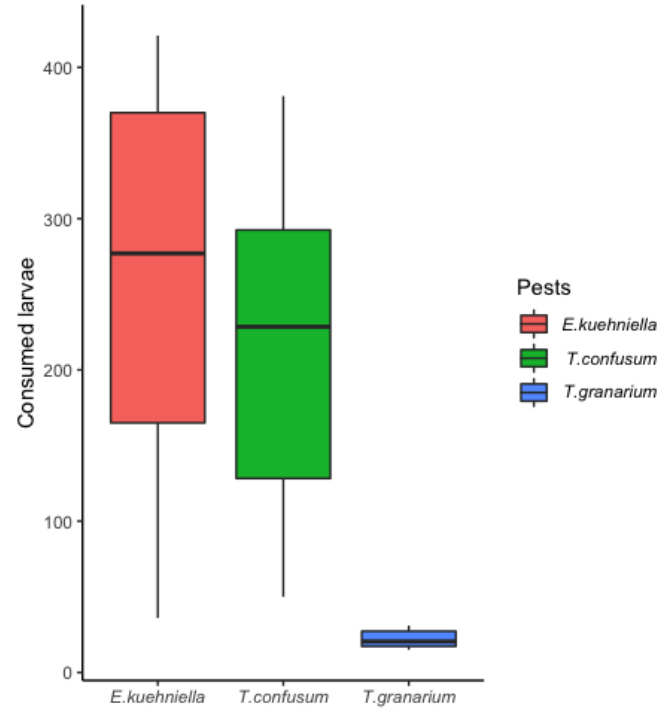


Figure 6. Boxplot showing the average consumed larvae of *Ephestia kuehniella*, *Tribolium confusum* and *Trogoderma granarium* by *Xylocoris flavipes* adults.

Şekil 6. *Xylocoris flavipes* ergin döneminin kutu diyagramına göre tükettiği *Ephestia kuehniella*, *Tribolium confusum* and *Trogoderma granarium* larva sayısı

laboratory for easy and abundant supply of the insect as a biological control agent. When the producers interviewed within the scope of research were asked to state the reasons for using the biological control method in combating pests in order of importance, their first two choices were, respectively, "Because I care about human health" and "To avoid polluting the natural environment" (Sayın et al., 2020). Support for biological control should be increased for human and environmental health.

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Authors' contributions

The authors contributed equally to the article. All authors read and approved the final manuscript.

Conflict of Interest Statement

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

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Taxonomic Studies on *Rhodocybe asyae* Specimens Discovered in a New Location

İlgaz AKATA¹, Gülce EDİŞ², Eda KUMRU², İsmail ACAR³⁻⁴, Ergin ŞAHİN⁵⁻⁶

¹ Ankara University, Faculty of Science, Department of Biology, Ankara/TÜRKİYE, ² Ankara University, Graduate School of Natural and Applied Sciences, Ankara/TÜRKİYE, ³Van Yüzüncü Yıl University, Başkale Vocational High School, Department of Organic Agriculture, Van/TÜRKİYE, ⁴Çanakkale Onsekiz Mart University Çanakkale Vocational School of Health Services, Department of Medical Services and Techniques, Çanakkale/TÜRKİYE, ⁵Dokuz Eylül University, Faculty of Science, Department of Biology, İzmir/TÜRKİYE, ⁶Dokuz Eylül University, Fauna and Flora Research and Application Center, İzmir/TÜRKİYE

¹<https://orcid.org/0000-0002-1731-1302>, ²<https://orcid.org/0000-0001-7038-9865>, ³<https://orcid.org/0009-0000-7417-6197>

⁴<https://orcid.org/0000-0002-6049-4896>, ⁵<https://orcid.org/0000-0003-1711-738X>

✉: akata@science.ankara.edu.tr

ABSTRACT

In 2023, fungal specimens were collected from the Tınaztepe Campus of Dokuz Eylül University (İzmir-Türkiye). These samples underwent a comprehensive and meticulous evaluation involving morphological examination and phylogenetic analysis based on nrITS rDNA sequencing. The DEU AKATA & SAHİN 148 samples exhibited microscopic and macroscopic characteristics that closely matched those of *Rhodocybe asyae*, initially described by Sesli & Vizzini. The genetic sequence analysis revealed over 99% similarity with *R. asyae* Sesli & Vizzini. This report represents the second recorded occurrence of *R. asyae* in Türkiye and the third globally. The study documents the collection site, geographical coordinates, habitat observations, the collection date, etc. Furthermore, the research provides macroscopic photographs, microscopic illustrations of the specimens, and detailed discussion, demonstrating the reliability of the present findings.

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Yeni Bir Lokasyonu Keşfedilen *Rhodocybe asyae* Örnekleri Üzerinde Taksonomik Çalışmalar

ÖZET

Mantar örnekleri 2023 yılında Dokuz Eylül Üniversitesi Tınaztepe Kampüsü'nden (İzmir-Türkiye) toplanmıştır. Bu örnekler, morfolojik inceleme ve nrITS rDNA dizilemesine dayalı filogenetik analizi içeren kapsamlı ve titiz bir değerlendirmeden geçirilmiştir. DEU AKATA & SAHİN 148 örnekleri, orijinal olarak Sesli & Vizzini tarafından tanımlanan *Rhodocybe asyae* ile yakından eşleşen mikroskopik ve makroskopik özellikler sergilemiştir. Genetik dizi analizi de *R. asyae* Sesli & Vizzini ile %99'un üzerinde benzerlik göstermiştir. Bu rapor, *R. asyae*'nin Türkiye'de kaydedilen ikinci ve Dünya'daki üçüncü kaydını temsil etmektedir. Çalışma, coğrafi koordinatlar, habitat gözlemleri, toplama tarihi ve benzeri bilgileri de içermektedir. Ayrıca, araştırma makroskopik fotoğraflar, örneklerin mikroskopik çizimleri ve bulguların güvenilirliğini gösteren ayrıntılı tartışmalar sunmaktadır.

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INTRODUCTION

The genus *Rhodocybe*, established by Maire (1926) as a part of the *Entolomataceae*, is distinguished by its variable-shaped, often dull-coloured basidiomata, which typically vary from conical to funnel-shaped with a central depression. The colours of the pileus

range from pinkish to vinaceous cinnamon. The lamellae are attached in ways that range from adnate to decurrent, occasionally exhibiting slight notches (Bas et al., 1988; Sun & Bau, 2023). The genus produces a spore print varying from salmon to brownish pink, and its basidiospores vary from

globose to ellipsoid or tear-shaped, characterized by a subtly nodulose or gently angular surface with pustulate details. In the polar view, the basidiospores are angular with 6–12 facets. Importantly, the genus lacks clamp connections. Hymenial cystidia may be absent or present as pseudocystidia with brightly coloured contents or as hyaline leptocystidia appearing as cheilocystidia and sometimes as pleurocystidia (Baroni, 1981; Vizzini et al., 2018). The genus members are primarily saprotrophic, thriving in soil among debris and occasionally on decaying wood. These species are found extensively across temperate and tropical regions in the northern and southern hemispheres. (Vizzini et al., 2016; Sesli & Vizzini, 2017).

Originally categorized in 1981 by Baroni, the genus *Rhodocybe* was organized into seven distinct sections, including *Claudopodes*, *Crepidotoides*, *Decurrentes*, *Rhodocybe*, *Rhodophana*, *Rufobrunnea*, and *Tomentosi*. The *Rufobrunnea* section is distinguished by its unique colouration, featuring shades of reddish-beige, salmon pink, pinkish-brown, or ochre on the pileus. Additionally, this section is noted for its adnate to decurrent gills and the notable absence of pseudocystidia (Vizzini et al., 2018). Further research, including molecular studies, has validated that this section is monophyletic, confirming its natural classification within the genus (Kluting et al., 2014; Sesli & Vizzini, 2017; Sun & Bau, 2023).

Around 50 species of the genus are recognized globally (Sun & Bau, 2023), but only six have been reported in Türkiye (Sesli, 2021; Sesli & Vizzini, 2017; Sesli et al., 2020; Vizzini et al., 2018). Within these, *R. asanii* Sesli & Vizzini and *R. asyae* Sesli & Vizzini, belong to *Rhodocybe* sect. *Rufobrunnea* (Sesli & Vizzini, 2017; Vizzini et al., 2018).

Rhodocybe asyae features distinctive clitocyboid basidiomata with exceptionally thin flesh. The species is noted for its smooth, dish-shaped pileus that exhibits a subtle salmon-pink hue. The lamellae are decurrent, transitioning in colour from whitish to light ivory, and may also have a hint of reddish beige. The stipe is pruinose and cylindrical. It possesses uniquely shaped basidiospores, which are ellipsoid to broadly ellipsoid, slightly angular, and have a warty texture. This species typically has two to four spored basidia and shows diversity in the form of its cheilocystidia (Sesli & Vizzini, 2017).

This study aims to enrich the knowledge of the rare species *R. asyae* by reporting a newly discovered location in Türkiye, thus contributing to the species' global distribution records.

MATERIALS and METHODS

The research employed a comprehensive approach, combining morphological assessments with molecular

methods to examine and categorize specimens collected from İzmir province, Türkiye. This study involved a thorough macroscopic and microscopic examination of the samples, which was enhanced by analyzing ribosomal DNA (rDNA) sequences using Internal Transcribed Spacer (ITS) sequencing techniques.

Morphological Characterization

The collection of the specimens was followed by an initial assessment of their macroscopic features and environmental conditions at the collection site. The samples were examined in detail using a binocular light microscope (Euromex Oxion). To ensure the reliability of the findings, each microscopic characteristic was measured using 30 different samples. 5% potassium hydroxide (KOH) and Congo red were used to prepare the material for examination.

For scanning electron microscopy (SEM), small fragments of the fungal samples were mounted on stubs using double-sided tape and coated with gold. These samples were then analyzed using an EVO 40XVP SEM from LEO Ltd., based in Cambridge, UK, operating at a 20 kV accelerating voltage.

The method for morphological identification of the samples followed the detailed protocols outlined in studies by Sesli & Vizzini (2017) and Aplin et al. (2022). These research papers were crucial in guiding the identification process and ensuring accuracy and adherence to established scientific standards. After accurately identifying the samples, they were carefully preserved in the Fungarium at Ankara University, Faculty of Science, Department of Biology.

Molecular Characterization

Genomic DNA Isolation From Fungal Specimens

In the genomic DNA isolation method, 50 mg of dry sporophore samples were mechanically powdered with a mill grinder, placed in 1.5 mL microcentrifuge tubes, and mixed with 700 μ L of CTAB lysis buffer (pH 8.0) containing 3% w v⁻¹ cetyl trimethyl ammonium bromide (CTAB), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris, 3% w v⁻¹ polyvinylpyrrolidone (PVP), and 0.2% v v⁻¹ β -mercaptoethanol. The samples were vortexed for 1 minute and incubated at 65 °C for 30 minutes. Following lysis, the samples were centrifuged at 13,000 rpm for 10 minutes, and 500 μ L of the supernatant was transferred to new tubes. An equal volume of chloroform-isoamyl alcohol (24:1) solution was added, vortexed briefly, and centrifuged again at 13,000 rpm for 5 minutes. The resulting supernatants were carefully transferred to new tubes, mixed with an equal volume of cold isopropanol, and stored at -20 °C for 30 minutes.

After this incubation, the samples were centrifuged at 13.000 rpm for 10 minutes to precipitate the genomic DNA as pellets. The supernatants were discarded, and the DNA pellets were washed twice with 70% ethanol. Residual ethanol was removed by incubating the DNA pellets at 60 °C. The partially dried DNA pellets were dissolved in an appropriate volume of nuclease-free distilled water. The concentration and purity of the isolated genomic DNA were determined spectrophotometrically using the Nanodrop Lite (Thermo Scientific) device. The integrity of the DNA was verified by agarose gel electrophoresis using a TAE buffer (40 mM Tris-acetate 1 mM EDTA; pH 8.3) with 0.8% agarose. Electrophoresis was performed at a standard voltage of 5 volts per centimetre. Imaging was done using a safe blue light transilluminator and safe green dye. A 1 kb Plus DNA Ladder was the DNA marker in the agarose gel electrophoresis.

PCR Amplification of the Internal Transcribed Spacer (ITS) rDNA Region and Determination of Nucleotide Sequences for Molecular Phylogeny of the Specimens

Genomic DNA samples isolated from macrofungal sporophores using the CTAB method were used as templates to amplify the ribosomal DNA region (ITS) for fungal molecular phylogeny studies. To prevent the formation of undesirable primer dimers during PCR, a hot start DNA polymerase enzyme was used. The PCR reaction was performed in 200 µL polypropylene tubes with a total volume of 50 µL. The reaction mixture included 5 µL of 10X DNA polymerase buffer (containing 25 mM MgCl₂), 1 µL of a deoxynucleoside triphosphate (dNTP) mixture (10 mM of each nucleotide), 300-400 ng of genomic DNA template, 1 µL of each 10 µL sequence-specific oligonucleotide primer, five units of DNA polymerase enzyme, and enough nuclease-free dH₂O to bring the

total volume to 50 µL. The sequences and melting temperatures (T_m) of the oligonucleotide primers used in PCR are provided in Table 1. PCR thermocycling conditions were optimized based on the T_m of each primer pair, the lengths of the amplified gene regions, and their copy number in the genome. The “Touchdown” approach was employed to minimize undesirable primer dimer formation.

The general PCR conditions were 30 seconds at 95 °C, 15 seconds at 65-50 °C (using the touchdown approach), and 15-30 seconds at 72 °C for 35 cycles, following an initial 2-minute denaturation step. The extension time at 72 °C was adjusted according to the length of the amplicon and the DNA polymerase enzyme used. The reaction concluded with a final extension step of 7 minutes at 72 °C. Thermal cycling was conducted using the MiniAmp Plus Thermal Cycler (Applied Biosystems). The resulting amplicons were analyzed by electrophoresis in a 1% agarose gel to assess the success and quality of the amplification, indicated by the presence of a single band in the gel and the absence of non-specific amplifications. Agarose gel electrophoresis was performed as described earlier. A GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific) was used as a size marker for the DNA amplicons.

Amplicons of confirmed quality were then cleaned and purified using the GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific) according to the manufacturer's instructions. The concentration and purity of the purified amplicons were measured spectrophotometrically using the Nanodrop Lite device. DNA sequencing was performed using the Sanger dideoxy chain termination method. The same oligonucleotide primers used for PCR were used to sequence the amplicons. DNA sequence analysis of the amplicons was outsourced to an external facility.

Table 1. Sequences and T_m temperatures of Oligonucleotide Primers Used in the PCR
Çizelge 1. PCR’da Kullanılan Oligonükleotid Primerlerin Dizileri ve T_m sıcaklıkları

LOCUS	Primer	Oligonucleotide Sequence (5’-3’)	T _m Temperature (°C)
ITS	ITS1	TCCGTAGGTGAACCTGCGG	62
	ITS4	TCCTCCGCTTATTGATATGC	53

Molecular Phylogenetic Analyses of the Fungal Specimen

Molecular phylogenetic analyses of the specimens were conducted using MEGA-X software (<https://www.megasoftware.net>) based on their nucleotide sequences. The amplicon sequences were initially analyzed with NCBI's Nucleotide BLAST (Basic Local Alignment Search Tool) to identify similar sequences. The sequences in the GenBank DNA database that showed the highest similarity to the analyzed amplicon sequences were selected as the ingroup for phylogenetic analyses. Additionally, sequences from distantly related macrofungi that did not show

similarity to the analyzed amplicon sequences were chosen as the outgroup for the analyses.

The sequences were first aligned with the ingroup and outgroup sequences using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithms to construct a phylogenetic tree. The most appropriate nucleotide substitution model was then determined, and phylogenetic trees were constructed using the Neighbor-Joining algorithm. To assess the reliability of the tree branches, 1000 bootstrap replicates were applied.

RESULTS

The specimens collected from Dokuz Eylül University Tınaztepe Campus were identified as *Rhodocybe asyae*. This species was briefly described, encompassing macroscopic and microscopic characteristics observed in the samples. The documentation includes the collection date, the precise location of the samples, habitat descriptions, geographic coordinates, and unique collection identifiers. Macroscopic photos, illustrations of microscopic structures, and Scanning Electron

Microscope (SEM) images of the basidiospores were also provided.

Taxonomic overview

Basidiomycota R.T. Moore

Agaricales Underw.

Entolomataceae Kotl. & Pouzar

Rhodocybe asyae Sesli & Vizzini (2017), (Figure 1-3).
Sesli & Vizzini (2017) thoroughly characterized the type specimens.



Figure 1. *Rhodocybe asyae*: a-d basidiomata (scale bars: 10 mm)
Şekil 1. *Rhodocybe asyae*: a-d basidiomata (ölçekler: 10 mm)

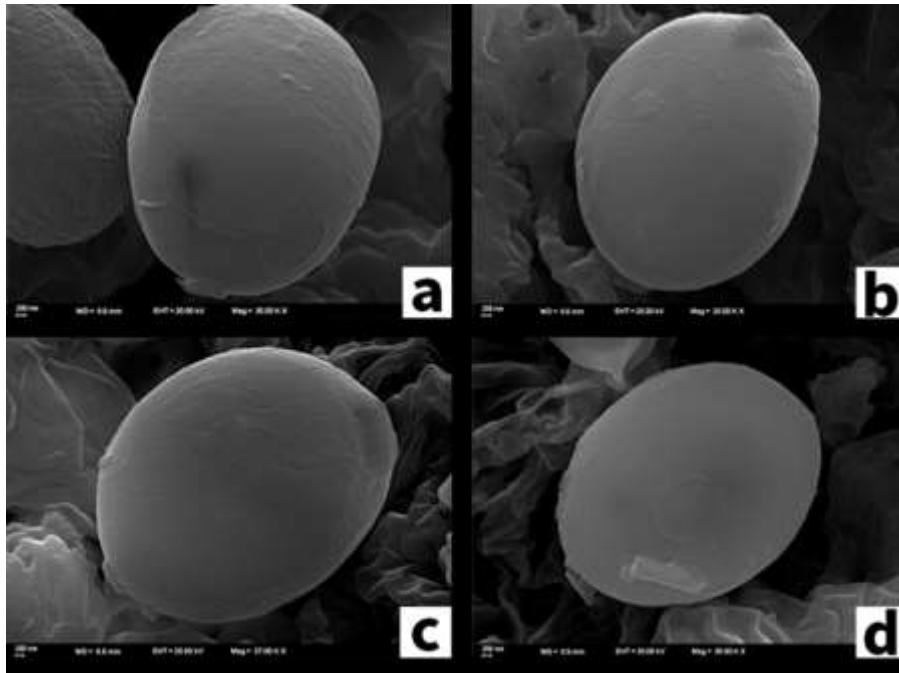


Figure 2. *Rhodocybe asyae* a-d. basidiospores (SEM), (scale bars: 200 nm)
Şekil 2. *Rhodocybe asyae*: a-d. sporlar (SEM), (ölçekler: 200 nm)

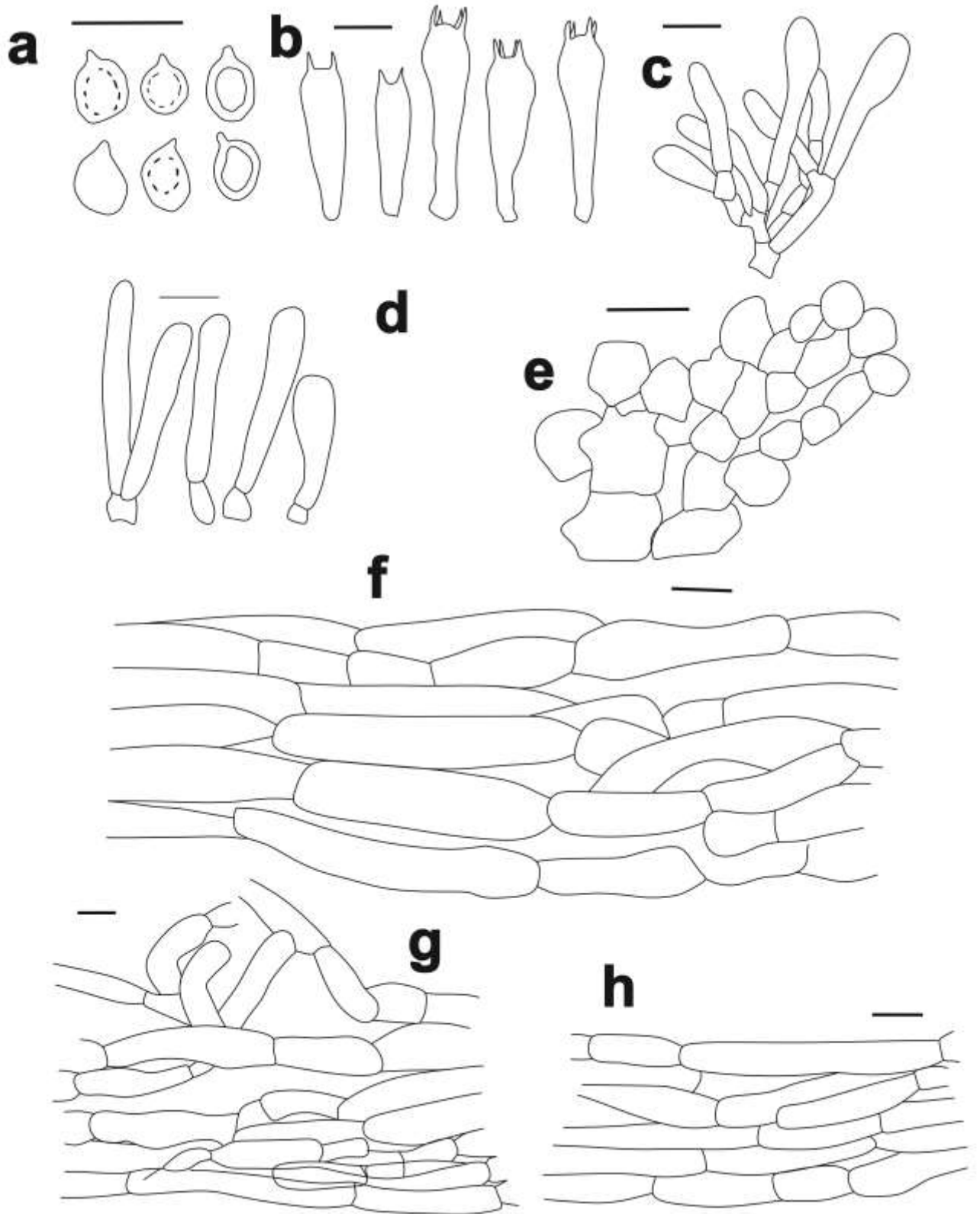


Figure 3. *Rhodocybe asyae*: a. basidiospores, b. basidia, c. basidioles, d. cheilocystidia, e. subhymenium, f. hyphae of the hymenophoral trama, g. pileipellis, h. stipitipellis (scale bars: 10 µm).

Şekil 4. *Rhodocybe asyae*: a. basidiosporlar, b. bazidiyumlar, c. bazidiyoller, d. keylosistidyumlar, e. subhimeniyum, f. himenoforal trama hifleri, g. pileipellis, h. stipitipellis (ölçekler:10 µm).

Macroscopic and microscopic features

Habit tricholomatoid. **Pileus** 15–30 mm broad, convex at first, soon convex to the plane, slightly depressed in the centre, salmon pink with ivory to beige hue, paler pinkish towards the margin, reddish brown in the centre, dry, smooth, slightly hygrophaneous. Margin slightly inrolled when young, soon plane, ivory to beige. **Lamellae** are adnate to subdecurrent, whitish, light ivory to beige orange, fragile and regular. **Stipe** 25–30 × 5 mm, cylindrical, hollow, intensely pruinose at apex, typically tapering towards base, light pinkish to light orange-brown, and with a white-tomentose bulb. **Odor** and **Taste** not distinctive. **Flesh** thin, whitish to ivory or grayish-beige. **Basidiospores** (4–)4.5–7.5 (–8) × (3–)3.2–5.3(–5.6) µm including apiculus [(n = 30), Q = 1.18–1.45 Q_{av} = 1.39], ellipsoid to broadly ellipsoid and subangular in profile view, containing tiny droplets or granules, hyaline, and inamyloid. **Basidia** 24–32 × 6.5–9 µm, 2–4 spored, slenderly clavate, thin-walled, and has 3–5 µm long sterigmata. Basal clamp not observed. **Basidioles** similar to basidia and 18–25 × 4.5–6.5 µm. **Subhymenium** ranges from lobed and rounded to nearly spherical, broadly elliptical, and occasionally even angular, 4.5–7 × 5.5–9 µm. **Hymenophoral trama** regular, consisting of cylindrical and septate hyphae, measuring 6.5–12.5 µm broad. **Pleurocystidia** not observed. **Cheilocystidia** rarely observed, 18–24 × 4–6 µm, slenderly cylindrical to slightly subclavate and thin-walled. **Pileipellis** is a cutis comprising two distinct layers: A thin upper layer of narrow, 3–4.5 µm cylindrical hyphae and a thicker lower layer consisting of multicellular structures of 6.5–10 µm broad. **Stipitipellis** is a cutis comprising cylindrical, septate, and thin-walled, 4–8 µm broad hyphae. **Clamp connections** not observed.

Ecology and distribution: The species was described from Türkiye (Trabzon) and recorded in the United Kingdom (East Sussex). It usually grows singly or in small groups within coniferous forests, including pine, spruce, and fir trees. It is often found amid fallen pine needles and grass tufts, predominantly emerging during autumn (Sesli & Vizzini, 2017; Aplin et al., 2022).

Material examined: TÜRKİYE—İzmir, Dokuz Eylül University Tınaztepe Campus, under Turkish pine (*Pinus brutia* Ten), 210 m, 38° 22' 13" N, 27° 12' 43" E, 01.12.2023, DEU AKATA & SAHİN 148 (nrITS rDNA sequence GenBank accession number: PP944722.1).

Evolutionary History of DEU AKATA & SAHİN 148

The evolutionary lineage of specimen DEU AKATA & SAHİN 148 was examined based on its nrITS rDNA sequence, which was obtained using standard molecular techniques and archived in the NCBI GenBank under accession number PP944722.1. To

explore its evolutionary relationships, nrITS rDNA sequences from various members of the *Rhodocybe* genus were selected for comparison, with *Tuber melanorufum*'s nrITS rDNA sequence serving as an outgroup. Molecular phylogenetic analysis identified ten distinct clades, including Clade 1, which contained different isolates of *Rhodocybe asyae* and DEU AKATA & SAHİN 148. The remaining clades (Clade 2-10) included other *Rhodocybe* species. *T. melanorufum* formed a separate branch, confirming its role as the outgroup. BLAST analyses showed over 99% similarity between the nrITS rDNA sequences of DEU AKATA & SAHİN 148 and various isolates of *R. asyae*. Phylogenetic analyses confirmed the close relationship between DEU AKATA & SAHİN 148 and *R. asyae*, with bootstrap branch support validating the reliability of their grouping.

DISCUSSION and CONCLUSION

The genetic diversity of fungal species exceeds their morphological diversity, prompting the integration of genetic information with traditional morphological methods for more precise species identification. Various genetic markers, such as rRNA gene regions like nrITS, nrSSU, and nrLSU, as well as protein-coding gene sequences, have been utilized in molecular systematic studies for decades (Raja et al., 2017; Akata et al., 2023; 2024a; 2024b; Altuntaş et al., 2021). The ITS region stands out in fungal molecular taxonomy, offering valuable insights. High-throughput sequencing technologies and bioinformatics tools have advanced whole genome comparisons and phylogenomic analyses among fungal taxa, potentially replacing molecular phylogenetic analyses based on a few marker genes (Marian et al., 2024). In the present study, nuclear ITS rDNA sequences were employed for the molecular identification of DEU AKATA & SAHİN 148, revealing a similarity of over 99% with the specimen (GenBank ID: PP944722.1) and *R. asyae* (Figure 4).

Rhodocybe asyae, a species within Section *Rufobrunnea*, shares several morphological and ecological traits with its close relatives. Notably, it exhibits similarities to species such as *R. asanii* E. Sesli & Vizzini, *R. fusipes* Silva-Filho, D.L. Komura & Wartchow, *R. fumanellii* R.J. Ferrari, Vizzini & Fellin and *R. subasyae* T. Bau & Y.L. Sun.

Rhodocybe asanii shares similar habitats (pine, spruce, and fir) and several characteristics with *R. asyae*, including a small pileus size (20–45 mm) and similar size of basidia (20–30 × 7–8 µm) and basidiospores (5.4–6.8 × 3.9–4.9 µm). Both species exhibit a change in the surface color of the pileus upon aging or when damaged. Despite these similarities, *R. asanii* is distinct in its tricholomatoid morphology. The pileus of *R. asanii*, described as light ivory to beige-red, displays a range of shapes from

convex to plane or even irregular and is notably fragile. The lamellae are adnexed to sinuate and transition from whitish to reddish beige, with a tendency to become more reddish when injured. The stipe of *R. asanii* is pruinose, contributing to its unique textural qualities and lack of cheilocystidia (Sesli & Vizzini, 2017).

R. fumanellii is recognized by its solid, tricholomatoid basidiomata, vibrant reddish-brown tinges, and

closely spaced adnate lamellae. Microscopically, it stands out due to its long cheilocystidia (35–95 × 3–6.5 μm) and its ellipsoid basidiospores. Caulocystidia are also present. *R. fumanellii* has larger basidiomata (pileus: 35–100 mm and stipe: 40–70 × 5–15 mm). Additionally, this species has rhizomorphs at the stipe base and 4-spored basidia and usually grows among the leaf litter under deciduous trees (Vizzini et al., 2018).

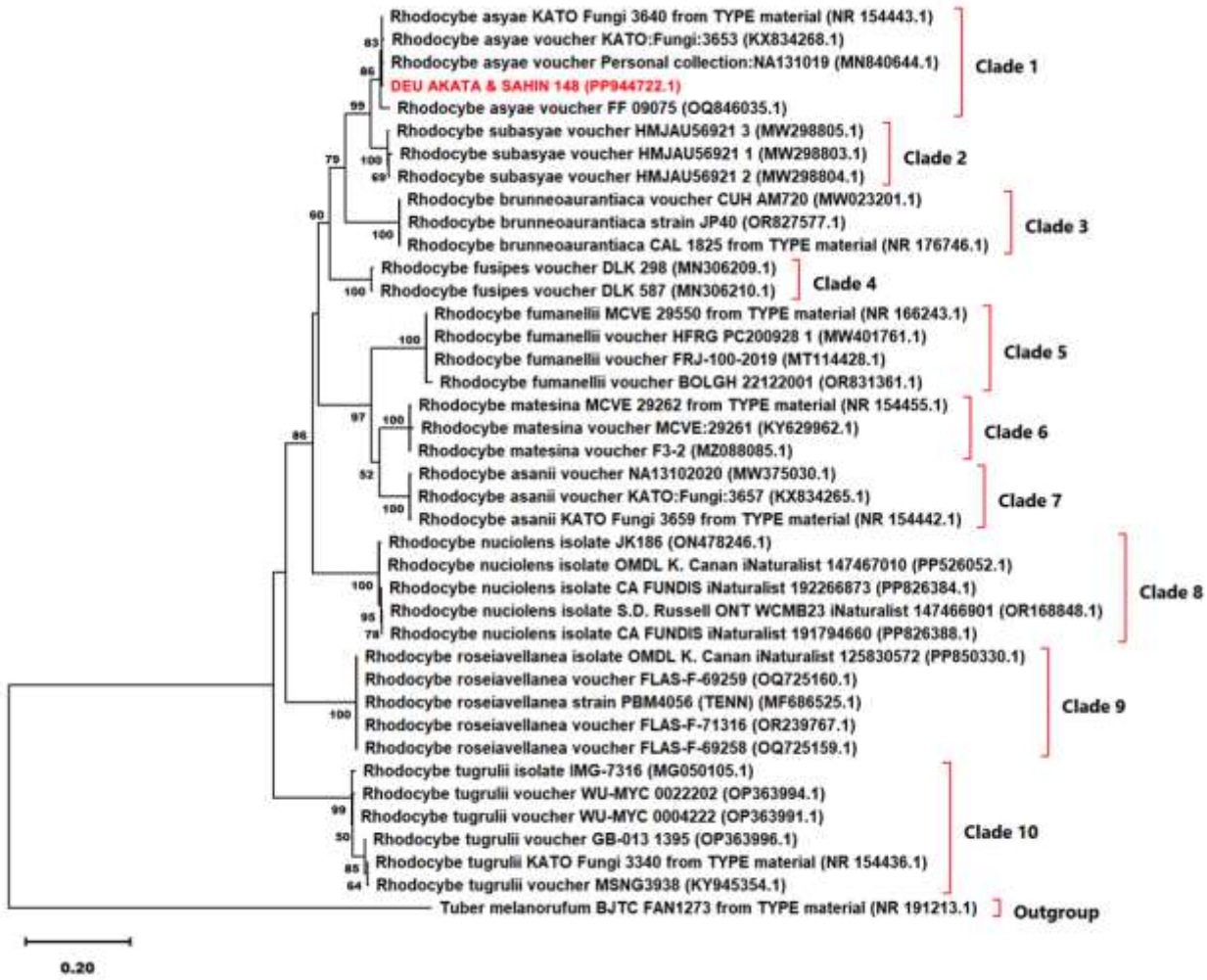


Figure 4. The phylogenetic tree, constructed using the nrITS rDNA region and the Neighbour Joining algorithm with the T92+G nucleotide substitution model, reveals the evolutionary relationships among 40 fungal specimens. Confidence levels were indicated by assigning bootstrap rates (≥ 50) to each branch. The sequences used in constructing the tree were obtained from the NCBI GenBank, except DEU AKATA & SAHIN 148. Additionally, *Tuber melanorufum* was included in the phylogenetic tree as the outgroup representative. A GenBank accession number accompanies each sequence, and a scale bar in the lower left corner represents a genetic distance of 0.20.

Şekil 4. nrITS rDNA bölgesi ve T92+G nükleotit yerini alma modeli ile Komşu Birleştirme algoritması kullanılarak oluşturulan filogenetik ağaç, 40 mantar örneği arasındaki evrimsel ilişkileri ortaya koyuyor. Güven seviyeleri, her şubeye önyükleme oranları (≥ 50) atanarak belirtildi. Ağacın oluşturulmasında kullanılan diziler, DEU AKATA & SAHIN 148 hariç, NCBI GenBank'tan elde edildi. Ek olarak *Tuber melanorufum*, dış grup temsilcisi olarak filogenetik ağaca dahil edildi. Ağaçtaki her diziyeye ait GenBank erişim numarası belirtilmiş ve sol alt köşedeki ölçek çubuğu 0.20'lik genetik mesafeyi temsil etmektedir.

Rhodocybe fusipes are distinguished by their orange to red colored pileus and prominently expansive umbonate center. Its lamellae are white, adnate to slightly decurrent, close or crowded, and sometimes forked. The stipe transitions from white to a subtle sordid orange and is characterized by a tapering that culminates in a distinctively fusiform base. It produces subglobose to broadly ellipsoid basidiospores and differs with the absence of cheilocystidia. Pileipellis is similar to a cutis and additionally, *R. fusipes* lacks hymenial pseudocystidia and clamp connections. The central stipe of this species displays a reddish hue. It is found in the biodiverse Amazonian forests, which are rich in families such as *Lecythidaceae*, *Sapotaceae*, *Burseraceae*, and *Fabaceae* members but do not include coniferous trees. *R. fusipes* can be distinguished from *R. asyae* with the color of pileus, larger stem, unique spore morphology, and the absence of cheilocystidia (Silva-Filho et al., 2020).

R. subasyae differs from orange-white to beige-red and 19–25 mm pileus. It grows under the canopy of mixed forests composed of pine, larch, and oak trees. It is distinguished with sinuate to adnate lamellae, ellipsoid to broadly ellipsoid, and $5.4\text{--}6.8 \times 3.9\text{--}4.9 \mu\text{m}$ basidiospores. Its lamellae range from adnexed to sinuate. The stipe is $22\text{--}37 \times 5\text{--}7 \text{ mm}$, cylindrical, fibrillose, and orange-white and with white rhizomorphs. Cheilocystidia are $22.4\text{--}28.2 \times 3.9\text{--}6.8 \mu\text{m}$, slenderly clavate, and occasionally branched (Sesli & Vizzini, 2017; Sun & Bau, 2023).

In the study conducted by Sesli and Vizzini (2017), the morphological traits of *Rhodocybe asyae* samples are thoroughly documented, detailing specific features of various macroscopic structures, the properties, and dimensions of basidiospores, basidia,

cheilocystidia, and other microscopic structures in the hymenium, pileus, and stipe. Similarly, Aplin et al. (2022) described some macroscopic structures and the properties and dimensions of basidiospores and basidia. Table 2. compares the specimens of *R. asyae* analyzed in the present study with those documented by Sesli and Vizzini (2017) and Aplin et al. (2022). This table thoroughly details the dimensions of various macroscopic and microscopic structures observed in the presented samples, providing a comprehensive overview of their measurements. The detailed comparison highlights the similarities and differences in measurements and characteristics between the present study's samples and those of the type specimens and the specimens analyzed by Aplin et al. (2022).

The comparative analysis of the macroscopic and microscopic features of *R. asyae* specimens with those documented by Sesli & Vizzini (2017) and Aplin et al. (2022) highlights a significant degree of congruence, particularly regarding dimensional attributes. This consistency reinforces the reliability of these characteristics as critical identifiers for the species. However, the present findings also reveal some notable discrepancies. Specifically, the habit of the present specimens diverges from the previously reported descriptions. While Sesli & Vizzini (2017) characterized the type specimen as having a different habit (clitocyboid), the present specimens exhibit a distinct tricholomatoid habit. This variation in habit could suggest potential intraspecific diversity or environmental influences affecting morphological expression. Additionally, the analysis of microscopic features aligns with previous studies, further validating the species identification.

Table 2. Comparison of various measurements of the morphological structures of *Rhodocybe asyae*
 Çizelge 2. *Rhodocybe asyae*'nin morfolojik yapılarının çeşitli ölçümlerinin karşılaştırılması

Dimensions	Sesli and Vizzini (2017)	Aplin et al. (2022)	Current study
Habit	clitocyboid	not provided	tricholomatoid
Pileus	10–30 mm broad	up to $35 \times 5 \text{ mm}$	15–30 mm broad
Stipe	$25\text{--}30 \times 2\text{--}5 \text{ mm}$	up to 35 mm	$25\text{--}30 \times 5\text{--}7 \text{ mm}$
Basidiospores (average)	$(4.8\text{--}5\text{--}7\text{--}8) \times 4\text{--}5\text{--}(5.5) \mu\text{m}$	$5.2\text{--}6.8 \times 3.5\text{--}4.6 \mu\text{m}$	$(4\text{--})4.5\text{--}7.5\text{--}(8) \times (3\text{--})3.2\text{--}5.3\text{--}(5.6) \mu\text{m}$
Basidiospores (Q-values)	Q = 1.1–1.4, Q _{av} = 1.3	not provided	Q = 1.18–1.45 Q _{av} = 1.39
Basidia	$20\text{--}30 \times 6.5\text{--}8.5 \mu\text{m}$	not provided	$24\text{--}32 \times 6.5\text{--}9 \mu\text{m}$
Sterigmata	3–8 μm long	not provided	3–5 μm long
Cheilocystidia	$20\text{--}30 \times 4\text{--}6 \mu\text{m}$	not observed	$18\text{--}24 \times 4\text{--}6 \mu\text{m}$
hymenophoral trama (hyphae)	6–15 μm broad	not provided	6.5–12.5 μm broad
Pileipellis (upper layer)	3–5 μm broad	not provided	3–4.5 μm broad
Pileipellis (lower layer)	6–12 μm broad	not provided	6.5–10 μm
Stipitipellis	7–16 μm broad	not provided	4–8 μm broad

Contribution Of The Authors As Summary

The authors declare that their contributions are equal.

Statement Of Conflict Of Interest

The authors have declared no conflict of interest.

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Contributions of Aphrophoridae (Hemiptera: Cicadomorpha) fauna of Sinop, Kastamonu and Black Sea Region of Türkiye

Rukiye TANYERİ¹, Ünal ZEYBEKOĞLU²

¹Sinop Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü. ²Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü.

¹<https://orcid.org/0000-0001-9994-8763>, ²<https://orcid.org/0000-0003-1646-5999>

✉: rtanyeri@sinop.edu.tr

ABSTRACT

In this study, Aphrophoridae (Hemiptera:) specimens collected from Sinop and Kastamonu between 2016 May-2018 October were evaluated. Specimens were collected by sweeping net and a hand aspirator over the plants during the daytime. Nine species belonging to 4 genera were identified in the study area: *Aphrophora alni* (Fallén, 1805), *A. salina* (Goeze, 1778), *A. geruzei* Tanyeri & Zeybekoğlu, 2021, *Lepyronia coleoptrata* (Linnaeus, 1758), *Neophilaenus albipennis* (Fabricius, 1798), *N. campestris* (Fallén, 1805), *N. lineatus* (Linnaeus, 1758), *N. minor* (Kirschbaum, 1868), *Philaenus spumarius* (Linnaeus, 1758). Information provided covering distributional data of the species in Türkiye and worldwide, collection localities, and number of species were given. Of the identified species *Neophilaenus albipennis* is the first record for the Black Sea Region of Türkiye. General photographs of *N. albipennis* and *N. lineatus* and drawings of genital structures are given.

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ÖZET

Bu çalışmada 2016 Mayıs-2018 Ekim döneminde Sinop ve Kastamonu'dan toplanan Aphrophoridae (Hemiptera: Cicadomorpha) familyasına ait örnekler değerlendirilmiştir. Örnekler gün içerisinde bitkilerin üzerinden atrap ve aspiratör kullanılarak toplanmıştır. Çalışma alanında 4 cins'e ait 9 tür tespit edilmiştir: *Aphrophora alni* (Fallén, 1805), *A. salicina* (Goeze, 1778), *A. geruzei* Tanyeri & Zeybekoğlu, 2021, *Lepyronia coleoptrata* (Linnaeus, 1758), *Neophilaenus albipennis* (Fabricius, 1798), *N. campestris* (Fallén, 1805), *N. lineatus* (Linnaeus, 1758), *N. minor* (Kirschbaum, 1868), *Philaenus spumarius* (Linnaeus, 1758). Tespit edilen türlerin Türkiye'deki ve dünyadaki yayılışları, toplandıkları lokaliteler, örnek sayıları verilmiştir. Tespit edilen türlerden *Neophilaenus albipennis*, Türkiye'nin Karadeniz Bölgesi'nden ilk kez kaydedilmiştir. *N. albipennis* ve *N. lineatus*'un genel fotoğrafları ve genital yapılarına ait çizimler verilmiştir.

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INTRODUCTION

Among the Hemiptera, the suborder Cicadomorpha is a diverse group of varied sizes, which may reduce crop yield by feeding on plant fluid and weakening the terminal young branches (Mozaffarian, 2018). The Cercopoidea superfamily (Hemiptera: Cicadomorpha)

is classified into five families Cercopidae, Aphrophoridae, Clastopteridae, Machaerotidae and Epipygidae. The superfamily comprises approximately 3000 species that have been described to date worldwide. The species of the superfamily Cercopoidea is known as spittlebugs because it produces a foamy mass during the nymphal periods of their development

(Cryan & Svenson, 2010). They are called froghoppers since the adults have jumping ability.

Aphrophoridae spittlebugs are the most abundant and widespread xylem-sap feeder insects and they have been mentioned as pests with economic importance on rice, sugarcane, strawberry, alfalfa, perennial plants and some pasture plants etc (Mozaffarian & Wilson, 2015). The plant pathogenic *Xylella fastidiosa* is transmitted to plants by several xylem-sap feeding insects belonging to the Cicadomorpha suborder, mainly the Aphrophoridae family and causes diseases such as Olive quick decline syndrome (Smaili et al., 2022). In this family member, *Aphrophora alni* is a potential vector for *Candidatus phytoplasma*. *P. spumarius* is the vector of *Candidatus phytoplasma* and *Xylella fastidiosa*. The genera *Neophilaenus* and *Aphrophora* are also considered potential vectors of *Xylella fastidiosa* (EPPO, 2017; Germain, 2016). Demir & Unver (2019) drew attention to the Cicadomorpha species which can be potential vectors in hazelnuts in Türkiye and *A. alni*, *P. spumarius* listed in that study. That's why local records are considered to be very important.

The first studies of this family in Türkiye are Fahringer (1922), Dlabola (1957) and Metcalf (1962). Lodos & Kalkandelen (1981) evaluated the materials collected from field and museum and listed 4 new records and species of this family from Türkiye. In the following years, limited local records were given. In Türkiye, research on Aphrophorid spittlebugs was focused on polymorphism, mainly the meadow spittlebug *P. spumarius* (Yurtsever, 2001; Yurtsever et al., 2010).

According to Önder et al. (2011), Türkiye Aphrophoridae fauna consists of 14 species belonging to 6 genera. This number has increased to 15 with new records and species reported from our country in subsequent studies (Tanyeri & Zeybekoğlu, 2021).

Although they are an economically and ecologically important insect group, it has been observed that there is no comprehensive study in this region. In this study, it was aimed to determine the Aphrophoridae species in Sinop and Kastamonu in the Western Black Sea region of Türkiye.

MATERIAL and METHOD

The material was collected from Sinop and Kastamonu provinces (North Türkiye) between May 2016 and October 2018 periodically during the daytime. In the sampling of the adults, a standard sweeping net and a hand aspirator were used. The specimens were prepared according to standard insect preparation. The genital capsule of males and pregenital sternite VII in females were separated from the body with the help of a dissecting needle. Features of the genital structures were examined and they were identified by the first author using previously published articles

(Emeljanov, 1967; Holzinger et al., 2003; Mozaffarian & Wilson, 2015). An overview photograph of dry samples was taken with a Canon EOS 70D model camera connected to a Zeiss Stem 2000-C stereomicroscope. The shapes of genital structures were drawn using Zeiss discovery V-20 stereomicroscope attached drawing attachment.

The material is deposited in Sinop University, Faculty of Arts and Sciences, Department of Biology, Invertebrata Laboratory.

RESULTS and DISCUSSION

Genus: *Aphrophora* Germar, 1821

***Aphrophora alni* (Fallén, 1805)**

Examined material:

Kastamonu: 22.07.2017: 41° 38' 14.7" N 33° 17' 28.6" E (1 ♂), 41° 37' 14.1" N 33° 07' 08.8" E (4 ♀♀) leg. RT; 06.08.2017: 41° 47' 35.8" N 34° 04' 53.2" E (4 ♂♂, 4 ♀♀) leg. RT&ÜZ; 15.06.2017: 41° 58' 11.3" N 34° 05' 25.4" E (2 ♂♂) leg. RT; 16.06.2017: 41° 39' 33.3" N 33° 08' 02" E (2 ♂♂, 2 ♀♀) leg. RT

Sinop: 09.06.2017: 41° 47' 06.0" N 35° 11' 08.4" E (2 ♂♂) leg. RT; 08.06.2017: 42° 00' 52.1" N 34° 58' 24.4" E (3 ♂♂, 3 ♀♀), 41° 59' 41.7" N 34° 54' 00.2" E (4 ♂♂, 4 ♀♀), 41° 57' 15.6" N 34° 48' 05.3" E (5 ♂♂, 4 ♀♀) leg. RT; 13.06.2017: 41° 45' 40.7" N 34° 58' 32.5" E (14 ♂♂, 11 ♀♀), 41° 35' 50.1" N 34° 51' 04.6" E (1 ♀) leg. RT; 30.07.2017: 41° 41' 58.1" N 34° 35' 42.1" E (2 ♂♂, 1 ♀), 41° 58' 02.4" N 34° 50' 32.3" E (1 ♂, 4 ♀♀) leg. RT; 01.08.2017: 41° 52' 23.8" N 34° 51' 57.0" E (2 ♂♂) leg. RT&ÜZ; 17.08.2017: 41° 46' 17.8" N 35° 12' 20.4" E (1 ♂, 2 ♀♀) leg. RT&ÜZ

Distribution in Türkiye: Adana, Afyon, Ankara, Antalya, Artvin, Aydın, Balıkesir, Bitlis, Bolu, Çanakkale, Çorum, Diyarbakır, Erzincan, Erzurum, Giresun, İstanbul, İzmir, Kayseri, Kırklareli, Konya, Kütahya, Mardin, Manisa, Muğla, Ordu, Rize, Samsun, Sinop, Tekirdağ, Trabzon, Yozgat (Önder et al., 2011; Demir, 2019).

Distribution in the world: Austria, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Crete Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece Hungary, Ireland, Italy, Netherlands, Spain, Sweden, Switzerland, Near East, North Africa, Latvia, Lithuania, Macedonia, Malta, Moldova, Norway, Poland, Portugal, Romania, Russia (Southern Europe, Northern Europe, Central Europe), Sardinia, Sicily, Slovakia, Slovenia, West Palearctic, Yugoslavia (Anonymous, 2021)

***Aphrophora salicina* (Goeze, 1778)**

Examined material:

Kastamonu: 20.07.2017: 41° 14' 15.1" N 33° 21' 57.0" E (15 ♂♂, 13 ♀♀) leg. RT

Sinop: 13.06.2017: 41° 36' 02.1" N 34° 51' 02.4" E (23

♂♂, 6 ♀♀) leg.RT

Distribution in Türkiye: Balıkesir, Çanakkale, Elazığ, Giresun, Gümüşhane, Kırklareli, Konya, Kütahya (Önder et al., 2011; Özgen et al., 2018; Demir, 2019)

Distribution in the world: Albania, Austria, Belgium, Britain I, Bulgaria, Central European Russia, Croatia, Czech Republic, Danish, East Palearctic, Estonia, French, Germany, Greek, Hungary, Ireland, Italian, Latvia, Lithuania, Moldova, Near East, North European Russia, Norwegian, Poland, Portuguese, Romania, Sardinia, Slovakia, Slovenia, South European Russia, Spanish, Sweden, Switzerland, The Netherlands, Ukraine, Vatican City (Anonymous, 2021)

***Aphrophora geruzei* Tanyeri & Zeybekoğlu, 2021**

Examined material:

Kastamonu: 15.06.2017: 41° 58' 11.3" N 34° 05' 25.4" E (4 ♀♀, 8 ♂♂), 48.7 N 34° 09' 16.1" E (5 ♀♀, 7 ♂♂) leg.RT

Sinop: 08.06.2017: 41° 46' 20.0" N 35° 12' 18.4" E (10 ♀♀, 6 ♂♂) leg. RT; 09.07.2017: 41° 44' 25.7" N 35° 13' 49.7" E (8 ♀♀, 9 ♂♂) leg.RT

Distribution in Türkiye: Kastamonu, Sinop (Tanyeri & Zeybekoğlu, 2021)

Distribution in the world: Türkiye (Tanyeri & Zeybekoğlu, 2021)

Genus: *Lepyronia* Amyot & Serville, 1843

***Lepyronia coleoprata* (Linnaeus, 1758)**

Examined material:

Kastamonu: 20.07.2017: 41° 36' 53.5" N 33° 07' 09.1" E (1 ♂), 41° 38' 29.7" N 33° 07' 05.2" E (1 ♂), 41° 43' 09.5" N 33° 27' 23.5" E (1 ♂), 41° 39' 33.3" N 33° 08' 02" E (3 ♀♀), 41° 39' 20.1" N 33° 35' 14.2" E (1 ♀) leg.RT; 16.06.2017: 41° 18' 01.2" N 33° 31' 54.4" E (1 ♀) leg. RT; 22.07.2017: 41° 38' 48.7" N 33° 35' 49.2" E (2 ♂♂, 1 ♀), 41° 38' 14.7" N 33° 17' 28.6" E (7 ♂♂, 2 ♀♀), 41° 37' 14.1" N 33° 07' 08.8" E (1 ♂, 2 ♀♀), 41° 42' 29.9" N 33° 04' 34.6" E (1 ♂, 3 ♀♀) leg. RT

Sinop: 27.05.2017: 41° 44' 52.0" N 34° 57' 40.9" E (2 ♂♂), 41° 32' 45.1" N 34° 47' 0.0" E (5 ♂♂, 9 ♀♀) leg. RT; 12.08.2017: 41° 41' 58.1" N 34° 35' 42.1" E (2 ♀♀) leg. RT&ÜZ; 30.07.2017: 41° 36' 03.6" N 34° 51' 28.4" E (5 ♂♂) leg. RT

Distribution in Türkiye: Adana, Afyon, Ankara, Antalya, Artvin, Aydın, Bilecik, Bursa, Çanakkale, Çankırı, Çorum, Diyarbakır, Edirne, Elazığ, Erzincan, Gümüşhane, İzmir, Kahramanmaraş, Kars, Kahramanmaraş, Konya, Kütahya, Manisa, Mardin, Muğla, Muş, Sakarya, Samsun, Siirt, Tokat (Fahringer, 1922; Dlabola, 1957; Kartal et al., 1994; Önder et al., 2011; Özgen et al., 2018; Demir, 2019)

Distribution in the world: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Central European

Russia, Croatia, Czech Republic, Danish, East Palearctic, Estonia, Finland, French, Germany, Greek, Hungary, Ireland, Italian, Latvia, Lithuania, Macedonia, Moldova, Near East, North Africa, North European Russia, Norwegian, Poland, Portuguese, Romania, Sardinia, Sicily, Slovakia, Slovenia, South European Russia, Spanish, Sweden, Switzerland, The Netherlands, Ukraine, Yugoslavia (Anonymous, 2021)

Genus: *Neophilaenus* (Haupt, 1935)

***Neophilaenus albipennis* (Fabricius, 1798)**

(Figures 1,2)

Examined material:

Kastamonu: 06.08.2017: 41° 55' 57.5" N 34° 11' 13.1" E (3 ♂♂, 4 ♀♀) leg. RT&ÜZ

Sinop: 30.07.2017: 41° 41' 58.1" N 34° 35' 42.1" E (1 ♂) leg.RT

Distribution in Türkiye: Ankara, Kırklareli (Demir, 2006; Önder et al., 2011)

Distribution in the world: Europe, North Africa, Eastern Palearctic (Anonymous, 2021)

***Neophilaenus campestris* (Fallén, 1805)**

(Figure 3,4)

Examined material:

Kastamonu: 14.06.2017: 41° 15' 12.2" N 33° 59' 53.8" E (4 ♂♂, 3 ♀♀) leg. RT; 16.06.2017: 41° 42' 09.1" N 33° 26' 41.9" E (2 ♂♂, 3 ♀♀) leg. RT; 20.07.2017: 41° 13' 21.9" N 33° 25' 38.0" E (4 ♂♂, 2 ♀♀) leg. RT

Sinop: 27.05.2017: 41° 47' 23.9" N 35° 09' 26.9" E (1 ♂, 1 ♀) leg. RT; 08.06.2017: 41° 46' 20.0" N 35° 12' 18.4" E (1 ♂) leg. RT; 13.06.2017: 41° 33' 55.8" N 34° 48' 49.9" E (4 ♂♂, 3 ♀♀) leg. RT; 12.08.2017: 41° 25' 13.1" N 34° 58' 44.2" E (4 ♂♂, 3 ♀♀) leg. RT&ÜZ

Distribution in Türkiye: Adana, Adıyaman, Afyon, Ankara, Antalya, Artvin, Bitlis, Bursa, Çanakkale, Eskişehir, Elazığ, Giresun, Hatay, İstanbul, İzmir, Kırklareli, Konya, Kütahya, Manisa, Muğla, Nevşehir, Samsun, Sinop, Siirt, Trabzon, Van (Lodos & Kalkandelen, 1981; Önder et al., 2011; Özgen et al., 2018; Demir, 2019)

Distribution in the world: Austria, Bulgaria, Czech Republic, East Palearctic, Estonia, France, Germany, Greece, Hungary, Italian, North Africa, Poland, Romania, Sardinia, Slovakia, Slovenia, Switzerland, Ukraine, Yugoslavia (Anonymous, 2021)

***Neophilaenus lineatus* (Linnaeus, 1758)**

Examined material:

Sinop: 14.06.2017: 41° 35' 17.4" N 34° 51' 01.3" E (1 ♂, 1 ♀) leg. RT

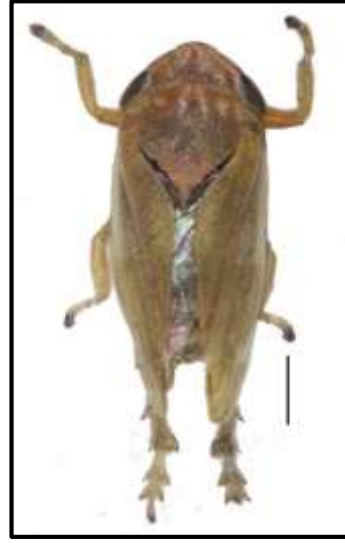
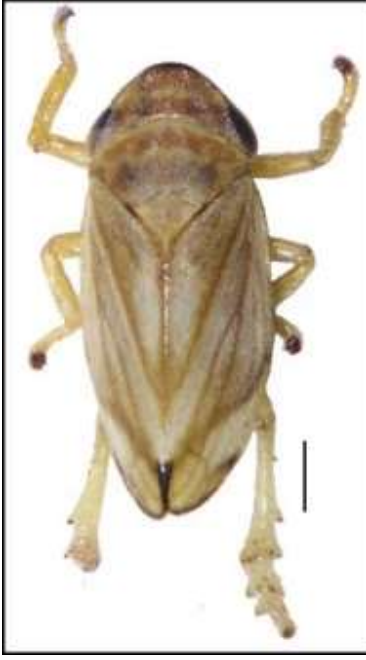


Figure 1. *Neophilaenus albipennis* (Fabricius, 1798) (♂)
(Scale: 1 mm)

Figure 3. *Neophilaenus campestris* (Fallén, 1805), (♂)
(Scale: 1 mm)

Şekil 1. *Neophilaenus albipennis* (Fabricius, 1798) (♂)
(Ölçek: 1 mm)

Şekil 3. *Neophilaenus campestris* (Fallén, 1805), (♂)
(Ölçek: 1 mm)

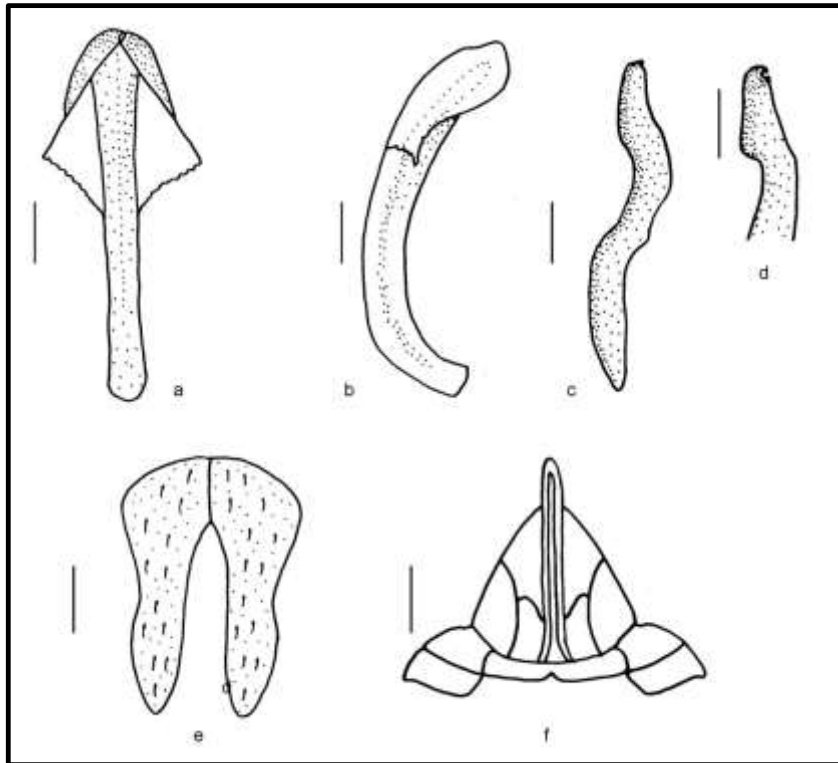


Figure 2. Genital structures of *Neophilaenus albipennis* an aedeagus from dorsal; b, aedeagus from lateral; c, stylus, d, the tip of the stylus; e, genital plates; f, the tip of the female abdomen from ventral (Scale a, b, c, d, e: 0,1 mm; f: 0,5 mm)

Şekil 2. *Neophilaenus albipennis*'te genital yapılar a) Aedeagus dorsal, b) Aedeagus lateral, c) Stilus, d) Stilusun uç kısmı, e) Genital plaklar, e) Dişi abdomenin uç kısmı (Ölçek a, b, c, d: 0,1 mm; e: 0,5 mm)

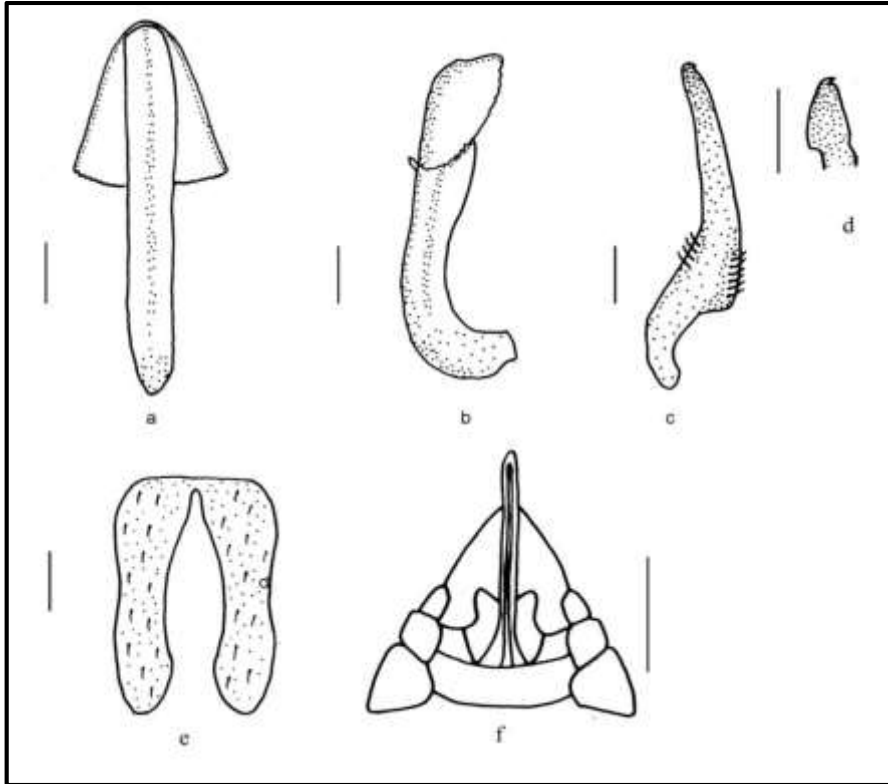


Figure 4. Genital structures of *Neophilaenus campestris* an aedeagus from dorsal; b, aedeagus from lateral; c, stylus, d, the tip of the stylus; e, genital plates; f, the tip of the female abdomen from ventral (Scale a, b, c, d, e: 0,1 mm; f: 0,5 mm)

Şekil 4. *Neophilaenus campestris*'te genital yapılar a) Aedeagus dorsal, b) Aedeagus lateral, c) Stylus, d) Stylusun uç kısmı, e) Genital plaklar, e) Dişi abdomenin uç kısmı (Ölçek a, b, c, d: 0,1 mm; e: 0,5 mm)

Distribution in Türkiye: Afyon, Ankara, Bursa, Erzincan, İstanbul, İzmir, Konya, Nevşehir, Van (Önder et al., 2011; Demir, 2019)

Distribution in the world: Albania, Austria, Balearic Islands, Belgium, Britain, Bulgaria, Central European Russia, Czech Republic, Danish, East Palearctic, Estonia, Finland, French, Germany, Greek, Hungary, Ireland, Italian, Latvia, Lithuania, Macedonia, Moldova, Near East, Nearctic Region, North Africa, North European Russia, Norwegian, Poland, Portuguese, Romania, Sardinia, Sicily, Slovakia, Slovenia, South European Russia, Spanish, Sweden, Switzerland, The Netherlands, Ukraine, Yugoslavia (Anonymous, 2021)

Neophilaenus minor (Kirschbaum, 1868)

Examined material:

Sinop: 13.06.2017: 41° 35' 17.4" N 34° 51' 01.3" E (1 ♂, 1 ♀) leg. RT

Distribution in Türkiye: Ankara, Antalya, Konya, Manisa, Mersin, Sivas (Dlabola, 1957; 1981; Önder et al., 2011, Demir, 2019)

Distribution in the world: Albania, Austria, Belgium, Bulgaria, Czech Republic, East Palearctic, Finland, France, Germany, Greece, Hungary, Italian, Latvia, Lithuania, Near East, Poland, Portuguese, Slovakia,

The Netherlands, Ukraine, Yugoslavia (Anonymous, 2021)

Genus: *Philaenus* Stal, 1864

Philaenus spumarius (Linnaeus, 1758)

Examined material:

Kastamonu: 15.06.2017: 41° 14' 03.9" N 34° 00' 45.7" E (5 ♂♂, 4 ♀♀) leg. RT; 16.06.2017: 41° 43' 09.5" N 33° 27' 23.8" E (2 ♂♂), 41° 42' 09.3" N 33° 26' 41.8" E (228 ♂♂, 80 ♀♀), 41° 40' 56.6" N 33° 23' 34.1" E (3 ♂♂), 41° 36' 53.5" N 33° 07' 09.1" E (3 ♂♂) leg. RT; 20.07.2017: 41° 18' 01.2" N 33° 31' 54.4" E (16 ♂♂, 13 ♀♀), 41° 14' 15.1" N 33° 21' 57.0" E (7 ♂♂, 4 ♀♀), 41° 13' 21.9" N 33° 25' 38.0" E (21 ♂♂, 20 ♀♀), 41° 38' 48.7" N 33° 35' 49.2" E (14 ♂♂, 4 ♀♀) leg. RT; 02.08.2018: 41° 37' 14.1" N 33° 07' 08.8" E (14 ♂♂, 25 ♀♀) leg. RT&ÜZ

Sinop: 31.05.2016: 41° 53' 07.9" N 34° 33' 52.6" E (4 ♂♂, 15 ♀♀) leg. RT; 07.06.2016: 42° 01' 21.5" N 35° 12' 06.7" E (16 ♂♂, 20 ♀♀), 42° 05' 41.9" N 34° 56' 50.8" E (3 ♂♂, 3 ♀♀) leg. RT; 06.06.2016: 42° 01' 03.9" N 34° 54' 03.7" E (2 ♂♂), 42° 03' 04.8" N 35° 02' 25.6" E (2 ♂♂, 1 ♀) leg. RT; 27.05.2017: 41° 52' 32.2" N 34° 59' 54.5" E (4 ♂♂, 6 ♀♀), 42° 00' 50.5" N 34° 56' 90.6" E (70 ♂♂, 12 ♀♀), 42° 01' 21.5" N 35° 12' 06.7" E (4 ♀♀) leg. RT; 01.06.2016: 41° 32' 45.1" N 34° 47' 0.01" E (10 ♂♂), 41° 48' 46.9" N 35° 10' 23.5" E (33 ♂♂, 34 ♀♀), 41° 50' 22.0" N 35° 03' 02.2" E (3 ♂♂, 7 ♀♀) leg. RT; 08.06.2017: 41° 46' 18" N 35° 11' 12"

E (118 ♂♂, 88 ♀♀), 41° 46' 20.0" N 35° 12' 18.4" E (35 ♂♂, 29 ♀♀), 41° 44' 25.7" N 35° 13' 49.7" E (3 ♂♂, 1 ♀), 41° 47' 24.4" N 35° 09' 27.4" E (18 ♂♂, 16 ♀♀), 41° 53' 21.8" N 35° 00' 52.0" E (21 ♂♂, 30 ♀♀), 42° 01' 21.6" N 35° 12' 06.8" E (49 ♂♂, 31 ♀♀); 01.06.2017: 41° 57' 15.6" N 34° 48' 05.3" E (133 ♂♂, 60 ♀♀) leg. RT; 09.06.2017: 41° 44' 21.9" N 35° 13' 53.1" E (23 ♂♂, 16 ♀♀) leg. RT; 01.08.2017: 42° 01' 21.5" N 35° 12' 06.7" E (3 ♂♂, 10 ♀♀); 12.08.2017: 41° 36' 03.5" N 34° 51' 28.3" E (3 ♀♀) leg. RT&ÜZ; 17.08.2017: 41° 46' 02.5" N 35° 12' 11.0" E (3 ♀♀) leg. RT&ÜZ

Distribution in Türkiye: Ağrı, Amasya, Ankara, Antalya, Artvin, Aydın, Balıkesir, Bilecik, Bitlis, Bolu, Bursa, Çanakkale, Elazığ, Erzincan, Erzurum, Eskişehir, Giresun, Gümüşhane, Hakkari, İstanbul, İzmir, Kars, Kırklareli, Kuzeydoğu Karadeniz Bölgesi, Kastamonu, Kocaeli, Konya, Kütahya, Malatya, Manisa, Mardin, Muğla, Ordu, Rize, Samsun, Siirt, Sinop, Tekirdağ, Trabzon, Tokat, Van (Dlabola, 1957; 1981; Lodos & Kalkandelen, 1981; Kartal et al., 1994 Önder et al., 2011; Tanyeri & Zeybekoğlu, 2018; Demir, 2019)

Distribution in the world: Afro-tropical Region, Albania, Australian Region, Austria, Azores Islands, Balearic Islands, Belgium, Bosnia and Herzegovina, Bulgaria, Canary Islands, Central Cyclades Is., European Russia, Channel Islands, Corsica, Croatia, Cyprus, Czech Republic, Danish, the Dodecanese Is., East Palearctic, Estonia, Finland, French, Germany, Gibraltar, Greek, Hungary, Ireland, Italian, Kriti, Latvia, Lithuania, Macedonia, Malta, Moldova, Near East, Nearctic Region, Neotropical Region, North Aegean Is., North Africa, North European Russia, Norwegian, Oriental Region, Poland, Portuguese, Romania, Sardinia, Sicily, Slovakia, Slovenia, South European Russia, Spanish, Sweden, Switzerland, The Netherlands, Ukraine, Yugoslavia (Anonymous, 2021).

During this study, it was found that 10 species were distributed in the provinces of Sinop and Kastamonu. These are; *Aphrophora alni*, *A. salicina*, *A. geruzei*, *Lepryonia coleoptrata*, *Neophilaenus albipennis*, *N. campestris*, *N. exclamationis*, *N. lineatus*, *N. minor* and *Philaenus spumarius*.

Aphrophora is the largest genus in the family Aphrophoridae. Three species were identified from the study area. Species included in the genus *Aphrophora* are similar to each other in terms of body structures. The ratio of the body parts to each other, the swelling of the frontoclypeus, and the prominence of the anterior wing veins differ in terms of colour and patterning. Male genital structure characteristics are quite different between species (Komatsu, 1997). In addition, it has been shown that the species identified from this study area differ in terms of host plants and are in a close relationship with the host plants. *A. alni* is a major species found in pastures. However, in Japan, it has also been detected on trees such as *Salix*

spp. and *Populus* spp. (Komatsu 1997). In the study area, samples were collected from pastures and were not found in the tree layer. Mozaffarian & Wilson (2015), reported that *A. salicina* has a host relationship with *Salix* spp. This species was also found on *Salix* spp. in the study area. *A. gauzei* was collected from *Pinus* spp. *A. corticea* which was reported to be found in the region according to Önder et al., (2011), but was not found in this area. It should be clearly stated that the species *Aphrophora geruzei* Tanyeri & Zeybekoğlu, 2021, which was given in this study and which is essentially part of the Aphrophoridae section of the first author's PhD thesis, was separated from this study and evaluated in a previous study for a new species article.

The hook-like at the tip of the stylus is one of the important taxonomic characters in specimens belonging to the genus *Neophilaenus*. The body structure, wing patterning and genital structure of the examined specimens in the region are consistent with the definitions and drawings of Holzinger et al., (2003). *N. campestris* in terms of colour and patterning is very similar to typical phenotype of *Philaenus spumarius*. Although there are differences in body sizes, genital structures should be examined for the diagnosis of samples belonging to these two species. *N. lineatus* and *N. minor* were collected from the tree layer, while other taxa were collected from pastures and herbaceous vegetation.

N. lineatus and *N. minor* are characterized by a longitudinal black band on the lateral parts of the wings and are very similar to each other. Also, there is a similarity in genital structures. This makes their diagnosis difficult. *N. lineatus* is relatively smaller than this taxon. (4-5mm). *N. minor* is 3-3.5 mm in size. It also differs in the structure of the stylus of *N. lineatus* and *N. minor*. The distance between the outer tooth and the inner tooth in the stylus is shorter in *N. minor* compared to the others. According to the measurements of the individuals obtained in the study, this distance in the stylus was 0.15 mm in *N. lineatus* and 0.08 mm in *N. minor*.

N. albipennis has a characteristic patterning. In the lower 1/3 of the anterior wing, there is a dark band extending from the clavus to the wing margin (Figure 1). 5 ♂♂, 2 ♀♀ individuals were collected from Kastamonu and Sinop. The genital structures of the diagnosed specimens are consistent with the drawings of Holzinger et al. (2003).

L. coleoptrata, known as a polyphagous species, is the only known taxon from Türkiye belonging to the *Lepryonia*. *L. coleoptrata* which is found in both herbaceous and tree layers, was collected from the herbaceous layer in this study. It has been determined that it is found on different plants belonging to the Apiaceae family. When the material was evaluated, it was determined that the female and male individuals

of this species exhibited a significant sexual dimorphism in terms of body size and the females were larger than the males. The average size is 6.1 mm in males and 7.6 mm in females.

P. spumarius, known as the meadow spittlebug, is common and is distributed in the warm regions of both hemispheres and also attracts attention with its colour and patterning polymorphism. 8 phenotypes of this species from Sinop province and 9 different phenotypes from Kastamonu were determined and evaluated in previous studies. (Tanyeri & Zeybekoğlu, 2018; 2019).

CONCLUSION

At the end of the study, six species; *A. salicina*, *L. coleoptrata*, *N. albipennis*, *N. lineatus*, and *N. minor* were first recorded from Sinop and Kastamonu provinces. *A. alni* and *N. campestris* were recorded for the first time from Kastamonu provinces. *N. albipennis* which is known to be distributed in Ankara and Kırklareli, was recorded for the first time in the Black Sea Region of Türkiye.

A. alni, *A. salicina*, *L. coleoptrata*, *N. albipennis*, *N. campestris*, *N. lineatus*, *N. minor*, *P. spumarius* are considered as potential vectors for the bacterium *Xylella fastidiosa* associated with serious diseases in a wide range of plants in Europe (European Food Safety Authority, 2013). Considering the damage potential of Aphrophoridae spittlebugs, taxonomic and faunistic records from different regions are thought to be important.

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Contribution of the Authors as Summary

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

There is no conflict of interest between the article authors.

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Yağlık ve Yeşil Sofralık Amaca Yönelik Erkençi Bir Zeytin Çeşidi: 'As Topakaşı'

Sabahittin ABAY¹, İpek SEZER², Adem DAL³, Yazgan TUNÇ⁴, Emel ABAY⁵

¹Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Müdürlüğü, 42020, Karatay Konya, Türkiye, ²Bursa İl Tarım ve Orman Müdürlüğü, 16170, Osmangazi, Bursa, Türkiye, ³Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Müdürlüğü, 42020, Karatay Konya, Türkiye, ⁴Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü, Hassa İstasyonu, 31700, Hassa, Hatay, Türkiye, ⁵Toprak Su ve Çölleşme ile Mücadele Araştırma Enstitüsü Müdürlüğü, 42010, Meram, Konya, Türkiye

¹<https://orcid.org/0000-0002-7483-6957>, ²<https://orcid.org/0000-0002-6326-2054>, ³<https://orcid.org/0000-0003-3033-0265>

⁴<https://orcid.org/0000-0002-3228-8657>, ⁵<https://orcid.org/0009-0001-1145-0272>

✉: Sorumlu Yazar e-posta: yazgan.tunc@tarimorman.gov.tr

ÖZET

Türkiyede zeytin genetik kaynaklarının ele alınması ve seleksiyon çalışmalarına 1968 yılında başlanmış olup, bu amaçla günümüze kadar farklı bölgelerde yer yer bu çalışmalara devam edilmiştir. Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü tarafından Hatay ili Arsuz ilçesinde 2017-2020 yılları arasında yapılan seleksiyon ıslahı kapsamında 'As Topakaşı' isimli yeni bir zeytin çeşidi tespit edilmiştir. Bu yeni yerel çeşidin, çeşit tanımlamasını yapmak amacıyla üretici bahçesinden 5 tane 'As Topakaşı' zeytin çeşidi seçilerek işaretlenmiştir. Çeşit tanımlama özellikleri 20.10.2011 tarihli TG/99/4 sayılı UPOV kriterlerine göre yapılmıştır. Erken olgunlaşan 'As Topakaşı' zeytin çeşidi, yeşil sofralık ve yağlık olarak tüketilmektedir. Ağacı orta kuvvette ve yayvan gelişim göstermektedir. Meyvesi orta irilikte olup, yağ oranı %31'dir. 'As Topakaşı' zeytin çeşidi 28 Ekim 2020 tarihinde T.C. Tarım ve Orman Bakanlığı Çeşit Tescil ve Tohumluk Sertifikasyon Merkezi Müdürlüğü tarafından tescil edilmiştir.

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Araştırma Makalesi

Makale Tarihçesi

Geliş Tarihi : 15.02.2024

Kabul Tarihi : 18.06.2024

Anahtar Kelimeler

Olea europaea L.

Seleksiyon ıslahı

Erken hasat

Yağlık zeytin

Sofralık zeytin

An Early Olive Variety for Oil and Green Table Purposes: 'As Topakaşı'

ABSTRACT

In Türkiye, olive genetic resources and selection studies were started in 1968, and for this purpose, these studies have continued in different regions until today. A new olive variety named 'As Topakaşı' was detected within the scope of the selection breeding carried out by the Hatay Olive Research Institute Directorate in the Arsuz district of Hatay between 2017 and 2020. To identify this new local variety, 5-piece 'As Topakaşı' olive varieties were selected and marked from the producer's garden. Variety identification features were made according to UPOV criteria numbered TG/99/4 dated 20.10.2011. The early ripening 'As Topakaşı' olive variety is consumed as green table and oil. The tree has medium vigour and spreading growth. The fruit is medium-sized and its oil content is 31%. The 'As Topakaşı' olive variety was registered by the Variety Registration and Seed Certification Center Directorate of the Ministry of Agriculture and Forestry of the Republic of Türkiye on October 28, 2020.

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Oil olive

Table olive

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GİRİŞ

Herdem yeşil ve kurakçıl bir bitki olan zeytin (*Olea europaea* L.), Akdeniz Bölgesinin doğal bitki örtüsüdür (Tunç & Yılmaz, 2022a). Tarihte zeytin yetiştiren ilk çiftçiler, doğadan üstün genotipleri seçip, bu genotipleri vejetatif yöntemlerle çoğaltarak doğal seleksiyon yapmışlardır. Böylece hem üstün

genotipleri korumuş hem de zeytin dikim alanını genişleterek zeytin yetiştiriciliğinin Akdeniz Havzası'na yayılmasını sağlamışlardır. Ayrıca doğal seleksiyon yöntemi kullanılarak zeytinde çeşitlendirme çalışmaları başlatılmış ve dünyada yetiştirilen birçok yeni çeşit ortaya çıkmıştır (Petruccelli ve ark., 2022).

Türkiye coğrafi konum olarak dünya üzerinde bulunan üç önemli gen havuzunun kesiştiği yerde bulunmasından dolayı zengin bitki genetik kaynaklarına sahiptir (Özbek, 1978; Anonim, 2010; Güner & Aslan, 2012; Pınar ve ark., 2017; Yaman, 2022a; Kaya ve ark., 2023). Öte yandan doğada bulunan bu bitki genetik kaynakları, yöredeki halk tarafından yerinde bilinen çeşitlerle aşılama, şehirleşme, yol yapımı, sanayileşmeye dönük organize sanayi bölgelerinin oluşturulması gibi farklı yatırım projeleri ile arazilerin imara açılması gibi etmenlerden dolayı kaybolma durumundadır. Bu genetik çeşitlilik içerisinde seleksiyon yapılarak üstün nitelikli bireylerin belirlenmesi, toplanması ve bunların koruma altına alınması bitki çeşitliliğinin sürdürülebilirliği açısından önem arz etmektedir (Uzun ve ark., 2021; Yıldız ve ark., 2023). Buna bağlı olarak koruma altına alınan üstün nitelikli bireylerin tohum ve bitki gen bankaları ile oluşturulan koleksiyon bahçelerinde ex-situ olarak muhafaza altına alınması, ayrıca yetiştiği doğal ortamlarında mevcut ekolojik koşullarında adaptasyonun sürdürülebilirliği için in-situ olarak muhafaza altına alınması, morfolojik, pomolojik, biyokimyasal, moleküler gibi çok yönlü karakterizasyonu, üretimde yenilenme ve ilgili araştırma kurum ve kuruluşlarının kullanımına sunulması oldukça önemlidir (Altındağ & Akgün, 2015; Yaman, 2022b). Çeşit geliştirme

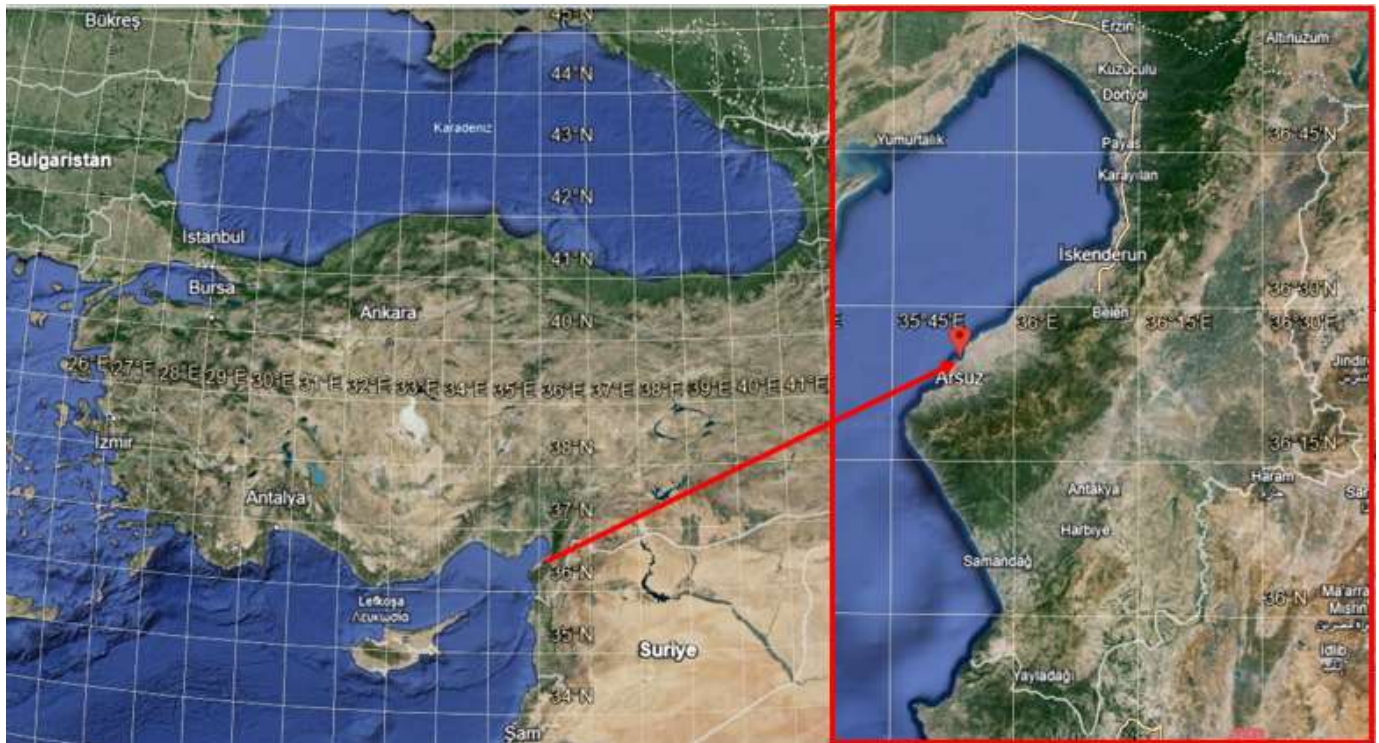
konusunda yapılan seleksiyon çalışmalarının asıl amacı, üretime yeni çeşitler kazandırmak, gen bankası oluşturmak, bu gen bankası içerisinde teknolojik ve agronomik olarak istenilen özelliklere sahip genetik çeşitliliği amaç doğrultusunda değerlendirmek ve ayrıca selekte bireyleri ıslah programlarına da dahil ederek gen bankası içerisindeki ebeveyn ağaçları seçmek olmalıdır (Rallo, 1995).

Bu çalışmanın asıl amacı, zeytin çeşitlerinin ayırt edilmesinde kullanılan morfolojik ve pomolojik tanımlama bilgilerine göre, erken olgunlaşan hem yağlık hem de yeşil sofralık olarak değerlendirilen 'As Topakaşı' zeytin çeşidinin özelliklerinin ortaya konulması ve çeşit tanımının ve tanıtımının yapıp yaygınlaştırılmasıdır.

MATERYAL ve METOD

Materyal

'As Topakaşı', Hatay ili Arsuz ilçesinde (Şekil 1) Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü tarafından 2017-2020 yılları arasında 4 yıl süreyle yürütülen seleksiyon ıslahı yöntemiyle elde edilen erkenci, yağlı ve yeşil sofralık amaca yönelik bir zeytin çeşididir (Anonim, 2020). Araştırma, 36°24'46"N 35°53'12"E koordinatlarında bulunan üretici bahçesinden seçilen 'As Topakaşı' zeytin çeşidine ait 5 ağaç üzerinde yürütülmüştür.



Şekil 1. Hatay ili Arsuz ilçesinde yürütülen doğal seleksiyon ıslahı alanına ait görüntü (Anonim, 2024)

Figure 1. Images of the natural selection breeding are carried out in the Arsuz district of Hatay province (Anonymous 2024)

Method

Fenolojik gözlemler ve pomolojik analizler

Fenolojik dönemler Turanoğlu (2015)'na göre belirlenmiştir.

Çiçeklenme başlangıcı: Çiçeklerin yaklaşık olarak %5'inin açtığı dönem.

Tam çiçeklenme: Çiçeklerin yaklaşık olarak %70'inin açtığı dönem.

Çiçeklenme sonu: Çiçeklerin tamamının açtığı dönem.

Çiçeklenme süresi: Çiçeklenme başlangıcı ile çiçeklenme sonu arasında geçen günlerin toplamı.

Meyve olgunlaşma dönemi Özdağ ve Koyuncu (2020)'ya göre belirlenmiştir.

Erken: Ekim ayı sonuna kadar.

Orta: 15 Kasım-15 Aralık ayları arası.

Geç: 15 Aralık ve sonrası.

Meyve ve çekirdek pomolojisine ait özellikler Özdemir ve ark. (2023)'na göre belirlenmiştir.

Meyve boyu (mm): Meyvelerin stil ucu ile meyvenin sapı arasında kalan mesafenin ölçülmesi ile tespit edilmiştir.

Meyve eni (mm): Meyvelerin orta eksenine dik olacak şekilde en geniş mesafenin ölçülmesi ile belirlenmiştir.

Meyve indeksi (boy/en): Meyve boyunun meyve enine oranlanması ile tespit edilmiştir.

Meyve ağırlığı (g): Meyvelerin tartılması ile belirlenmiştir.

Meyve eti ağırlığı (g): Meyvenin toplam ağırlığından çekirdeğin ağırlığı çıkarılarak saptanmıştır.

Çekirdek boyu (mm): Çekirdek stil ucu ile çekirdeğin en tepesi arasındaki mesafenin ölçülmesi ile tespit edilmiştir.

Çekirdek eni (mm): Çekirdeklerin orta eksenine dik olacak şekilde en geniş mesafenin ölçülmesi ile belirlenmiştir.

Çekirdek indeksi (boy/en): Çekirdek boyunun çekirdek enine oranlanması ile tespit edilmiştir.

Çekirdek ağırlığı (g): Çekirdeklerin tartılması ile belirlenmiştir.

Yaprak morfolojisine ait özellikler Kaya ve ark. (2014); Hakan (2017); Gözel (2018); Özdağ & Koyuncu (2020)'ya göre belirlenmiştir.

Yaprak uzunluğu (cm): Yaprakların ucundan sap kısmına kadar olan mesafenin ölçülmesi ile belirlenmiştir.

Yaprak genişliği (cm): Yaprakların orta eksenine dik olacak şekilde en geniş mesafenin ölçülmesi ile belirlenmiştir.

Yaprak indeksi (uzunluk/genişlik): Yaprak boyunun çekirdek enine oranlanması ile tespit edilmiştir.

Çiçek salkımına ait özellikler Özdağ ve Koyuncu (2020)'ya göre belirlenmiştir.

Çiçek salkımı uzunluğu (cm): Çiçek salkımı ucundan dip kısma kadar ölçülmesiyle belirlenmiştir.

Çiçek salkımı genişliği (cm): Çiçek salkımının orta eksenine dik olacak şekilde en geniş mesafenin

ölçülmesi ile belirlenmiştir.

Morfolojik özellikler ve yağ oranı analizi

Çalışmada bitki, yaprak, çiçek yapısı, çiçek sürgünü, çiçek salkımı, meyve ve çekirdek özellikleri belirlenmiştir. Çeşit tanımlamasında kullanılan söz konusu morfolojik özellikler UPOV (TG/99/4, 2011) kriterlerine göre yapılmıştır (Tablo 1). Ağacın 160-180 cm arasındaki yükseklikten 8 ile 10 adet yıllık sürgünün orta kısımlarından alınan 40 adet yaprakta yaprak özellikleri (Özdağ & Koyuncu, 2020), çeşidi temsil eden ağacın güney cephesinden alınan 40 adet meyvede de meyve ve çekirdek özellikleri belirlenmiştir (Kaya ve ark., 2018; 2023). Yaprak, meyve ve çekirdek örneğinde INSIZE marka 0.01 mm hassasiyete sahip dijital kumpas kullanılarak ölçümler yapılmıştır (Kaya ve ark., 2018; 2023). Meyve ağırlığı 40 adet meyvenin 0.0001 g hassasiyete sahip DENSİ HZK-110FA markalı dijital terazide tartılması sonucu belirlenmiştir (Asayesh ve ark., 2023). Çiçek salkımı özellikleri ağacın dört bir yönünden 8 ile 10 adet meyve dalı üzerinden, çiçekler somak evresindeyken alınan 40 adet çiçek salkımının ölçülmesiyle saptanmıştır (Özdağ ve Koyuncu, 2020). Ayrıca çeşidin yağ oranı soxhlet ekstraksiyon cihazında Anonim (1973) ve Kaya ve ark. (2018; 2023)'na göre belirlenmiştir. Hasat döneminde toplanan meyveler tartılmıştır. Taze meyveler sabit ağırlığa gelene kadar etüvde kurutulmuştur. Sabit ağırlığa gelen kuru meyve örnekleri tartılmıştır. Kuru ağırlığı belirlenen meyve örnekleri çekirdeği ile ezilerek soxhlet ekstraksiyon cihazında hekzan yardımıyla yağ ekstraksiyon işlemi yapılmıştır. Sonra soxhlet ekstraksiyon cihazındaki balonların içerisinde bulunan hekzan uçurulup, geriye kalan ham yağ tartılmıştır. Elde edilen ham yağ miktarı, ilk başta tespit edilen yaş meyve örneğinin ağırlığına bölünerek yaş meyve örneğindeki yağ oranı tespit edilmiştir.

Yağ oranı sınıflandırılması Kaya ve ark. (2018; 2023)'e göre yapılmıştır.

Düşük yağ oranı: <%18, **orta yağ oranı:** %18-%22, **yüksek yağ oranı:** >%22.

Pomolojik analizler, morfolojik değerlendirmeler ve yağ oranı analizi Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü, Hassa İstasyonunda bulunan laboratuvarında gerçekleştirilmiştir.

BULGULAR ve TARTIŞMA

2017-2020 yılları arasında gerçekleşen fenolojik evrelerin ortalaması Tablo 2'de detaylı olarak verilmiştir. Fenolojik evreler yıllar arasında iklimsel olaylara göre değişmekle birlikte, çiçeklenme başlangıcı 18-25 Nisan; tam çiçeklenme 26 Nisan ile 2 Mayıs; çiçeklenme sonu 5-12 Mayıs; çiçeklenme süresi 18-19 gün; yeşil olum evresi 1-5 Ekim; pembe olum evresi 10-16 Ekim; siyah olum evresi 9-13 Kasım tarihleri arasında gerçekleşmiştir.

Tablo 1. 20.10.2011 tarihli ve TG/99/4 sayılı zeytin (*Olea europaea* L.) UPOV kriterleri (UPOV, 2011)
Table 1. Olive (*Olea europaea* L.) UPOV criteria dated 20.10.2011 and numbered TG/99/4 (UPOV, 2011)

Özellikler		Açıklamalar
Ağaç	Gelişme kuvveti	Zayıf, orta, kuvvetli
	Büyüme şekli	Sarkık, yayvan, dik
	Taç yoğunluğu	Seyrek, orta, yoğun
Çiçek sürgünü	Yan sürgünlerin sayısı	Az, orta, çok
Yaprak ayası	Uzunluk	Kısa (<5 cm), orta (5-7 cm), uzun (>7 cm)
	Genişlik	Dar (<1 cm), orta (1-1,5 cm), geniş (>1,5 cm)
	Uzunluk genişlik oranı	Hafif uzun (eliptik) (<4), orta derecede uzun (uzun eliptik) (4-6), çok uzun (mızrak) (>6)
	Üst düzeyde yeşil rengin yoğunluğu	Açık, orta, koyu
	Uzunlamasına eksenin eğriliği	Kavisli, düz, kıvrık
Çiçek salkımı	Dönme	Yok veya zayıf, orta, güçlü
	Uzunluk	Kısa (<25 mm), orta (25-35), uzun (>35 mm)
	Genişlik	Dar, orta, geniş
Çiçek	Korolla lobunun duruşu	Dik, yatay, refleksli
Meyve	Uzunluk	Çok kısa, kısa, orta, uzun, çok uzun
	B konumunda genişlik	Çok dar, dar, orta, geniş, çok geniş
	Ağırlık	Küçük (<2 g), orta (2-4 g), iri (4-6 g), çok iri (>6 g)
	A konumunda şekil	Yuvarlak (<1.25), orta (1.25-1.45), uzun (>1.45)
	A konumunda uzunluk/genişlik oranı	Hafif uzun, orta derecede uzun, çok uzun
	Tam olgunlukta üst renk	Açık, orta, koyu
	A konumunda simetri	Simetrik, zayıf asimetrik, güçlü asimetrik
	A konumunda uç şekli	Sivri, geniş, yuvarlak
	Uç	Yok veya zayıf, orta, geniş
	A konumunda taban şekli	Yuvarlak, yuvarlak küt, küt
Olgunlaşmamış meyve	Yüzeyin püslülüğü	Zayıf, orta, güçlü
	Yeşil rengin yoğunluğu	Açık, orta, koyu
	Lentisellerin büyüklüğü	Küçük, orta, büyük
Çekirdek	Lentisellerin sayısı	Az, orta, çok
	B konumunda şekil	Oval, dikdörtgen, eliptik, dairesel, obovat
	Uzunluk	Kısa, orta, uzun
	B konumunda genişlik	Dar, orta, geniş
	Uzunluk/genişlik oranı	Hafif uzun, orta derecede uzun, çok uzun
	Ağırlık	Küçük (<0.3 g), orta (0.33-0.45 g), iri (0.45-0.70 g), çok iri (>0.70 g)
	A konumunda simetri	Simetrik, zayıf asimetrik, güçlü asimetrik
	B konumunda simetri	Simetrik, zayıf asimetrik, güçlü asimetrik
	Bazal uçtaki yiv sayısı	7'den az, 7-10 arası, 10'dan fazla
	Bazal uçtaki yivlerin dağılımı	Eşit dağılmış, sütür etrafında zayıf gruplanmış, sütür etrafında güçlü gruplanmış
A konumundaki uç şekli	Sivri, geniş, yuvarlak	
Uç	Yok veya zayıf, orta, geniş	
B konumunda taban şekli	Yuvarlak, yuvarlak küt, küt	
Yüzeyin buruşukluğu	Zayıf, orta, güçlü	

Tablo 2. 2017-2020 yılları arasında gerçekleşen fenolojik evreler ve ortalaması

Table 2. Phenological stages and average between 2017-2020

Yıllar ve ortalaması	Çiçeklenme başlangıcı	Tam çiçeklenme	Çiçeklenme sonu	Çiçeklenme süresi (gün)	Yeşil olum evresi	Pembe olum evresi	Siyah olum evresi
2017	18 Nisan	26 Nisan	5 Mayıs	18	1 Ekim	10 Ekim	9 Kasım
2018	22 Nisan	1 Mayıs	11 Mayıs	19	2 Ekim	11 Ekim	12 Kasım
2019	25 Nisan	2 Mayıs	12 Mayıs	18	5 Ekim	16 Ekim	13 Kasım
2020	20 Nisan	28 Nisan	9 Mayıs	19	4 Ekim	14 Ekim	8 Kasım
Ortalama	21 Nisan	29 Nisan	9 Mayıs	19	03 Ekim	13 Ekim	10 Kasım

'As Topakaşı' zeytin çeşidinin çeşit özelliklerine (pomolojik ve morfolojik) ilişkin detaylı bilgiler Tablo 3'de sunulmuştur.

Tablo 3. 'As Topakaşı' zeytin çeşidinin çeşit tanımlama sertifikası (Anonim, 2020)

Table 3. Variety identification certificate of 'As Topakaşı' olive variety (Anonymous, 2020)

Özellikler	Açıklamalar
Ağaç	Gelişme kuvveti Büyüme şekli Taç yoğunluğu
Çiçek sürgünü	Yan sürgünlerin sayısı
Yaprak ayası	Uzunluk Genişlik Uzunluk genişlik oranı Üst düzeyde yeşil rengin yoğunluğu Uzunlamasına eksenin eğriliği Dönme
Çiçek salkımı	Uzunluk Genişlik
Çiçek	Korolla lobunun duruşu
Meyve	Uzunluk B konumunda genişlik Ağırlık A konumunda şekil A konumunda uzunluk/genişlik oranı Tam olgunlukta üst renk A konumunda simetri A konumunda uç şekli Uç A konumunda taban şekli Yüzeyin püslülüğü
Olgunlaşmamış meyve	Yeşil rengin yoğunluğu Lentisellerin büyüklüğü Lentisellerin sayısı
Çekirdek	B konumunda şekil Uzunluk B konumunda genişlik Uzunluk/genişlik oranı Ağırlık A konumunda simetri B konumunda simetri Bazal uçtaki yiv sayısı Bazal uçtaki yivlerin dağılımı A konumundaki uç şekli Uç A konumunda taban şekli Yüzeyin buruşukluğu
Meyve olgunlaşma zamanı	Erken
Meyve yağ oranı	%31

Genel olarak ağaç büyüme şekli yayvan olup, yoğun bir taç yapısına sahiptir (Şekil 2). Meyve şekli yuvarlak, meyve ağırlığı orta ağırlıktadır. Çiçek salkımının boyu kısa olup, çiçek salkımı orta genişliktedir (Şekil 3). Kısmi periyodisite gösteren 'As Topakaşı' çeşidinin, T.C. Tarım ve Orman Bakanlığı Çeşit Tescil ve

Tohumluk Sertifikasyon Merkezi Müdürlüğü tarafından diğer çeşitlerden farklı, yeterince homojen ve stabil olduğu rapor edilmiştir (Anonim, 2020). 'As Topakaşı' zeytin çeşidinin yağ oranı %31 olarak tespit edilmiştir. Çeşide ait yaprak, meyve ve çekirdek görüntüleri Şekil 4'te sunulmuştur.



Şekil 2. 'As Topakaşı' zeytin çeşidinin ağaç büyüme şekli ve taç yoğunluğu (Sabahittin ABAY'dan alınmıştır)
Figure 2. Tree growth habit and canopy density of 'As Topakaşı' olive variety (taken from Sabahittin ABAY)

Çanakkale'nin Ezine ilçesinde seleksiyon ıslahı sonucunda 'Hanım Parmağı' isimli yeni bir çeşitte tescil çalışması gerçekleştirilmiştir (Kaya ve ark., 2018). Benzer şekilde başka bir çalışmada da Antalya'nın Manavgat ilçesinde 'Beylik' isimli yeni bir çeşitte tescil çalışması gerçekleştirilmiştir (Kaya ve ark., 2023). Lavee (1978), İsrail'de gerçekleştirdiği seleksiyon ıslahı çalışması kapsamında 'Kadesh' isimli sofralık yeni bir çeşit geliştirdiğini bildirmiştir. 'Kadesh' çeşidinin çekirdek ağırlığı meyve ağırlığının %16,6'sını oluşturmasından dolayı meyve et oranının yüksek olduğunu rapor etmiştir. Lavee ve ark. (1999), İsrail'de yürüttükleri bir çalışma kapsamında "Maalot" isimli yeni bir zeytin çeşidini selekte ederken, Rallo ve ark. (2008) İspanya'da 'Chiquitita' isimli yeni bir zeytin çeşidini tespit edip tanıtmışlardır. Camposea ve ark. (2021), İtalya'da yaptıkları bir çalışmada 'Lecciana' isimli zayıf dallanan, süper yüksek sık dikime uygun zeytin çeşidini rapor

etmişlerdir. Valverde ve ark. (2024), İspanya'da yaptıkları çalışma kapsamında 'Sultana' ismini verdikleri çit bahçeleri için uygun bir zeytin çeşidi geliştirdiklerini bildirmişlerdir. Konu hakkında çalışma yapan araştırmacıların bulguları Tablo 4.'de detaylı bir şekilde verilmiştir. Bu bağlamda, bulgular araştırmacıların bulguları ile kıyaslandığında bazı özelliklerin benzer bazı özelliklerin farklı olduğu görülmektedir. Ortaya çıkan bu farklılığın sebebi çeşidin genetik yapısının ve bulunduğu yörenin farklı olması ile açıklanabilir. Ayrıca, elma (Sansavini ve ark., 2004), alıç (Pan, 2011), kayısı (Asma, 2012; Wu ve ark., 2024), üzüm (Park ve ark., 2020), Japon eriği (Guevara ve ark., 2021), kiraz (Jiang ve ark., 2022), armut (Bell ve ark., 2023), badem (Dicenta ve ark., 2023), ceviz (Özcan ve ark., 2023), fındık (Mehlenbacher ve ark., 2023), yaban mersini (Stringer ve ark., 2023), aronya (Brand, 2024), nektarin (Kwon ve ark., 2024), şeftali (Chen & Okie, 2024), kivi (Scorza

ve ark., 2024), kızılılık (Bogges & Trigiano, 2024) gibi farklı meyve tür ve çeşitlerinde de ıslah metoduyla yeni çeşit(ler) geliştirilmiş ve dünyaya tanıtılmıştır. Türkiye'de seleksiyon yoluyla çeşit çalışmaları birçok

çeşidin üretime kazandırılmasına katkı sağlamıştır. Geniş genetik kaynakların kazandırmış olduğu bu çeşitlerin ardından günümüzde klonal zeytin anaç çalışmaları da başlatılmıştır (Tunç & Yılmaz, 2022a; 2022b).



Şekil 3. 'As Topakaşı' zeytin çeşidinin çiçek salkımına ait görüntü (Sabahittin ABAY'dan alınmıştır)
Figure 3. Image of the inflorescence of the 'As Topakaşı' olive variety (taken from Sabahittin ABAY)



Şekil 4. 'As Topakaşı' zeytin çeşidinin yaprak, meyve ve çekirdek yapısı (Sabahittin ABAY'dan alınmıştır)
Figure 4. Leaf, fruit and stone structure of 'As Topakaşı' olive variety (taken from Sabahittin ABAY)

Tablo 4. Konu hakkında çalışma yapan araştırmacıların bulguları
Table 4. Findings of researchers working on the subject

Özellikler	Açıklamalar	Lavee (1978)	Lavee ve ark. (1999)	Rallo ve ark. (2008)	Kaya ve ark. (2018)	Camposeo ve ark. (2021)	Kaya ve ark. (2023)	Valverde ve ark. (2024)
Ağaç	Gelişme kuvveti	-	Orta	Zayıf	Kuvvetli	Zayıf	Kuvvetli	Zayıf
	Büyüme şekli	-	Sarkık	Sarkık	Yayvan	Dik	Yayvan	Sarkık
	Taç yoğunluğu	-	-	Yoğun	Orta	Orta	Orta	Yoğun
Çiçek sürgünü	Yan sürgünlerin sayısı	-	-	-	-	-	-	Orta
Yaprak ayası	Uzunluk	-	-	Uzun	5.36 cm (Orta)	Uzun	6.12 cm (Orta)	Uzun
	Genişlik	-	-	Dar	1.19 cm (Orta)	-	1.39 cm (Orta)	Orta
	Uzunluk genişlik oranı	-	-	Uzun eliptik	4.50 cm (Uzun eliptik)	-	4.40 cm (Uzun eliptik)	Uzun eliptik
	Üst düzeyde yeşil rengin yoğunluğu	-	-	-	-	-	-	Orta
	Uzunlamasına eksenin eğriliği	-	-	Düz	Düz	Düz	Düz	Düz
	Dönme	-	-	-	-	-	-	Yok veya zayıf
Çiçek salkımı	Uzunluk	-	-	-	-	Kısa	-	Orta
	Genişlik	-	-	-	-	-	-	Orta
Çiçek	Korolla lobunun duruşu	-	-	-	-	-	-	Refleksli
Meyve	Uzunluk	2.4 cm	Uzun	-	-	Orta	-	Orta
	B konumunda genişlik	1.9 cm	-	Orta	-	Dar	-	Orta
	Ağırlık	6 g	<2 g	Orta	4.65 g (İri)	Orta	6.38 g (Çok iri)	Orta
	A konumunda şekil	-	-	Oval	1.35 (Oval)	Eliptik	1.52 (Uzun)	Oval
	A konumunda uzunluk/genişlik oranı	-	-	-	-	-	-	Orta derecede uzamış
	Tam olgunlukta üst renk	-	-	Siyah	Koyu menekşe	Koyu menekşe	Koyu menekşe	Açık
	A konumunda simetri	-	-	Simetrik	Simetrik	Zayıf asimetrik	Asimetrik	Güçlü Asimetrik
	A konumunda uç şekli	-	-	Yuvarlak	Yuvarlak	Yuvarlak	Sivri	Kesik
	Uç	-	-	Yok	-	Yok	-	Yok
	A konumunda taban şekli	-	-	Yuvarlak	-	Küt	-	Kesik
	Yüzeyin püslülüğü	-	-	-	-	-	-	Orta
Olgunlaşmamış meyve	Yeşil rengin yoğunluğu	-	-	-	-	-	-	Açık
	Lentisellerin büyüklüğü	-	-	-	Küçük	Orta	Küçük	Büyük
	Lentisellerin sayısı	-	-	-	Az sayıda	Az sayıda	Az sayıda	Orta

Çekirdek	B konumunda şekil	-	-	Eliptik	-	Eliptik	-	Eliptik
	Uzunluk	-	-	-	-	Orta	-	Orta
	B konumunda genişlik	-	-	Orta	-	Dar	-	Orta
	Uzunluk/genişlik oranı	-	-	-	1.91 (Eliptik)	-	1.91 (Eliptik)	Orta derecede uzamış
	Ağırlık	-	-	Orta	0.62 g (İri)	Orta	1.02 g (Çok iri)	Orta
	A konumunda simetri	-	-	Simetrik	Simetrik	Zayıf asimetrik	Simetrik	Simetrik
	B konumunda simetri	-	-	Simetrik	Simetrik	-	Simetrik	Simetrik
	Bazal uçtaki yiv sayısı	-	-	7-10 arası (Orta)	-	7-10 arası (Orta)	-	7-10 arası (Orta)
	Bazal uçtaki yivlerin dağılımı	-	-	-	-	-	-	Sütür etrafında zayıf bir şekilde gruplanmış
	A konumundaki uç şekli	-	-	Sivri	Sivri	Sivri	Sivri	Sivri
Uç	-	-	-	Var	Var	Var	Var	
A konumunda taban şekli	-	-	Yuvarlak	-	-	-	Yuvarlak	
Yüzeyin buruşukluğu	-	-	Güçlü	Orta	Güçlü	Orta	Zayıf	
Meyve olgunlaşma zamanı	-	-	-	-	Geç	-	Çok erken	
Meyve yağ oranı	<%18 (düşük)	%20- 22 (orta)	-	19.6% (orta)	19% (orta)	18% (orta)	-	

SONUÇ ve ÖNERİLER

Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü tarafından yapılan doğal seleksiyon ıslahı çalışmasında, 'As Topakaşı' zeytin çeşidi, T.C. Tarım ve Orman Bakanlığı Çeşit Tescil ve Tohumluk Sertifikasyon Merkezi Müdürlüğü tarafından 28 Ekim 2020 tarihinde tescil edilerek ulusal çeşitler listesine eklenmiştir. Geçmiş dönemlerden günümüze kadar bölgede yetiştirilen 'As Topakaşı' zeytin çeşidi yörede Ekim ayının ortasına kadar olgunlaşması nedeni ile erkenci, yeşil olum evresinde hem meyvesinin orta irilikte hem de yağ oranının yüksek olmasından dolayı alternatif çift yönlü (yeşil sofralık/yağlık) bir çeşit olarak kayıtlara geçmiştir. Genel olarak ağaç büyüme şekli yayvan olup, yoğun bir taç yapısına sahiptir. Meyve şekli yuvarlak, meyve ağırlığı orta ağırlıktadır. Çeşit üzerinde yürütülecek yayım çalışmaları kapsamında bölgede daha geniş alanlara yayılma şansı bulacaktır. Ayrıca 'As Topakaşı' zeytin çeşidi hakkında daha detaylı araştırmalar yürütülerek farklı özellikleri de belirlenip literatüre kazandırılacaktır. 'As Topakaşı' zeytin çeşidi fidanları çoğaltılarak Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğü

tarafından koruma altına alınmış olup, Hatay Zeytincilik Araştırma Enstitüsü Müdürlüğünden fidanlar temin edilebilmektedir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Hidroponik Fesleğen Yetiştiriciliğinde Vermikompost Uygulamalarının Etkileri

Gölgen Bahar ÖZTEKİN^{1,2,3}, Ali Kemal DEMİRCAN², Tunç DURDU³

^{1,2,3}Ege Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Bornova-İzmir

¹<https://orcid.org/0000-0001-6023-013x>, ²<https://orcid.org/0000-0003-4225-014x>, ³<https://orcid.org/0000-0002-9837-7775>

✉: golgen.oztekin@ege.edu.tr

ÖZET

Hidroponik fesleğen (*Ocimum bacilium*) yetiştiriciliğinde solucan gübresi (vermikompost) uygulamasının bitki gelişimi, verim ve bazı kalite parametreleri üzerine etkilerinin araştırıldığı bu çalışmada, vermikompost yapraktan püskürtme, kökten besin solüsyonu ile uygulama ve hem kökten hem yapraktan verilme şeklinde uygulanmıştır. Kontrol uygulamasında sadece besin solüsyonu uygulanmış, yapraklardan sadece su püskürtülmüştür. Tohumlar file saksılarda her saksıya 10 tohum olacak şekilde torf ortamına elle ekilmiş, tohum ekiminden 84 gün sonra bitkiler yaklaşık 25 cm boylandığında tek seferde hasat yapılarak deneme sonlandırılmıştır. Ekim sonrası 45'lik viyollere konan file saksılar, 22±2°C sıcaklık ve %85 nemde çimlendirme odasında 4 gün tutulmuştur. Fideler 2 gerçek yapraklı oldukları zaman hidroponik sisteme (Besleyici Film Tekniği, NFT) yetiştirme kanallarına aktarılmıştır. Kapalı sistem yetiştiricilikte besleme+drenaj tankları her konu için ayrılarak, tanklardaki EC ve pH değerlerinin kontrolü yapılarak yetiştiricilik yapılmıştır. Araştırmada bitki morfolojik gelişim parametreleri, biyokütlesi, bitki ağırlığı ve verim, yaprak renk değerleri, vitamin C ve nitrat içerikleri belirlenmiştir. Elde edilen veriler değerlendirildiğinde, vermikompost uygulamasının bitki gelişimi ve verimini arttırdığı; uygulama yeri olarak yapraktan yapılan püskürtme şeklinde uygulamanın diğer uygulamalara göre daha iyi sonuç verdiği görülmüştür. Bu nedenle hidroponik fesleğen yetiştiriciliğinde kaliteden ödün vermeden mevcut verimi arttırmak ve erkencilik sağlamak adına sıvı vermikompostun yapraktan uygulaması önerilmektedir.

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler

Ocimum bacilium

NFT

Solucan gübresi

Verim

Biyokütle

Effect of Vermicompost Applications on Hydroponic Basil Cultivation

ABSTRACT

In this study, the effects of vermicompost (earthworm compost) application on plant growth, yield, and some quality parameters were investigated in hydroponic basil (*Ocimum bacilium*) cultivation. The vermicompost was applied through foliar spray, nutrient solution (to root), and root and foliar application methods. In the control group, only a nutrient solution was applied, and water was sprayed on the leaves. Seeds were manually planted in peat medium in mesh pots, with 10 seeds per pot. The experiment was finished when the plants reached approximately 25 cm in height, 85 days after seeding, with a single harvest. Mesh pots placed in 45-cell trays after seeding were kept in a germination chamber at a temperature of 22±2°C and 85% humidity for 4 days. When the seedlings had two true leaves, they were transferred to the hydroponic system (Nutrient Film Technique, NFT). In closed-system cultivation, feeding, and drainage tanks were separated for each treatment, and cultivation was carried out by monitoring the EC and pH values in the tanks. Plant morphological development parameters, biomass, plant weight and yield, leaf colour values, vitamin C, and nitrate contents were determined. The results indicated that vermicompost application increased plant growth and yield. Among the application methods, foliar spray yielded better results compared to other applications. Therefore, for hydroponic basil cultivation, it is

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recommended to apply liquid vermicompost through foliar spray to increase current yield without compromising quality and to ensure early harvesting.

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GİRİŞ

Gerek tadı ve gerekse görselliği nedeni ile sevilen bir bitki olan fesleğen, en çok üretilen ve tüketilen tıbbi ve aromatik bitki gurubunda yer almaktadır. Fesleğen birçok sektör (gıda, ilaç, baharat vb.) tarafından yoğun bir halde yaş/taze kesme (yaygın kullanımı) ya da kurutulmuş şekillerde (konserve yiyeceklerde, bitki çaylarında, parfümeri materyali, baharat, sağlık, aromaterapi, kozmetik ve geleneksel tıp alanında da uçucu yağ) değerlendirilmektedir (Labra et al., 2004; Telci ve ark., 2005; Naghibi et al., 2022). Bunların yanında ek olarak pesto sos olarak ve de peyzaj alanında süs bitkisi olarak evlerde, minik saksılar içerisinde yetiştirilmektedir (Dumanoglu & Mokhtarzadeh, 2021; Karagöz, 2020). Türkiye de saksılarda küçük yapraklı fesleğenlerin görüntü ve hoş kokusu nedeni ile mutfaklarda yerini aldığı, tıbbi ve aromatik bitki olarak da büyük alanlarda iri yapraklı fesleğenlerin yetiştirildiği görülmektedir.

Yetiştirildiği iklime bağlı olarak değişik renklerde olabilen ve 30-80 cm boylana bilen (Darrah, 1988; Ceylan, 1997) fesleğen, *Ocimum* cinsinde Ballıbabagiller (*Lamiaceae*) familyasına ait olup, tek ve çok yıllık yetiştirilebilen bir bitkidir (Ekren ve ark., 2009). Geniş bir varyasyonu bulunan bitki, pek çok alt tür ve varyetelere ayrılmaktadır (Çamlıca & Yıldız, 2017). Fesleğenin *Ocimum bacilium* L. türü ekonomik öneme sahip ve tek yıllık kültürü yapılan türdür ve bu türün tatlı, mor renkli (reyhan), küçük veya büyük yapraklı, limon veya anason kokulu alt türleri mevcuttur (Telci ve ark., 2005).

Besin değeri ve içerdiği vitaminler açısından zengin bir aromatik bitki (Anonymous, 2020) olan fesleğenin sağlık açısından da faydalı olduğu bilinmektedir (Anonymous, 2022). Dünyada yılda ortalama 90-95 ton civarında üretiminin yapıldığı tahmin edilmektedir

Türkiye’de fesleğen çeşitleri yayılım göstermemesine rağmen genel olarak Batı ve Güney Anadolu’da (Ceylan, 1997; Ekren ve ark., 2009), temel olarak çeşnilik, baharat ve süs bitkileri olarak yetiştirilmektedir. Türkiye’de fesleğen yetiştiriciliği yapılan tarım arazilerinin az miktarda olduğu bilinmektedir. Ekonomik getirisi yüksek bir ürün olan fesleğen, her geçen gün üreticilerin dikkatini daha da çekmektedir.

Topraksız tarımda su kültüründe ticari olarak en çok yetiştirilen ürünler yeşillikler olarak da bilinen yaprakları yenilen sebzeler olup, ilk sırayı marul almaktadır. Fesleğen maruldan sonra topraksız

tarımda su kültüründe en çok üretilen üründür ve Besleyici Film Tekniği (NFT) ile Derin Akan Su Kültürü Tekniği (DFT) bu amaçla en fazla kullanılan sistemlerdir. Ticari olarak topraksız tarımda fesleğen yetiştiriciliğin topraksız tarımın ticarileşmesi kadar eski olduğu, 1970’li yıllara dayandığı düşünülmektedir. Fesleğenin topraksız tarımda yetiştiriciliği konvensiyonel üretime göre daha temiz, daha kısa sürede ve yüksek verimli ürün alınabilmesine neden olmaktadır (Walters & Currey, 2015). Bunlara ilaveten, yıl içerisinde daha fazla üretim yapılmasına imkân sağlamaktadır. Yeşilliklerin su kültüründe yetiştirilme kolaylığı nedeni ile de araştırmanın topraksız tarım sistemlerinden su kültüründe yapılması düşünülmüştür.

Vermikompost, diğer bir ifade ile solucan gübresi, topraklardaki solucanların beslenmek için vücutlarından geçirdikleri ve dışarı attıkları besin değeri yüksek materyaldir ve tarımda çevre dostu ve sürdürülebilir özellikleri nedeni ile gübre olarak oldukça fazla kullanılmaktadır. Vermikompost yapımında en çok Kaliforniya Solucanı, *Humbricus rubellis* ve *Eisenia foetida*, *Dendrobena veneta* türlerindeki solucanlar kullanılmaktadır (Singh, 1997; Karaçalı & Tüfenkçi, 2010). Küçük ve orta ölçekli tarım işletmelerinde de uygulanabilirliği olan vermicompost ile tarımsal üretim sürecinde oluşan atık ve/veya atık sınıfındaki materyaller ticari değeri yüksek bir ürüne dönüştürülmektedir (Şimşek-Erşahin, 2007).

İnce dokulu, torf benzeri gözenekli bir yapısı olan vermicompost, yüksek havalanma, drenaj, su tutma kapasitesine ve mikrobiyal aktiviteye sahiptir. Vermikompostun bitki tarafından ihtiyaç duyulan bitki besin maddelerini elverişli bir şekilde temin ettiği ve bu besin maddelerinin bitki tarafından alınımını artırdığı (Masciandaro et al., 1997; Çitak ve ark., 2011), bitki verimini olumlu etkilediği (Sahni et al., 2008; Mısırlıoğlu, 2011), bitki kök gelişimine katkı sağladığı (Sahni et al., 2008; Mısırlıoğlu, 2011), tuzluluğun bitki üzerindeki toksik etkisini azalttığı (Demir & Kıran, 2020), hastalık ve zararlıları baskıladığı (Arancon et al., 2005; Şimşek-Erşahin, 2007), topraktaki mikroorganizma popülasyonunu artırdığı (Arancon et al., 2006), toprağın fiziko-kimyasal ve biyolojik özelliklerini iyileştirdiği (Singh et al., 2008; Parthasarathi et al., 2008; Zaller, 2007a,b; Warman & Anglopez, 2010) önceki çalışmalar ile ortaya konmuştur.

Vermikompost katı ve sıvı halde piyasada ticari olarak bulunmakta ve tarımsal üretimde çokça kullanılmaktadır. Yürütülen bu çalışmada son günlerin yüksek trendi tıbbi ve aromatik tek yıllık bir bitki olan fesleğenin (*Ocimum bacilium*) hidroponik yetiştiriciliğinde verim ve kaliteye etkilerinin belirlenmesi, en uygun uygulama yerinin saptanması amaçlanmıştır.

MATERIAL ve METOD

Çalışma Ege Üniversitesi (EÜ) kampüsü içerisinde (Bornova/İzmir) yer alan EÜ Ziraat Fakültesi Bahçe Bitkileri Bölümü Örtüaltı Sebze Yetiştirme Bilim Dalı'na ait eğitim ve araştırma seralarında yürütülmüştür. 16.56 x 50 m ölçülerinde ve plastik (PE) örtü materyali ile örtülmüş bitünel seranın, yan ve çatı havalandırmaları böcek neti ile kaplıdır. Isıtmasız seranın yaklaşık 10 m²lik bir bölümünde yetiştiricilik yapılmıştır.

Bitkisel materyal olarak iri yapraklı fesleğen türünün Yeşil (standart) çeşidi (Mitofarm Tohumculuk, Gaziantep) kullanılmıştır. Tohumlar 25 g hacimli file saksılarda ithal torf ortamına, her bir saksıya 10 adet tohum gelecek şekilde 31.10.2022 tarihinde elle ekilmiştir. Saksılar, kendileri için özel hazırlanmış 45'lik viyollere yerleştirilmiştir. Viyollerde ortamların nem kaybını önlemek için viyoller streç film ile kaplanılmış ve tohumların çimlenmesi için çimlendirme odasına (karanlık, gece/gündüz 22±2°C, %85 nem) konularak ve burada 4 gün tutulmuştur. Süre sonunda streç filmleri açılarak fide yetiştirmek için özelleşmiş fide adaptasyon serasına alınan viyoller, 1-2 gerçek yaprağı çıkana kadar burada tutulmuş (yaklaşık 15 gün); fidelere, ortamların nem durumuna göre seradaki su rampası (boom) sistemi ile yaprak üstünden sadece su verilerek sulama yapılmıştır. 1-2 adet gerçek yapraklarını oluşturan fidelere, saksılarıyla beraber serada hidroponik sistemine aktarılmıştır.

Bitkiler serada "besleyici film tekniği (NFT: Nutrition Film Technique)"nde, kapalı besleme sistemi ile yetiştirilmiştir. NFT sistemi %4 eğimle yerleştirilmiş 350 cm uzunluğunda, 10 cm genişliğinde ve 4 cm yüksekliğindeki 16 adet PVC yetiştirme kanallarından oluşmuştur. Her bir kanal, üzerinde 10 cm aralıklar ile açılmış 32 adet deliğe sahiptir. Her 4 kanal (128 saksı fide⁻¹) bir uygulamayı oluşturmuştur. File saksılardaki fidelere, saksısıyla beraber bu deliklere yerleştirilmiştir. Saksıların kanallara yerleştirilmesinden itibaren besin solüsyonu uygulamasına başlanmıştır. Her 4 kanal/uygulama için NFT kanalları altında 1 besleme tankı yer almış ve her bir uygulama kendi tankından beslenmiştir. Sistem kapalı sistem olduğu için, kanallardan drene olan besin çözeltisi kendi tankına toplanmış ve tank içindeki dalgıç pompa ile (35 W, H-max 1.5 m, saatte

2000 L su basma kapasiteli) kanal başlarına tekrar basılmıştır. Besin solüsyonu NFT kanallarından ince bir film şeridi halinde 24 saat boyunca kesintisiz olarak döndürmüştür.

Besin solüsyonu olarak Alberici et al. (2007)'nin yeşilliklerin hidroponik üretimi için önerdiği, gerekli makro ve mikro besin elementlerini içeren besin reçetesi modifiye edilerek [N:160, P:80, K:180, Ca:140, Mg:34, S:86, Fe:2.2, Mn:0.50, Zn:0.05, B:0.50, Cu:0.02, Mo:0.01 ppm] kullanılmıştır (pH: 5.5-6.5, EC: 1.8-2.0 dS m⁻¹). Besin reçetesi üzerinden hidroponik sisteme uygun suda eriyebilir gübreler ile gübrelerin karışa bilirlilik durumuna göre makro ve mikro elementler için stok solüsyonlar hazırlanmış, bu stok solüsyonlar ile her bir uygulamanın kendisine ait 50 L hacimli besleme tankları, haftada 2 defa eksilen kısmın tamamlanması şeklinde doldurulmuştur. Eksilen kısım yükseklik olarak ölçülüp, hacim hesabı üzerinden litreye çevrilerek ve bu litre su için stok solüsyonlar ilave edilip, elektriksel iletkenlik (EC) ve hidrojen potansiyeli (pH) kontrolü taşınabilir EC ve pH metre ile yapılmıştır. Üretim dönemi sonuna yaklaşıldığında besleme tanklarının yeni besin solüsyonu ile doldurulması yapılmamış; mevcut besin solüsyonunun sıfır atık mantığı ile bitene kadar kullanılması sağlanmıştır.

Deneme konusunu oluşturan vermikompost olarak piyasada ticari olarak bulunabilen Ekosolfarm: %100 organik sıvı solucan gübresi (Ekosol Tarım, Manisa) kullanılmıştır. Katı formda (toz-granül) %100 solucan gübresinin sıvılaştırma sürecinden geçirilerek üretilen, konsantre, yoğun kıvamda ve sıvı formda olan solucan gübresi için garanti edilen içerik şöyle belirtilmiştir: organik madde %35, toplam azot %1.2, organik azot %1, C/N 14, toplam humik+fulvik asit %20, pH:6.5-8.5, EC_{max} 5 dS m⁻¹ (ECOSEL, 2023).

Vermikompost bitkilere (1) kökten: besin solüsyonuna karıştırma, (2) yapraktan: yapraklara püskürtme, (3) kökten ve yapraktan birlikte olacak şekilde uygulanmıştır. Vermikompost uygulaması yapılmayan bitkiler (4) kontrol grubunu oluşturmuştur. Yapraktan püskürtme yapıldığı zaman kontrol bitkilerine sadece su spreyleneceği yapılmıştır. Vermikompost uygulama dozu firma beyanına göre 3 mL Ekosolfarm L⁻¹ olmuştur. Her 4 NFT kanalı (128 saksı) bir deneme konusunu oluşturmuştur.

Araştırma süresi boyunca sera içinde sıcaklık ve nem değerleri HOB0 cihazıyla otomatik olarak kayıt altına alınmıştır. Bitkiler tohum ekiminden 84 gün sonra 23.01.2023 tarihinde hasat olgunluğuna gelmiş her konudan 40 saksı alınarak laboratuara getirilmiştir. Örnek bitkiler saksı üzerinden kesilerek kök ve vejetatif aksam olarak ayrılmışlardır. Şerit metre yardımı ile boğum arası (cm), bitki boyu (cm), dijital kumpas yardımı ile gövde kalınlığı (mm) ölçülmüştür. Bir bitkideki yaprak sayıları (adet) sayılmıştır. Aynı örneklerde yaprak rengi renk ölçerle (Konica Minolta

CR-400 Chroma Meter, Japonya) ölçülmüş L^* , a^* , b^* üzerinden ölçülmüş, a^*/b^* , hue (h°) ve kroma (C) değerleri hesaplanmıştır (Mcguire, 1992). Daha sonra örneklerin vejetatif aksam ve kök yaş ağırlıkları (g bitki $^{-1}$) hassas terazi ile tartılıp 65 °C etüvde kurutulduktan sonra kuru ağırlıkları (g bitki $^{-1}$) alınmış; elde edilen veriler yaş ve kuru ağırlık (g) ve kuru madde (%) olarak verilmiştir. Bitkilerin nitrat içeriği ($mg\ kg^{-1}$) Cataldo et al. (1975)'e göre, vitamin C içeriği ($mg\ 100\ g^{-1}$) Pearson (1970)'e göre spektrofotometrik yöntemle belirlenmiştir. Alınan yaş ağırlık ve saksıdaki bitki sayısı üzerinden verim değeri hesaplanmıştır. Besin solüsyonu örneklerinin EC ve pH ölçümleri EC metre (Mettler Toledo, MC-126) ve pH metre (Mettler Toledo, Seven Easy) yardımı ile ölçülmüştür.

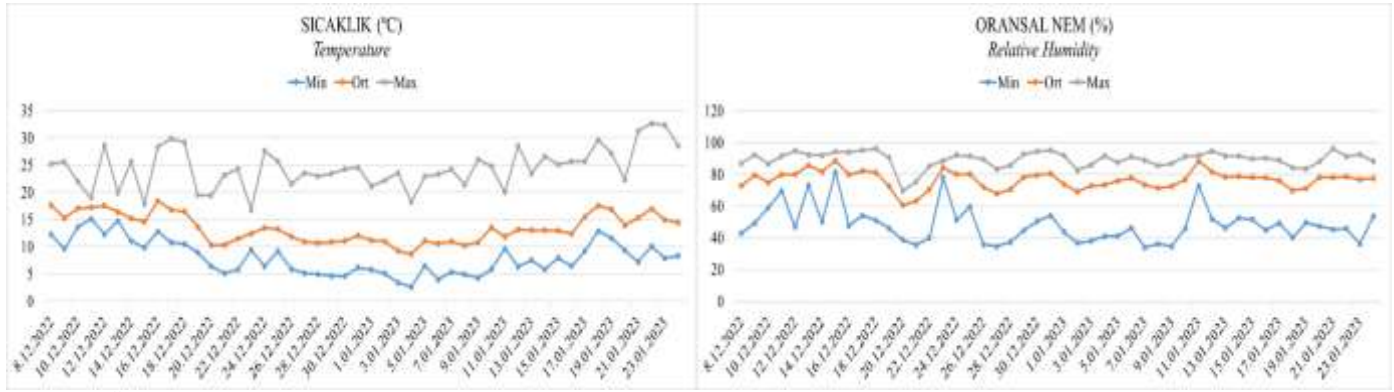
Deneme Tesadüf Parselleri Deneme Deseni'ne göre kurulmuş ve tek faktörlü olarak (uygulama yeri)

değerlendirilmiştir. Elde edilen verilere bilgisayarda JMP (sürüm 5.0.1) istatistiksel analiz paket programı kullanılarak varyans analizi yapılmıştır. Ortalamalar arasındaki farklılıkları belirlemek için %5 önem düzeyinde Tukey testi kullanılmıştır.

BULGULAR

Sera İçi İklim Verileri

Üretim dönemi boyunca sera içinde ölçülen minimum, ortalama ve maksimum sıcaklık ve oransal nem değerlerinin değişimi Şekil 1'de verilmiştir. Maksimum sıcaklıklar 16.8-32.7 °C (ortalama 24.5°C), minimum sıcaklıklar 2.6-15.2 °C (ortalama 7.9°C), ortalama sıcaklıklar 8.7-18.4°C (ortalama 13.4°C) arasında değişmiştir. Oransal nem değerleri minimum %33.8-81.6 (ortalama %48.2), maksimum %69.6-96.2 (ortalama %89.5) ve ortalama %60.5-88.6 (ortalama %76.5) arasında değişmiştir.



Şekil 1. Üretim dönemi boyunca sera içinde ölçülen sıcaklık ve nem değerleri değişimi

Figure 1. Variation of temperature and relative humidity values measured in the greenhouse during the production period

Bitki Gelişimi

Uygulamaların boğum arası uzunluğu, bitki boyu ve gövde çapı üzerine etkileri istatistiksel olarak önemli bulunmuştur (Çizelge 1). Boğum arası 5.50 ± 0.25 cm (yapraktan) ile 3.07 ± 0.08 cm (kontrol), bitki boyu 29.62 ± 0.44 cm (yapraktan) ile 16.24 ± 0.86 cm (kontrol), gövde çapı 3.24 ± 0.08 mm (yapraktan) ile 1.99 ± 0.09 mm (kontrol), yaprak sayısı 10.32 ± 0.17 adet (yapraktan) ile 9.20 ± 0.29 adet (kontrol) arasında değişim göstermiştir. Vermikompost uygulamalarının boğum arasını dolayısıyla bitki boyunu ve gövde çapını arttırdığı gözlemlenmiştir. Bitki yaprak sayısı uygulamalardan etkilenmemiştir. Uygulama yeri olarak yapraktan yapılan vermikompost uygulamasının diğer uygulamalara göre daha iyi sonuç verdiği, kontrole göre boğum arasını %66.4, bitki boyunu %82.4, gövde çapını %62.12 ve yaprak sayısını %12.2 oranında arttırdığı saptanmıştır.

Vermikompost uygulamalarının vejetatif aksam ve kök, yaş ve kuru ağırlığı ile kuru madde içeriği üzerine etkileri önemli bulunmuştur (Tablo 2). Vermikompost uygulaması hidroponik fesleğen yetiştiriciliğinde yeşil

aksam ve kök biyokütlesini (yaş ve kuru ağırlığını) arttırmıştır. Bitkinin yeşil aksam yaş ağırlığı $5.10\pm 0.34 - 1.36\pm 0.19$ g bitki $^{-1}$ arasında, kök yaş ağırlığı $6.56\pm 0.22 - 19.91\pm 0.70$ g bitki $^{-1}$ arasında değişmiştir. En yüksek biyokütle yapraktan vermikompost uygulaması ile elde edilmiştir. Benzer şekilde vejetatif aksam kuru ağırlığı da en fazla yapraktan vermikompost uygulamasından elde edilmiştir. Kök kuru ağırlığında en yüksek değer kök+yaprak uygulamasından elde edilirken, kökten ve yapraktan uygulama ile aynı istatistiksel sınıfta kök kuru ağırlıklarına sahip olmuştur. Yeşil aksam ve kök kuru madde içeriği ise tersi durumda en fazla kontrol uygulamasından elde edilmiştir, vermikompost uygulamaları kuru madde içeriğini azaltmıştır (Çizelge 2).

Verim Değerleri

Bir fide saksıdaki bitkilerin ağırlığı, ortalama bitki ağırlığı ve toplam verim uygulamalardan istatistiksel olarak etkilenmiştir. Vermikompost uygulaması verim değerlerini arttırmakla beraber, en yüksek verim

değerleri yapraktan yapılan uygulamadan elde edilmiştir. Yapraktan uygulamayı aynı istatistiksel grupta yer alan kökten ve kökten+yapraktan uygulaması izlemiştir. Kontrol uygulamasına göre

yapraktan yapılan vermikompost uygulaması saksıda bitki ağırlığını %277.9, ortalama bitki ağırlığını %328.4, toplam verimi %297.3 oranında arttırmıştır (Çizelge 3).

Çizelge 1. Uygulamaların bitki morfolojik gelişim parametrelerine etkileri

Table 1. Effects of treatments on plant morphological development parameters

Uygulamalar (Applications)	Boğum arası (cm) Internode (cm)	Bitki boyu(cm) Plant height (cm)	Gövde çapı (mm) Stem diameter (mm)	Yaprak sayısı (adet) Leaf number (pcs)
Kontrol (Control)	3.07±0.08 ^c	16.24±0.86 ^b	1.99±0.09 ^c	9.20±0.29
Kökten (Root)	5.11±0.27 ^{ab}	27.34±0.63 ^a	2.90±0.06 ^{ab}	9.67±0.30
Yapraktan (Leaf)	5.50±0.25 ^a	29.62±0.44 ^a	3.24±0.08 ^a	10.32±0.17
Kökten+Yapraktan (Root+Leaf)	4.31±0.14 ^b	26.56±0.05 ^a	2.53±0.10 ^b	9.32±0.38
<i>P</i>	<0.0001	<0.0001	<0.0001	0.0819

Çizelge 2. Uygulamaların bitki biyokütlesi üzerine etkileri

Table 2. Effects of treatments on plant biomass

Uygulamalar (Applications)	Vejetatif / Yeşil Aksam (Gövde+Yaprak) Vegetative / Green Parts (Stem+Leaf)			Kök (Root)		
	YA (g bitki ⁻¹) <i>FW</i>	KA (g bitki ⁻¹) <i>DW</i>	KM (%) <i>DM</i>	YA (g bitki ⁻¹) <i>FW</i>	KA (g bitki ⁻¹) <i>DW</i>	KM (%) <i>DM</i>
	(g plant ⁻¹)	(g plant ⁻¹)	(%)	(g plant ⁻¹)	(g plant ⁻¹)	(%)
Kontrol (Control)	1.36±0.19 ^c	0.132±0.02 ^c	9.71±0.58 ^a	6.56±0.22 ^b	1.282±0.04 ^b	19.56±0.08 ^a
Kökten (Root)	3.45±0.20 ^b	0.214±0.01 ^b	6.23±0.14 ^b	13.47±0.49 ^{ab}	1.822±0.17 ^{ab}	13.64±0.36 ^b
Yapraktan (Leaf)	5.10±0.34 ^a	0.364±0.01 ^a	7.19±0.20 ^b	19.91±0.70 ^a	1.793±0.07 ^{ab}	10.79±0.27 ^b
Kökten+Yapraktan (Root+Leaf)	2.77±0.11 ^b	0.211±0.01 ^b	7.61±0.26 ^b	14.71±0.83 ^{ab}	2.157±0.10 ^a	14.92±0.54 ^b
<i>P</i>	<0.0001	<0.0001	<0.0001	0.0209	0.0064	0.0093

YA: yaş ağırlık, KA: kuru ağırlık, KM: kuru madde (FW: fresh weight, DW: dry weight, DM: dry matter)

Çizelge 3. Uygulamaların verim değerlerine etkileri

Table 3. Effects of applications on yield values

Uygulamalar (Applications)	Saksıda Bitki Ağırlığı* (g saksı ⁻¹) Plant Weight in Pots (g pot ⁻¹)	Ort. Bitki Ağırlığı (g bitki ⁻¹) Avg. Plant Weight (g plant ⁻¹)	Toplam Verim (kg m ⁻²) Total Yield (kg m ⁻²)
Kontrol (Control)	12.27±1.40 ^c	1.27±0.12 ^c	1.10±0.15 ^d
Kökten (Root)	30.22±0.85 ^b	3.68±0.27 ^b	3.17±0.04 ^b
Yapraktan (Leaf)	46.38±1.04 ^a	5.44±0.37 ^a	4.37±0.16 ^a
Kökten+Yapraktan (Root+Leaf)	26.43±0.80 ^b	3.05±0.08 ^b	2.62±0.10 ^c
<i>P</i>	<0.0001	<0.0001	<0.0001

*Her bir file saksıdaki ortalama bitki ağırlığı (saksıda bitki sayısı 7-10 bitki saksı⁻¹ arasında değişmiştir)

Kalite Değerleri

Yeşil renk değerini belirleyen a renk değeri hariç, uygulamaların renk parametreleri üzerine etkileri önemli bulunmuştur (Çizelge 4). En parlak renkli yapraklar yüksek L değeri ile kökten yapılan uygulamadan alınmış, yapraktan yapılan uygulamalar fesleğen yapraklarının parlaklığını azaltmıştır. a* renk değeri uygulamalardan etkilenmemiş, yaprak yeşil renk değerleri birbirine yakın bulunmuştur. a* ve hue değerleri birlikte incelendiğinde yaprakların hafif sarımtırak bir yeşil renge sahip olduğu görülmüştür. a*/b* renk oransal

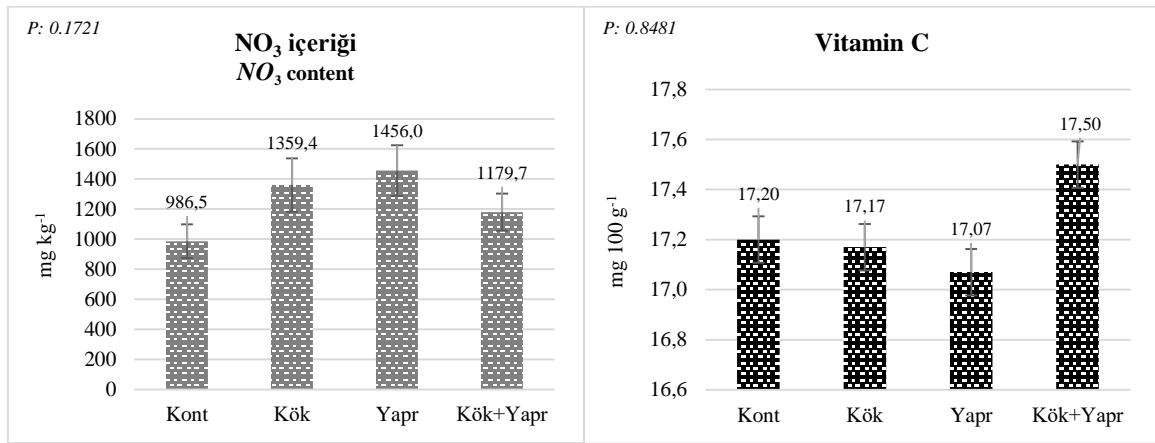
değeri ve kroma değeri kökten yapılan uygulamada yüksek bulunmuş, bu uygulamadaki bitkiler en doygün yeşil renkli yapraklara sahip olmuşlardır.

Vermikompostun farklı uygulama yerlerinin fesleğen yapraklarının nitrat ve vitamin c içeriği üzerine etkisi önemsiz bulunmuştur. Nitrat içeriği 986.5±112.20 (kontrol) ile 1456.0±167.61 (yapraktan) mg kg⁻¹ arasında değişmiş; Vitamin C içeriği ise 17.07±0.35 (yapraktan) ile 17.50±0.34 (kökten+yapraktan) mg 100 g⁻¹ arasında değişim göstermiştir (Şekil 2)

Çizelge 4. Uygulamaların renk değerlerine etkileri
Table 4. Effects of applications on color values

Uygulamalar (Applications)	L	a*	b*	a*/b*	h°	C°
Kontrol (Control)	51.15±0.70 ^b	-17.61±0.19	33.97±1.22 ^b	-0.52±0.01 ^{bc}	117.45±0.59 ^{ab}	38.26±0.37 ^b
Kökten (Root)	57.97±0.43 ^a	-16.98±0.34	38.77±0.96 ^a	-0.44±0.01 ^a	113.69±0.85 ^c	42.34±0.81 ^a
Yapraktan (Leaf)	50.35±0.70 ^b	-18.08±0.22	33.77±0.49 ^b	-0.54±0.01 ^c	118.18±0.60 ^a	38.31±0.35 ^b
Kökten+Yapraktan (Root+Leaf)	53.65±1.07 ^b	-17.54±0.56	37.95±0.54 ^a	-0.46±0.02 ^{ab}	114.82±0.93 ^{bc}	41.82±0.38 ^a
<i>P</i>	<i>0.0009</i>	<i>0.2579</i>	<i>0.0019</i>	<i>0.0037</i>	<i>0.0039</i>	<i>0.0028</i>

L, siyah:0'dan beyaz:100'a olacak şekilde rengin parlaklığını; negatif a* yeşili; pozitif b* sarıyı; hue açısı (h°) rengin temel bileşenlerini (0°: kırmızı, 90°: sarı, 180°: yeşil ve 270°: mavi); kroma (C*) rengin doygunluğunu ve canlılığını belirler.



Kont: Kontrol, Kök: Kökten, Yapr: Yapraktan, Kök+Yapr: Kökten+Yapraktan

Şekil 2. Uygulamaların nitrat ve vitamin C değerlerine etkileri
Figure 2. Effects of treatments on nitrate and vitamin C values

Besin solüsyonu EC ve pH Değerleri

Besin solüsyonu EC değeri tüm konular için 1.30-2.25 dS m⁻¹ arasında değişmiş, ortalama EC değeri 1.94 dS m⁻¹ olmuştur. Konulara ait besleme tanklarında yapılan 5 günlük ölçümlerde besin solüsyonu EC değeri kontrol uygulamasında 1.46-2.25 dS m⁻¹, kökten yapılan uygulamada 1.30-2.25 dS m⁻¹, yapraktan yapılan uygulamada 1.49-2.15 dS m⁻¹, kökten+yapraktan yapılan uygulamada 1.49-2.24 dS m⁻¹ arasında değişmiş, ortalama değerler sırası ile 1.96, 1.94, 1.90 ve 1.95 dS m⁻¹ olmuştur (Şekil 3). Besin solüsyonu pH değeri ise 6.12-7.50 arasında değilmiş ve ortalama 6.74 olmuştur. Besleme tanklarının pH değerleri ise kontrol uygulamasında 6.16-7.18, kökten yapılan uygulamada 6.47-7.50, yapraktan yapılan uygulamada 6.12-7.07, kökten+yapraktan yapılan uygulamada 6.37-7.36 arasında değişmiş, ortalama değerler sırası ile 6.57, 6.90, 6.60 ve 6.90 olmuştur (Şekil 3).

TARTIŞMA ve SONUÇ

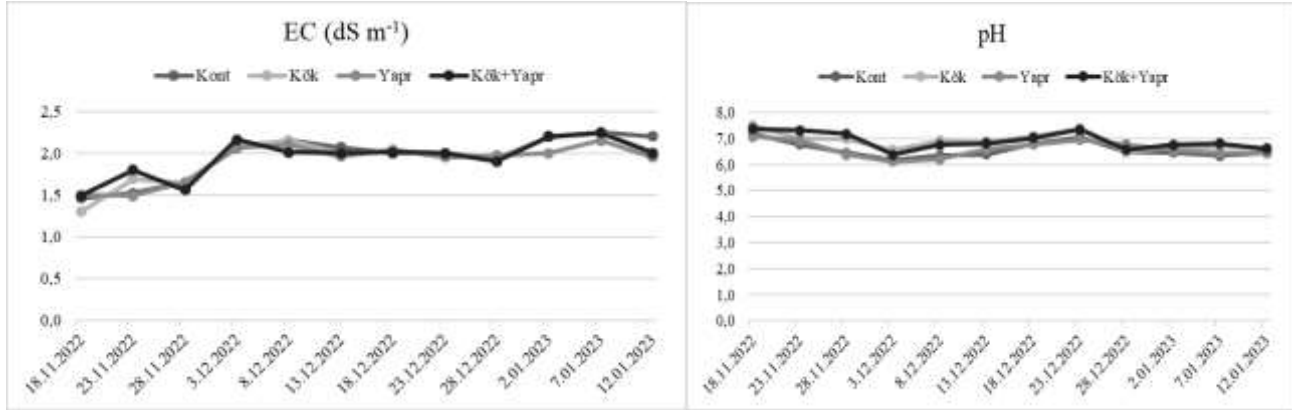
Hidroponik fesleğen yetiştiriciliğinde farklı uygulama yerlerinden verilen sıvı vermikompostun bitki gelişimi, verim ve kaliteye olan etkilerinin araştırıldığı bu çalışmada, vermikompost uygulamasının özellikle

bitkinin morfolojik gelişimi ve verimini arttırdığı saptanmıştır. Elde edilen bu sonuç, tarla bitkilerinde (Sahni et al., 2008), tahıllarda (Mısırlıoğlu, 2011), baklagillerde (Jeyabal & Kuppaswamy, 2001) ve değişik sebze türlerinde (Atiyeh et al., 2000; Arancon et al., 2006; Gutiérrez-Miceli et al., 2007; Singh et al., 2008; Warman & Anglopez, 2010) yapılan çalışmalar ile de desteklenmiştir. Bununla birlikte vermikompostun fide yetiştirme ortamına eklenerek kullanıldığı çalışmalarda fide kalitesini iyileştirdiği de belirlenmiştir (Zaller, 2007a; Matta et al., 2008; Zhang et al., 2009; Ivenish, 2011).

Araştırmadan elde edilen sonuçlar kökten besleme şeklinde verilen vermikompost uygulamasının da kontrole göre iyi sonuçlar ortaya koyduğunu göstermiştir. Ancak en yüksek sonuçlar yapraktan yapılan uygulamalardan elde edilmiştir. Vermikompost, içinde bulunan fenolik ve hümitik maddeler varlığı ile de bitki gelişimini etkilemektedir. Bitkilerin etkilenme derecesi kullanılan vermikompost miktarı, kaynağı ve bitki tür ve çeşidine bağlı olarak değişmektedir (Ivenish, 2011; Zaller, 2007b). Vermikompostun uygulandığı ortama bitki gelişimi düzenleyici maddeler sağlayarak da bitki gelişimine etkisi olduğu yönünde çalışmalar da

mevcuttur (Atiyeh et al., 2001). Bu maddelerin yapraktan alınım kolaylığı nedeni ile etkileri daha çabuk görülmüş ve yapraktan yapılan uygulamalarda

bitki gelişimi ve buna bağlı olarak verim değerleri yüksek çıkmıştır.



Kont: Kontrol, Kök: Kökten, Yapr: Yapraktan, Kök+Yapr: Kökten+Yapraktan

Şekil 3. Besin solüsyonu EC ve pH değerlerinin üretim dönemi içindeki 5 günlük değişimi

Figure 3. 5-day variation of nutrient solution EC and pH values during the production period

Denemede m²'deki verim kontrol uygulamasında 1.10 kg m⁻² olmuş, vermikompost uygulamalarında 2.62-4.37 kg m⁻² arasında değişmiştir. Topraksız tarımda yapılan önceki çalışmalarda ise verim değerlerinin 3.14 kg m⁻² (Miceli et al., 2003) ile 14.41 kg m⁻² (Ferrarezi & Bailey, 2019) arasında değiştiği belirtilmiştir. Öztekin ve Sayın (2023) NFT sisteminde yetiştirdikleri fesleğenlerin toplam verimini uygulamalara göre değişmekle birlikte 3.03 ile 5.25 kg m⁻² olarak belirlemişlerdir. Bu bağlamda kontrol uygulamasında verim değerlerinin düşük kaldığı, ancak vermikompost uygulamalarında verim değerlerinin önceki çalışmalardan elde edilen verilerle uyumlu olduğu görülmüştür. Burada verim değerlerinin kullanılan çeşide, yetiştirme şekline, yetiştirme dönemindeki sıcaklıklara, birim alandaki (bitki m⁻²) bitki sayısına, hasat edilen bitki uzunluğuna, üretim sürecinde kaç defa hasat yapıldığına ve yetiştirilen coğrafyaya göre değişebileceği unutulmamalıdır (Öztekin & Sayın, 2023). Bu kriterler içerisinde "hasat edilen bitki boyu" verimi önemli ölçüde etkilemektedir. Ticari fesleğen yetiştiren firmalar yurtdışına yaptığı taze fesleğen ticaretinde kullanılan nakliye paketlerine bitkilerin sığması için bitki boyunu ortalama 25 cm olarak tercih etmektedirler. Yürütülen denemede de fide boyları 25 cm civarına (ortalama 24.9 cm) ulaştığında kök bölgesinden 2-4 cm yukarıdan hasat yapılmıştır. Vermikompost uygulaması bitki boyunu uzatmıştır ve gübre uygulamalarında boy 26.5±0.05 ile 29.6±0.44 arasında değişmiştir. Ancak topraklı tarımda yapılan önceki çalışmalarda bitki boyu daha uzun (31.4-61.0 cm) olabilmıştır (Lachowicz et al., 1997; Kaçar ve ark., 2009).

Topraklı tarımda taze/yeşil herba verimi (kg da⁻¹) olarak ifade edilen fesleğen verimini Vömel ve Ceylan (1977) bitkileri 28-33 cm'den hasat ettiklerinde 315-

672 kg da⁻¹ olarak; Verma et al. (1989) 3679 kg da⁻¹; Cabar (2016) ise 2318.1-2874.3 kg da⁻¹ arasında bulmuşlardır. Bu açıdan bakıldığında elde edilen verim değerleri topraklı fesleğen yetiştiriciliğinden ise oldukça yüksek bulunmuştur. Öztekin ve Sayın (2023) bu sonucu topraklı yetiştiricilikte sıra arası ve üzeri mesafelerin, dolayısıyla birim alandaki bitki yoğunluğunun ve bir dikim noktasında bulunan bitki sayısının (çalışmada 10 bitki saksı⁻¹) düşük olmasına bağlamışlardır.

Fesleğenin topraklı tarımda yetiştirme süresini Aslan (2014) 81-99 gün olarak belirtmişlerdir. Araştırmada bitkiler NFT sistemine aktarıldıktan 68 gün sonra hasat edilmiştir ve fesleğenin klasik yetiştiriciliğine göre önemli bir erkencilik elde edilmiştir (Öztekin & Sayın, 2023). Yetiştirildiği dönem baz alındığında da sera içi sıcaklıkların (ortalama max.: 24.5°C, min.: 7.9 °C, ort.: 13.4 °C) fesleğenin optimum yetiştirme sıcaklığından (25-30°C) (Barickman et al., 2021) düşük olduğu, bitkilerin sorunsuz yetiştirildiği ancak iklimsel faktörlere bağlı olarak yavaş gelişim gösterdiği, yine de topraklı tarım etkisi ile topraklı tarıma göre erkencilik sağladığı görülmüştür.

Yaprakları yenilen sebzelerde önemli bir albeni kriteri olan yaprak rengi, yürütülen çalışmada uygulamalardan etkilenmiştir. Moncada et al. (2021) topraklı tarımda fesleğen yapraklarının renk değerlerini L*: 46.6, a*: -20.7, b*: 38.8; h°: 120.1 ve C°: 42.2 olarak; Alibas ve ark. (2021) ise taze fesleğenlerde L*, a*, b*, h° ve C° değerlerini sırası ile 43.55, -7.27, 18.19, 111.79 ve 19.60 olarak; Öztekin ve Sayın (2023) ise L* değeri 44.21 ile 46.42; a* değeri -16.62 ile -17.33, b* değeri 24.39 ile 25.97 arasında; h° değeri 123.59 ile 124.70 arasında; C° değeri ise 29.60 ile 31.18 arasında ölçmüşlerdir. Yürütülen çalışmadan elde edilen renk değerlerinin (Çizelge 4) önceki çalışmalarda belirtilen ölçümler ile uyumlu olduğu görülmüştür.

Vermikompost uygulamalarında kökten uygulamaların gözle görülür bir fark yaratmadan yaprak renklerinde kısmen açıklık yarattığı görülmüştür.

Yaprakları tüketilen sebzelerin içsel kalite özelliklerinde vitamin C ve nitrat miktarı önemli bir kalite kriteridir. Bilindiği gibi yeşillikler iyi bir vitamin C kaynağıdır. Nitekim, taze fesleğen yapraklarında vitamin C içeriği 18 mg 100 g⁻¹ olarak belirtilmiştir (USDA, 2023). Topraksız tarımda yürütülen önceki çalışmalarda fesleğen yapraklarının vitamin C içeriğini Aldiyab (2020) 47.39-64.14 mg 100 g⁻¹ arasında, Daşgan ve ark. (2022) 40.7 ile 55.0 mg 100 g⁻¹ arasında, Öztekin ve Sayın (2023) 20.51 ile 24.43 mg 100 g⁻¹ arasında bulmuştur. Yürütülen bu çalışmada fesleğen bitkilerinde vitamin C içeriği 17.07±0.35 - 17.50±0.34 mg 100 g⁻¹ arasında değişmiş ve daha önceki topraksız tarımdan elde edilen sonuçlardan düşük olduğu görülmüştür. Bu durumun bitkilerin vitamin C içeriğinin üretim zamanı ve yetiştirme koşullarına göre değişebilmesinden kaynaklandığı düşünülmektedir (Eşiyok ve ark., 2006). Vermikompost uygulaması fesleğen yapraklarının vitamin C içeriğini etkilememiştir.

Beslenmek için tüketilen yapraklarla alınan nitrat, midede nitrite dönüşerek toksik etki yaratmakta, bu durum insan sağlığını tehdit ederek kansere kadar gidebilmektedir. Bu nedenle yaprakların yüksek nitrat içeriği alınan nitrat miktarını arttırdığı için tehlike yaratmaktadır (Kara, 1993; Özdekan & Üren, 2010). Avrupa Birliği Komisyon Tüzüğü taze yeşillikler için maksimum nitrat sınır değerlerini 3500 mg kg⁻¹ olarak belirlemiştir (EUR-LEX, 2017). Hidroponik sistemde yetiştirilen fesleğenlerde ise nitrat miktarını Rouphael et al. (2017) 2584-4102 mg kg⁻¹, Orsini ve Pascale (2006) en düşük 1650 mg kg⁻¹, Aldiyab (2020) 2645-4524 mg kg⁻¹, Moncada et al. (2021) 55 ile 558 mg kg⁻¹, Öztekin ve Sayın (2023) 172.11 ile 322.62 mg kg⁻¹ arasında bulmuştur ve değişiklikler yetiştirme koşulları, yetiştirme zamanı ve özellikle ışık şiddeti farkından kaynaklanmıştır. Yürütülen çalışmada elde edilen nitrat miktarı (986.5±112.20 - 1456.0±167.61 mg kg⁻¹) insan sağlığını tehdit edecek nitrat seviyesinin çok altında ve önceki çalışmalardan elde edilen sınır değerler içinde çıkmıştır. Vermikompost uygulaması fesleğen yapraklarının nitrat içeriğini etkilememiştir (Paul & Metzger, 2005). Ancak kontrole göre vermikompost uygulamaları ile nitrat içeriği istatistiksel anlamda önemsiz olsa da ortalama %35 artış göstermiştir.

Topraksız tarımda özellikle kapalı sistemde yetiştiricilik için besin solüsyonu EC ve pH değerleri oldukça önemlidir. Yeşillikler için EC'nin 1.8-2.0 dS m⁻¹ arasında, pH'nın 5.5-6.5 arasında olması istenir. Böylece bitkilerin besin elementlerinden maksimum düzeyde faydalanmaları sağlanır. Yürütülen bu çalışmada uygulamalara göre değişmekle beraber

ortalama EC değerleri 1.94 dS m⁻¹, pH değerleri 6.74 olmuştur ve önerilen değerlere uyumlu/yakın bulunmuştur. Uygulamalar arasında besin solüsyonu EC ve pH değeri açısından önemli bir farklılık görülmemiştir. Beklenenin aksine, sıvı vermikompostun (EC_{ort} 3.85 dS m⁻¹) besin solüsyonuna katılarak uygulanması (kökten yapılan uygulamalar) solüsyonun EC ve pH değerlerinde önemli bir değişiklik yaratmamıştır. Bu durumun uygulama dozunun (1/300 oranında seyreltme) ve ortam sıcaklığının yüksek olmamasından kaynaklandığı düşünülmektedir. Ayrıca üretici firma, ticari preparat içinde bulunan zengin faydalı mikroorganizma varlığının, su ile birleştiğinde aktif hale geldiğini ve EC ve pH değerlerini düşürebildiklerini beyan etmiştir (Ekosel, sözlü görüşme). Araştırma süresince vermikompost uygulaması ile bitkilerde herhangi bir tuz zararı görülmemiştir. Yürütülen araştırmada elde edilen besin solüsyonu EC_{max} değeri 2.25 dS m⁻¹ olarak belirlenmiştir. Her ne kadar fesleğen tuza hassas bitki olarak kategorize edilse de yetiştirme koşulu ve çeşide göre değişmekle birlikte 3.75 dS m⁻¹'ye kadar sulama suyu elektriksel iletkenliğine toleranslı olabildiği de literatürde belirtilmiştir (de Sousa et al., 2021).

Elde edilen tüm veriler birlikte değerlendirildiğinde, vermikompost uygulamasının bitki gelişimi ve verimini arttırdığı; uygulama yeri olarak yapraktan yapılan püskürtme şeklinde uygulamanın diğer uygulamalara göre daha iyi sonuç verdiği görülmüştür. Bu nedenle hidroponik fesleğen yetiştiriciliğinde kaliteden ödün vermeden mevcut verimi arttırmak ve erkencilik sağlamak adına sıvı vermikompostun yapraktan uygulaması önerilmektedir.

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.

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Detection of the Presence of Powdery Mildew Resistance -Associated Genes (*Ren1*, *Ren3*, and *Ren9*) in *Vitis labrusca* L. Genotypes

Adem YAĞCI^{1*}, Selda DALER², Abdurrahim BOZKURT³, Davut Soner AKGÜL⁴

¹Department of Horticulture, Faculty of Agriculture, University of Tokat Gaziosmanpaşa, Tokat, Türkiye, ²Department of Horticulture, Faculty of Agriculture, University of Bozok, Yozgat, Türkiye, ³Erzincan Horticultural Research Institute, Erzincan, Türkiye, ⁴Plant Protection Department, Agriculture Faculty, Çukurova University, Adana, Türkiye

¹ <https://orcid.org/0000-0002-3650-4679>, ² <https://orcid.org/0000-0003-0422-1444>, ³ <https://orcid.org/0000-0001-7315-202X>,

⁴ <https://orcid.org/0000-0002-9990-4194>,

✉: adem.yagci@gop.edu.tr

ABSTRACT

Powdery mildew disease (*Erysiphe necator* Schwein) is a significant threat to grape cultivation in vineyards. Severe yield and quality losses could occur in vineyards when this pathogen is not managed correctly. Several commercial grape varieties are highly susceptible to powdery mildew. Therefore, large quantities of fungicides are applied throughout the growing season. In addition to yields and quality, new grapevine varieties that are genetically resistant to powdery mildew are required for sustainable viticulture. This study was conducted through molecular screening of powdery mildew resistance genes in nine different *Vitis labrusca* L. genotypes (TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8, and TEG-VI-9) grown in the Black Sea Region of Türkiye. After PCR amplifications using *Ren1*, *Ren3* and *Ren9* locus-specific primers, *Ren1*, and *Ren 9* genes were detected in three genotypes (TEG-VI-1, TEG-VI-3, and TEG-VI-4). However, the *Ren3* gene was not detected in any genotypes. It was concluded based on present findings that *Vitis labrusca* L. genotypes with resistance genes could be used as genetic resources in grapevine breeding programs and significant economic benefits can be provided accordingly.

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Vitis labrusca L. Genotiplerinde Küllemeye Dirençle İlişkili Genlerin (*Ren1*, *Ren3* ve *Ren9*) Varlığının Tespiti

ÖZET

Bağlarda görülen külleme hastalığı (*Erysiphe necator* Schwein) üzüm yetiştiriciliği için büyük bir tehdittir. Bu patojene karşı mücadele edilmediğinde, üzüm verim ve kalitesinde önemli düşüşler meydana gelmektedir. Çoğu ticari üzüm çeşidi, küllemeye karşı oldukça hassastır. Bu nedenle yetiştirme dönemi boyunca fazla miktarlarda fungusit uygulanmaktadır. Sürdürülebilir bir bağcılık için verim ve kalitenin yanında, külleme hastalığına karşı genetik olarak dirençli yeni asma çeşitlerine ihtiyaç duyulmaktadır. Bu çalışmada, Türkiye'nin Karadeniz Bölgesi'nde yetiştiriciliği yapılan dokuz farklı *Vitis labrusca* L. genotipinde (TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8 ve TEG-VI-9) külleme hastalığına dirençli genlerin moleküler taraması yapılmıştır. *Ren1*, *Ren3* ve *Ren9* lokuslarına özgü primerler kullanılarak gerçekleştirilen PCR amplifikasyonu sonrasında üç genotipte (TEG-VI-1, TEG-VI-3 ve TEG-VI-4) *Ren1* ve *Ren9* genlerinin bulunduğu tespit edilmiştir. Ancak *Ren3* geni hiçbir örnekte saptanamamıştır. Araştırma sonuçlarında direnç genlerine sahip oldukları belirlenen *Vitis labrusca* L. genotiplerinin, gelecekte dirençli asma çeşitlerinin ıslahında genetik kaynaklar olarak kullanılacakları ve bu sayede önemli ekonomik faydalar sağlanabileceği düşünülmektedir.

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INTRODUCTION

Cultivation is a process in which humans select and genetically modify organisms for their desired traits (Jiao et al., 2021). This process often leads to a reduction in genetic diversity in the breeding population and the loss of parental genes (Doebley et al., 2006). The occurrence of grapevine species dates to 28 million years ago, and today, there are more than 60 species belonging to the genus *Vitis* (Wan et al., 2013). Of these, *V. vinifera*, native to the Mediterranean and Central Europe, is known to be the most widely cultivated vine species and was cultivated ~8,000 years ago during the Neolithic era (This et al., 2006; Jaillon et al., 2007; Zhou et al., 2017). However, *V. vinifera* is highly susceptible to powdery mildew caused by '*Erysiphe necator* Schw.' (Gadoury et al., 2003; Jiao et al., 2021). *E. necator* infects all green tissues of vines. It is easily recognized through white-grayish powdery mildew symptoms on the surface of shoots, stems, leaves, buds, cluster skeletons, flowers, peduncles, and young berries (Bendek et al., 2002; Calonnec et al., 2004). Moreover, powdery mildew negatively affects cluster weight, ripening, photosynthetic activity, transpiration (Sosa-Zuniga et al., 2022), the sugar/acid ratio, and anthocyanin levels (Calonnec et al., 2004).

Although *V. vinifera* is the most widely cultivated *Vitis* species, the resistance of this species to powdery mildew is lower than that of *Muscadinia* species with germplasms of wild *Vitis* spp. from North America or Central Asia (Riaz et al., 2013; Pap et al., 2016; Mermer Doğu et al., 2022; Sosa-Zuniga et al., 2022). As described in previous studies, the natural powdery mildew resistance of North American and Central Asian genotypes is largely related to the evolutionary history of these genotypes (Hoffmann et al., 2008; Dry et al., 2010; Blanc et al., 2012; Pap et al., 2016). These resistant genotypes can be included in grapevine breeding programs and become valuable germplasm sources (Atak, 2023). In recent years, especially in table grape breeding, this system has increased in demand and is spreading worldwide (Montaigne et al. 2021).

The *Vitaceae* family has two primary gene families responsible for powdery mildew resistance: *Run* (resistance to *Uncinula necator*) and *Ren* (resistance to *Erysiphe necator*) (Sosa-Zuniga et al., 2022). To date, 15 loci belonging to the *Run* and *Ren* gene families associated with the defence response of grapevines against powdery mildew have been described (Maul, 2023). Several of these *Ren* loci are used in different breeding programs to strengthen the defence response of plants and increase plant resistance (Li et al., 2013; Feechan et al., 2015; Agurto et al., 2017). *Ren1* loci have been detected in some genotypes of *V. vinifera*

from Central Asia (e.g., 'Kishmish Vatkana' and 'Dzhandzhal Kara' varieties from Uzbekistan). The *Ren3* locus was discovered in the 'Regent' variety, which has resistant parents such as *V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. lincecumii* and *V. rupestris* (Eibach & Töpfer, 2003; Welter et al., 2007). Zendler et al. (2017) characterized the *Ren9* locus as a second resistance-coding region during a detailed genetic mapping study of *Ren3* on chromosome 15. However, loci on the same chromosome in different regions can be separated into different genotypes.

Türkiye has reasonably available ecological conditions for various plant species and thus has an important position in world agriculture. It also has genetic potential in terms of plant genetic resources (Ergül & Ağaoglu 2001; Ergül et al., 2011, Dilli et al., 2014). The humid climate of the Black Sea Region of Türkiye, which has an average annual rainfall of more than 1000 mm, limits the viticulture of *Vitis vinifera* L. Therefore, *Vitis labrusca* species or hybrids resistant to fungal diseases can grow in this region (Cangi et al., 2006; Çelik et al., 2008; Tahmaz et al., 2022). These species can be grown without pesticides in the Black Sea Region; thus, the must obtained from these grapes contains resveratrol and antioxidants, which are extremely important for human health and nutrition (Üneş, 2016; Atak & Şen, 2021; Tahmaz et al., 2022). Although it is not known exactly how and when the *Vitis labrusca* L. species, originating from North America, arrived in the region, it was reported that there were several varieties and genotypes of this species in the region, and most of them were resistant to fungal diseases (Cangi et al., 2006; Çelik et al., 2008; Atak, 2017; Tahmaz et al., 2022).

Various studies have revealed that *V. labrusca* genotypes generally exhibit greater mildew resistance than *V. vinifera* varieties. However, the level of resistance may vary from variety to variety (Atak et al., 2017; Sargolzaei et al., 2021). Yıldırım et al. (2019) reported that a genotype derived from hybridization between *V. labrusca* and *V. vinifera* possessed the *Rpv3* gene, which is associated with resistance to mildew. Among these genotypes, '57 Gerze 04' (*V. labrusca* × *V. vinifera*) and 'Mortensen' (*V. labrusca* × *V. vinifera*) demonstrated resistance to mildew, whereas the 'Köfteci Üzüümü' (*V. labrusca* × *V. vinifera*) genotype exhibited a greater level of resistance. Furthermore, this study revealed that resistance to grapevine anthracnose in *V. labrusca* is governed by three independent genes. While *An1* and *An2* are the dominant susceptibility genes, *An3* is the only dominant resistance gene (Mortensen, 1981; Gao et al., 2012).

However, in several studies on the pathogen resistance genes of different *Vitis* spp. Species, there are almost

no studies on *Vitis labrusca* L. Therefore, there is a need for research to elucidate the molecular basis of powdery mildew resistance in genotypes of *V. labrusca* L. species. In this study, molecular analyses were conducted to detect the presence of the *ren1*, *ren3*, and *ren9* genes in nine different *Vitis labrusca* L. genotypes grown in the Black Sea Region of Türkiye.

MATERIAL and METHOD

Plant Material

Grapevine genotypes were collected from the Erbaa district of Tokat Province (Türkiye) (Figure 1, Figure 2, Figure 3 and Figure 4). The grapevine genotypes used in this study and their collection sites are

delineated in Table 1. During the winter dormancy period, 20 cuttings were obtained from each of nine different *Vitis labrusca* genotypes. These genotypes were then cultivated in a greenhouse at the Faculty of Agriculture, Tokat Gaziosmanpaşa University, for further study. For molecular analysis, bud samples were collected from all cuttings of the nine genotypes. Three shoot tips and young leaf samples were selected from each genotype to serve as plant material for the study.

Shoot tips taken from plant materials were delivered to the Sivas Cumhuriyet University Advanced Technology Research and Application Center in cooler boxes, frozen at -20°C and stored until analysis.



Figure 1. Naturally-grown *V. labrusca* genotypes
Şekil 1. Kendiliğinden yetişen *V. labrusca* türüne ait genotipler



Figure 2. Genotype marking process
Şekil 2. Genotiplerde işaretleme işlemleri



Figure 3. Shoot sampling from marked genotypes after defoliation.
Şekil 3. Yaprak dökümü sonrası işaretli genotiplerden sürgün örneklerinin alınması

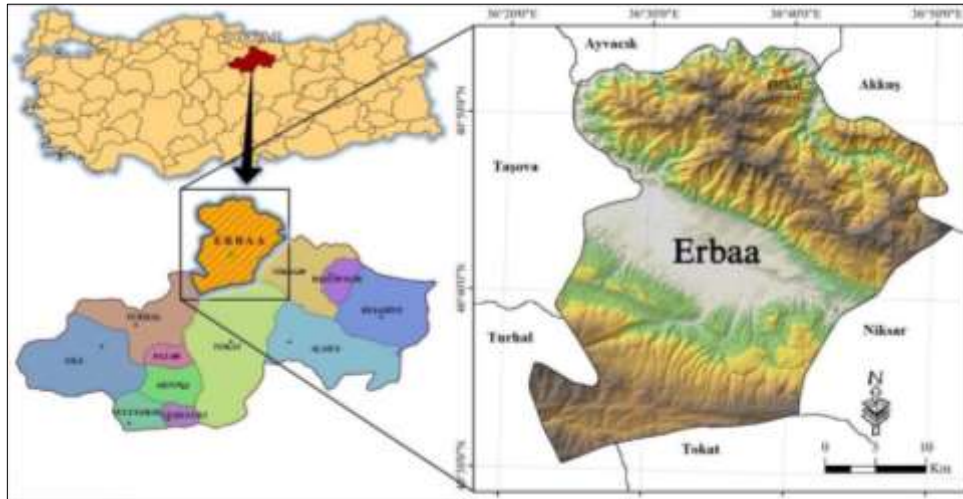


Figure 4. The region from which grapevine genotypes were collected
Şekil 4. Çalışmada kullanılan asma genotiplerinin toplandığı bölge

DNA Isolation

Genomic DNA isolation was performed according to Doyle and Doyle (1990) with slight modifications as outlined below:

1. Tissues stored at -80°C were crushed with a pestle and transferred to Eppendorf tubes

(advantageously accelerating this step will provide an advantage in terms of DNA quantity). In cases of low tissue volume, tissue can be crushed inside the Eppendorf tube to prevent DNA loss).

2. Each sample was homogenized by adding 700 µl of preheated 2% CTAB buffer (100 mM Tris-HCl,

- 25 mM EDTA, pH 8.0, and 2.5 M NaCl).
- The tubes were incubated in a water bath at 65°C for 60 minutes with occasional inversion.
 - After incubation, the samples were centrifuged at 7378 ×g for 10 minutes at 4°C, and the supernatant was carefully transferred to a new tube.
 - An equal volume of chloroform:isoamyl alcohol (24:1) was added to the samples and mixed by inversion.
 - The samples were centrifuged at 7378 ×g for 10 minutes at 4°C, and the supernatant was transferred to a new tube.
 - To precipitate the DNA, an equal volume of isopropanol (-20°C) was added to the samples and incubated at -20°C for 30 minutes.
 - The precipitated DNA was centrifuged at 14462 ×g for 10 minutes at 4°C, after which the supernatant was removed.
 - For washing, the pellets in the tubes were centrifuged at 14462 ×g for 5 minutes at 4°C after adding 500 µl of 70% ethanol (-20°C).
 - The resulting pellets were air-dried at room temperature and dissolved in 30/60 µl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).
 - RNase A was added to each sample (1/100 µl DNA sample) and incubated at 37°C for 1 hour.

DNA quality and quantity were evaluated by electrophoresis in a 1% (w/v) agarose gel and using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE), respectively. The isolated DNA was stored at -80°C until PCR was performed.

Table 1. Grapevine genotypes and sampling locations

Çizelge 1. Çalışmada kullanılan asma genotipleri ve toplandıkları lokasyonlar

No	Genotype	Location
1	TEG-VI-1	40°86'10"N 36°65'38"E
2	TEG-VI-2	40°86'17"N 36°68'73"E
3	TEG-VI-3	40°84'49"N 36°66'77"E
4	TEG-VI-4	40°83'03"N 36°66'40"E
5	TEG-VI-5	40°82'93"N 36°66'53"E
6	TEG-VI-6	40°83'94"N 36°67'41"E
7	TEG-VI-7	40°84'09"N 36°67'50"E
8	TEG-VI-8	40°84'50"N 36°66'56"E
9	TEG-VI-9	40°32'40"N 36°44'97"E

Polymerase Chain Reaction (PCR)

To determine whether the 9 DNA samples used in the study were resistant to powdery mildew, three resistance gene regions, namely, *ren1*, *ren3*, and *ren9* were selected. The sequences of the primers used for these gene regions were obtained from previous studies (Akkurt et al., 2007; van Heerden et al., 2014; Pozharskiy et al., 2020). Information on the primers used for resistance-related marker amplification in *Vitis labrusca* L. genotypes is presented in Table 2.

Table 2. Markers used to describe alleles corresponding to loci associated with resistance to powdery mildew in *Vitis labrusca*

Tablo 2. *Vitis labrusca*'da külemeye dirençle ilişkili lokuslara karşılık gelen alelleri tanımlamak için kullanılan markerler

Locus	Marker	Forward / Reverse primer	Mildew	Fragment Length (bp)	Annealing Temperature (T _A)	Reference
<i>ren1</i>	GF13-13R	GTGCATCTTCTTCCCAACC/ GCATTTGTCAAAGTCGTGTACTTC	+	214	60	Pozharskiy et al., 2020
<i>ren3</i>	ScORA7-760R	GAAACGGGTGTGAGGCAAAGGTGG/ GGCCATTAGGAAATCAACATTAC	+	760	60	Akkurt et al., 2007
<i>ren9</i>	CenGen6R	TGAATTTTGTCTTTAGGATTTGGA/ CACAAGAACAATTTCTACGCACA	+	287	55	van Heerden et al., 2014

Table 3. PCR amplification conditions for markers associated with powdery mildew resistance in *Vitis labrusca*

Çizelge 3. *Vitis labrusca*'da külemeye dirençle ilişkili markerler için PCR amplifikasyon koşulları

Initial denaturation	5 min at 94°C	
Denaturation	30 s at 94°C	
Annealing	30 s at 55-60°C	30 cycles
Extension	2 min at 72 °C	
Final Extension	10 min at 72°C	

Powdery mildew-related genes and PCR conditions were evaluated with the primers given in Table 3.

All PCRs were prepared in a final volume of 25 µl. The 25 µl reaction volume contained: 0.125 U of Taq DNA polymerase (Fermentas), 2.5 µl of reaction buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, and 0.8% Nonidet P-40), 1 µl each of 10 pmol primer, 2.5 µl of 2.5 mM dNTPs (MBI Fermentas), 2.5 µl of 25 mM MgCl₂ and 1 µl of 100-500 ng of template DNA, which was added to a final volume of 25 µl with dH₂O. PCRs were performed in a Blue-Ray Biotech thermocycler under the following conditions.

The amplification products were subjected to 1.5% agarose gel electrophoresis containing ethidium bromide (2 µg/ml) and imaged under a UV transilluminator.

RESULT and DISCUSSION

PCR amplification results for markers associated with the *ren1*, *ren3*, and *ren9* loci in DNA samples

To determine the genotypes associated with powdery mildew resistance, a comprehensive analysis was conducted on nine samples utilizing polymerase chain reaction (PCR) to detect the presence of the *Ren1*, *Ren3*, and *Ren9* loci. Subsequently, the genotypes were classified based on their resistance or sensitivity to powdery mildew, which was determined through visualization of the amplified products via gel electrophoresis.

Analysis via molecular markers identified specific alleles correlated with resistance to powdery mildew within *V. labrusca* genotypes, as detailed in Table 4. Figure 5 shows the results of PCR amplification employing primers tailored for the *Ren1*, *Ren3*, and *Ren9* loci. For the *Ren1* gene, 214 bp PCR products encompassing TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8, and TEG-VI-9 were discerned in all examined samples,

constituting a 100% detection rate, as depicted in Figure 6. Conversely, for the *Ren9* gene, a 287 bp PCR product including TEG-VI-1, TEG-VI-3, and TEG-VI-4 was identified in three samples, representing a 33.33% occurrence rate, as highlighted in Figure 7. Notably, the *Ren3* gene was absent in all the samples analyzed.

This investigation corroborates and extends upon the established body of knowledge, highlighting the significant genetic diversity within the *Vitis* genus, especially concerning powdery mildew resistance. The majority of commercial grape cultivars exhibit vulnerability to this pathogen, yet resistance often occurs within wild populations, suggesting substantial potential for breeding. Previous studies have identified *V. labrusca* for its distinctive resistance properties against fungal diseases (Cangi et al., 2006; Çelik et al., 2008; Tahmaz et al., 2022), although the precise molecular mechanisms conferring this resistance remain largely unexplored. In this context, this research aimed to identify the presence of the *Ren1*, *Ren3*, and *Ren9* genes within nine *Vitis labrusca* L. genotypes from the Black Sea region of Türkiye.

Table 4. Distribution of the *ren1*, *ren3* and *ren9* genes in the investigated genotypes

Çizelge 4. İncelenen genotiplerde *ren1*, *ren3* ve *ren9* genlerinin dağılımı

Genotype	Loci		
	<i>ren1</i>	<i>ren3</i>	<i>ren9</i>
TEG-VI-1	+	-	+
TEG-VI-2	+	-	-
TEG-VI-3	+	-	+
TEG-VI-4	+	-	+
TEG-VI-5	+	-	-
TEG-VI-6	+	-	-
TEG-VI-7	+	-	-
TEG-VI-8	+	-	-
TEG-VI-9	+	-	-

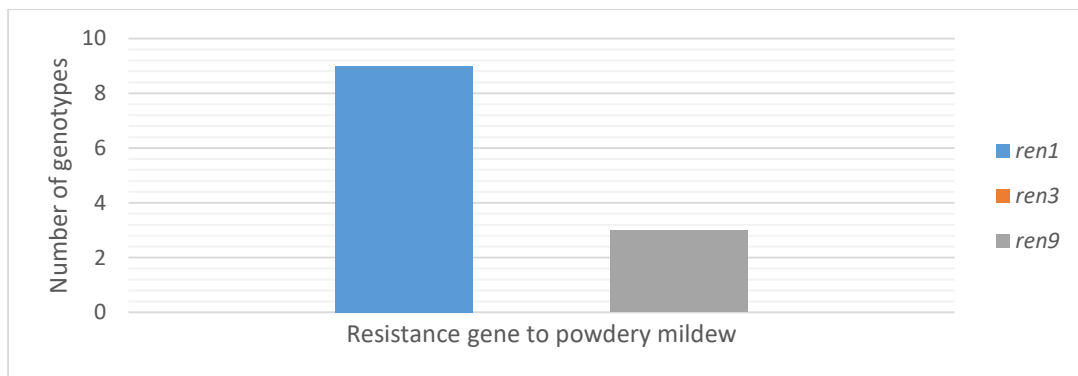


Figure 5. Distribution of the *ren1*, *ren3* and *ren9* genes in the investigated genotypes
 Şekil 5. İncelenen genotiplerde *ren1*, *ren3* ve *ren9* genlerinin dağılımı

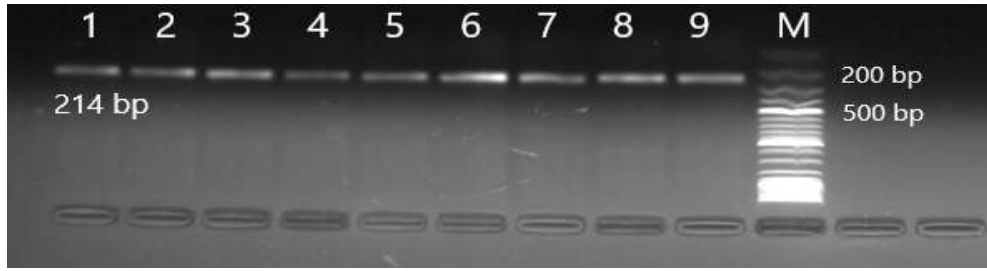


Figure 6. Electrophoretic separation of PCR amplicons of the *Ren1* gene obtained from the GF13-13F / GF13-13R primer pair

Şekil 6. GF13-13F / GF13-13R primer çiftlerinden elde edilen *Ren1* geninin PCR ampliconlarının elektroforetik ayrımı

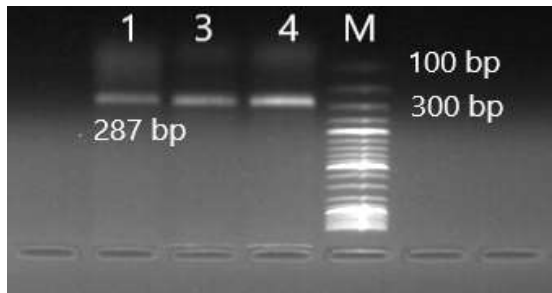


Figure 7. Electrophoretic separation of PCR amplicons of the *Ren9* gene obtained from the CenGen6F / CenGen6R primer pair

Şekil 7. CenGen6F / CenGen6R primer çiftlerinden elde edilen *Ren9* geninin PCR ampliconlarının elektroforetik ayrımı

Molecular analyses in this research revealed that all nine genotypes harbor the *Ren1* gene. The dominant locus *Ren1* (resistance to *Erysiphe necator* 1) belongs to 'Kishmish Vatkana' and 'Dzhandzhal Kara', two Central Asian *V. vinifera* cultivars (Korbuly, 1999; Kozma et al., 2006; Reisch et al., 2014). However, the *Ren3* gene was conspicuously absent in all genotypes examined in this study, consistent with findings from other investigations indicating the gene's sparse distribution among grape varieties (Welter et al., 2007; Pozharskiy et al., 2020). On the other hand, the *Ren9* gene was detected in three out of the nine genotypes analyzed, which suggests a nuanced distribution of resistance genes within *V. labrusca*, indicating the complexity of the mechanisms underlying resistance to powdery mildew.

Interestingly, this study documents the identification of the *Ren1* locus within North American-derived *V. labrusca* genotypes. The detection of the *Ren1* gene in the examined *V. labrusca* genotypes highlights the possibility of natural hybridization between *V. labrusca* and *V. vinifera*, especially in Türkiye, where the cultivation of *V. vinifera* cultivars has historically been widespread. This finding suggests that the examined genotypes may not be pure *V. labrusca* but likely represent hybrids with *V. vinifera*. A similar study was conducted by Cadle-Davidson et al. (2011a)

with different *Vitis* species at two different locations and the results were similar to the results of this study. In this study, two different treatments, natural infection and artificial inoculation with a single isolate, were applied for powdery mildew disease on grapevine leaves and the results revealed significant differences between the species. One of the most susceptible species was *V. vinifera*, while interspecific hybrids were found to be more resistant to powdery mildew. *V. labrusca* was found to be one of the most resistant species. Similar to the results of this study, Reisch et al. (1993) reported that the interspecific hybrid variety Alden was tolerant to powdery mildew and resistant to mildew, while another interspecific hybrid variety, 'Kay Gray', had good resistance to mildew and powdery mildew. Almost all *V. vinifera* cultivars are highly susceptible to powdery mildew; nevertheless, several Vitaceae species have developed resistance mechanisms against this fungus but lack commercial qualities (Riaz et al., 2007; Glawe, 2008; Dry et al., 2010; Gadoury et al., 2012). In this context, resistant genotypes have become valuable germplasm for inclusion in grapevine breeding programs. These natural powdery mildew resistance sources correspond to some North American and Asian genotypes, and the resistance trait is related to their evolutionary history, as described by several works (Riaz et al., 2007; Hoffmann et al., 2008; Coleman et al., 2009; Dry et al., 2010; Feechan et al., 2011; Ramming et al., 2011; Blanc et al., 2012; Gadoury et al., 2012; Qiu et al., 2015; Pap et al., 2016). Resistance to pathogenic microorganisms is a common and important trait to be incorporated into new plant cultivars. Many sources of resistance to grapevine powdery mildew have been identified, including some North American and Chinese species, and even some Asian *V. vinifera* cultivars, which exhibit different levels of resistance but lack commercial qualities (Barker et al., 2005; Welter et al., 2007; Ramming et al., 2011; Blanc et al., 2012; Feechan et al., 2015; Pap et al., 2016). An important issue in the development of new pathogen-resistant cultivars is the emergence of new virulent isolates with the ability to overcome R gene recognition (Peressotti et al., 2010; Cadle-Davidson et al., 2011b).

Hence, pyramiding two or more R genes from different *Vitis* species has become a durable and secure strategy; even if any mutation or loss of an avirulence factor occurs, the pathogen will still be recognized by at least one R gene (Feechan et al., 2015; Armijo et al., 2016; Pap et al., 2016). In a hybridization study, Agurto et al. (2017) used segregating plants from *V. vinifera* 'Dzhandzhal Kara' × *V. vinifera* 'Laszta' and the fifth pseudobackcross of *M. rotundifolia* × *V. vinifera* as two genetically different sources of resistance against the biotrophic fungus *E. necator* carrying *Ren1* and *Run1* loci, respectively, and pyramided them in single grapevine plants until the seventh pseudobackcross with *V. vinifera* 'Crimson Seedless'. Such dual-purpose hybrids can significantly contribute to the diversity of genetic resources in the viticulture industry. By providing valuable genetic material for the development of high-quality grape varieties and offering natural resistance to diseases, these hybrids can support sustainable viticulture practices (Atak & Göksel, 2019). The genotypes examined in the present study have the potential to carry the commercial traits of certain *V. vinifera* high-quality grape varieties, while also harboring the disease resistance characteristics of *V. labrusca*.

Therefore, the discovery and utilization of hybrid genotypes hold considerable promise for transforming and improving the viticulture industry. The outcomes of this study emphasize the strategic significance of amalgamating multiple resistance loci to forge robust and enduring resistance against powdery mildew. The co-occurrence of the *Ren1* and *Ren9* genes within the same genotypes supports the ability of diverse resistance mechanisms to bolster disease resistance. This approach is in harmony with contemporary breeding objectives that aim for sustainable resistance through the incorporation of various modes of action, thereby reducing evolutionary pressures on pathogens and delaying the emergence of virulent fungal strains.

The molecular analysis of the *Vitis labrusca* genotypes TEG-VI-1, TEG-VI-3, and TEG-VI-4 highlighted the co-occurrence of the *Ren1* and *Ren9* genes, marking a noteworthy discovery for grapevine breeding endeavors. The identification of two distinct powdery mildew resistance loci within the same genotype represents a pivotal achievement. This dual locus presence aligns with the broader consensus among researchers advocating for the integration of *Ren* genes from varied genetic backgrounds as a strategy to establish durable resistance in agricultural settings. By pyramiding resistance genes from diverse sources, the intention is to reduce the selection pressure on pathogen populations, thereby decelerating the emergence of virulent fungal strains (Feechan et al., 2015; Pap et al., 2016). Such strategic breeding is deemed crucial for preventing mutations within pathogen effector molecules that might otherwise

elude recognition by the *Ren* proteins, ensuring the continued efficacy of resistance mechanisms (Pap et al., 2016). These findings underscore the strategic importance of considering the geographical origins and genetic diversity of resistance loci. The aim is to compile a mosaic of resistance sources, thereby broadening the genetic base upon which new grapevine cultivars resistant to powdery mildew are developed (Sosa-Zuniga et al., 2022). Zender et al. (2020) further support this approach by suggesting that resistance traits identified in the *Ren3* and *Ren9* loci from North American grapevine species, when coupled with those exhibiting strong resistance from European origins, can lead to enhanced durability and efficacy of resistance against powdery mildew. This collective body of work advocates for a nuanced and globally informed approach to grapevine breeding. By embracing genetic diversity and the integration of resistance genes from internationally distinct *Vitis* species, breeders can forge new paths toward the cultivation of grapevine varieties endowed with comprehensive and enduring resistance to powdery mildew. This endeavor not only holds promise for safeguarding the viticulture industry against current and future pathogenic challenges but also exemplifies a forward-thinking commitment to the sustainable management of plant health and productivity.

The findings of this study endorse a strategic breeding methodology that capitalizes on genetic diversity and integrates resistance alleles from disparate sources. By prioritizing the combination of resistance genes from geographically and genetically diverse *Vitis* species, breeders can cultivate grapevine varieties with enhanced and lasting resistance to powdery mildew, contributing significantly to the sustainable future of viticulture (Feechan et al., 2015; Pap et al., 2016; Sosa-Zuniga et al., 2022; Zender et al., 2020). This strategy not only leverages the intrinsic genetic potential within the *Vitis* genus but also serves as a bulwark against the rapid evolution of pathogenic threats, ensuring the longevity and productivity of grapevine populations worldwide.

CONCLUSION

The findings of the present study may help to elucidate the molecular basis of resistance mechanisms against powdery mildew across various *V. labrusca* genotypes. This research indicated that the distribution of resistance genes differed among the genotypes, suggesting underlying genetic diversity as a potential influencer. These identified grapevine genotypes present an opportunity for registration as new grape varieties that exhibit resistance to *Erysiphe necator*, thereby contributing to the diversification and enhancement of disease-resistant cultivars. The genotypes characterized by these resistance genes are poised to illuminate future research aimed at

augmenting resistance to powdery mildew across different species and varieties. These findings could serve as valuable resources for researchers striving to devise optimal combinations of genes and loci for enhanced disease resistance. This knowledge can significantly accelerate the development of grapevine varieties with improved resilience against powdery mildew, aligning with the broader goals of sustainable viticulture and crop protection. Comprehensive studies involving physiological, biochemical, and transcriptomic analyses are highly recommended to better understand and decipher the intricate effects of these resistance genes. Such in-depth investigations will pave the way for a more nuanced appreciation of the resistance mechanisms at play, potentially leading to the discovery of novel strategies for managing powdery mildew in grapevines. Moreover, it is imperative that future evaluations of these genotypes be conducted under field conditions or controlled environments. Proper experimental setups are essential for validating resistance traits and ensuring the practical applicability of these genotypes in real-world viticultural practices. This approach will not only corroborate the initial findings but also facilitate the integration of these resistant genotypes into breeding programs, thus bolstering global efforts to mitigate the impact of powdery mildew on grape production.

Researchers' Contribution Rate Statement Summary

AY prepared the original manuscript. SD, AB, and DSA contributed to supervision, editing, and conceptualization. AY, SD, and AB gathered relevant research articles and reviewed the manuscript. DSA also contributed to reviewing and editing the manuscript. All the authors have read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Kahramanmaraş Dulkadiroğlu İlçesinde Yetiştirilen Üzüm Çeşitlerinin Belirlenmesi ve Ampelografik Özelliklerin Araştırılması

Turhan YILMAZ¹, Mehmet TAKAS²

¹Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Kahramanmaraş, Türkiye

¹<https://orcid.org/0000-0002-3756-4497>, ²<https://orcid.org/0000-0001-6304-8843>

✉: turhanyilmaz@ksu.edu.tr

ÖZET

Üzüm üreticilerinin kendi bölgelerinde tercih edilen üzüm çeşitlerinin tanımlanması ve koruma altına alınması genetik kaynakların gelecek nesillere aktarılabilmesi için önem arz etmektedir. Bu çalışmada, Kahramanmaraş Dulkadiroğlu'nda yetiştiriciliği yapılan üzüm çeşitlerinden 32 adet genotip incelenmiştir. Bu çeşitler; Horuzyüreği (Sinonim= İman Üzümü), Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Ağ Üzüm, Hatun Parmağı, Kıbrıs Üzümü, Red Globe, Kokulu Üzüm, Kirkit Üzümü, Şeker Üzümü, Yediveren, Bandırma, Pafi, Azezi, Künefi, Kırmızı Gaziantep Künefisi, Sarı Sergilik, Honi Kabarcık (Sinonim= Tosbağa Kabarcığı, Kuzucak Kabarcığı), Tüylü Kabarcık, Cırt Kabarcık, İzmir Kabarcığı, Kızaran Kabarcık, Sararan Kabarcık, Siyah Kabarcık, Kilis Kara Sergilik, Orak Karası, Kara Sergilik, Deve Gözü (Sinonim= Öküzgözü), Sultani Çekirdeksiz, Dökülgen ve Perpil olarak sıralanmıştır. Çalışmadaki asma genotipleri uluslararası anlamda kabul görmüş ve araştırmacılar tarafından yoğun bir şekilde kullanılan OIV (The International Organisation of Vine and Wine, Uluslararası Bağ ve Şarap Örgütü) kriterlerine (sürgün özellikleri, genç yaprak özellikleri, olgun yaprak özellikleri, tane özellikleri, fenolojik ve pomolojik özellikler) göre tanımlanmıştır. Bu çalışma ile bölgedeki asma genotiplerinin literatüre kazandırılması ve mevcut gen kaynaklarının korunması amaçlanmıştır. Çalışma sonucunda, Kahramanmaraş Dulkadiroğlu ilçesinin zengin bir asma genetik kaynağına sahip olduğu anlaşılmıştır. Yapılan çiftçi ziyaretleri sonucunda, Kabarcık en fazla üretimi yapılan çeşit olarak tespit edilmiştir. Perpil çeşitinin *V. labrusca* L. türüne ait olduğu düşünülürken diğer çeşitlerin tamamının *Vitis vinifera* L. türüne ait olduğu tespit edilmiştir. Tespit edilen çeşitlerin benzerlik ilişkilerini belirlemek için moleküler bazlı analizlerin yapılması gerekmektedir.

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Determination of Grape Varieties Growing in Kahramanmaraş Dulkadiroğlu District and Investigation of Ampelographic Characteristics

ABSTRACT

Identification and protection of grape varieties preferred by grape producers in their regions are important to transfer genetic resources to future generations. In this study, 32 genotypes of grape varieties grown in Kahramanmaraş Dulkadiroğlu were examined. These varieties; Horuzyüreği (Synonym= İman üzümü), Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Ağ Üzümü, Hatun Parmağı, Kıbrıs Üzümü, Red Globe, Kokulu Üzüm, Kirkit Üzümü, Şeker Üzümü, Yediveren, Bandırma, Pafi Üzümü, Azezi, Künefi, Kırmızı Gaziantep Künefisi, Sarı sergilik, Honi Kabarcık (Synonym = Tosbağa Kabarcığı, Kuzucak Kabarcığı), Kara Sergilik, Cırt Kabarcık, İzmir Kabarcığı, Kırmızılı Kabarcık, Sararan Kabarcık, Siyah Kabarcık, Kilis Kara Sergilik, Orak Karası, Kara Sergilik, Deve Gözü (Synonym= Öküzgözü), Sultani Çekirdeksiz, Dökülgen and Perpil. Grapevine genotypes were compared according to OIV (The

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International Organisation of Vine and Wine) criteria (shoot characteristics, young leaf characteristics, mature leaf characteristics, berry characteristics, phenological and pomological characteristics), which are internationally accepted and used extensively by researchers. With this study, it was aimed to introduce the grapevine genotypes in the region to the literature and to protect the existing genetic resources. As a result of our study, it was understood that Kahramanmaraş Dulkadiroğlu district has a rich grapevine genetic resource. As a result of the grower's visits, Kabarcık was determined to be the most produced variety. While the Perpil variety was thought to belong to the *V. labrusca* L. species, all other varieties were determined to belong to the *Vitis vinifera* L. cultivar. It is recommended to perform molecular-based analyzes to determine the similarity relationships of the detected varieties.

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GİRİŞ

Türkiye’de 2022 yılında toplam 384537 ha alandan yaklaşık 4165000 ton üzüm üretimi yapılmıştır (FAOSTAT, 2024). Kahramanmaraş’ ta ise 2022 yılında Dulkadiroğlu (25265 ton), Onikişubat (17304 ton), Pazarcık (12860 ton) ilçeleri başta olmak üzere toplam 64681 ton üzüm üretimi yapılmıştır (TUİK, 2024). Asmanın tanımlanması ile ilgilenen bilim dalına Ampelografi adı verilmektedir. İlk olarak Sachs tarafından 1661 yılında yayınlanan “Ampelographia” adlı eserde kullanılan ampelografi terimi; Yunanca "ampelos" (asma) ve "graphe" (nitelemek, tasnif etmek) kelimelerinden türetilmiştir (Oraman, 1959). Asma gen kaynaklarının belirlenmesi ve tanımlanmasında uluslararası yöntem birliği sağlamak amacıyla “Uluslararası Bağcılık ve Şarapçılık Ofisi” (OIV- Office International de la vigne et du Vin), “Uluslararası Bitki Gen Kaynakları” (IBPGR- International Board for Plant Genetic Resources) ve “Uluslararası Yeni Bitki Çeşitlerinin Korunması Birliği” (UPOV- International Union For the Protection of New Varieties of Plants) tarafından oluşturulan “Üzüm Tanımlayıcıları” (Descriptor for Grape) yaygın olarak kullanılmaktadır (Anonim, 1983; OIV, 1983). Tanımlayıcılar geleneksel olarak sürgün ucu (şekil, tüylülük ve renklenme), yaprak (sap cebi, dilimler ve dişler ile aya şekli), meyve salkımları (büyüklük ve şekil) ve taneler (büyüklük, şekil ve renklenme) gibi görsel özellikleri kapsamaktadır (Keller, 2020; OIV, 1983). Türkiye Asma (*Vitis vinifera* L.)’nın anavatanı olan coğrafya içerisinde yer almaktadır. Yüzyıllardır süregelen bağcılık kültürü sonucunda Türkiye dünya asma gen potansiyeli bakımından söz sahibi ülkelerden biridir. Türkiye’nin hemen her bölgesinde üzüm yetiştiriciliği yapılmaktadır. Bu nedenle homonim (farklı) ve sinonim (aynı) bakımdan geniş bir varyasyon gösteren

çok zengin ve değişik özelliklere sahip yerel çeşit ve tipler bulunmaktadır. Bu çeşit ve tiplerin doğru tanımlanması, toplam çeşit sayısının belirlenmesi ve çeşit standardizasyonu açısından büyük önem taşımaktadır (Ergül ve ark., 2006). Türkiye asma genetik kaynaklarının toplanması ve muhafazası 1965 yılında Tekirdağ Bağcılık Araştırma Enstitüsü tarafından yürütülmeye başlayan “Türkiye Asma Genetik Kaynaklarının Belirlenmesi, Muhafazası ve Tanımlanması” isimli proje ile olmuştur. Proje çalışmaları bugüne kadar İl Tarım ve Orman Müdürlükleri, üniversitelerin ilgili bölümleri/akademisyenleri ve doğrudan üreticiler ile irtibat sağlanarak yürütülmüştür. İlk yıllarda yapılan envanter çalışmaları sonucu Türkiye’de yaklaşık 1.600 adet üzüm çeşidinin bulunduğu tespit edilmiş olup, günümüzde Milli koleksiyon bağında 1.435 çeşit muhafaza edilmektedir (Uysal & Yaşasın, 2017). Bu çalışmanın amacı Kahramanmaraş ili Dulkadiroğlu ilçesinde yetiştirilen üzümlerin çeşit varlıklarının uluslararası normlara göre tanımlanmasını sağlamaktır.

MATERYAL ve METOD

Materyal

Bu çalışma Kahramanmaraş Dulkadiroğlu ilçesi Merkez, Bertiz Bölgesinde bulunan üretici bağlarında yürütülmüştür. Çalışmada tespit edilen 32 üzüm çeşidinin OIV kriterlerine göre ampelografik özellikleri ortaya konulmuştur. Çalışmada tespit edilen üzüm çeşitleri, üretim alanı ve rakım bilgileri Çizelge 1’de gösterilmiştir.

Metot

Bu çalışma Dulkadiroğlu ilçesini temsil edecek şekilde Yusufçacı, Peynirdere, Çokyaşar, Yenişehir, Çobanlı

ve Hacıyüplü bağlarında yürütülmüştür. Yetiştiricilerden ayrıntılı alınan bilgiler doğrultusunda, 15-20 yaşları arasında, sağlıklı ve verimli omcalar yaz gelişme döneminde yaprak ve salkım örnekleri alınmış ve işaretlemeler yapılmıştır. Çalışmada üzüm çeşitlerinin ampelografik olarak tanımlanmasında uluslararası yöntem birliği sağlamak amacı ile 'Üzüm

tanımlayıcıları' adı altında yayınlanan kriterler kullanılmıştır (OIV, 1983). OIV kriterleri doğrultusunda her bir çeşitten alınan örnekler sürgün, genç yaprak, olgun yaprak, salkım, fenolojik ve pomolojik özellikleri Kahramanmaraş Sütçü İmam Üniversitesi Bahçe Bitkileri Laboratuvarlarında incelenmiştir.

Çizelge 1. Kahramanmaraş Dulkadiroğlu ilçesinde tespit edilen üzüm çeşitleri ve toplandıkları alan ve rakım bilgileri

Table 1. Grape varieties identified in Kahramanmaraş Dulkadiroğlu district and information about the area and altitude where they are collected

Çeşit Adı (Cultivar Name)	Rakım (Attitude)
Horoz Yüreği (Sinonim; İmam Üzümü)	1249
Kırmızılı Mahrabaşı	820
Kararan Mahrabaşı	893
Ağ Mahrabaşı	893
Ağ Üzüm	851
Hatun Parmağı	718
Kıbrıs Üzümü	1249
Red Globe	1249
Kokulu Üzüm	1249
Kirkit Üzümü	1249
Şeker Üzümü	820
Yediveren	851
Bandırma Üzümü	1249
Pafi Üzümü	820
Azezi Üzümü	851
Künefi Üzümü	1249
Kırmızı Gazi Antep Künefisi	1249
Sarı Sergilik	744
Honi Kabarcık (Sinonim; Tosbağa Kabarcığı, Kuzucak Kabarcığı)	851
Tüylü Kabarcık	820
Cırt Kabarcık	820
İzmir Kabarcığı	1249
Kızaran Kabarcık	1249
Sararan Kabarcık	1249
Siyah Kabarcık	851
Kilis Kara Sergilik	820
Orak Karası	763
Kara Sergilik	893
Deve Gözü (Sinonim; Öküz Gözü)	851
İzmir Sultani Çekirdeksiz	851
Dökülgen	851
Perpil	531

BULGULAR ve TARTIŞMA

Üzüm Çeşitlerinin Sürgün Özellikleri

Sürgün ucunun şekli bakımından (OIV 001) Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Hatun Parmağı, Red Globe, Kirkit, Şeker Üzüm, Yediveren, Bandırma üzüm çeşitleri "açık", Ağ Üzüm, Siyah Kabarcık ve Kilis Kara Sergilik üzüm çeşitleri 'yarı açık' olarak

tespit edilmiştir. Sülüklerin sürgündeki dizilişi (OIV 016) bakımından Horoz Yüreği, Kıbrıs Üzüm, Red Globe, Kirkit, Yediveren, Bandırma, Pafi, Kırmızı Gaziantep Künefisi, Honi Kabarcık, Tüylü Kabarcık, Cırt Kabarcık, İzmir Kabarcık, Kızaran Kabarcık, Perpil çeşitleri "kesikli", diğer çeşitler "devamlı" olarak tespit edilmiştir. Bu sonuçlar önceki çalışmalarda paralellik göstermektedir (Kara, 1990; Ünal, 2000;

Uyak, 2010). Sürgün ucundaki yatık tüylerin yoğunluğu (OIV 004) bakımından Ağ Üzüm, Kıbrıs Üzümü, Kokulu Üzüm, İzmir Kabarcık, Kilis Kara Sergilik, Orak Karası, Kara Sergilik, Deve Gözü, İzmir Sultani Çekirdeksiz, Dökülgen üzüm çeşitleri “seyrek”, Horuz Yüreği, Kirkit, Perpil üzüm çeşitleri “orta”, Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Pafi, Kızaran Kabarcık, Sararan Kabarcık, Siyah Kabarcık çeşitleri “sık”, Tüylü Kabarcık, “çok sık”, diğer çeşitler ise “çok seyrek” olarak belirlenmiştir. Sürgün ucunda dik tüylerin yoğunluğu (OIV 005) bakımından Ağ Üzüm, Red Globe, Kokulu Üzüm, Şeker Üzüm, Yediveren, Bandırma çeşitleri “çok seyrek”, Sararan Kabarcık ve Siyah Kabarcık üzüm çeşitleri “seyrek”, Pafi ve Tüylü Kabarcık çeşitleri “sık”, diğer çeşitler “yok” olarak değerlendirilmiştir. Çalışmanın sonucunda elde edilen bu bulgular önceki çalışmalarla uyumluluk göstermektedir (Kara, 1990; Ünal, 2000; Uyak, 2010). Boğum aralarının sırt rengi (OIV 007) bakımından Ağ Üzüm, Hatun Parmağı, Red Globe, Kirkit, Şeker Üzüm, Yediveren, Bandırma, Pafi üzüm çeşitleri “kırmızı-çizgili yeşil”, Ağ Mahrabaşı, Sarı Sergilik, Honi Kabarcık üzüm çeşitleri “yeşil”, diğer çeşitler “kırmızı” sınıfına girmiştir. Boğum aralarının karın tarafının rengi (OIV 008) bakımından Kıbrıs Üzümü, Azezi, Künefi, Tüylü Kabarcık, Cırt Kabarcık, İzmir Kabarcık çeşitleri “kırmızı”, Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Red Globe, Kirkit, Şeker Üzüm, Yediveren, Bandırma, Pafi, Sarı Sergilik, Honi Kabarcık üzüm çeşitleri “yeşil”, diğer çeşitler “kırmızı-çizgili yeşil” sınıfına girmiştir. Boğumların sırt rengi (OIV 009) bakımından Ağ Mahrabaşı, Ağ Üzüm, Red Globe, Kirkit, Yediveren, Bandırma, Pafi, Kırmızı Gaziantep Künefisi, Honi Kabarcık çeşitleri “kırmızı-çizgili yeşil”, Sarı Sergilik çeşidi “yeşil”, diğer çeşitler “kırmızı” olarak tespit edilmiştir. Boğumlardaki dik tüyler bakımından Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Ağ Üzüm, Red Globe, Kirkit, Şeker Üzüm, Yediveren, Bandırma, Pafi çeşitleri “çok seyrek”, Tüylü Kabarcık çeşidi “sık”, diğer çeşitler “yok” olarak değerlendirilmiştir. Sülüklerin uzunluğu (OIV 017) bakımından Ağ Üzüm ve Kızaran Kabarcık “orta”, Ağ Mahrabaşı çeşidi “uzun”, diğerler çeşitler “kısa” sınıfına girmiştir.

Üzüm Çeşitlerinin Genç Yaprak Özellikleri

Üst yüzün rengi (OIV 051) bakımından Ağ Mahrabaşı, Ağ Üzüm, Kıbrıs Üzümü, Kırmızı Gaziantep Künefisi, Sarı Sergilik, Honi Kabarcık, Tüylü Kabarcık, Cırt Kabarcık, İzmir Kabarcık, Kızaran Kabarcık çeşitleri “bronz benekli yeşil”, Kirkit üzüm çeşidi “bakır sarısı”, Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Hatun Parmağı üzüm çeşitleri “yeşil”, Azezi ve Künefi çeşitleri “kırmızı”, diğer çeşitler “bronz benekli sarı” sınıfına girmiştir. Damar aralarındaki yatık tüyler

(OIV 053) bakımından Kırmızılı Mahrabaşı, Kararan Mahrabaşı, Ağ Mahrabaşı, Ağ Üzüm çeşitleri “yok”, Kıbrıs Üzümü, Kokulu Üzüm, İzmir Kabarcık, Kızaran Kabarcık, Sararan Kabarcık, Siyah Kabarcık, Orak Karası, Kara Sergilik, Deve Gözü, İzmir Sultani Çekirdeksiz, Dökülgen çeşitleri “seyrek”, Horoz Yüreği ve Perpil çeşitleri “orta”, Tüylü Kabarcık ve Cırt Kabarcık çeşitleri “çok sık” diğer çeşitler “çok seyrek” sınıfına girmiştir. Damar aralarındaki dik tüyler (OIV 054) bakımından, Tüylü Kabarcık ve Cırt Kabarcık çeşitleri “sık”, Kıbrıs Üzümü, Kirkit ve İzmir Kabarcık üzüm çeşitleri “seyrek”, diğer üzüm çeşitleri “yok” sınıfına dahil olmuştur.

Üzüm Çeşitlerinin Olgun Yaprak Özellikleri

Olgun yaprak uzunluğu (OIV 066) bakımından Kırmızılı Mahrabaşı, Ağ Mahrabaşı, Kıbrıs Üzüm, Kokulu Üzüm, Kirkit, Pafi, Azezi, Kırmızı Gaziantep Künefisi üzüm çeşitleri “orta”, Horuz Yüreği, Ağ Üzüm, Sarı Sergilik, Honi Kabarcık, Tüylü Kabarcık, Cırt Kabarcık üzüm çeşitleri “uzun”, diğer çeşitler “kısa” sınıfına girmiştir. Olgun yaprak ayasının şekli (OIV 067) bakımından Kıbrıs Üzüm, Red Globe, Kokulu Üzüm, Kirkit, Şeker Üzümü, Azezi, Tüylü Kabarcık, Cırt Kabarcık ve Perpil üzüm çeşitleri “beşgen”, Kırmızılı Mahrabaşı, Ağ Mahrabaşı, Yediveren, Künefi ve Kırmızı Gaziantep Künefisi üzüm çeşitleri “yuvarlak”, İzmir Sultani Çekirdeksiz ve Dökülgen üzüm çeşitleri “böbrek”, diğer çeşitler “kama şeklinde” sınıfına girmiştir. Olgun yaprak dilim sayısı (OIV 068) bakımından İzmir Sultani Çekirdeksiz ve Perpil üzüm çeşitleri “dilimsiz”, Ağ Mahrabaşı üzüm çeşidi “üç dilim”, diğer üzüm çeşitleri ise “beş dilim” olarak tespit edilmiştir. Olgun yaprak dilim sayısının ve olgun yaprak ayasının şeklinin ekolojik faktörlerden etkilenmeyeceği düşünülürse bu parametrelerin üzüm tanımlamalarında doğrucu bilgileri sunacağı önceki araştırmacılar tarafından bildirilmiştir (Diri, 1996). Olgun yaprağın üst yüzünün rengi (OIV 069) bakımından Ağ Mahrabaşı ve Ağ Üzüm üzüm çeşitleri “çok açık yeşil” diğer çeşitler “yeşil” olarak tespit edilmiştir. Olgun yaprağın üst tarafının rengi yöreden yöreye veya beslenme programlarına göre değişiklik gösterebileceği göz önünde bulundurulursa, bu parametrenin yetiştiriciliği yapılan bölgede çeşit ayırımında önemli bir parametre olduğu düşünülmektedir (Anonim, 1983).

Üzüm Çeşitlerinin Tane Özellikleri

Tane şekli (OIV 223) bakımından Pafi, Sarı Sergilik, Deve Gözü, İzmir Sultani Çekirdeksiz, Dökülgen üzüm çeşitleri “yumurta şeklinde”, Kararan Mahrabaşı üzüm çeşidi “ters yumurta şeklinde”, Ağ Mahrabaşı ve Ağ Üzüm çeşitleri “uzun oval”, Kıbrıs Üzüm, Kokulu Üzüm, Şeker Üzüm, Künefi, Kırmızı Gaziantep Künefisi üzüm çeşitleri “silindirik”, Kirkit Üzüm, Orak

Karası, Kara Sergilik üzüm çeşitleri “kısa oval”, Bandırma ve Kilis Kara Sergilik üzüm çeşitleri “parmak şeklinde”, diğer çeşitler “yuvarlak” sınıfına girmiştir. Farklı araştırmacılar tarafından ekolojik isteklerin, kültürel uygulamalardaki farklılıklar ve farklı tozayıcıların tane şekli üzerinde önemli etkileri olduğu bildirilmiştir. Araştırmacılara göre tane şekli çevre faktörlerinden çok etkilenmeyen bir kriter olarak bildirilmektedir ve bu sayede çeşit tanımlama kriterlerinde kullanılmasında önemli bir yeri vardır (Fidan, 1985). Tane enine kesiti (OIV 224) bakımından üzüm çeşitlerin tamamı “yuvarlak” sınıfına girmişlerdir. Tane kabuk rengi (OIV 225) bakımından Horoz Yüreği ve Kırmızı Mahrabaşı üzüm çeşitleri “kırmızı-gri”, Kararan Mahrabaşı, Künefi Üzüm, Siyah Kabarcık, Kilis Kara Sergilik, Kara Sergilik üzüm çeşitleri “siyah”, Ağ Üzüm çeşidi “sarı”, Red Globe, Kırmızı Gaziantep Künefisi ve Perpil üzüm çeşitleri “koyu kırmızı-mor”, Pafi, Kızaran Kabarcık, Orak Karası, Deve Gözü ve İzmir Sultani Çekirdeksiz üzüm çeşitleri “diğer”, diğer çeşitler ise “yeşil-sarı” sınıfına girmiştir. Tane meyve etinin rengi (OIV 230) bakımından Bandırma, Pafi, Azezi, Kızaran Kabarcık, Deve Gözü, İzmir Sultani Çekirdeksiz, Dökülgen üzüm çeşitleri “çok hafif renkli”, Horoz Yüreği, Kırmızı Mahrabaşı, Ağ Mahrabaşı, Red Globe üzüm çeşitleri “hafif renkli”, Siyah Kabarcık ve Orak Karası üzüm çeşitleri “orta renkli” Kararan Mahrabaşı, Künefi, Kırmızı Gaziantep Künefisi, Kilis Kara Sergilik, Kara Sergilik ve Perpil üzüm çeşitleri “çok kuvvetli renkli”, diğer çeşitler “renksiz” sınıfına girmiştir. Ben düşme döneminden önce tanedeki yeşil renk belirginliğini sürdürürken bu dönemden sonra çeşit rengine bağlı olarak tanedeki renk değişimi başlamaktadır. Tane rengi çeşide özgü olarak değişim göstermekle birlikte etkili sıcaklık toplamı gibi iklim şartları dolayısıyla çoğu zaman aynı çeşitler içerisinde bile farklılık gösterdiği tespit edilmiştir. Tane çekirdek varlığı (OIV 241) bakımından çeşitlerin çekirdekli olup olmadığı bakımından Sultani Çekirdeksiz üzüm çeşidi “yok”, diğer otuzbir üzüm çeşitlerinde 1-3 arasında “var” olarak değerlendirilmiştir.

Üzüm Çeşitlerinin Fenolojik ve Pomolojik Özellikleri

Üzüm çeşitlerinde gözlerin uyanması (OIV 301) genel anlamda Mayıs ayının birinci yarısı olarak tespit edilmiştir. Erkenci çeşitler ise Nisan 15 gibi erken uyandığı gözlemlenmiştir. En erken uyanma 01.04.2022 tarihinde Orak Karası ve Ağ Üzüm çeşitlerin de gözlemlenmiştir. Yediveren ise 18.05.2022 tarihinde en geç uyanan üzüm çeşidi olarak tespit edilmiştir. Olgunlaşma zamanı bakımından (OIV 304) genel anlamda çeşitler haziran ayının ilk yarısında gerçekleşmektedir. En erken olgunlaşan çeşit 15.06.2023 tarihinde Orak Karası ve Ağ Üzüm çeşitlerinde gözlemlenirken, en geç olgunlaşan çeşit 05.10.2022 tarihinde Yediveren çeşidi olarak tespit edilmiştir. Olgunluk dönemine etki eden faktörler

gözlerin sürmesinden sonraki alınan toplam etkili sıcaklık toplamı, bağıın bulunduğu yer ve yöney, asmanın yaşı, terbiye şekli, toprağın genel yapısı ve içsel hormonların üzüm tanesindeki dağılımı etkilerinin bir toplamı olduğu bildirilmektedir (Fidan, 1985). Salkım ağırlığı bakımından (OIV 502) Perpil çeşidi “çok küçük”, Kırmızı Mahrabaşı, Ağ Mahrabaşı, Red Globe, Kokulu Üzüm, Kirkit, İzmir Kabarcık, Kızaran Kabarcık, Sararan Kabarcık, Siyah Kabarcık, Kilis Kara Sergilik, İzmir Sultani Çekirdeksiz çeşitleri “orta”, Künefi ve Kırmızı Gaziantep Künefisi çeşitleri “büyük” ve diğer çeşitler “küçük” sınıfına girmiştir. Tane ağırlığı bakımından (OIV 503) Pafi, Kilis Kara Sergilik, İzmir Sultani Çekirdeksiz ve Perpil üzüm çeşitleri “küçük”, Kokulu Üzüm ve Bandırma üzüm çeşitleri “büyük”, Red Globe üzüm çeşidi “çok büyük” ve diğer çeşitler “orta” sınıfına girmişlerdir. Üzüm çeşitlerinin hasat döneminde yapılan analizlere göre suda çözünebilir kuru madde (SÇKM) miktarları (OIV 505) bakımından Kararan Mahrabaşı, Ağ Mahrabaşı, Kıbrıs Üzüm, Pafi, Honi Kabarcık, Tüylü Kabarcık, Cırt Kabarcık, Kızaran Kabarcık, Sararan Kabarcık, Dökülgen ve Perpil üzüm çeşitlerinde “orta”, Hatun Parmağı, Bandırma ve Künefi üzüm çeşitlerinde “yüksek”, Kirkit, Azezi, Orak Karası, Deve Gözü ve İzmir Sultani Çekirdeksiz üzüm çeşitlerinde “çok yüksek”, diğer çeşitler ise “düşük” sınıfında yer almıştır. Tespit edilen bütün çeşitler asit içeriği bakımından (OIV 506) “düşük” olarak nitelendirilmiştir. Üzüm tanesindeki şıranın şeker ve asit miktarı kriterleri iklim koşullarına ve çeşide göre farklılık göstermektedir. Üzüm tanelerindeki su miktarlarından tespit edilen SÇKM miktarları genetik faktörler ile farklılık göstermesinin yanında, ayrıca denemenin yapıldığı yıllar arasında her yıl aynı zamanın denk getirilememesi olarak da sonuçlandırılabilir (Fidan, 1985; Kara, 1990).

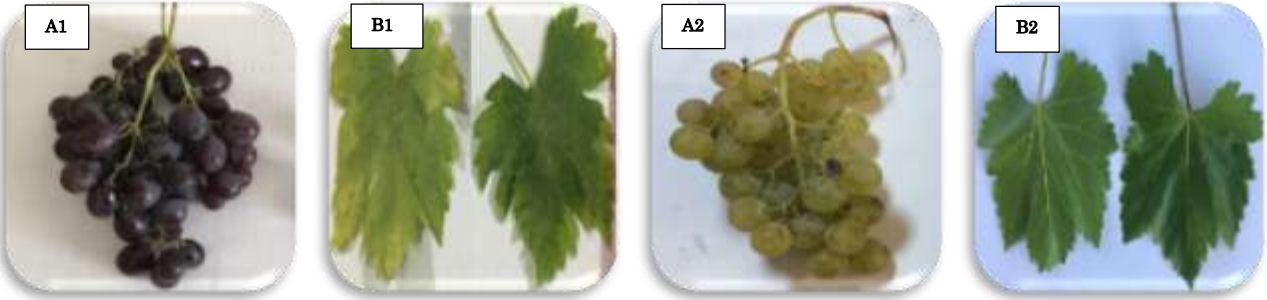
SONUÇ ve ÖNERİLER

Bu çalışma sonucunda Kahramanmaraş Dulkadiroğlu ilçesi asma gen kaynağı bakımından zengin bir yöre olarak kabul edilmiştir. Kabarcık çeşidi Dulkadiroğlu ilçesinde %80 oranında en fazla üretimi yapılan çeşit olarak belirlenmiştir. Çalışmada en yüksek SÇKM miktarı Deve Gözü (%29), en yüksek salkım ağırlığı Künefi (850 g) üzüm çeşitinden elde edilmiştir. En erken uyanma ve olgunlaşma Ağ Üzüm çeşidinde 05.04.2023 ve 15.07.2023 tarihlerinde tespit edilmiştir. Bu çalışmada tespit edilen üzüm çeşitlerinden bazıları (Kabarcık, Sultani Çekirdeksiz, Dökülgen, Öküz gözü, Red Globe, Yediveren, Azezi, Antep üzümü, Mahrabaşı, Red Globe) daha önceden farklı araştırmacılar tarafından tanımlamaları yapılmıştır (Sabır ve ark., 2009; Bilen, 2017; İşçi & Altındışli, 2017; Karataş ve ark., 2019; Candar ve ark., 2021; Baykul & Söylemezoğlu, 2023).



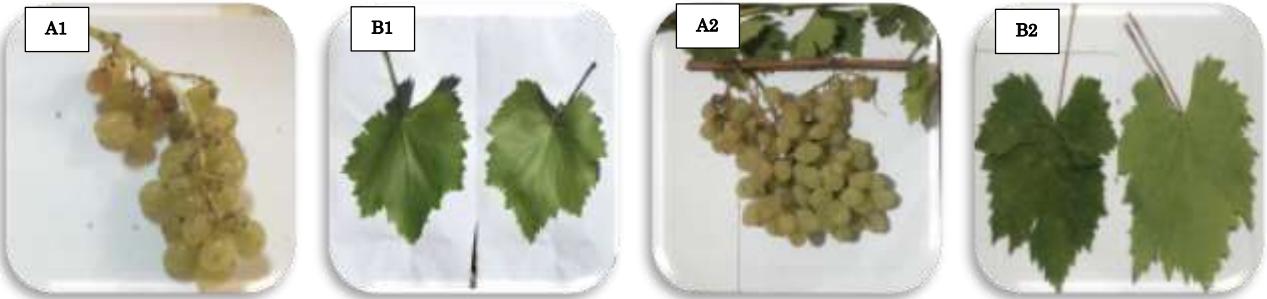
Şekil 1. Horozyüreği salkım (A1) ve olgun yaprak (B1), Kırmızılı Mahrabaşı salkım (A2) ve olgun yaprak (B2) görüntüleri

Figure 1. Images of Horozyüreği cluster (A1) and mature leaf (B1), Kırmızılı Mahrabaşı cluster (A2) and mature leaf (B2)



Şekil 2. Kararan Mahrabaşı salkım (A1) ve olgun yaprak (B1), Ağ Mahrabaşı salkım (A2) ve olgun yaprak (B2) görüntüleri

Figure 2. Images of Kararan Mahrabaşı cluster (A1) and mature leaf (B1), Ağ Mahrabaşı cluster (A2) and mature leaf (B2)



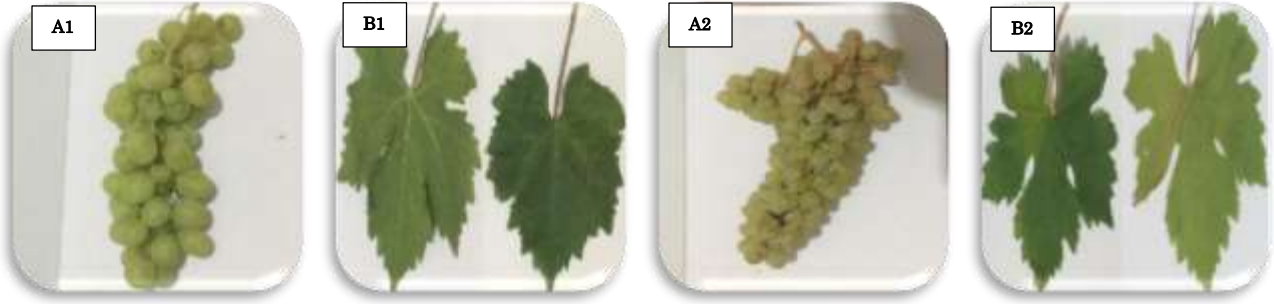
Şekil 3. Ağ Üzüm salkım (A1) ve olgun yaprak (B1), Hatun Parmağı salkım (A2) ve olgun yaprak (B2) görüntüleri

Figure 3. Images of Ağ Üzüm cluster (A1) and mature leaf (B1), Hatun Parmağı cluster (A2) and mature leaf (B2)

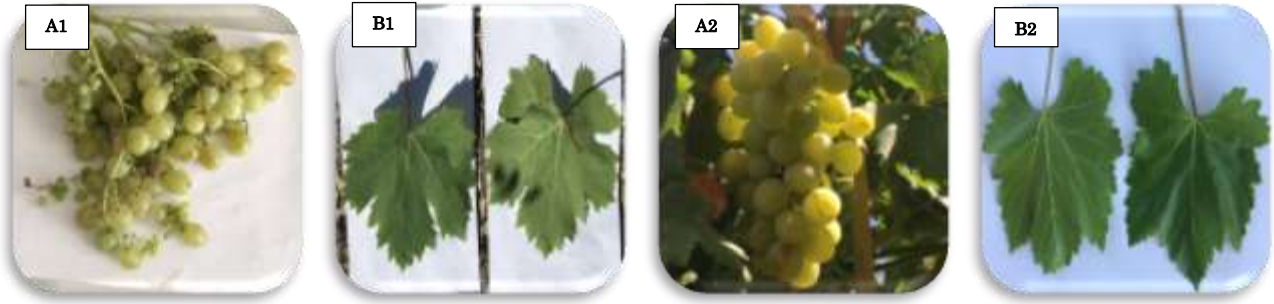


Şekil 4. Kıbrıs Üzümü salkım (A1) ve olgun yaprak (B1), Red Globe salkım (A2) ve olgun yaprak (B2) görüntüleri

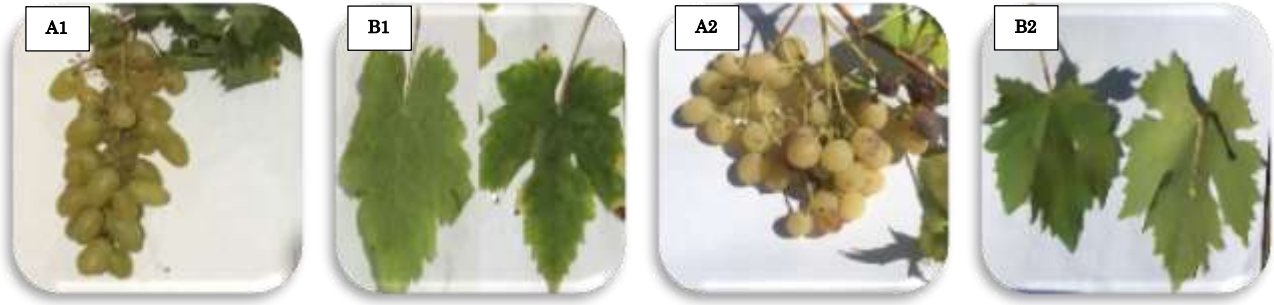
Figure 4. Images of Kıbrıs Üzümü cluster (A1) and mature leaf (B1), Red Globe cluster (A2) and mature leaf (B2)



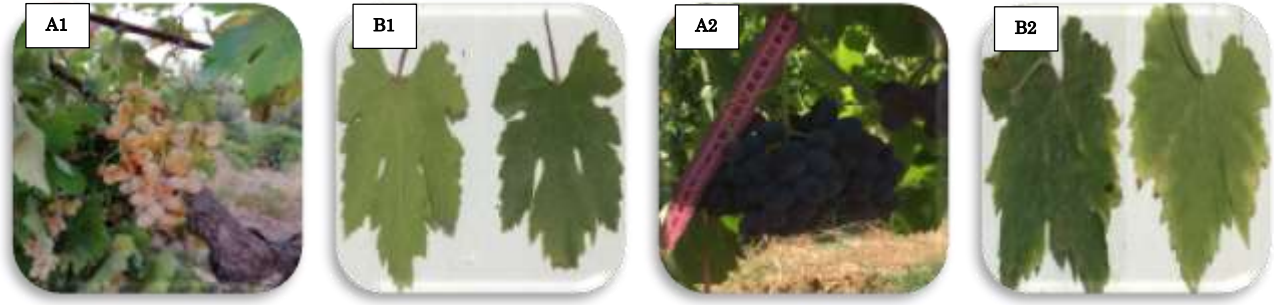
Şekil 5. Kokulu Üzüm salkım (A1) ve olgun yaprak (B1), Kirkit Üzümü salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure5. Images of Kokulu Üzüm cluster (A1) and mature leaf (B1), Kirkit Üzümü cluster (A2) and mature leaf (B2)



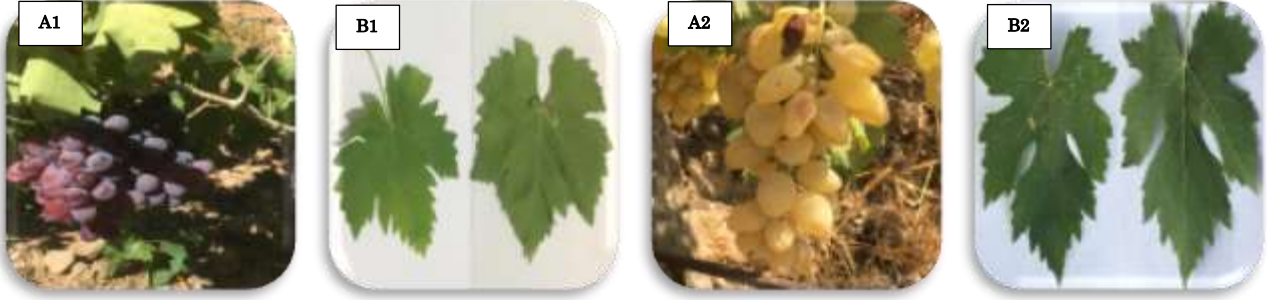
Şekil 6. Şeker Üzümü salkım (A1) ve olgun yaprak (B1), Yediveren salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure6. Images of Şeker Üzümü cluster (A1) and mature leaf (B1), Yediveren cluster (A2) and mature leaf (B2)



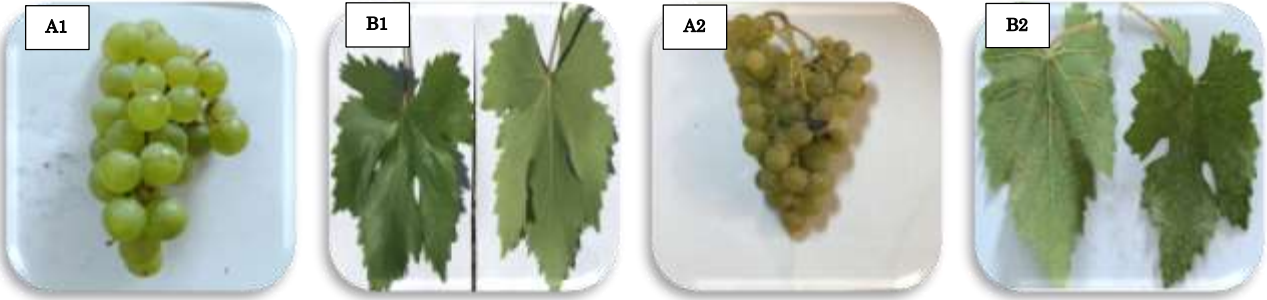
Şekil 7. Bandırma salkım (A1) ve olgun yaprak (B1), Pafi salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure7. Images of Bandırma cluster (A1) and mature leaf (B1), Pafi cluster (A2) and mature leaf (B2)



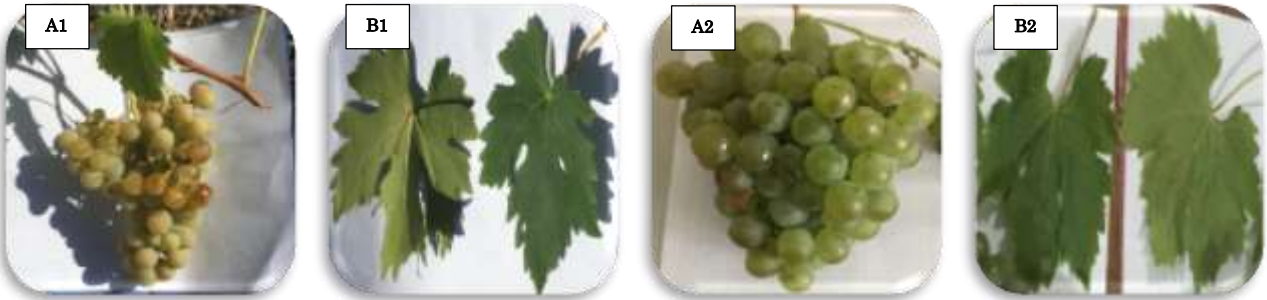
Şekil 8. Azezi salkım (A1) ve olgun yaprak (B1), Künefi salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure8. Images of Azezi cluster (A1) and mature leaf (B1), Künefi cluster (A2) and mature leaf (B2)



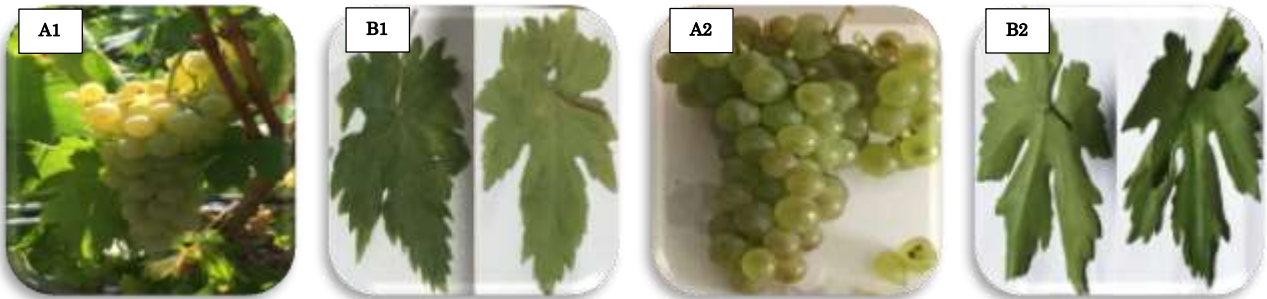
Şekil 9. Kirmızı Gaziantep Künefisi salkım (A1) ve olgun yaprak (B1), Sarı Sergilik salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure9. Images of Kirmızı Gaziantep Künefisi cluster (A1) and mature leaf (B1), Sarı Sergilik cluster (A2) and mature leaf (B2)



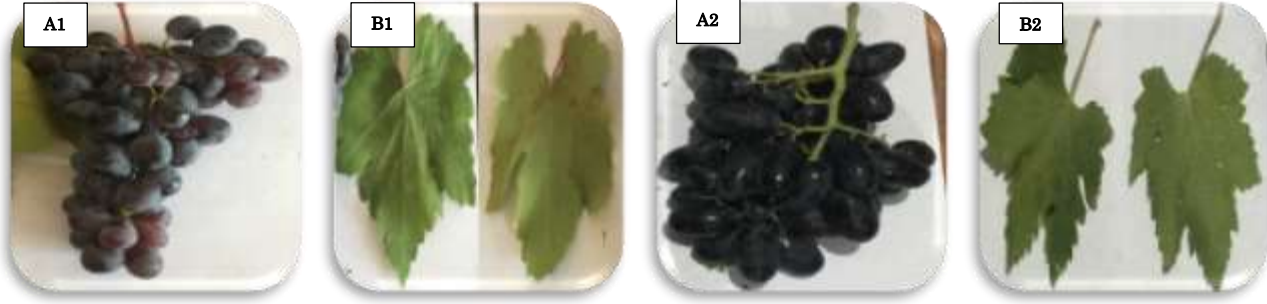
Şekil 10. Honi Kabarcık salkım (A1) ve olgun yaprak (B1), Tüylü Kabarcık salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure10. Images of Honi Kabarcık cluster (A1) and mature leaf (B1), Tüylü Kabarcık cluster (A2) and mature leaf (B2)



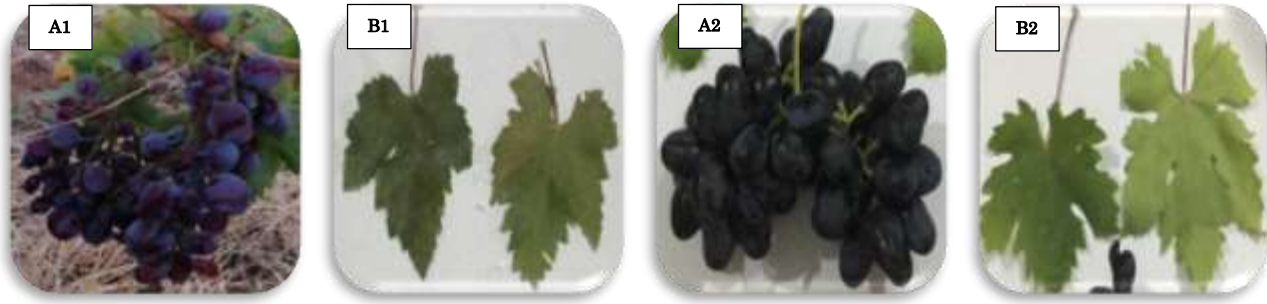
Şekil 11. Cırt Kabarcık salkım (A1) ve olgun yaprak (B1), İzmir Kabarcığı salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure11. Images of Cırt Kabarcık cluster (A1) and mature leaf (B1), İzmir Kabarcığı cluster (A2) and mature leaf (B2)



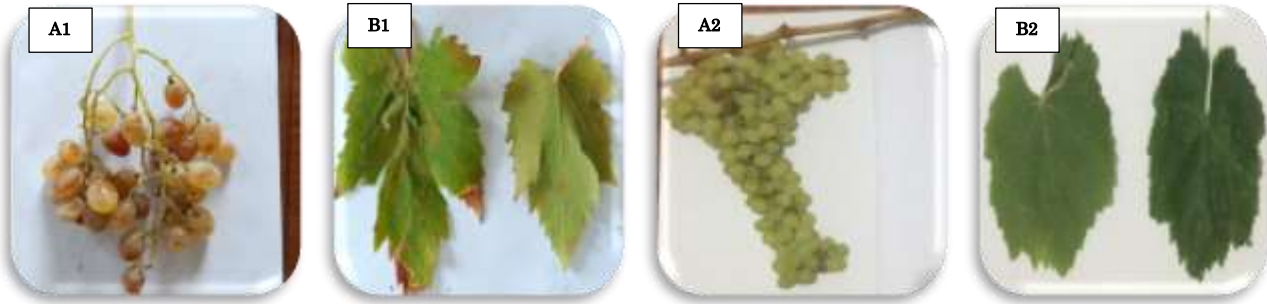
Şekil 12. Kızaran Kabarcık salkım (A1) ve olgun yaprak (B1), Sararan Kabarcık salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure12. Images of Kızaran Kabarcık cluster (A1) and mature leaf (B1), Sararan Kabarcık cluster (A2) and mature leaf (B2)



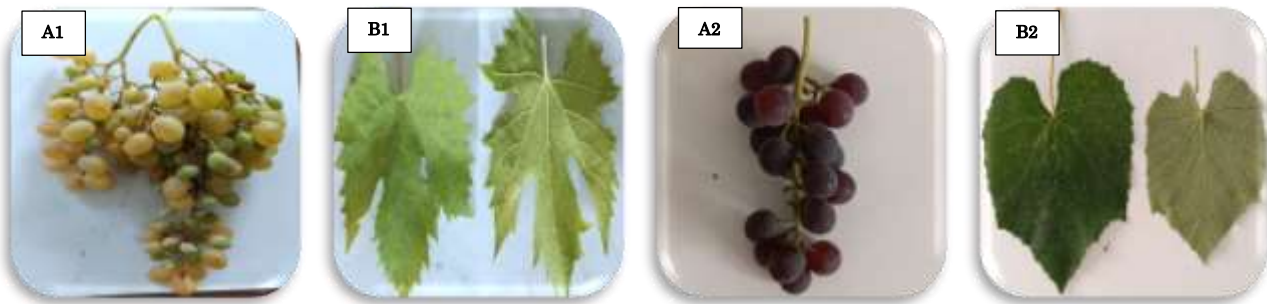
Şekil 13. Siyah Kabarcık salkım (A1) ve olgun yaprak (B1), Kilis Kara Sergilik salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure13. Images of Siyah Kabarcık cluster (A1) and mature leaf (B1), Kilis Kara Sergilik cluster (A2) and mature leaf (B2)



Şekil 14. Orak Karası salkım (A1) ve olgun yaprak (B1), Kara Sergilik salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure14. Images of Orak Karası cluster (A1) and mature leaf (B1), Kara Sergilik cluster (A2) and mature leaf (B2)



Şekil 15. Deve Gözü salkım (A1) ve olgun yaprak (B1), Sultani Çekirdeksiz salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure15. Images of Deve Gözü cluster (A1) and mature leaf (B1), Sultani Çekirdeksiz cluster (A2) and mature leaf (B2)



Şekil 16. Dökülgen salkım (A1) ve olgun yaprak (B1) ve Perpil salkım (A2) ve olgun yaprak (B2) görüntüleri
Figure16. Images of Dökülgen cluster (A1) and mature leaf (B1), Perpil cluster (A2) and mature leaf (B2)

Ayrıca Tekirdağ Bağcılık Araştırma Enstitüsü tarafından yayınlanan Türkiye Asma Genetik Kaynakları Kataloğunda Kahramanmaraş İli genelinde yetiştirilen çeşitler 'Bandırma, Kirkit, Kara Sergi, Ağ Sergi, Kabarcık, Ağmahrabaşı, Horoz Yüreği,

Devegözü, Mahrabaşı ve Azezi' olarak sıralanmıştır (Anonim, 2024). İl genelinde 1995-96 yıllarında yürütülmüş olan tek bir ampelografi çalışmasında ise 23 beyaz ve 10 renkli üzüm tespit edilmiştir. (Yalnkılıç, 1996). Yalnkılıç (1996) tarafından tespit

edilen Ata şansı, San yıldız, Miskiye, Kıbrıs, Hasani ve Yıldız çeşitleri mevcut çalışmada yer almamaktadır. Fakat bu çalışma sonucunda 7 farklı Kabarcık (Kızaran Kabarcık, Honi Kabarcık (Sinonim: Tosbağa Kabarcığı, Kuzucak Kabarcığı), Siyah Kabarcık, Sararan Kabarcık, Tüylü Kabarcık, Cırt Kabarcık, İzmir Kabarcığı) ve 3 farklı Mahrabaşı (Ağ Mahrabaşı, Kırmızılı Mahrabaşı, Kararan Mahrabaşı) çeşitleri benzer isimler altında fakat farklı üzüm çeşitleri olarak literatüre kazandırılmıştır. Perpil üzüm çeşitinin *Vitis labrusca* ve diğer çeşitlerinin *Vitis Vinifera*'ya ait olduğu tespit edilmiştir. Çiftçi ziyaretleri sonrasında yörede modern bağcılıkta kullanılan telli terbiye sistemleri gibi verim ve kaliteyi artırıcı unsurların çok sık kullanılmadığı gözlenmiştir. Modern bağcılıkta kullanılan sistemlerin çiftçiye tanıtılması önerilmektedir. Böylece hem bölge halkına hem de ülke ekonomisine katkıda bulunacağı düşünülmektedir. Ayrıca Dünyada ve Türkiye'de etkili olan filoksera zararlısının asmalara olan zararı hala devam ettiği düşünüldüğünde bu tür çalışmaların devamı, çeşitlerin tespiti ve korunmasına olan önemi bir kat daha artırmaktadır. Bu çalışmada kullanılan çoğu kriterlerin ekolojik faktörlere, üretilen bölgeye ve bakım koşullarına göre farklılıklar göstereceği bir gerçektir. Bu sebeple, çalışmada tespit edilen üzüm çeşitlerinin yeni nesil moleküler teknikler kullanılarak akrabalık ilişkilerinin belirlenmesi gerekmektedir.

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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

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Opuntia ficus-indica (L.) Mill. Bitkisinden Hazırlanan Farklı Ekstrelerin Antidiyabetik, Antitirozinaz, Antioksidan ve Sitotoksik Etkisinin Araştırılması

Leyla PAŞAYEVA^{1*}, Sena KICALI², Ayşe Kübra KARABOĞA ARSLAN³

^{1,2}Erciyes Üniversitesi, Eczacılık Fakültesi, Farmakognozi Anabilim Dalı, 38039, Kayseri, ³Erciyes Üniversitesi, Eczacılık Fakültesi, Farmakoloji Anabilim Dalı, 38039, Kayseri

¹<https://orcid.org/0000-0003-3860-7222>, ²<https://orcid.org/0009-0008-6179-6387>, ³<https://orcid.org/0000-0002-4689-0657>

✉: leylapasayeva@erciyes.edu.tr

ÖZET

Türkiye farklı iklim ve ekolojik koşullara sahip olması nedeniyle floranın çok sayıda bitki türü ve çeşidi içermesi bakımından doğadan toplanan ve kültürü yapılan tıbbi bitkiler açısından büyük bir ekonomik potansiyele sahiptir. Çalışmada *Opuntia ficus-indica* bitkisinin meyve kısmından farklı ekstraksiyon yöntemleriyle elde edilen ekstraktların antidiyabetik, antitirozinaz, antioksidan ve hücre canlılığı üzerine etkileri incelenmiştir. Bu amaçla meyve kısmı kurutulduktan sonra %70'lik metanol ile maserasyon ve ses dalgaları-destekli sıvı ekstraksiyonu yöntemleriyle hazırlanan ekstraktların α -amilaz, α -glikozidaz, tirozinaz inhibitör etkileri yanında antioksidan kapasiteleri DPPH ve ABTS yöntemleri ve hücre canlılığı üzerine etkileri ise RL95-2 ve A549 kanser hücrelerinde tayin edilmiştir. Sonuç olarak ekstraktlardan ses dalgaları-destekli sıvı ekstraksiyonu yöntemiyle hazırlanan ekstraktın α -amilaz ($IC_{50}=395.123\pm 3.477 \mu g ml^{-1}$) ve tirozinaz enzimi üzerinde inhibisyon etkisinin ($IC_{50}=551.633\pm 1.159 \mu g ml^{-1}$), ABTS radikal süpürücü aktivitesinin ($0.522\pm 0.041 \mu M Trolox/g_{ekstre}$) diğer ekstraktlardan daha yüksek olduğu görülmüştür. Ayrıca aynı ekstraktın toplam fenolik madde ve flavonoid miktarının da daha yüksek olduğu belirlenmiştir. Ekstraktların α -glikozidaz, antitirozinaz ve hücre canlılığı üzerine etkileri orta düzeyde bulunmuştur. Bu çalışmanın sonuçları ile özellikle ses dalgaları-destekli sıvı ekstraksiyonu yöntemiyle hazırlanan ekstraktın α -amilaz inhibitör etki ve antioksidan etkisinin hangi bileşiklerden kaynaklandığını ve etki mekanizmalarını belirlemek için daha ileri çalışmalara ihtiyaç duyulmaktadır.

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Anahtar Kelimeler

Dikenli incir,

Hipoglisemik etki

Hücre canlılığı

Investigation of the Antidiabetic, Antitirozinaz, Antioxidant, and Cytotoxic Effects of Different Extracts Prepared from *Opuntia ficus-indica* (L.) Mill. plant

ABSTRACT

The Flora of Türkiye contains many plant species and varieties due to its different climate and ecological conditions. It has great economic potential in terms of cultivated and natural medicinal plants. In this study, the effects of extracts obtained from the fruit part of the *O. ficus-indica* plant by different extraction methods were examined on antidiabetic, antityrosinase, antioxidant, and cell viability. For this purpose, after drying the fruits, the extracts were prepared with %70 methanol by maceration and ultrasound-assisted extraction methods. Then the α -amylase, α -glucosidase, and tyrosinase inhibitory effects as well as antioxidant capacities with DPPH and ABTS methods and cell viability of extracts were determined on RL95-2 and A549 cancer cells. As a result, it was shown that, the inhibitory effect of the extract prepared by ultrasound-assisted extraction method on α -amylase ($IC_{50}=395.123\pm 3.477 \mu g ml^{-1}$) and tyrosinase enzyme ($IC_{50}=551.633\pm 1.159 \mu g ml^{-1}$ and ABTS radical scavenging activity ($0.522\pm 0.041 \mu M Trolox/g_{extract}$) was found to be higher than the other extract. It was also determined that the total phenolic and flavonoid

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content of the same extract was higher ((181.189±4,576 mgGAE/extract and 125.635±1.946 mgCA/extract). The effects of the extracts on α -glucosidase and tyrosinase enzymes and cell viability were found to be moderate. In conclusion, further studies are needed to understand which compounds were related to α -amylase inhibitory effect and antioxidant activity and mechanisms of action.

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GİRİŞ

Diyabet dünya genelindeki başlıca sağlık sorunlarından biri olup insülin salgısındaki bozukluklardan, insülin etkisinden veya her ikisinden kaynaklanan hiperglisemi ile karakterize metabolik hastalıklardan biridir (Association, 2010; Petersmann ve ark., 2018). Diyabet tedavisinde temel yaklaşım yaşam tarzı değişikliği, artan fiziksel aktivite ve besin alınımına dikkat edilmesidir. Mevcut olan tedavi yaklaşımlarında oral antidiyabetik ajanlar, insülinler ve insülin analogları, sülfonilüreler, glinidler, biguanidler, glitazonlar (tiazolidindionlar) ve glikozidaz inhibitörleri bulunmaktadır (Skyler, 2004). Diyabet hastalarındaki temel amaç erken postprandial hiperglisemiyi azaltmak, geç postprandial hiperglisemiyi kontrol altına almaktır. Bu kontrolü sadece diyetle kontrol altına alamayan kişilerde karbonhidrat absorpsiyonunu yavaşlatacak ajanların etkili olması mümkündür. Bu nedenle gastrointestinal sistemdeki enzimlerin aktivitesini geçici olarak inhibe edebilen ilaçların postprandial glikoz yükselmeleri üzerinde etkili olması beklenir. Bu duruma bağlı olarak α -glikozidaz ve α -amilaz enzim inhibitörleri diyabetik hipergliseminin düzenlenmesinde etkili bir ilaç grubu olarak geliştirilmişlerdir. Bu grup ilaçlar, insülin sekresyonu ve insülin etkisi üzerinde doğrudan etkileri olmayıp, daha çok lokal etkileri ile glikozun absorpsiyonunu yavaşlatarak dolaylı yoldan hipergliseminin önlenmesine yardımcı olurlar (Skelin ve ark., 2010).

Tirozinaz melanin sentezinde yer alan bir enzimdir. Melanin sentezi yoluyla hidrojen peroksit ve hidroksil radikalleri gibi yüksek ara reaktif molekülleri üretilir. Bundan dolayı, tirozinaz inhibitörlerinin, antioksidan özellikleri ve anti-tirozinaz aktivitelerine dayanarak cilt beyazlatma ve cilt kanserinde kozmetik ve klinik olarak faydalı olabileceği düşünülmektedir. Ayrıca son zamanlarda yüksek tirozinaz inhibitörü aktivitesinin Parkinson hastalığının tedavisinde uygulanması da dikkat çekmektedir (Kaewnarin ve ark., 2016; Nanok & Sansenya, 2020).

İnsanlığın çeşitli rahatsızlıklarının tedavisinde tıbbi bitkileri kullanımının tarihi eskiye dayanmaktadır. Günümüzde bu tedavi yaklaşımlarının bilimsel temele

oturtulması amaçlı yapılan çalışmalar hız kazanmaktadır. Özellikle halk arasında kullanımı olan bitkiler bu açıdan önem taşımaktadır (Arıtuluk, 2012; Güven & Gülçin, 2020).

Dikenli incir (*O. ficus-indica* (L.) Mill.) Akdeniz ve Ege bölgelerinin özellikle kıyı şeridinde doğal olarak yetişebilme yeteneğine sahip olup Cactaceae familyasına ait bir bitkidir. Yetiştirildiği bölgeye göre "hint inciri, papaz yemişi, frenk inciri, dikenli incir ve babutsa" gibi farklı yerel isimlerle anılmaktadır. Dikenli incirin vatanı Meksika olmakla birlikte genel olarak Akdeniz Bölgelerinde yaygın olan ve günümüzde pek çok ülkede kendiliğinden ya da kültüre alınarak yetiştirilmektedir. Kurak iklimlerin yanında daha çok subtropik iklim özelliklerine sahip olan bölgelerde yaygın olarak görülmektedir. Dikenli incire yol kenarlarında, tarım arazilerinin ya da konutların etraflarında rastlama olasılığı oldukça yüksektir (Dumanoglu ve ark., 2020). *O. ficus-indica* bitkisinin doğal olarak yetiştiği iller arasında Mersin, Adana, Osmaniye yer almaktadır. Bitkinin halk arasında farklı kısımlarının spazm çözücü, yumuşatıcı, idrar söktürücü olarak kullanıldığı bilinmektedir. Çiçeklerinde gözlenen astrenjan özelliğinden dolayı kanamaları azaltmak için kullanımı mevcuttur. Dikenli incirin meyve ve gövdesinin geleneksel olarak diyabet tedavisinde, çiçeklerinin ise prostat tedavisinde halk tarafından kullanıldığı bildirilmiştir (Saenz, 2000).

Bu çalışma kapsamında *O. ficus-indica* bitkisinin meyve kısımlarından ekstre verimi ve fenolik bileşikleri daha yüksek oranda elde etmek amaçlı maserasyon ve ses dalgaları-destekli sıvı ekstraksiyonu metotları kullanılarak %70'lik metanol ekstresi hazırlanmış ve bu ekstrelerin α -amilaz, α -glikozidaz, tirozinaz enzimlerini inhibisyon etkisi, antioksidan kapasite ve sitotoksik etkisi incelenmiştir.

MATERYAL ve METOD

Bitkisel materyal ve ekstraksiyon

Çalışmada kullanılan *O. ficus-indica* bitkisinin meyve kısmı (OF) Mersin'in Silifke ilçesi civarından (Karahacılı köyü 165m) 2022 yılında toplanmıştır. Toplanan bitkisel materyal gölgeli ve havalı ortamda 1

hafta süreyle kurutulmuş deęirmen yardımıyla toz edildikten sonra %70'lik metanol ile oda sıcaklığında maserasyon (Atiya ve ark., 2023) ve ultrasonik banyoda %50 sonikasyon amplitudu ve 30 dk süreyle ses dalgaları-destekli sıvı ekstraksiyonu yöntemiyle (Gouws ve ark., 2019) ekstre edilmiştir. Ekstreler daha sonra süzgeç kâğıdı yardımıyla süzülerek çözücüsü Rotary evaporatörde 38 °C'de düşük basınç altında uçurulmuştur. Sonuç olarak maserasyon yöntemiyle OFM ekstresi, ve ses dalgaları-destekli sıvı ekstraksiyonu yöntemi ile OFU ekstresi elde edilmiştir. Kuruluğa kadar uçurulan ekstreler Labconco 7750020 cihazı kullanılarak -51°C sıcaklık ve 0,018 mBar basınç altında liyofilize edilerek çalışmalara bu ekstreler üzerinden devam edilmiştir. Çalışma anına kadar ekstreler -20°C'de saklanmıştır.

Enzim inhibitör aktivite testleri

Ekstrelerin α -amilaz ve α -glukozidaz inhibe edici etkisi Paşayeva ve ark. 2021 metoduna göre yapılmıştır (Paşayeva ve ark., 2021). Her iki deneyde akarboz referans madde olarak kullanılmış ve ekstreler olmaksızın kontrol hazırlanarak her deney üçlü tekrar halinde yapılmıştır. Çalışma kapsamında ekstrelerin tirozinaz enzimi inhibisyon etkisinin incelenmesi amacıyla hem OFM hem de OFU ekstrelerinin farklı konsantrasyonları hazırlanarak mikroplate 20 μ l olarak tatbik edilmiştir (Paşayeva ve ark., 2021). Daha sonra 20 μ l enzim çözeltisi (250 U/ml) ve 100 μ l fosfat tamponu çözeltisi (100 μ M, pH 6.8) eklenerek 25°C'de 10 dk inkübe edilmiştir. Devamında 20 μ l substrat çözeltisi (L-tirozin 3mM) ilave edilerek 30 dk daha inkübasyon yapılmıştır. Sonuçlar 492 nm'de mikroplak okuyucuda okunmuştur. Deneyde pozitif kontrol olarak kojik asit kullanılmıştır. Ekstreler olmaksızın kontrol hazırlanarak her deney üçlü tekrar halinde yapılmıştır.

Ekstrelerin kimyasal bileşimi

Toplam fenolik madde (TPC) ve toplam flavonoid miktarı (TFC)

Ekstrelerin içerdikleri toplam fenolik madde ve toplam flavonoid miktarı Fatullayev ve ark. (2023)'nın çalışmalarında kullandıkları metot kullanılarak yapılmıştır (Fatullayev ve ark., 2023). Ekstrelerin TPC miktarını belirlemek için 1 ml distile su içeren 25 mL'lik kap içerisine 1 ml örnek çözelti ve 1 ml Folin-Ciocalteu reaktifi ilave edilmiştir. 5 dakika karanlıkta bekletildikten sonra 10 mL %7'lik Na₂CO₃ 25 mL'ye su ile tamamlanmıştır. Kontrol olarak ekstre içermeyen reaktif karışımı kullanılmıştır. 25 °C'de 90 dk inkübe edildikten sonra 750 nm'de absorbansı ölçülmüş ve gallik asit kalibrasyon eğrisi ile karşılaştırılmıştır. Toplam fenolik madde miktarı gallik asite eşdeğer olarak hesaplanmıştır.

Çalışma kapsamında ekstrelerin TFC değerlerini belirlemek için, 1 mL ekstre t=0. dakikada 4 mL su ve

0.3 mL %5'lik NaNO₂ çözeltisi ile karıştırılmış ve 5 dakika bekletilmiştir. t=5. dakika'da 0.3 mL % 10'luk AlCl₃.6H₂O çözeltisi ilavesinden sonra, t=6. dakika'da 2 mL 1 M NaOH çözeltisi eklenmiş ve toplam hacim 10 mL'ye su ilave edilerek tamamlanmıştır. 510 nm'de köre karşı absorbans ölçülmüştür. Ekstrelerin içerdikleri toplam flavonoid miktarları katesine eşdeğer olarak mg CE/g_{ekstre} olarak hesaplanmıştır.

Antioksidan kapasite tayini

Bu çalışmada ekstrelerin antioksidan etkileri DPPH ve ABTS yöntemleri ile gerçekleştirilmiştir (Paşayeva ve ark., 2022).

Ekstrelerin DPPH (1,1-difenil-2-pikrilhidrazil) radikalini süpürücü etki tayini için 50 μ L numune çözeltisi, 950 μ L Tris-HCl tamponu (50 nM, pH 7,4) ve 1mL 0,1 mM metanolde hazırlanmış DPPH çözeltisi ile karıştırılmıştır. Kontrol olarak ekstre içermeyen reaktif karışımı ve pozitif kontrol olarak BHA kullanılmıştır. Oda sıcaklığında ve karanlıkta 30 dakika inkübe edildikten sonra absorbanslar 517 nm'de okunmuştur.

Çalışma kapsamında ekstrelerin ABTS (2,2'-azino-bis (3-etilbenzotiazolin-6-sulfonik asit) radikalini süpürücü kapasitesini belirlemek amacıyla ABTS radikali (7 mM), ABTS' in sulu çözeltisi ile K₂S₂O₈ (2.6 mM, son konsantrasyon)' un karanlıkta 12 saat bekletilmesiyle hazırlanmış ve absorbansı oda sıcaklığında 734 nm'de 1.1 \pm 0.02 olacak şekilde ayarlanmıştır. Bu şekilde hazırlanan radikal çözeltisi (2850 μ L) ile numune çözeltisi (150 μ L) karıştırılarak oda ısısında 30 dk. inkübe edilmiştir. Adsorbanslar 734 nm'de ölçülerek inhibisyon yüzdeleri Troloks'a eşdeğer olarak (TEAC) hesaplanmıştır.

Hücre kültürü

Çalışma kapsamında RL95-2 (ATCC, CRL-1671™) hücreleri %5 CO₂ ve 37 °C sıcaklıktaki inkübatörde inkübe edilerek, %10 (h/h) fetal sığır serumu (FBS), 200 mM L-glutamin, %1 (h/v) penisilin-streptomisin ve 0.005 mg/ml insülin içeren DMEM:F12 besiyeri ile A549 (ATCC, CCL-185™) ise %10 (h/h) FBS ve %1 (h/v) penisilin-streptomisin içeren F-12K hücre medyumunda çoğaltılmıştır. Çoğalmakta olan hücre pasajları, %70-80 doygunluğa ulaşıncaya yeniden pasajlanmıştır. Pasaj alınırken %0.25 tripsin-EDTA solüsyonu uygulanmış ve 3-4 dk. süreyle inkübatörde bırakılmıştır. Hücre sayım cihazı kullanılarak tripan mavisi ile hücre sayımı yapılmıştır (Karaboğa Arslan ve ark., 2018).

MTT (3-(4,5-dimetiltiazol-2-il)2,5-difenil tetrazolyum bromür) testi

Tetrazolyum bazlı kolorimetrik analiz olan MTT yöntemi, *in vitro* hücre canlılığının belirlenmesinde kullanılan bir sitotoksikite testidir (Ferrari ve ark.,

1990). Bu çalışmada OFM ve OFU ekstralarının RL95-2 (insan endometriyum kanser hücre hattı) ve A549 (insan akciğer kanseri hücre hattı) kanser hücrelerinin canlılığını azaltıcı etkisi MTT yöntemi ile belirlenmiştir. Bunun için öncelikle OFM ve OFU ekstraları DMSO içerisinde çözülerek stok çözelti oluşturulmuştur. Hücreler 10.000 hücre/kuyu olacak şekilde ekilmiş ve 24 saat sonra hücrelere OFM ve OFU ekstralarının farklı konsantrasyonları (0, 3, 10, 30, 45, 60, 90, 140, 180, 240, 300 ve 400 µg/ml) uygulanmıştır. Hücreler, 96'lı plakının her bir kuyusuna 0.5 g/ml MTT eklenerek 37°C'de 2 saat inkübe edilmiştir. Kuyucuklarda oluşan formazan tuzu kristallerini çözmek için 100 µL dimetilsülfoksit (DMSO) uygulanarak mikropilaya okuyucuda 570 nm'de absorbanlar ölçülmüştür. OFM ve OFU'nun uygulandığı kuyuların absorbanları kontrol absorbanına bölünerek % canlılık üzerinden hesaplama yapılmıştır.

İstatistiksel analiz

Hücre kültürü çalışma gruplarından elde edilen sonuçlar, GraphPad Prism 8.3.0 (San Diego-California, ABD) yazılımı kullanılarak analiz edilmiştir. Sonuçların analizinde, deney ve kontrol grupları arasındaki anlamlılığı saptamada farklılıklara uygun olarak tek yönlü varyans analizi ile istatistiksel farklılığa sahip parametreleri saptamada post-hoc test olarak Dunnet testi kullanılmıştır. Veriler, 3 ayrı deneyden hesaplanan ortalama ± ortalamanın standart hatası olarak verilmiştir.

Enzim inhibisyon ve antioksidan etki testlerinde ölçümler üçer kez yapıp sonuçlar ortalama ± standart sapma cinsinden ifade edilmiştir (Paşayeva ve ark., 2022).

BULGULAR ve TARTIŞMA

Enzim inhibitör aktivite test sonuçları

Ekstrelerin α-amilaz, α-glikozidaz ve tirozinaz enzimi inhibitör aktivitesi sonuçları Çizelge 1. ve Şekil 1'de verilmiştir. Tüm bulgular değerlendirildiğinde ekstralar arasında OFU ekstrasının α-amilaz (IC₅₀=395.123±3.477 µg ml⁻¹) ve tirozinaz enzimi üzerinde inhibisyon etkisinin (IC₅₀=551.633±1.159 µg ml⁻¹) diğer ekstreye göre daha yüksek olduğu belirlenmiştir. Çalışılan ekstralar birbiri arasında ve referans maddeler (akarboz ve kojik asit) ile istatistiksel olarak anlamlı bulunmuş (sırasıyla, P=0.049 ve P=0.041) ve ekstralardan hiçbirinin α-glikozidaz enzimi üzerinde inhibisyon etkisinin olmadığı görülmüştür.

TPC ve TFC sonuçları

Çalışma kapsamında iki farklı yöntemle hazırlanan ekstralardan ses dalgaları-destekli sıvı ekstraksiyonu ile hazırlanan OFU ekstrasının daha yüksek ekstre verimine sahip olduğu bulunmuştur (%35) (Çizelge 2.).

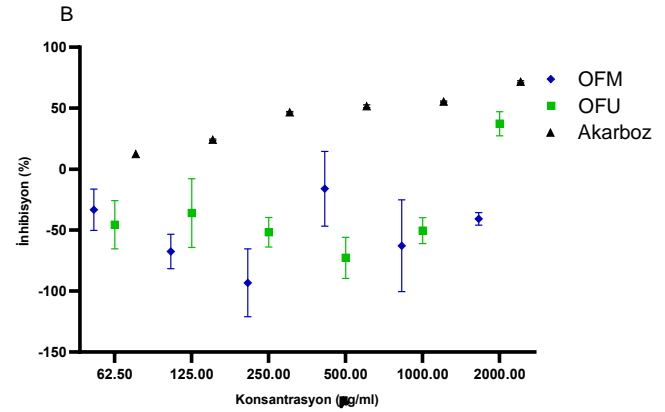
Bunun dışında ekstralardan OFU ekstrasında toplam fenolik madde ve flavonoid miktarının da sırasıyla 181.189±4.576 mg_{GAE}/g_{ekstre} ve 125.635±1.946 mg_{CA}/g_{ekstre} olduğu belirlenmiştir.

Çizelge 1. Ekstrelerin enzim inhibitör etkisine ait IC₅₀ değerleri

Table 1. IC₅₀ values of the enzyme inhibitory effect of extracts

Ekstreler	α-amilaz (IC ₅₀ µg/ml)	Tirozinaz (IC ₅₀ µg/ml)
OFU	395.123±3.477 ^a	551.633±1.159 ^a
OFM	802.101±2.128 ^b	741.267±2.285 ^b
Akarboz	228.367±2.318 ^c	-
Kojik asit	-	34.997±1.963 ^c

Değerler mean±SD (n=3) şeklinde ifade edilmiştir. Her sütunda farklı harflerle gösterilen değerler arasındaki farklılık istatistiksel olarak anlamlı bulunmuştur.



Şekil 1. OF ekstralarının α-glikozidaz inhibitör etkisinin tayini. Ortalama ± SS olarak verilen değerler ±%95 güven aralığında belirtilmiştir.

Figure 1. Determination of the α-glycosidase inhibitory effect of OF extracts. Values are expressed as the mean ± SS and stated within ±95% confidence

Antioksidan etki sonuçları

Bu çalışmada ekstraların antioksidan etkisi ABTS ve DPPH yöntemleri ile ölçülmüş ve sonuçlara göre OFU ve OFM ekstralarının yalnızca ABTS deneyinde birbirine yakın değerlerle (sırasıyla, 0.522±0.041 ve 0.555±0.028 µM_{Trolox}/g_{ekstre}) radikal süpürücü etki gösterdiği belirlenmiştir (Çizelge 2.). Ekstrelerin standart madde olarak kullanılan BHA ile istatistik olarak anlamlı olduğu görülmüştür (sırasıyla, P=0.026 ve P=0,032). Çalışma kapsamında ekstralardan hiçbirinin DPPH radikal süpürücü etki deneyinde etkili olmadığı belirlenmiştir (Şekil 2.).

MTT sonuçları

Çalışmada RL95-2 ve A549 hücrelerinde 48 saat sonra artan konsantrasyonlarda (0, 3, 10, 30, 45, 60, 90, 140, 180, 240, 300 ve 400 µg/ml) OFM ve OFU'nun canlılığa

etkisi değerlendirilmiştir. Sonuç olarak OFM ve OFU ekstraktlarının RL95-2 hücre canlılığını en yüksek konsantrasyon olan 400 µg/ml'de sırasıyla %73.33'e ve %74.98'e düşürdüğü görülmüştür (Şekil 3a.). Bunun

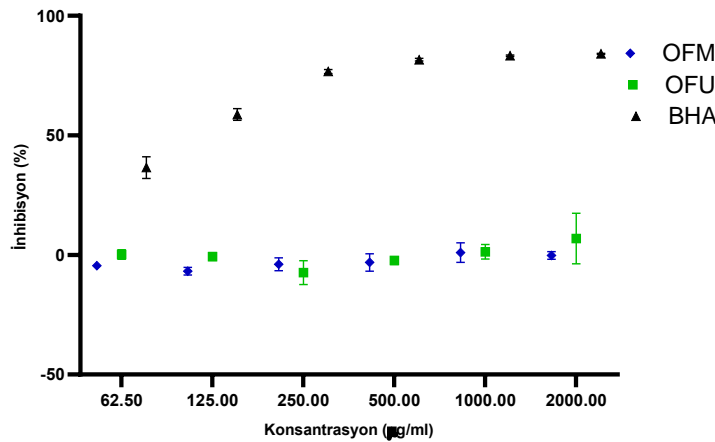
dışında ekstraktlardan OFM ekstraktının A549 hücre canlılığını en yüksek konsantrasyon olan 400 µg/ml'de %83.49'e, OFU ekstraktının ise %89.63'e düşürdüğü bulunmuştur (Şekil 3b).

Çizelge 2. Ekstrelerin antioksidan etki sonuçları

Table 1. Antioxidant effect results of extracts

Ekstreler	TPC (mg _{GAE} /g _{ekstre})	TFC (mg _{CA} /g _{ekstre})	ABTS (µM Trolox/g _{ekstre})	Ekstre verimi (%)
OFU	181.189±4.576	125.635±1.946	0.522±0.041 ^c	%35
OFM	156.471±0.908	111.734±0.554	0.555±0.028 ^{b,c}	%20
BHA	-	-	9.871±0.019 ^a	

Değerler mean±SD (n=3) şeklinde ifade edilmiştir. Her sütunda farklı harflerle gösterilen değerler arasındaki farklılık istatistiksel olarak anlamlı bulunmuştur.



Şekil 2. OF ekstraktlarının DPPH inhibitör etkisinin tayini.

Figure 2. Determination of the DPPH inhibitory effect of OF extracts.



Şekil 3. OFM ve OFU'nun 3-400 µg/mL arasındaki konsantrasyonlarının 48 saatlik inkübasyon süresinde (a) RL95-2 ve (b) A549 hücre canlılığına etkisi. Gruplar kontrole göre kat değişimi olarak verildi (n=3). Sonuçlar, ortalama ± ortalamanın standart hatası olarak sunuldu.

Figure 3. Effect of OFM and OFU concentrations between 3-400 µg/mL on (a) RL95-2 and (b) A549 cell viability during 48 hours of incubation period. Groups are presented as fold change compared to the control (n=3). Results are presented as mean ± standard error of the mean.

Literatürde *O. ficus-indica* bitkisinin farklı kısımları ile yapılan antidiyabetik etki çalışmaları bulunmaktadır. Yapılan bir çalışmada bitkinin tohumlarından elde edilen yağın alloksanla indüklenmiş diyabetik farelerde koruyucu etkisi incelenmiş ve yağın 2 mL/kg dozuyla tedavi edilen farelerde alloksan kaynaklı ölüm ve hiperglisemiyi önemli ölçüde azalttığı bulunmuştur (Berraaouan ve

ark., 2015). Bir başka çalışmada bitkinin meyve ve etli gövdesinden hazırlanan su ekstresi streptozotisin ile indüklenen yüksek yağlı diyetle beslenen diyabetik sıçanlara oral yolla verilmiş ve sonuç olarak ekstrenin kan glukoz düzeylerini önemli ölçüde azalttığı ve α-glikozidaz enzimini 67.33 µg/ml IC₅₀ değeri ile inhibe ettiği görülmüştür (Hwang ve ark., 2017). Diğer bir çalışmada *O. ficus-indica* bitkisinin kladodlarından

hazırlanan su ekstresi ve tescilli gövde/meyve kabuğu karışımının (75/25 oranında) kan glukoz düzeyleri ve plazma insulin seviyelerine etkisi araştırılmıştır. Sonuç olarak 6–176 mg/kg dozda oral yolla verilen su ekstresinin kan glukoz düzeylerini ve plazma insulinini azalttığı görülmüştür (Butterweck ve ark., 2011). Ydjedd ve ark. (2021) yaptığı bir çalışmada *O. ficus-indica* bitkisinin kırmızı ve sarı varyetelerinden elde edilen betalain ekstresinin α -amilaz inhibitör etkisi araştırılmış ve sarı varyetenin su ekstresi ve kırmızı varyetenin metanol ekstresinin güçlü inhibitör etkiye sahip olduğu bulunmuştur (sırasıyla, %57.28 ve %43.52). Bunun yanında yapılan farklı klinik çalışmalarda bitkinin gövdesinden hazırlanan preparatların tip 2 diyabetli hastalarda kan glukoz seviyelerini önemli ölçüde azalttığı kaydedilmiştir (Fрати ve ark., 1990; López-Romero ve ark., 2014). Diğer bir çalışmada bitkinin meyve kabuklarından farklı çözücüler ve ekstraksiyon yöntemleri kullanarak hazırlanan ekstrelerin anti-tirozinaz etkisi değerlendirilmiştir. Sonuç olarak Soxhlet yöntemi ve metanol ile elde edilen ekstrenin antitirozinaz etkisi %72 inhibisyon değeri ile diğerlerine göre daha yüksek bulunmuştur (Atiya ve ark., 2023). Tunus menşeli *O. ficus-indica* bitkisinin çiçeklerinden farklı çözücülerle elde edilen ekstrelerin tirozinaz enzimi üzerine inhibisyon etkisinin incelendiği bir çalışmada ise aseton ekstresinin diğer ekstrelerden daha etkili olduğu bulunmuştur ($IC_{50}=0.285 \pm 0.03$ mg/ml) (Masmoudi ve ark., 2024). Bu verilerden yola çıkarak OFU ekstresinin hipoglisemik etkisinin literatür verileri ile örtüştüğü halde anti-tirozinaz etkisinin literatür verilerinden daha düşük bulunmasının sebebinin ekstraksiyon yöntemi, bitkinin yetiştiği koşullar ve bitki türü ile ilişkili olduğu düşünülmektedir.

O. ficus-indica bitkisinin sitotoksik etkisinin incelendiği çalışmalar değerlendirildiğinde çeşitli kanser hücre hatlarında bitkinin farklı ekstrelerinin incelendiği ancak OFU ekstresinde de olduğu gibi sitotoksik etki bulunamadığı görülmüştür. Bir çalışmada Meksika *O. ficus-indica* bitkisinden elde edilen meyve suyunun MCF-7, PC-3 ve HepG2 kanser hücre hatlarında hücre canlılığını sırasıyla %7.6, %5.1 ve %12.6 oranda indirdiği görülmüştür (Chavez-Santoscoy ve ark., 2009). Serra ve ark. (2013) tarafından yapılan çalışmada Portekiz orijinli bitkinin meyve suyunun HT29 hücre hattında denenen bütün konsantrasyonlarda anti-proliferatif etki gösterdiği bildirilmiştir (%2-20) (Serra ve ark., 2013). Ayrıca *O. ficus-indica* sarı meyve kısımlarından elde edilen su ekstresinin insan kolon kanseri hücrelerinde canlılığı %50 oranında (Naselli ve ark., 2014), Mısır menşeli bitkinin meyve kabuğu ve etli kısmından hazırlanan etanol ekstresinin HepG2, Caco-2 ve MCF-7 kanser hücre hatlarında canlılığı artan konsantrasyonlarda indirdiği görülmüştür (El-Beltagi ve ark., 2019). Yapılan bir doktora tezi kapsamında *O. ficus-indica*

bitkisinden elde edilen meyve suyu, posası ve kladotları kimyasal içerik ve aktivite yönünden incelenmiştir. Çalışma kapsamında bitkiden elde edilen ana ekstre ve izole edilmiş bileşiklerin (flavonol aglikonu, izoramnetin glikoziti) antioksidan aktiviteleri, sitotoksik, antidiyabetik ve antiobezite etkileri çalışılmıştır. Sonuçlar olarak DPPH radikaline karşı hem ekstrenin hem de bileşiklerden izoramnetin glikozitinin antioksidan aktivite gösterdikleri, sitotoksik açıdan kullanımlarının doza bağlı olarak güvenli olduğu, antidiyabetik ve antiobezite etkilerinin ise istatistiksel olarak anlamlı çıktığı bulunmuştur (Keleş, 2023).

Literatürde dikenli incirin meyve kabuğu ve etli kısmıyla ilgili çok sayıda antioksidan çalışması bulunmaktadır. Genel olarak, dikenli incir meyvelerinin, süperoksit radikalleri, hidrojen peroksit, hidroksil radikalleri ve tekli oksijen dahil olmak üzere serbest radikal türlerine karşı yüksek bir antioksidan kapasite sergilediği bilinmektedir (Giraldo-Silva ve ark., 2023). Bununla ilgili yapılmış bir çalışmada bitkinin farklı lokasyonlardan toplanan meyvelerinden hazırlanan metanol ekstrelerinin DPPH radikal süpürücü etki değeri %52-59 arasında bulunmuşken (Belviranlı ve ark., 2019) bir başka çalışmada meyve kısmından elde edilen metanol ekstresinin DPPH radikal süpürücü etki değeri 6.70 μ M Troloks/g m.a., ABTS radikal süpürücü etki değeri ise 5.22 μ M Troloks/g m.a. olarak belirlenmiştir (Fernández-López ve ark., 2010). Ayrıca diğer bir çalışmada bitkinin meyve kısmından elde edilen metnaollü sulu ekstresinin 2.32-2.88 mg/ml oranında, bir başka çalışmada ise 0.5 mg/ml konsantrasyonda DPPH radikal süpürücü etkisi %71.6 olarak bulunmuştur (Ishtiaque ve ark., 2014; Siriwardhana ve ark., 2006). Bunun dışında El Mannoubi'nin (2021) yaptığı çalışmada sarı meyveden elde edilen aseton fraksiyonunun radikal süpürücü etkisi DPPH deneyi için 784 μ g/ml EC_{50} değeri olarak bulunduğu halde ABTS antioksidan etki deneyi için ise 40 μ g/ml olarak bulunmuştur. Bulunan bu sonuçların OFU ekstresinin antioksidan etkisini destekler nitelikte olduğu görülmektedir.

Antioksidan etkinin genellikle farklı ekstrelerde bulunan askorbik asit, tokoferol, glutatyon, karotenoidler ve fenolik bileşiklerle ilişkili olduğu bilinmektedir (Krishnaiah ve ark., 2011). Yapılan güncel literatür taraması sonucunda farklı ekstraksiyon yöntemlerinin kullanılmasının dikenli incir meyvelerinin TPC verimi üzerinde doğrudan etkisi olduğu kanısına varılmıştır (Cano ve ark., 2019; Zeghad ve ark., 2019). Şöyle ki yapılan bir çalışmada fırında kurutulmuş meyve kabuğunun TPC değerlerinin varyeteye bağlı olarak 1.7 ile 2.4 gGA/100g_{ekstre} arasında değiştiği görülmüştür (Bourhia ve ark., 2020). Bir başka çalışmada yüksek hidrostatik basınca (HHP) maruz bırakılan Meksika meyvesinin kabuk ve etli kısmının TPC değerinin 11

ila 4 gGA/g_{ekstre} (Gómez-Maqueo ve ark., 2020), Meksika yeşil ve kırmızı meyve kabuklarının ise 648 ila 734 mg GA/100 g_{ekstre} arasında değişen TPC değerlerine karşılık geldiği görülmüştür (Manzur-Valdespino ve ark., 2020). Dört farklı kurutma tekniğinin (dondurarak kurutma, mikrodalgada kurutma, 35 ve 55°C'de fırında kurutma) kullanıldığı bir çalışmada ise Avustralya dikenli incirinin meyve kısımları kullanılmış ve sonuç olarak dondurarak kurutma tekniğinin sonucunda en yüksek TPC değeri elde edildiği gösterilmiştir (Gouws ve ark., 2019). Yapılmış olan bu çalışmada *O. ficus-indica* türünün ses dalgaları destekli-ekstraksiyon yöntemi ile hazırlanan ekstresinin hem fenolik madde hem de flavonoid içeriğinin zengin olduğu, dolayısıyla da bu bitkinin meyvelerinden fenolik ve flavonoid bileşiklerini elde etmek için en uygun ekstraksiyon yönteminin ses-dalgaları destekli ekstraksiyon olduğu düşüncesine varılmıştır. Elde edilen sonuçlara göre OFU ve OFM ekstresinin ABTS antioksidan testinde daha iyi etki gösterirken DPPH testinde bu etkinin görülememesi ekstrelerin sadece hidrofobik antioksidan sistemlerinde değil, aynı zamanda hidrofilik antioksidan sistemlerinde de değerlendirilebileceğini göstermekle birlikte bu etkinin sadece flavonoidlerden değil flavonoid yapısında olmayan fenolik bileşiklerden kaynaklandığını da ortaya koymaktadır (Floegel ve ark., 2011). Tüm bu verilerden yola çıkarak özellikle halk arasında yaygın olarak kullanımı olan dikenli incirden hazırlanan ekstreler için daha ileri çalışmaların yapılması ve standartize ekstresinin elde edilmesi gerektiği düşünülmektedir.

SONUÇ ve ÖNERİLER

Yapılan çalışma kapsamında *O. ficus-indica* bitkisinin meyve kısmından 2 farklı ekstraksiyon yöntemiyle hazırlanan metanol ekstrelerinin hipoglisemik, antioksidan ve hücre canlılığı üzerine etkileri yanında fenolik madde ve flavonoid içeriği incelenmiştir. Ekstrelerden ses dalgaları-destekli sıvı ekstraksiyonu yöntemiyle hazırlanan ekstrenin daha yüksek miktarda fenolik bileşik taşıdığı (181.189±4.576 mg_{GAE}/g_{ekstre}) görülmüş, aynı ekstrenin α -amilaz (IC₅₀=395.123±3.477 μ g ml⁻¹) ve tirozinaz enzimini inhibe edici etkisinin (IC₅₀=551.633±1.159 μ g ml⁻¹) ve özellikle ABTS yönteminde antioksidan kapasitesinin 0.555±0.028 μ M_{Trolox}/g_{ekstre} değeri ile daha yüksek olduğu bulunmuştur. Böylelikle ses dalgaları-destekli sıvı ekstraksiyonu yöntemiyle hazırlanan ekstrenin daha yüksek miktarda fenolik bileşik taşıması ve hipoglisemik ve antioksidan etkinin de fenolik bileşiklerden kaynaklandığı düşünülmekle birlikte bu bileşiklerin belirlenip standartizasyon çalışmalarının yapılmasına ve etki mekanizmalarının aydınlatılmasına önayak olacağı düşünülmektedir.

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Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Türkiye ve Kırgızistan Yerel Sanayilik Domates Genotiplerinin Morfolojik ve Agronomik Özelliklerinin Belirlenmesi

Zülfiye YARDIM¹, Rufeyde Nur ÖZEN², Esen BOZABA³, Banu Çiçek ARI⁴, Mustafa PAKSOY⁵

¹Selçuk Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Konya/TÜRKİYE, ²Selçuk Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Konya/TÜRKİYE, ³Selçuk Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Konya/TÜRKİYE, ⁴Selçuk Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Konya/TÜRKİYE, ⁵Selçuk Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Anabilim Dalı, Konya/TÜRKİYE
¹<https://orcid.org/0009-0006-5460-1196>, ²<https://orcid.org/0009-0003-8538-5973>, ³<https://orcid.org/0009-0002-0517-159X>,
⁴<https://orcid.org/0000-0002-1578-8561>, ⁵<https://orcid.org/0000-0002-6852-8659>

✉: yardimzulfiye@gmail.com

ÖZET

Bu çalışma, S2 kademesindeki Kırgızistan ve Türkiye yerli domates genotiplerinin UPOV kriterlerine göre morfolojik ve agronomik özelliklerinin belirlenmesi amacıyla yapılmıştır. Çalışmanın temeli 2022 yılında atılmış 14 genotip ile çalışmaya başlanmış, bütün genotipler ayrı ayrı incelenmiştir. Yapılan incelemeler sonucunda, 2 adet Kırgızistan ve 2 adet Türkiye kökenli sanayi domatesi ümit var olarak tespit edilmiştir. Çalışmada seçilmiş genotiplerde bazı bitki ve meyve özellikleri incelenmiştir. Araştırma sonucunda genotipler arasında meyve olgunluk rengi bakımından önemli farklılıklar bulunmamıştır. Genotiplerin ortalama yaprak uzunluğu değerleri 23.3 cm (KR202) ile 25 cm (TR123-1) arasında, ortalama yaprak genişliği ise 17.5 cm (TR95) ile 22.3 cm (KR207) arasında değişmiştir. Genotiplerin ikisi sırk ikisi de oturak tip olarak belirlenmiştir. TR123-1, KR202 ve KR207 genotiplerinde fide döneminde antosiyanin gözlemlenmiş, ancak TR95’de gözlemlenmemiştir. Fide döneminde sap kırılması yalnızca üç genotipde görülmüştür. TR95 ve KR207 numaralı genotiplerde meyvede ortalama parlaklık az ve gövdede tüylülük orta seviyede iken TR123-1 ve KR202 numaralı genotiplerde meyvede parlaklık ve gövdede tüylülük yoğun şekilde gözlemlenmiştir. KR207 genotipi 5.116 kg bitki⁻¹ verim değeriyle en yüksek bitki başına verimi vermiştir. Yapılan değerlendirmeler sonucunda bu genotiplerin yeni çeşit geliştirmek için ümit var olduğu söylenebilir.

Bahçe Bitkileri

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UPOV

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Determination of Morphological and Agronomic Characteristics of Local Industrial Tomato Genotypes of Turkey and Kyrgyzstan

ABSTRACT

This study was carried out to determine the morphological and agronomic characteristics of Kyrgyzstan and Turkey native tomato genotypes at the S2 stage according to UPOV criteria. The study laid its foundation in 2022. The study started with the 14 genotypes; all genotypes were examined separately. As a result of the examinations, two industrial tomatoes of Kyrgyzstani origin and two industrial tomatoes of Turkish origin were determined to be promising. In the study, some plant and fruit characteristics were examined in selected genotypes. As a result of the research, no significant differences were found between genotypes in terms of fruit ripeness or colour. The average leaf length values of the genotypes varied between 23.3 cm (KR202) and 25 cm (TR123-1), and the average leaf width varied between 17.5 cm (TR95) and 22.3 cm (KR207). The plant growth type of the genotype was determined to be two-pole and two-seated. Anthocyanin was observed in seedling stages in TR123-1, KR202, and KR207, but not in TR95. Stem breakage during the seedling period was observed in three genotypes. In genotypes numbered TR95 and KR207, the average brightness of the fruit was low and the hairiness

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of the stem was moderate, while in genotypes numbered TR123-1 and KR202, the glossiness of the fruit and hairiness on the stem were intense. The KR207 genotype gave the highest yield per plant, with a yield value of 5.116 kg plant⁻¹. As a result of the evaluations, it can be said that there is hope for developing new varieties of these genotypes.

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GİRİŞ

Çin Halk Cumhuriyeti, yıllık 68 341 799.62 ton domates üretimi ile dünyanın en çok domates üreten ülkesi konumundadır. Hindistan 20 694 000 ton üretimi ile Çin Halk Cumhuriyetinden sonra yerini almaktadır. Türkiye, yıllık 13 milyon ton üretim ile üçüncü sırada yer almaktadır (FAO, 2022). Domatesin anavatanı Türkiye olmamasına rağmen, Türkiye’de Karadeniz Bölgesi’nin çok yağışlı olan bölgeleri dışındaki her yerde domates yetiştirilebilmektedir. Özellikle Marmara, Ege ve Akdeniz Bölgeleri’nde büyük ölçeklerde domates yetiştirilmektedir (Vural ve ark., 2000).

Türkiye’de üretilen domateslerin %3-4’lük bir kısmı yurtdışına ihraç edilmektedir. Yüzde yirmisi endüstriyel alanda kullanılmakta, geri kalan kısmı ise doğrudan tüketilmekte ya da güneşte kurutulmuş domates vb. ürünlere işlenmektedir. Sanayi domates yetiştiriciliğinde Marmara Bölgesinde ve Ege Bölgesi ilk sıralarda yer almaktadır (Günay, 1992; Vural ve ark, 2000).

Tüm bitki türlerinde olduğu gibi domateste de değişen talepler doğrultusunda yeni ıslah programlarının geliştirilmesi bir zorunluluk haline gelmiştir. Islah programlarında üretici ve tüketici taleplerine uygun yeni çeşitler ortaya çıkarırken, diğer taraftan bu üstün çeşitlerle rekabet şansı düşük olan yerel gen kaynaklarının yok olmasına neden olmaktadır (Altıntaş ve ark., 2016). “Ege Tarımsal Araştırma Enstitüsü (ETAE) Bitki Genetik Kaynakları Bölümü Gen Bankası’nda ülkemizin değişik yerlerinden toplanmış 80’den fazla genotip muhafaza edilmektedir (Oğuz, 2010). Bitki gen kaynaklarının karakterizasyon çalışmaları yapılarak değerlendirilmesi gerekmektedir (Bliss, 1981).

Sebze ıslahında, agronomik özelliklerin oluşturduğu genetik varyasyon önem taşımakta, genotipler arasındaki farklılıklar ile populasyonların genetik özelliklerinin belirlendiği çalışmaların yapılması önemlidir (Balkaya ve ark., 2010; Bozokalfa & Eşiyok, 2010). Bu açıdan domates yerel gen kaynakları usulüne uygun olarak toplanmakta ve kayıt ve koruma altına alınmaktadır. Bitkisel gen kaynaklarının korunması ve ıslah çalışmalarında etkili bir şekilde kullanılmasında ki önemli başarı, materyalin cins ve

türlerine göre gruplandırılmasıyla birlikte genetik ve agronomik özelliklerin belirlenmesine materyaldeki genetik değişimin izlenmesine ve kullanım için gerekli özelliklerin belirlenmesine bağlıdır (Kayak, 2017). Domatesin en dikkat çekici morfolojik karakterizasyonları; meyve şekli, meyve sıklığı, meyve et rengi veya meyve suyu pH’sı gibi özelliklerdir (Altıntaş ve ark, 2016). Araştırmacılar amaçları doğrultusunda “Yeni Bitki Çeşitlerini Koruma Birliği (The International Union for the Protection of New Varieties of Plants = UPOV)” kriterlerinde çeşitli modifikasyonlar yaparak çalışmaktadırlar (Kurt, 2019).

Bu çalışmanın amacı Türkiye’nin ve Kırgızistan’ın farklı yerlerinden toplanan ve iki kademe kendilenmiş sanayilik domates tipindeki genotiplerin bazı agronomik özelliklerinin belirlenmesidir.

MATERYAL ve METOT

Materyal

Şekil 1’de Türkiye ve Kırgızistan’dan toplanan yerel domates genotiplerine ait görseller verilmiştir. Bu çalışmada bitki materyali olarak; Kırgızistan’dan 2 yerel genotip, Türkiye’den 2 yerel genotip olmak üzere toplam 4 adet domates genotipi kullanılmıştır.

Metot

Çalışmada kullanılan genotiplerin tohumları 13 Nisan 2023 tarihinde torf ile doldurulmuş 70 ml kök hacmine sahip viyollere ekilmiştir. Tohumların çimlenmesinden fideler dikim büyüklüğüne gelene kadar her türlü bakım işlemi eksiksiz yerine getirilmiştir. Deneme arazisi tınlı yapıda, pH’ sı 7.51 (hafif alkalin), tuzsuz (199 $\mu\text{S cm}^{-1}$), çok fazla kireçli (%39.4), organik madde ve inorganik azot miktarı azdır. Ayrıca toprakta az miktarda N, P, K ve Fe elementleri bulunmaktadır. Gübrelemede Azot (N) 15 kg da⁻¹, Fosfor (P) 8 kg P₂O₅ da⁻¹, Potasyum (K) 15 kg K₂O da⁻¹ dozlarında damlama sulama ile uygulanmıştır. Demir gübrelemesi olarak demir sülfat (%19 Fe) 4 kg da⁻¹ olacak şekilde ikinci çapayla toprağın 8-10 cm derinliğine karıştırılmıştır. Dikim büyüklüğüne gelen fideler 22 Mayıs 2023 tarihinde Selçuk Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü’ne ait araştırma arazisinde, sıra arası 1 m,

sıra üzeri 66 cm olacak şekilde açık araziye her genotipten on iki fide olacak şekilde dikilmiştir. Fide dikiminden sonra sulama damla sulama ile yapılmış ve vejetasyon dönemi içinde çapalama, boğaz doldurma, gübreleme, hastalık ve zararlılarla

mücadele gibi kültürel işlemler düzenli yapılmıştır (Vural ve ark, 2000). Bitkilerde dikimden 1 ay sonra ise görülen Fusarium hastalığına karşı ise mancozeb etkili bir ilaç ile mücadele yapılmıştır.



Şekil 1. Türkiye ve Kırgızistan'dan toplanan yerel domates genotipleri
Figure 1. Local tomato genotypes collected from Türkiye and Kyrgyzstan

Bitki gelişimi döneminde yaprak, çiçek, meyve özellikleri Uluslararası Yeni Bitki Çeşitlerini Koruma Birliği (UPOV) özellik belgesine göre genotiplerin morfolojik özellikleri belirlenmiştir (Upov, 2019). Her genotipi temsil eden 3 adet meyve seçilerek gözlem, ölçüm ve analizler yapılmıştır. Çizelge 1'de sanayilik domates popülasyonunun morfolojik karakterizasyonunda kullanılan kriterler verilmiştir.

Genotiplerin yaprak uzunluğu (cm) ve genişliği (cm) bir cetvel yardımı ile ölçülmüştür. Çekirdek evi (Karpel) sayısı adet olarak belirlenmiştir, SÇKM (%) miktarı kırmızı olum döneminde hasat edilen meyvelerden elde edilen meyve suyunun refraktometre yardımı ile ölçülmesiyle belirlenmiştir. Meyvelerin pH miktarı elde edilen meyve sularından pH metre yardımıyla ile tespit edilmiştir. Meyve eni (mm), meyve boyu (mm), kaliks boyu (mm), kaliks eni (mm), perikarp kalınlığı (mm), meyve çekirdek evi büyüklüğü (mm), meyve sapı büyüklüğü (mm) ve çiçek burnu büyüklüğü (mm) bir kumpas yardımı ile ölçülmüştür. Meyve eti sertliğine bakılırken, meyvelerinin kabuğu kaldırılıp ve meyve etinin üzerine dijital penetrometre ile bastırılarak belirlenmiştir. Bitkilerden elde edilen bütün meyveler bir terazi yardımı ile tartılarak verim (kg bitki⁻¹) olarak bulunmuştur. Meyvelerde renk ölçümü D25A-9 model hunter marka renkölçer ile üç meyvede orta bölgeden birer okuma şeklinde yapılmış ve L, a ve b olarak belirlenmiştir.

İstatistik Analizler

Çalışma sonucunda elde edilen veriler, IBM SPSS Statistics 27 istatistik paket programı kullanılarak istatistiksel olarak değerlendirilmiş ve varyans analizine tabi tutulmuş genotiplerin birbirleri arasındaki farklılıklar Duncan testi ($P \leq 0.05$) ile belirlenmiştir.

BULGULAR ve TARTIŞMA

Meyve Özelliklerine Ait Bulgular

Çizelge 2'de domates genotiplerinin meyve boyu ve meyve eni değerleri arasındaki istatistiksel farklılıklar belirlenmiştir. Meyve boyu değerleri KR207 (75.51 mm) ve KR202 (70.15 mm) genotipleri ilk grubu oluşturmuş, TR123-1 genotipi son grupta yer almıştır. Kuzucu ve ark. (2004) sanayi domates tipindeki Uno'da meyve boyunun 5.11 cm ve Rio Grande'de ise 6.42 cm olduğunu bildirmişlerdir. Meyve eni değerleri bakımından TR123-1 (74.25 mm) en yüksek değere sahipken, KR207 (48.19 mm) en düşük meyve enine sahip olmuştur. Genotipler arasında TR123-1 numaralı genotipin basık meyve yapısı ile diğer genotiplere göre meyve eninin daha geniş olduğu gözlemlenmiştir (Çizelge 2). Levent ve ark. (2015) yaptıkları çalışmada domates meyve genişliğinin 73 mm olduğunu bildirmişlerdir. Bu çalışma elde edilen bulguları destekler niteliktedir.

Çizelge 1. Sanayilik domates popülasyonunun morfolojik karakterizasyonunda kullanılan kriterler
Table 1. Criteria used in the morphological characterization of the industrial tomato population

Kriterler	Sınıf değeri	Sınıf puanı	Kriterler	Sınıf değeri	Sınıf puanı	
Meyve olgunluk rengi	Beyaz	1	Çiçek rengi	Çok büyük	9	
	Sarı	2		Sarı	1	
	Turuncu	3		Turuncu	2	
	Pembe	4		Yarı dik	3	
	Kırmızı	5		Yatay	5	
	Kahverengi	6		Yarı sarkık	7	
	Yeşil	7				
Meyve boyuna kesit şekli	Düzleştirilmiş	1	Bitki gelişim şekli	Sırk	1	
	Yassı	2		Oturak	2	
	Dairesel	3	Meyve kabuk rengi	Turuncu	1	
	Dikdörtgen	4		Beyaz	2	
	Silindirik	5		Kırmızı	3	
	Eliptik	6		Sarı	4	
	Cordate	7		Pembe	5	
	Oval	8	Fide dönemi antosiyanin	Var	1	
	Obovate	9		Yok	2	
	Pyriform	10	Bitki büyüme gücü	Az	1	
Çok zayıf	1	Orta		2		
Zayıf	2	Çok		3		
Meyve sapında damarlanma	Orta	3	Yaprak yeşil rengin yoğunluğu	Açık	1	
	Güçlü	4		Orta	2	
	Çok güçlü	5		Yoğun	3	
	Meyve sapında çukur	Çok zayıf	1	Yaprak ayası	Var	1
		Zayıf	3		Yok	2
Orta		5	Yaprak parlaklığı	Az	1	
Güçlü		7		Orta	2	
		Yoğun		3		
Meyve çiçek burnu şekli	Girintili	1	Meyve sapı kırılması	Var	1	
	Düz girintili	2		Yok	2	
	Düz	3	Meyve olgunluğundan önce yeşil yakalılık	Çok küçük	1	
	Sivrimsi	4		Küçük	3	
	Sivri	5		Orta	5	
Meyve parlaklığı	Zayıf	1	Büyük	7		
	Orta	2	Gövdede tüylülük	Az	1	
	Güçlü	3		Orta	2	
	Çok küçük	1		Yoğun	3	
Meyve boyutu	Küçük	3				
	Orta	5				
	Büyük	7				

Çizelge 2. Domates genotiplerinde meyve eni ve meyve boyu (mm)
Table 2. Fruit width and fruit length of tomato genotypes (mm)

Genotipler	N	Meyve boyu (mm) (Ortalama±SH)	Standart sapma	Meyve eni (mm) (Ortalama±SH)	Standart sapma
TR95	3	59.85±0.11 ^b	0.19	52.96±0.28 ^b	0.49
TR123-1	3	45.98±1.44 ^c	2.50	74.25±1.70 ^a	2.95
KR202	3	70.15±3.43 ^a	5.94	55.23±0.69 ^b	1.21
KR207	3	75.51±3.44 ^a	5.97	48.19±1.65 ^c	2.86
P değeri		0.001		0.001	

P ≤ 0.05 düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir (P ≤ 0.05)
Different letters (a,b,c) shown in the same column indicate statistically significant differences (P ≤ 0.05)

Domates genotiplerine ait kaliks boyu, kaliks eni ve perikarp kalınlığı değerleri Çizelge 3'de sunulmuştur. Genotiplerin kaliks eni ve kaliks boyu değerlendirildiğinde, KR202 (11.55 mm) genotipinin diğer genotiplere göre kaliks boyunun daha kısa olduğu tespit edilmiştir. Kaliks eninde ise genotipler arasında önemli farklılıklar bulunmamıştır ($P=0.689$). Çalışmada en yüksek perikarp kalınlığına KR202

(8.54 mm) ve KR207 (7.35 mm) genotiplerinin sahip olduğu tespit edilmiş en düşük perikarp kalınlığına ise TR123-1 (4.28 mm) genotipinin sahip olduğu gözlemlenmiştir (Çizelge 3). Benzer bir çalışmada Demir ve Ünlü (2023) hatların meyve eti kalınlığını ortalama en düşük 6.81 mm ve en yüksek 10.54 mm olarak belirlemişlerdir.

Çizelge 3. Domates genotiplerine ait kaliks boyu, kaliks eni ve perikarp kalınlığı değerleri (mm)
Table 3. Calyx width, calyx length and pericarp thickness values of tomato genotypes (mm)

Genotipler	N	Kaliks boyu (mm) (Ortalama±SH)	Standart sapma	Kaliks eni (mm) (Ortalama±SH)	Standart sapma	Perikarp kalınlığı (mm) (Ortalama±SH)	Standart sapma
TR95	3	14.22±0.14	0.25	3.36±0.26	0.46	6.73±0.58 ^b	1.01
TR123-1	3	14.67±0.92	1.59	3.60±0.25	0.44	4.28±0.36 ^c	0.63
KR202	3	11.55±0.99	1.72	3.78±0.36	0.63	8.54±0.12 ^a	0.22
KR207	3	13.52±0.60	1.04	4.03±0.60	1.05	7.35±0.23 ^{ab}	0.41
P değeri		0.074	Ö.D.	0.689	Ö.D.	0.001	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)
Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Çizelge 4'de domates genotiplerine ait bazı ölçümler yer almaktadır. Çalışmada TR123-1 genotipi 50.01 mm ile en büyük meyve çekirdek evi büyüklüğüne sahiptir. Meyve çekirdek evi büyüklüğü tohum üretimi için önemli bir özellik taşıdığı için, bu genotipin diğer genotiplere göre tohum üretiminde daha yüksek verim sağlayacağı öngörülmektedir. Çalışmada meyve sapı

büyüklüğüne bakıldığında en uzun meyve sapına TR123-1 (8.64 mm) genotipinin sahip olduğu gözlemlenmiştir. Genotiplerin çiçek burnu büyüklüğüne bakıldığında ise en büyük çiçek burnu büyüklüğü TR123-1 (11.32 mm) genotipinin en küçük çiçek burnu büyüklüğü ise KR207 (0.89 mm) genotipinde belirlenmiştir (Çizelge 4).

Çizelge 4. Domates genotiplerine ait çekirdek evi büyüklüğü, meyve sapı büyüklüğü ve çiçek burnu büyüklüğü özellikleri (mm)
Table 4. Seed chamber size, fruit stem size and flower nose size characteristics of tomato genotypes (mm)

Genotipler	N	Çekirdek evi büyüklüğü (mm) (Ortalama±SH)	Standart sapma	Meyve sapı büyüklüğü (mm) (Ortalama±SH)	Standart sapma	Çiçek burnu büyüklüğü (mm) (Ortalama±SH)	Standart sapma
TR95	3	25.00±0.64 ^b	1.11	7.82±0.35 ^{ab}	0.61	1.68±0.08 ^b	0.13
TR123-1	3	50.01±0.53 ^a	0.93	8.64±0.25 ^a	0.44	11.32±0.25 ^a	0.43
KR202	3	24.46±1.71 ^b	2.97	6.25±0.33 ^c	0.57	1.04±0.05 ^c	0.10
KR207	3	24.13±1.00 ^b	1.74	6.96±0.13 ^{bc}	0.23	0.89±0.04 ^c	0.07
P değeri		0.001		0.002		0.001	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)
Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Çizelge 5'te, domates genotiplerinin meyvelerindeki SÇKM ve pH değerleri sunulmuştur. Çalışmada kullanılan genotiplerin SÇKM değerleri %5.1-7.9 arasındadır ve bu değerler ile sanayik domates için iyi bir kaynak niteliğindedirler. Yapılan çalışmalarda sanayik domateslerde SÇKM miktarını Paksoy (2003) %5.0-5.50 arasında, Kazak ve ark. (2018) %4.30 – 5.67 arasında bulmuştur. Genotiplerde pH değeri 4.1-5.1 arasında bulunmuştur. Adeniji ve ark. (2020) 4

domates genotipi ile 2 domates çeşidinde, yaptıkları çalışmada pH değerini 4.65-5.17 olarak bulmuşlardır. Her iki çalışmada da pH değerlerinin benzer aralıkta yer aldığı gözlemlenmektedir (Çizelge 5). Çalışmada en uzun meyve boyuna sahip KR207 genotipinin en düşük SÇKM değerine sahip olduğu belirlenmiştir. Öte yandan, en kısa meyve boyuna sahip TR123-1 genotipinin en yüksek SÇKM değerine sahip olduğu gözlemlenmiştir. Benzer sonuçlar Adeniji ve ark.

(2020)'nın yapmış olduğu çalışmada da elde edilmiştir. Çalışmada, domates genotiplerinin meyve kalite özellikleri açısından yaptıkları karşılaştırma

sonucunda meyve yüksekliği ile SÇKM arasında negatif anlamda bir ilişki olduğu söylenmiştir.

Çizelge 5. Domates genotiplerinin meyvelerinde SÇKM ve pH değerleri

Table 5. Brix and pH values in fruits of tomato genotype

Genotipler	N	SÇKM (%) (Ortalama±SH)	Standart sapma	PH (Ortalama±SH)	Standart sapma
TR95	3	5.70±0.11 ^c	0.20	4.10±0.05 ^b	0.10
TR123-1	3	7.90±0.05 ^a	0.10	5.09±2.64 ^a	0.04
KR202	3	6.20±0.15 ^b	0.26	5.00±0.02 ^a	0.04
KR207	3	5.10±0.05 ^d	0.10	5.12±0.18 ^a	0.32
P değeri		0.001		0.001	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)

Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Çizelge 6'da domates genotiplerinin Lab renk değerleri verilmiştir. Genotiplerin Lab renk ölçümüne bakıldığında L değeri 36.07-41.33 aralığında, a değeri 20.85-30.90 aralığında b değeri ise 17.73-27.26 aralığında olduğu görülmektedir. Elde edilen veriler sonucunda, Kırgızistan genotiplerinin Türkiye

genotiplerine oranla daha yüksek Lab değerlerine sahip olduğu belirlenmiştir. Bu değerler Renna ve ark. (2019)'ın yaptığı çalışmadaki değerler ile rakamsal olarak benzerlik göstermektedir. Araştırmalarında L değerini 36.4-44.3 , a değerini 27.70-30.9 , b değerini 31.2-37.7 arasında tespit etmişlerdir.

Çizelge 6. Domates genotiplerinde L, a ve b renk değerleri

Table 6. L, a and b color values of tomato genotypes

Genotipler	N	L (Ortalama±SH)	Standart sapma	A (Ortalama±SH)	Standart sapma	B (Ortalama±SH)	Standart sapma
TR95	3	38.80±1.15 ^b	1.99	27.31±0.20 ^b	0.35	25.07±0.08 ^b	0.14
TR123-1	3	36.07±0.15 ^c	0.27	20.85±0.27 ^c	0.48	17.73±0.48 ^c	0.84
KR202	3	41.33±0.26 ^a	0.45	28.75±0.53 ^b	0.93	26.85±0.71 ^a	1.23
KR207	3	40.55±0.11 ^{ab}	0.20	30.90±0.77 ^a	1.34	27.26±0.33 ^a	0.57
P değeri		0.001		0.001		0.001	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)

Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Çizelge 7'de domates genotiplerinin genç, orta olum ve olgun meyvelerindeki sertlik değerleri verilmiştir. Genotiplerin meyve eti sertliği gözlemlendiğinde genç olum meyve sertliğinde genotipler arasında önemli farklılıklar bulunmamıştır ($P=0.332$). Orta olumda en sert meyve KR202 (%16.66) ve olgun meyvede en sert meyve etine sahip genotip KR207 (%4.50) olarak

gözlemlenmiştir. En yumuşak meyve etine sahip genotipler ise orta olumda TR95 (%6.03) ve olgun meyvede KR207 (%1.76) olarak tespit edilmiştir (Çizelge 7). Saka (2023) yaptığı araştırmada birinci yetiştiricilik yılında meyve eti sertliğini %10-36 arasında ve ikinci yılında meyve eti sertliğini %10-32 arasında tespit etmiştir.

Çizelge 7. Domates genotiplerine ait meyve eti sertliği özellikleri (%)

Table 7. Fruit flesh firmness characteristics of tomato genotypes (%)

Genotipler	N	Genç meyve (%) (Ortalama±SH)	Standart sapma	Orta olum (%) (Ortalama±SH)	Standart sapma	Olgun (%) (Ortalama±SH)	Standart Sapma
TR95	3	16.03±1.88	3.26	6.03±0.67 ^b	1.17	1.77±0.07 ^b	0.12
TR123-1	3	23.77±2.85	4.93	9.88±0.29 ^b	0.50	3.20±0.64 ^{ab}	1.12
KR202	3	20.58±5.01	8.68	16.67±2.02 ^a	3.51	4.23±0.88 ^a	1.52
KR207	3	24.40±2.70	4.68	6.87±0.68 ^b	1.18	4.50±0.20 ^a	0.35
P değeri		0.332	Ö.D.	0.001		0.031	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)

Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Domates genotiplerine ait bazı meyve özellikleri çizelge 8'de verilmiştir. Genotiplerin meyve özellikleri incelendiğinde bütün genotiplerin olgunluk rengi kırmızı olarak belirlenmiştir. Oğuz (2010) yaptığı çalışmada meyve rengi açısından inceleme yaptığına kırmızı, koyu kırmızı ve pembe olarak gözlemlenen materyalleri dikkate değer bulmuştur. Genotiplerin boyuna kesit şekilleri TR95 ve KR207'de eliptik, TR123-1'de yassı ve KR202'de oval olarak tespit edilmiştir. Meyve sapında damarlanma bütün genotiplerde orta düzeyde görülürken TR123-1 de çok güçlü olarak gözlemlenmiştir. Bütün meyvelerde;

Meyve sapında çukur orta düzeydeyken TR123-1'de çok büyük bir çukur tespit edilmiştir. Meyvelerin çiçek burnu şekline bakıldığında Türkiye genotiplerinin düz girintili KR202'nin düz KR207'nin ise sivrimsi bir çiçek burnu şekline sahip olduğu gözlemlenmiştir. Henaerh ve ark. (2014) yaptığı çalışmada genotiplerinin yarısının çiçek burnu şeklinin nokta olduğunu tespit etmiştir. Meyve parlaklığı kriterine bakıldığında TR95 genotipinin parlaklığının az olduğu diğer genotiplerin ise orta düzeyde bir parlaklığa sahip olduğu gözlemlenmiştir (Çizelge 8).

Çizelge 8. Domates genotiplerine ait meyve özellikleri
Table 8. Fruit characteristics of tomato genotypes

Genotipler	Meyve olgunluk rengi	Meyve boyuna kesit şekli	Meyve sapında damarlanma	Meyve sapında çukur	Meyve çiçek burnu şekli	Meyve parlaklığı
TR95	Kırmızı(5)	Eliptik(6)	Orta(3)	Orta(3)	Düz girintili(2)	Zayıf(1)
TR123-1	Kırmızı(5)	Yassı(2)	Çok güçlü(5)	Çok büyük(5)	Düz girintili(2)	Orta(2)
KR202	Kırmızı(5)	Oval(8)	Orta(3)	Orta(3)	Düz(3)	Orta(2)
KR207	Kırmızı(5)	Eliptik(6)	Orta(3)	Orta(3)	Sivrimsi(4)	Orta(2)

Çizelge 9'da domates genotiplerine ait bazı meyve özellikleri verilmiştir. Gözlemlenen meyve boyutu kriterine bakıldığında KR202 numaralı genotip diğerlerinden farklı olarak büyük olarak belirlenmiştir. Morfolojik gözlemlerden meyve kabuk rengine bakıldığında herhangi bir farklılık görülmemiştir. Bütün genotipler turuncu olarak gözlemlenmiştir. Bu çalışmada meyve olgunluğundan önce yeşil yakalılık Türkiye genotiplerinde orta Kırgızistan genotiplerinde çok küçük olarak bulunmuştur. Tembe ve ark. (2018) yaptıkları bir çalışmada yerli domates genotiplerinin yeşil yakalılıklarını incelemiş, genotiplerin yaklaşık %80'inde meyvelerde yeşil yakalılık tespit etmişlerdir.

Genotiplerin meyve eti rengine bakıldığında Türkiye genotiplerinin kırmızı Kırgızistan genotiplerinin ise pembe et rengine sahip olduğu gözlemlenmiştir (Çizelge 9). Çukadar (2011) yaptığı çalışmada bütün yerli domates genotiplerinin meyve eti rengini kırmızı olarak belirlemiştir. Çekirdek evi sayısı TR95, KR202 ve KR207 de 2 ila 3 arasında değişirken TR123-1 genotipinde 10 olarak bulunmuştur. Şekil 2'de domates genotiplerinin enine kesit şekli görülmektedir. Çalışma sonucunda elde edilen veriler benzer çalışmalara örnek niteliğindedir. Pradeepkumar ve ark. (2006) yaptığı çalışmada çekirdek evi sayısını 2-7 arasında belirlemiştir.

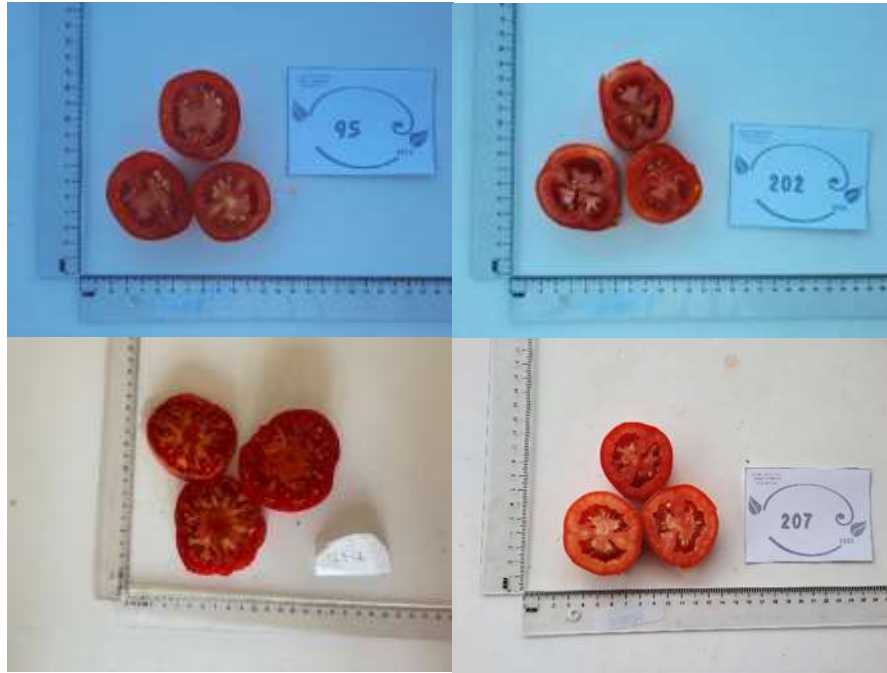
Çizelge 9. Domates genotiplerine ait meyve özellikleri
Table 9. Fruit characteristics of tomato genotypes

Genotipler	Meyve eti rengi	Meyve boyutu	Meyve kabuk rengi	Meyve olgunluğundan önce yeşil yakalılık	Çekirdek evi sayısı
TR95	Kırmızı(5)	Orta(5)	Turuncu(1)	Orta(5)	2
TR123-1	Kırmızı(5)	Orta(5)	Turuncu(1)	Orta(5)	10
KR202	Pembe(4)	Büyük(7)	Turuncu(1)	Çok küçük(1)	3
KR207	Pembe(4)	Orta(5)	Turuncu(1)	Çok küçük(1)	2

Yaprak Gözlemlerine Ait Bulgular

Domates genotiplerine ait bazı yaprak özellikleri çizelge 10'da verilmiştir. Yapılan değerlendirmeler sonucunda yaprak uzunluğunda genotipler arasında önemli farklılıklar bulunmamıştır (P=0.571). Yaprak genişliğine bakıldığında ise TR95 17.50 cm olarak

bulunup genotipin diğerlerine göre daha dar yaprak yapısına sahip olduğu tespit edilmiştir. Pradeepkumar ve ark. (2006)'na ait çalışmada domates hatlarındaki yaprak uzunluğunun 41.33-59.33 cm, yaprak genişliğinin ise 34.00-57.33 cm arasında olduğu belirlenmiştir.



Şekil 2. Türkiye ve Kırgızistan'dan toplanan yerel domates genotiplerinin enine kesiti
Figure 2. Cross-section of local tomato genotypes collected from Türkiye and Kyrgyzstan

Çizelge 10. Domates genotiplerine ait yaprak özellikleri (cm)
Table 10. Leaf characteristics of tomato genotypes (cm)

Genotipler	N	Yaprak uzunluğu (cm) (Ortalama±SH)	Standart sapma	Yaprak genişliği (cm) (Ortalama±SH)	Standart sapma
TR95	3	24.90±1.47	2.55	17.50±1.21 ^b	2.10
TR123-1	3	25.00±1.05	1.81	20.10±0.45 ^a	0.79
KR202	3	23.30±1.66	2.87	20.80±0.11 ^a	0.20
KR207	3	23.00±0.34	0.60	22.30±0.45 ^a	0.78
P değeri		0.571 Ö.D.		0.007	

$P \leq 0.05$ düzeyinde anlamlıdır.

Aynı sütunda gösterilen farklı harfler (a,b,c) istatistiksel olarak anlamlı farklılıkları göstermektedir ($P \leq 0.05$)
Different letters (a,b,c) shown in the same column indicate statistically significant differences ($P \leq 0.05$)

Çizelge 11'de domates genotiplerine ait bazı yaprak gözlemleri verilmiştir. Çalışmada genotiplerin yaprak özelliklerine bakıldığında TR95, KR202 ve KR207 numaralı genotiplerin yarı dik yaprak duruşuna sahip olduğu, TR123-1 genotipinin ise yatay yaprak duruşuna sahip olduğu gözlemlenmiştir. Benzer şekilde Turhan ve Şeniz (2009) çalışmasında genotiplerin yaprak duruş şeklini yarı dik ve yatay olarak gözlemlenmiştir (Çizelge 11). Her iki çalışma da genotiplerin yaprak duruşlarının benzer özellikler gösterdiği tespit edilmiştir. Yapılan gözlemler sonucunda bütün genotiplerde yaprak ayası bulunmakta olup yapraklarda yeşil renk yoğunluğu KR207 hariç bütün genotiplerde orta düzeyde ancak KR207 de yoğun olarak gözlemlenmiştir. Zhou ve ark. (2015) yaprak özelliklerinin fenotipik varyasyonun önemli bir kısmını oluşturduğunu çalışmalarla ortaya koymuştur. Saka (2023) ise yaptığı çalışmada

genotipler arasında yaprakların 17 tanesinin açık, 6 tanesinin koyu ve 35 tanesinin orta koyulukta yeşil renk yoğunluğuna sahip olduğunu belirtmektedir. Yaprak parlaklığı TR95 ve KR207 genotiplerinde az, ancak TR123-1 ve KR202'de yoğun olarak gözlemlenmiştir.

Bitki Gözlemlerine Ait Bulgular

Domates genotiplerinin bitki özellikleri çizelge 12'de verilmiştir. Bu çalışmada genotipler arasında çiçek rengi bakımından farklılıklar gözlemlenmemiş ve TR95 hariç bütün genotiplerde antosiyanin gözlemlenmiştir. Saka (2023) yaptığı yerel domates popülasyonu çalışmasında bu çalışmayı destekler nitelikte bulgular elde etmiştir, fide döneminde antosiyanin oluşumu ile bitkideki çiçek rengi özelliğinin bütün genotiplerde benzer olduğunu söylemiştir. Bitki büyüme gücü TR123-1 genotipinde

az ancak diğer genotiplerde orta seviyede görülmüştür. Meyve sapı kırılması TR95, TR123-1 ve KR202’de görülmüştür ancak KR207’de meyve sapı kırılması yoktur. Gövdede tüylülük TR95 ve KR207 de orta düzeyde iken TR123-1 ve KR202’de yoğun olarak tüylülük belirlenmiştir. Sönmez ve ark. (2015) yerli domates genotiplerinde yaptıkları çalışmada 15

genotipte az, 44 genotipte orta ve 2 genotipte yoğun tüylülük olduğunu söylemişlerdir. Bitki gelişim şekli Türkiye genotiplerinde sırım, Kırgızistan genotiplerinde oturak olarak görülmüştür (Çizelge 12). Demir ve Ünlü (2023) çalışmalarında kullandıkları hatların tamamının sırım büyüme tipine sahip olduğu tespit etmişlerdir.

Çizelge 11. Domates genotiplerine ait yaprak özellikleri
Table 11. Leaf characteristics of tomato genotypes

Genotipler	Yaprak duruş şekli	Yaprak parlaklığı	Yaprak ayası	Yaprak yeşil rengin yoğunluğu
TR95	Yarı dik(3)	Az(1)	Var(1)	Orta(2)
TR123-1	Yatay(5)	Yoğun(3)	Var(1)	Orta(2)
KR202	Yarı dik(3)	Yoğun(3)	Var(1)	Orta(2)
KR207	Yarı dik(3)	Az(1)	Var(1)	Yoğun(3)

Çizelge 12. Domates genotiplerine ait bitki özellikleri
Table 12. Plant characteristics of tomato genotypes

Genotipler	Çiçek rengi	Fide dönemi antosiyanin	Bitki büyüme gücü	Meyve sapı kırılması	Gövdede tüylülük	Bitki gelişim şekli
TR95	Sarı(1)	Yok(2)	Orta(2)	Var(1)	Orta(2)	Sırım(1)
TR123-1	Sarı(1)	Var(1)	Az(1)	Var(1)	Yoğun(3)	Sırım(1)
KR202	Sarı(1)	Var(1)	Orta(2)	Var(1)	Yoğun(3)	Oturak(2)
KR207	Sarı(1)	Var(1)	Orta(2)	Yok(2)	Orta(2)	Oturak(2)

Verim Bulguları

Domates genotiplerine ait verim değerleri çizelge 13’de verilmiştir. Bu çalışmadaki domates genotiplerinin bitki başına verim değerleri, 5.116 kg ile 2.514 kg arasında belirlenmiştir. Verim değerlerinin yıllar ortalaması incelendiğinde ise 0.20-1.65 kg bitki⁻¹ arasında belirlenmiştir (Saka, 2023). Çalışmadaki genotiplerinin meyve verimleri sırasıyla KR207 (5.116 kg), KR202 (4.151 kg), TR123-1 (3.178 kg) ve TR95 (2.514 kg) olarak tespit edilmiştir (Çizelge 13). Paksoy (2003) yaptığı çalışmada bazı sanayilik çeşitlerin verim değerlerini; 5740.1-8900.4 kg da⁻¹ arasında tespit etmiştir. Scarano ve ark. (2020) yaptıkları çalışmada bitki başına verim değerlerini 1.67-5.67 kg arasında ve Cebolla-Cornejo ve ark. (2013) yaptıkları çalışmada 0.5-5.5 kg arasında belirlemişlerdir.

Çizelge 13. Domates genotiplerinin bitki başına verimi

Table 13. Yield per plant of tomato genotypes

Genotipler	Verim(kg bitki ⁻¹)
TR95	2.51
TR123-1	3.19
KR202	4.15
KR207	5.11

SONUÇ ve ÖNERİLER

Ülkemiz yerel domates genotipleri stres koşullarına toleranslı ve meyve özellikleri bakımından nitelikli popülasyonları bünyesinde barındırmaktadır. Bu genetik çeşitliliğin korunması, sürdürülebilmesi ve bu kaynakların farklı özellikler açısından incelenerek ıslah programlarına alınması önemlidir. Böylelikle yerel kaynaklardan elde edilen farklı genetik özelliklere sahip materyallerden üstün nitelikli ve verimli çeşitlerin elde edilmesi mümkün olabilecektir. Bu çalışmada bazı yerel domates genotiplerinin morfolojik özellikleri tespit edilmiştir. Elde edilen veriler ışığında yerel genotiplerinin farklı ıslah çalışmalarında genetik birer kaynak olarak kullanılabilirliği düşünülmektedir. Genotiplerden verimli ve kaliteli sanayi tipi F1 hibrit domates çeşitlerinin çıkarılabileceği, bu çeşitlerin yerel ve uluslararası pazarlarda rekabet edebileceği düşünülmektedir.

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Antioxidant, Antimicrobial, and Antialzheimer Activities of *Tagetes patula* (Asteraceae)

Mansur Seymen SEĞMENOĞLU¹, Oguzhan KOÇER², Mustafa SEVİNDİK³, Nuh KORKMAZ⁴

Mehmet Ali YÜZBAŞIOĞLU⁵, Imran UYSAL⁶

¹Department of Nursing, Faculty of Health Sciences, Osmaniye Korkut Ata University, Osmaniye, Turkey, ²Osmaniye Korkut Ata University, Department of Pharmacy Services, Vocational School of Health Services, Osmaniye, Türkiye, ³Osmaniye Korkut Ata University, Faculty of Engineering and Natural Science, Department of Biology, Osmaniye, Türkiye, ⁴Osmaniye Korkut Ata University, Faculty of Engineering and Natural Science, Department of Biology, Osmaniye, Türkiye, ⁵Gaziantep University, Vocational School of Oguzeli, Gaziantep, Türkiye, ⁶Osmaniye Korkut Ata University, Bahçe Vocational School, Department of Food Processing, Osmaniye, Türkiye

¹<https://orcid.org/0000-0003-2743-6245>, ²<https://orcid.org/0000-0002-0104-7586>, ³<https://orcid.org/0000-0001-7223-2220>

⁴<https://orcid.org/0000-0001-8299-910X>, ⁵<https://orcid.org/0000-0002-0245-751X>, ⁶<https://orcid.org/0000-0003-0942-9658>

✉: sevindik27@gmail.com

ABSTRACT

Plants are natural products used in the prevention and treatment of many diseases. In this study, antioxidant, antimicrobial, anticholinesterase activities and total phenolic and flavonoid contents of *Tagetes patula* L. samples collected from Iraq were determined. The aerial parts of the plant were extracted with ethanol in a soxhlet device. The antioxidant potential of the extracts was measured with Rel Assay kits. Antimicrobial activity was determined by the agar dilution method against standard bacterial and fungal strains. To determine anticholinesterase activity acetyl and butyrylcholinesterase inhibitions were tested. The total phenolic content of the samples was carried out using the Folin-Ciocalteu reagent. The quantification of flavonoids was conducted using an aluminum chloride assay. As a result of the analyses, the total antioxidant value of the plant extract was determined as 5.386 ± 0.142 mmol Trolox equiv./L, the total oxidant value was 8.287 ± 0.146 $\mu\text{mol H}_2\text{O}_2$ equiv./L and the oxidative stress index was determined as 0.154 ± 0.003 . Plant extracts showed the highest activity against *Candida* species. It was also effective against bacterial and fungal strains at concentrations between 50-400 $\mu\text{g/mL}$. Acetylcholinesterase activity of the plant extract was determined as 24.97 ± 0.98 , and butyrylcholinesterase activity was determined as 35.65 ± 0.94 . Additionally, its total phenolic content was determined as 63.64 ± 0.74 mgGAE/g and its total flavonoid content was 108.9 ± 1.55 mgQE/g. It has been determined that the plant has antioxidant, antimicrobial, and antiallergic potential.

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Tagetes patula'nın (Asteraceae) Antioksidan, Antimikrobiyal ve Antialzheimer aktiviteleri

ÖZET

Bitkiler birçok hastalığın önlenmesinde ve tedavisinde kullanılan doğal ürünlerdir. Bu çalışmada *Tagetes patula*'nın Irak'dan toplanan örneklerinin antioksidan, antimikrobiyal, antikolinesteraz aktiviteleri ve toplam fenolik ve flavonoid içerikleri belirlenmiştir. Bu kapsamda bitkinin toprak üstü kısmının etanol ile soxhlet cihazında özütlenme işlemi yapıldı. Özütlerin antioksidan potansiyeli Rel Assay kitleri ile ölçüldü. Antimikrobiyal aktivite standart bakteri ve fungus suşlarına karşı agar dilüsyon metodu ile belirlendi. Antikolinesteraz aktivite için asetil ve bütirilcholinesteraz inhibisyonları test edildi. Numunelerin toplam fenolik içeriğinin miktarının belirlenmesi, Folin-Ciocalteu reaktifi kullanılarak gerçekleştirildi. Flavonoidlerin miktarının belirlenmesi, bir alüminyum klorür tahlili kullanılarak gerçekleştirildi. Yapılan analizler sonucunda bitki özütünün toplam antioksidan değeri 5.386 ± 0.142 mmol Trolox equiv./L, toplam oksidan değeri 8.287 ± 0.146 $\mu\text{mol H}_2\text{O}_2$ equiv./L ve oksidatif stress indeksi 0.154 ± 0.003 olarak belirlendi. Bitki özütleri en yüksek aktiviteyi *Candida* türlerine karşı

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gösterdi. Ayrıca bakteri ve fungus suşlarına karşı 50-400 µg/mL arasındaki konsantrasyonlarda etkili olduğu tespit edildi. Bitki özütünün asetilkolinesteraz aktivitesi 24.97±0.98 µg/mL, bütirikolinesteraz aktivitesi 35.65±0.94 µg/mL olarak belirlendi. Ayrıca toplam fenolik içeriği 63.64±0.74 mgGAE/g, toplam flavonoid içeriği 108.9±1.55 mgQE/g olarak tespit edildi. Bu kapsamda bitkinin antioksidan, antimikrobiyal ve Antialzheimer potansiyelinin olduğu tespit edilmiştir.

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INTRODUCTION

Traditional medicine has a very important place in human history. Different natural products are used in the fight against many diseases, based on old information (Çömlekçioğlu et al., 2022). The most commonly used among these natural products are plants. Many plants are used in different communities for many purposes such as burning, shelter, food, or medicine (Dogan et al., 2023). In many studies conducted on plants, it has been reported that plants have many activities such as antioxidant, anticancer, antiaging, anti-inflammatory, antiproliferative, hepatoprotective, antioxidant, antimicrobial, and DNA protective (Tutus et al., 2010; Mohammed et al., 2020a; Madani et al., 2022; Unal et al., 2022; Kalkan et al., 2023; Sevindik et al., 2023; Uysal et al., 2023). For this reason, determining the biological activities of plants is very important in terms of their usage potential. One of these plants is *T. patula*, which originates from America and is widespread around the World (Rueda et al., 2018). *Tagetes sp.* has demonstrated antibacterial, antifungal, and insecticidal activity in the control of many pests and diseases, especially in nematode management programs (Ismail et al., 2019; Gongalla, 2020).

It is an annual plant species of *T. patula* (Asteraceae) known as French marigold. It is easily grown with thousands of different varieties in bright shades of yellow and orange. It blooms from July to October. Its flowers are used to color foods (Romagnoli et al., 2005). *Tagetes* has approximately 30 species and is also used in traditional medicine in many regions of the world (Xu et al., 2012). The flowers and leaves of marigolds are used as folk medicine in the treatment of fever, liver diseases, diarrhea, vomiting, colic, and skin diseases (Kafaltiya et al., 2019). Plant extracts and essential oils are bioactive and potentially allelopathic against many pathogenic organisms such as bacteria, fungi, nematode viruses, acarid, and insects (Rueda et al., 2018). In this study, while determining the total phenolic content, total flavonoid content, and antimicrobial activity of the plant, we also aimed to

determine the total antioxidant and total oxidant values and anticholinesterase activity of *T. patula* for the first time.

MATERIAL and METHOD

T. patula species were collected from Iraq (Erbil). Muddy and dusty aboveground parts of the plant were cleaned using distilled water. It was then ground into powder in a mechanical grinder. Then, thirty grams of the powder samples were weighed and extracted in a soxhlet apparatus. The extraction procedure was applied to 30 g of sample in 250 mL ethanol at 50°C for 6 hours. Then, the solvents of the extracts were evaporated to obtain crude extract.

Antialzheimer tests

The anticholinesterase activity of the plant was determined for its potential anti-Alzheimer effect. For this, the Ellman method was used (Ellman et al., 1961). Galantamine was used as a standard. The plant extract was prepared at concentrations of 3.125-200 µg/mL. Then, 130 µL of 0.1 M pH=8 phosphate buffer, 10 µL of stock solution, and 20 µL of enzyme (AChE or BChE enzyme solution) were added to the microplate and incubated for 10 min at 25 °C in the dark. 20 µL of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added. Reading was made with a spectrometer at a wavelength of 412 nm. The absorbance readings of the samples were repeated 3 times. IC₅₀ values of percent inhibition of the samples was expressed as µg/mL (Kurt et al., 2020).

$$\text{Inhibition (\%)} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100$$

Antimicrobial Activity Tests

Stock solutions were prepared from the plant extract at concentrations ranging from 125.5-800 µg/mL. From these extract concentrations, the lowest concentrations that prevented the growth of bacterial and fungal strains were determined. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli*

ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as bacterial strains. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030 were used as fungal strains. Bacteria were grown in Hinton Broth medium. The growth of fungi was carried out in RPMI 1640 Broth medium (Baba et al., 2020).

Total Phenolic and flavonoid Tests

A stock solution was prepared from the plant extract at a concentration of 1 mg/mL. After 250 mL of this solution was taken and mixed with 1 mL of Folin-Ciocalteu reagent (1:9, v/v), 0.75 mL of 1% Na₂CO₃ was added. Then incubated for 2 hours at room temperature and finally measured at 760 nm. According to the calibration curve of the gallic acid standard solution, the total phenolic content (TPC) was expressed as mgGAE/g (Bal et al., 2023).

The total flavonoid content (TFC) of the plant extract was analyzed by aluminium chloride assay. 0.1 mL 10% Al (NO₃)₃, 0.1 mL 1 M NH₄CH₃COO, 4.3 mL methanol, 0.5 mL quercetin and 0.5 mL plant extracts were mixed. It was then incubated for 40 minutes, and absorbance was measured at 415 nm. According to the calibration curve of the quercetin standard solution, the total flavonoid content (TFC) was expressed as mgQE/g (Korkmaz et al., 2023).

Total antioxidant and oxidant analyze

Rel Assay kits were used to determine the antioxidant potential of the plant extract. Trolox was used as a calibrator in the total antioxidant (TAS) test, and hydrogen peroxide was used as a calibrator in the total oxidant (TOS) test. TAS values were expressed as mmol Trolox equiv./L. TOS values were expressed as μmol H₂O₂ equiv./L (Erel, 2004; Erel 2005). OSI (oxidative stress index) value was determined by dividing TOS values to TAS values and taking their percentages (Sevindik, 2019).

RESULTS AND DISCUSSION

Anticholinesterase activity

Alzheimer's is the most common neurodegenerative disease. The number of cases, which has been increasing in recent years, is expected to increase further in the coming years. Due to the increasing age scale, approximately 5 million new cases are seen

every year around the world. On the other hand, the pathogenesis of the disease is still not fully known (Konrath et al., 2013). However, the most common treatment options include inhibition of cholinesterases. In this study, the anti-AChE and anti-BChE potentials of the ethanol extract of *T. patula* were determined. The obtained IC₅₀ values are shown in Table 1.

Table 1 Anti-AChE and anti-BChE values of *T. patula*
 Çizelge 1. *T. patula*'nın anti-AChE ve anti-BChE değerleri

Extract and Control	AChE μg/mL	BChE μg/mL
Ethanol extract	24.97±0.98	35.65±0.94
Galantamine	9.46±0.18	15.86±0.41

In the literature, the AChE inhibition values of the ethanol extract of *T. patula* were reported to be 22.37-25.33 μ mol/mg (Ramakrishnan et al., 2015). In this study, anti-AChE and anti-BChE potentials were determined using the ethanol extract of *T. patula*. Additionally, galantamine was used as a standard. It was determined that *T. patula* used in this study showed lower activity than galantamine. It is very important to determine the presence of enzymes that cause the etiology of diseases. In addition, inhibition of these enzymes can make important contributions to the treatment of diseases (Świątek et al., 2021). It appears that *T. patula* used in this study has acetyl and butyrylcholinesterase inhibition potential. These findings suggest that *T. patula* may be a natural source for the treatment of neurodegenerative diseases.

Antimicrobial activity

Today, microorganisms are at the root of many diseases. The effects of antimicrobial drugs used against microorganisms in the market are insufficient (Eraslan et al., 2021). Among the general reasons for this is the increase in the number of resistant microorganisms due to unconscious use of antibiotics. The researchers have turned to the discovery of new antimicrobial drugs. Possible side effects of synthetic drugs have led researchers to natural antimicrobial sources (Bal et al., 2022; Lomberg et al., 2023; Mohammed et al., 2023). In this study, the antimicrobial potential of the ethanol extract of *T. patula* was determined. The findings obtained are shown in Table 2.

Table 2 MIC values of ethanol extract of *T. patula*
 Çizelge 2. *T. patula*'nın etanol ekstraktının MIC değerleri

	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>
Ethanol	200	200	100	400	400	200	50	50	50

*50, 100, 200, 400 μg/mL represents the lowest concentration that inhibits the growth of microorganisms.

In this study, it was determined that the ethanol extract of *T. patula* was effective against standard bacterial and fungal strains at concentrations between 50-400 µg/mL. It has previously been reported that methanol extract of *T. patula* is effective against *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus* (Latifian et al., 2021). In a different study, it was reported that different parts of *T. patula* were effective against *Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Micrococcus luteus*, *Micrococcus lysodeikticus*, *Mycobacterium fortuitum*, *Staphylococcus aureus*, *Staphylococcus aureus* AB, *Staphylococcus saprophyticus*, *Streptococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi* and *Shigella flexneri* (Faizi et al., 2008). In another study, it was reported that different parts of *T. patula* have antimicrobial activities against *Serratia fonticola*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus agalactiae*, and *Streptococcus oralis* (Safar et al., 2020). In this study, it was determined that *T. patula* was effective against standard bacterial and fungal strains using ethanol extract. It was observed that the plant extract exhibited the highest activity against *C. albicans*, *C. glabrata*, and *C. krusei* at a concentration of 50 µg/mL. It later became effective against *E. faecalis* at a concentration of 100 µg/mL. Subsequently, it was effective against *S. aureus*, *S. aureus* MRSA, and *A. baumannii* at a concentration of 200 µg/mL. It also showed activity against *E. coli* and *P. aeruginosa* at a concentration of 400 µg/mL. Latifian et al. (2021) found in their study that the ethanolic extract of *T. patula* showed good antibacterial effects against human pathogens. Rueda et al. (2018) examined the effect of methanolic flower extract and essential oil of *T. patula* on *R. solanacearum* bacteria and determined that the bacteria were highly sensitive to both components at a concentration of 100 mg/mL. In line with literature data and this study data, it has been observed that *T. patula* has antimicrobial potential.

Total phenolic and total flavonoid contents

As a result of their defense systems, plants produce secondary metabolites that are not nutritional but medically important (Türkmen and Koçer, 2021). In this study, total phenolic and total flavonoid contents of *T. patula* were determined. The findings obtained are shown in Table 3.

It is thought that the phenolic compound contents in plants, including flavonoids, have the ability to eliminate free radicals (Rueda et al., 2018). In the literature, it has been reported that the total phenolic

Table 3 TPC and TFC values of *T. patula*
 Çizelge 3. *T. patula*'nın TPC ve TFC değerleri

	TPC (mgGAE/g)	TFC (mgQE/g)
Ethanol extract	63.64±0.74	108.9±1.55

Values are given as mean ± standard deviation. (n=3)

contents of the flower and leaf parts of *T. patula* are 30 and 80 mg/g, and the total flavonoid content is 30 and 65 mg/g (Kushwaha and Verma, 2017). While the total phenolic contents of *T. patula* used in this study were similar compared to this study, the total flavonoid contents were determined to be higher. Tiwari et al. (2023) determined the total flavonoid content of the ethanolic extract of *T. patula* flower as 119.08 mg/g and the total flavonoid content as 71 mg/g. When we compared this study with Tiwari et al. (2023) study, we found that phenolic values were lower and flavinoid values were higher. In a different study, the total phenolic contents of the flower and leaf parts of *T. patula* were reported as 11.54-103.70 mg/g (Salachna et al., 2021). Rueda et al. (2018) determined the total phenolic content in the methanol extract of the leaves and flowers of *T. patula* as 227.67 mg/g and 153.48 mg/g, respectively. In another study, the total phenolic content of different fractions of *T. patula* was reported as 14.85-67.44 mg/g (Kuddus et al., 2012). Compared to these studies, the total phenolic contents of the aboveground parts of *T. patula* used in this study are similar, although there are some differences. It is thought that this difference arises from the difference in soil structure in the regions where the plants are collected and the variability of the stress conditions they encounter.

Antioxidant activity

Oxidant compounds are unstable compounds produced in routine metabolic processes. Oxidant compounds play a role at low doses in defending against infections or promoting the death of cancer cells (Bal et al., 2019). But as levels of oxidant compounds increase, they can become membrane bound. Antioxidants protect living organisms from diseases by reducing or suppressing the effects of oxidant compounds in living organisms (Krupodorova and Sevindik, 2020; Mushtaq et al., 2020). However, if the balance between oxidant compounds and the antioxidant defense system shifts towards oxidant compounds, oxidative stress occurs. As a result of oxidative stress, diseases such as degenerative diseases (Parkinson, Alzheimer's, AMD), rheumatic, pulmonary, digestive, cardiovascular, metabolic progressive chronic diseases, and cancer are linked to oxidative stress. They can promote chronic diseases such as cataracts, cancer, coronary heart disease, diabetes, and kidney failure (Manda et al., 2009; Sevindik et al., 2018; Gürgen et al., 2020). Supplemental antioxidants may serve to reduce the

possible effects of oxidative stress (Hawas et al., 2013). Plants are very important natural products in terms of their supplementary antioxidant potential. In this

study, the antioxidant potential of *T. patula* was determined. The findings obtained are shown in Table 4.

Table 4 TAS, TOS and OSI values of *T. patula*
 Çizelge 4. *T. patula*'nın TAS, TOS ve OSI değerleri

	TAS (mmol Trolox equiv./L)	TOS ($\mu\text{mol H}_2\text{O}_2$ equiv./L)	OSI (TOS/TASx10)
Ethanol extract	5.386±0.142	8.287±0.146	0.154±0.003

Values are given as mean ± standard deviation. (n=3)

Many previous studies have reported the antioxidant potential of *T. patula* using different methods (Negi et al., 2013; Munhoz et al., 2014; Kashif et al., 2015; Riaz et al., 2020). In this study, the antioxidant potential of *T. patula* was determined for the first time using TAS and TOS kits. There are studies on different plant species using TAS and TOS kits. In these studies, TAS values of *Mentha longifolia* ssp. *longifolia*, *Allium calocephalum*, *Helianthemum salicifolium*, *Silybum marianum*, *Ferulago platycarpa*, *Galium aparine*, and *Glycyrrhiza glabra* were reported as 3.628, 5.853, 9.490, 5.767, 5.688, 5.147 and 8.770, respectively. TOS values were reported as 4.046, 16.288, 14.389, 12.144, 15.552, 18.679 and 14.590, respectively. OSI values have been reported as 0.112, 0.278, 0.157, 0.211, 0.273, 0.346 and 0.167, respectively (Sevindik et al., 2017; Mohammed et al., 2019a; Mohammed et al., 2019b; Mohammed et al., 2020b; Korkmaz et al., 2021; Mohammed et al., 2021a; Mohammed et al., 2021b). Compared to these studies, the TAS value of *T. patula* used in this study was found to be higher than *Mentha longifolia* ssp. *longifolia* and *Galium aparine*, and lower than *Allium calocephalum*, *Helianthemum salicifolium*, *Silybum marianum*, *Ferulago platycarpa*, and *Glycyrrhiza glabra*. TAS value is an indicator of the totality of antioxidant compounds found in natural products (Selamoglu et al., 2020). In this context, it appears that *T. patula* used in this study has antioxidant potential. TOS value is an indicator of the oxidant-active compounds produced within natural products. The OSI value shows the percentage of oxidant compounds suppressed by antioxidant compounds. High OSI value shows that natural products are insufficient to suppress oxidant compounds. (Selamoglu et al., 2020). It was observed that the TOS and OSI values of *T. patula* used in this study were higher than *Mentha longifolia* ssp. *longifolia* and lower than *Allium calocephalum*, *Helianthemum salicifolium*, *Silybum marianum*, *Ferulago platycarpa*, *Galium aparine* and *Glycyrrhiza glabra*. In this context, it was observed that *T. patula* used in this study produced fewer oxidant compounds due to environmental effects. In addition, it appears that the antioxidant defense system functions well in suppressing oxidant compounds. In this context, it is thought that *T. patula* may be a natural antioxidant source.

CONCLUSION

In this study, the biological activities of *T. patula*, which is an easy-to-cultivate, fast-growing, and widespread species, were determined. Antioxidant, antimicrobial, and anticholinesterase activities, as well as total phenolic and flavonoid contents of *T. patula* were determined. As a result of the analyses, it is thought that the ethanol extract of the plant can be used both as a food preservative and against human pathogenic microorganisms due to its high antioxidant and antimicrobial potential. It is also thought that the plant may support the treatment of neurodegenerative diseases due to its antioxidant and anticholinesterase potential. Although the findings are compatible with the *T. patula* data used in folk medicine studies, the positive properties of this plant on living things can be detailed in different studies.

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None

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

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

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Determination of Nutrient Elements Content, Essential Oils Ratio and Biochemicals Composition of *Origanum rotundifolium* Boiss. and *Origanum syriacum* L.

Lutfi NOHUTCU¹, Murat TUNCTURK², Ezelhan SELEM³, Ruveyde TUNCTURK⁴, Orçun ÇINAR⁵

¹University of Van Yuzuncu Yil, Faculty of Agricultural, Van, Türkiye, ²University of Van Yuzuncu Yil, Faculty of Agricultural, Van, Türkiye,

³ University of Van Yuzuncu Yil, Department of Landscape and Ornamental Plants Program, Muradiye Vocational School, Van, Türkiye,

⁴University of Van Yuzuncu Yil, Faculty of Agricultural, Van, Türkiye, ⁵Batı Akdeniz Agricultural Research Institute, Department of Food Technology and Medicinal and Aromatic Plants, Antalya, Türkiye

¹<https://orcid.org/0000-0003-2250-2645>, ²<https://orcid.org/0000-0002-7995-0599>, ³<https://orcid.org/0000-0003-4227-5013>

⁴<https://orcid.org/0000-0002-3759-8232>, ⁵<https://orcid.org/0000-0002-8356-384X>

✉: ezelhanslem@yyu.edu.tr

ABSTRACT

In the study, the biochemical composition of bioactive ingredients, essential oils ratio and compounds, mineral elements content, and antioxidant activities of two *Origanum* species (*O. rotundifolium* Boiss. and *O. syriacum* L.) were investigated. It has been observed that the total flavonoid and phenolic contents vary, with the amounts being 6.57 mg QE g⁻¹ and 225.79 mg GAE g⁻¹ for *O. rotundifolium* Boiss., and 184.65 mg QE g⁻¹ and 114.46 mg GAE g⁻¹ for *O. syriacum* L., respectively. The highest element contents have been determined for macroelement Calcium (Ca) > Potassium (K) > Magnesium (Mg); and for microelement Iron (Fe) > Manganese (Mn) > Zinc (Zn) > Copper (Cu), respectively. The essential oil yield was determined between 2.39% (*O. rotundifolium* Boiss) and 5.29% (*O. syriacum* L.). Essential oil compositions were determined by GC-MS (Gas Chromatography–Mass Spectrometry analysis). Besides, the major components found in two species were carvacrol, γ -terpinene and cymene. As a result of the study, *O. rotundifolium* Boiss. and *O. syriacum* L. species are rich in nutrients and biochemical content and can be used in many areas as an alternative food source.

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Origanum rotundifolium Boiss. ve *Origanum syriacum* L. Türlerinin Besin Elementleri İçeriği, Uçucu Yağ Oranı ve Biyokimyasal Bileşiminin Belirlenmesi

ÖZET

Yürütülen çalışmada, iki *Origanum* türünün (*Origanum rotundifolium* Boiss. ve *Origanum syriacum* L.) biyoaktif bileşiklerin biyokimyasal içeriği, uçucu yağ miktarı ve bileşenleri, mineral element içeriği ve antioksidan aktiviteleri araştırılmıştır. Gözlemlenen toplam flavonoid ve fenolik içeriklerin değişiklik gösterdiği ve içeriklerin miktarları sırasıyla *O. rotundifolium* Boiss. için 6.57 mg QE g⁻¹ ve 225.79 mg GAE g⁻¹, *O. syriacum* L. için 184.65 mg QE g⁻¹ ve 114.46 mg GAE g⁻¹ olduğu tespit edilmiştir. En yüksek element içerikleri makro elementlerde Kalsiyum (Ca) > Potasyum (K) > Magnezyum (Mg); mikro elementlerde ise Demir (Fe) > Manganez (Mn) > Çinko (Zn) > Bakır (Cu) olarak belirlenmiştir. Uçucu yağ oranı *O. rotundifolium* Boiss. için %2.39 ve *O. syriacum* L. için %5.29 olmuştur. Uçucu yağların kimyasal bileşimi Gaz Kromatografi-Kütle Spektrometresi analizi (GC-MS) ile belirlenmiş ve her iki türde bulunan başlıca bileşenler karvakrol, γ -terpinen ve simen olmuştur. Yapılan çalışmanın sonucunda, *O. rotundifolium* Boiss. ve *O. syriacum* L. türlerinin besin ve biyokimyasal içerikler açısından zengin olduğu ve birçok alanda alternatif bir gıda kaynağı olarak kullanılabileceği görülmüştür.

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INTRODUCTION

Many species of oregano, belonging to the Lamiaceae family, are known *Coridothymus*, *Thymus*, *Thymbra*, *Satureja*, and *Origanum* genera in Turkey. The endemism rate of this family, which is among the three richest families in Turkey, is 44.2% (Fakılı, 2010). The Lamiaceae (Labiatae) family, which also includes the *Origanum* L. genus, includes approximately 200 genera and 3500 species. There are 45 genera and more than 546 species belonging to this family in Turkey. The genus *Origanum* has 41 species in the world. 75% of these species naturally spread in the Mediterranean region, especially in the Eastern Mediterranean region. *Origanum* L. genus is represented by 23 species and 5 subspecies in the flora of Turkey. 15 of these species are endemic. Turkey is the most important origin in the world for many species belonging to the genus *Origanum* L. (Baytop, 1984; Işık, 1995; Bayar and Çınar, 2020; Maral ve Kırıcı, 2022). Many species in the genus *Origanum* are in an important position due to their secondary metabolites, essential oils, nutrients, and biochemical contents.

Plants serve as a vital source of essential elements for human beings. The quantitative or qualitative assessment of mineral elements found in plants holds significance as the concentration and types of minerals are often required to be specified on food labels. The nutritional quality of many foods relies on the concentration and types of minerals they contain. Moreover, these minerals play a crucial role in combating various degenerative diseases and processes, mitigating the effects of environmental pollutants, and enhancing cognitive function and productivity. Certain minerals such as Phosphorus, Sodium, Potassium, and Calcium are indispensable for maintaining a healthy diet (Momin and Kadam, 2011). Hence, determining the nutrient profile of plant species holds paramount importance.

Oregano essential oils obtained from the genera *Origanum* are rich in carvacrol. Turkey is the biggest exporter of oregano herb and oil to the world markets. Oregano is mainly used in the spice, food, and pharmaceutical industries. Carvacrol is responsible for the biological activities of oregano. Many diverse activities of carvacrol such as antitumor, antimicrobial, analgesic, antimutagenic, antispasmodic, antigenotoxic, antiplatelet, angiogenic, antiparasitic, anti-inflammatory, anti-elastase, anti-hepatotoxic, insecticidal and hepatoprotective activities use such as feed additive, in honeybee breeding and gastrointestinal ailments have been

shown (Can Baser, 2008).

The study was carried out to detect total ash, dry matter, total antioxidant, total phenolic and total flavonoids content, nitrogen balance index (NBI), chlorophyll, flavonol, anthocyanin, nutrient elements, essential oil composition, and yield of *O. rotundifolium* Boiss. and *O. syriacum* L., which is cultivated in the Van region and Turkey.

MATERIAL and METHOD

Plant material

The study materials consist of *Origanum* species grown in the Medicinal and Aromatic Plants Garden of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crops. Diagnosed at the species level of plants were grown in the Medicinal and Aromatic Plants Garden to determine the adaptation ability. The study materials consist of two species. These are *O. rotundifolium* Boiss. and *O. syriacum* L.. Two years after the plants were planted, analyses were made in a single year. The harvest was made during the full bloom period. Samples were harvested from 9:00 to 10:00 in the morning.

Determination of ash, dry matter, heavy metal, and nutrient contents

The nutritious values such as total ash, dry matter, some heavy metals (As, Cd, Co, Ni, Cr, and Pb), and minerals (microelements, Fe, Zn, Cu, and Mn; macro elements, Mg, Ca, and K) and were measured in plant parts. For the total ash determination, an Electrical Muffle furnace set at 550 °C was used. Dry matter was determined with the drying of the samples for 24 hours at 105 °C in the oven. The mineral constituents of the plant samples were investigated as follows: at first, the dried samples were ashed in a furnace with hydrochloric acid and nitric acid (AR) (AOAC 2000). Then, distilled water (50 ml) was added to samples in a volumetric flask. All assays were performed in triplicate and the standard materials were utilized for chemical analyses. Atomic Absorption Spectrometry (AAS) was used to determine K, Ca, Mg, and Fe contents. ICP-OES (Inductively coupled plasma-optical emission spectrometer) was also used to determine other microelements and heavy metals (Cu, Zn, Ni, Mn, As, Cd, Co, Cr, and Pb).

Total antioxidant, total phenolic, and total flavonoid content

Total phenolic compound content was measured

according to Obanda et al. (1997) method. The antioxidant activity was also determined based on the Antioxidant Power (FRAP) (Iron (III) antioxidant power reduction) method (Benzie, Strain 1996) followed by readings of the absorbance at 593 nm, and antioxidant activity values were recorded as Trolox equivalent (TE) mg^{-1} . The total flavonoid content was determined with some modifications according to the method developed by Quettier-Deleu et al. (2000). The total amount of flavonoid was measured at 415 nm and calculated in mg quercetin equivalent (QE) 100 g^{-1} DM by using the calibration curve prepared using standard quercetin.

Determination of Nitrogen Balance Index, chlorophyll, flavonols, and anthocyanin

The Nitrogen balance index (NBI), chlorophyll, flavonol, and anthocyanin content were measured on the leaf non-destructively using and in real-time the Dualex scientific+ (FORCE-A, France) device before harvesting. Dualex measures flavonols and anthocyanins by analyzing their screening effect on chlorophyll fluorescence. The content of flavonols and anthocyanins is given in relative absorbance units ranging from 0 to 3 for flavanols and from 0 to 1.5 for anthocyanins.

Isolation of the Essential Oils

The leaves of *O. rotundifolium* Boiss. and *O. syriacum* L. species were used for the extraction of essential oils. All the plant parts (100 g for each plant species) were extracted separately by hydrodistillation using a Clevenger Apparatus for 3h at $100 \pm 5^\circ\text{C}$ (Gezici et al., 2017). The obtained oils were dried over anhydrous sodium sulfate and stored at $+4^\circ\text{C}$ in the dark until

analyzed and tested.

GC-MS analysis

Essential oil component analysis was performed using Gas chromatography (GC/GC-MS (Agilent 7890A)).

Sistem: Agilent 5975 GC-MSD sistemi

Essential oils 1:50 ratio hexane carrier gas: 0.8 mL/min flow rate helium gas

Split: 40:1

Injector temperature: at 250°C

Column temperature: 60°C (10 minutes) - 60°C to 220°C $4^\circ\text{C}/\text{minute}$ - 220°C (10 minutes)

Total analysis time: 60 minutes

Mass detection scan range: (m/z) 35-450

Library: Wiley 7n, Nist 05 and Flavor and Fragrance Natural and Synthetic Compounds (ver. 1.3).

All analyses carried out in the study were performed in three repetitions and standard deviations were determined. The data obtained as a result of the research were subjected to variance analysis according to the Randomized Parcel Trial Design. Statistical calculations were made using the computer analysis program COSTAT (Version 6.3). Differences between averages were determined according to the Duncan Multiple Comparison Method.

RESULTS and DISCUSSION

The dry matter (%), total ash (%), total flavonoid content (mg QE/100 g), total antioxidant activity ($\mu\text{mol TE/g}$), total phenolic content (mg GAE g^{-1}), NBI, chlorophyll, flavonol and anthocyanin contents of *O. rotundifolium* Boiss. and *O. syriacum* L. species are given in Table 1.

Table 1. Biochemical and bioactive compounds of *Origanum* species.

Çizelge1. Origanum türlerinin biyokimyasal ve biyoaktif bileşikleri.

	<i>O. rotundifolium</i> \pm SD	<i>O. syriacum</i> \pm SD	CV
Total Ash (%)	9.51 \pm 0.88	10.84 \pm 0.48	6.87 ns
Dry Matter (%)	34.49 \pm 1.56	32.87 \pm 0.56	2.09 ns
Total Flavonoid Content (mg QE 100 g^{-1})	6.57 \pm 0.27 b	184.65 \pm 56.44 a	2.22 **
Total Antioxidant Content ($\mu\text{mol TE g}^{-1}$)	102.12 \pm 1.01 b	172.03 \pm 2.43 a	0.01 **
Total Phenolic Content (mg GAE g^{-1})	225.79 \pm 0.72a	114.46 \pm 12.85 b	1.25 **
NBI	11.17 \pm 4.07	10.47 \pm 0.40	6.56 ns
Chlorophyll	19.63 \pm 8.12	21.13 \pm 0.80	3.43 ns
Flavonol	1.73 \pm 0.11 b	2.02 \pm 0.02 a	0.38 **
Anthocyanin	0.11 \pm 0.01	0.13 \pm 0.01	5.89 ns

* Significant at $P < 0.05$ level, ** Significant at $P < 0.01$ level, and there is no statistical difference between the means indicated with letters. CV: Coefficient of Variation, SD: Standart daviation, ns: not significant.

The parameters investigated, including total flavanol content, total antioxidant activity, total phenolic content, and the number of flavonoids, were found to be statistically significant at the 1% level among the

species. It was determined that the other parameters were not statistically significant. The dry matter from *O. rotundifolium* Boiss. and *O. syriacum* L. was 34.49% and 32.87%, respectively. Total ash was determined as

10.84% (*O. syriacum*) and 9.51% (*O. rotundifolium*). Biochemical content was determined as total flavonoid content, from 6.57 mg QE 100 g⁻¹ (*O. rotundifolium* Boiss.) to 184.65 mg QE 100 g⁻¹ (*O. syriacum* L.), total phenolic content from 114.46 mg GAE g⁻¹ (*O. syriacum* L.) to 225.79 mg GAE/g (*O. rotundifolium* Boiss.). *O. rotundifolium* Boiss. and *O. syriacum* L. are also high in total antioxidant content (102.12 and 172.03 µmol TE g⁻¹ dry mass, respectively).

The NBI contents were from 10.47 dualex index (*O. syriacum* L.) to 11.17 dualex index (*O. rotundifolium* Boiss.), chlorophyll from 19.63 (*O. syriacum* L.) to 21.13 (*O. rotundifolium* Boiss.), flavonol from 1.73 dualex index (*O. syriacum* L.) to 2.02 dualex index (*O. rotundifolium* Boiss.) and anthocyanin from 0.11 dualex index (*O. syriacum* L.) to 0.13 dualex index (*O. rotundifolium* Boiss.).

Macro and microelement contents of *O. rotundifolium*

Boiss. and *O. syriacum* L. species are given in Table 2 and Figure 1. This study determined the existence of seven elements in two *Origanum* (*O. rotundifolium* Boiss. and *O. syriacum* L.) species. It has been determined that the elements K, Fe, Zn, Cu, and Mn are statistically significant at the 1% level in terms of nutrient elements. It was found that the other elements are statistically insignificant. The concentrations of micronutrients were within the ranges of 234.775-280.100 mg kg⁻¹ for Fe, 32.415-36.150 mg kg⁻¹ for Zn, 11.660-15.610 mg kg⁻¹ for Cu, 54.755-65.040 mg kg⁻¹ for Mn. It was observed that the micro and macroelement values of *O. syriacum* L. were high, especially those of K (11.728 mg kg⁻¹), Fe (280.100 mg kg⁻¹), Ca (11.981 mg kg⁻¹) and Mg (4.032 mg kg⁻¹), in comparison with the other species. According to the results, *O. rotundifolium* Boiss. and *O. syriacum* L. appeared to have high nutritional value for consumption.

Table 2. Macro and micro element contents of *Origanum* species

Çizelge2. *Origanum* türlerinin makro ve mikro element içeriği

	<i>O. rotundifolium</i> ± SD	<i>O. syriacum</i> ± SD	CV
K(g/kg)	8,60±2.21 b	11,73±2.15 a	0.20 **
Ca(g/kg)	9,09±1.60	11,98±1.54	13.49 ns
Mg(g/kg)	2,96± 0.32	4,03±1.06	19.82 ns
Fe (mg/kg)	234,77±15.48 b	280,10±65.71 a	0.27 **
Zn(mg/kg)	36,15±0.90 a	32,41±0.82 b	0.06 **
Cu(mg/kg)	15,61±0.17 a	11,66±0.13 b	0.05 **
Mn(mg/kg)	65,04±2.40 a	54,75±3.81 b	0.02 **

* Significant at P<0.05 level, ** Significant at P<0.01 level, and there is no statistical difference between the means indicated with letters. CV: Coefficient of Variation, SD: Standard deviation, ns: not significant.

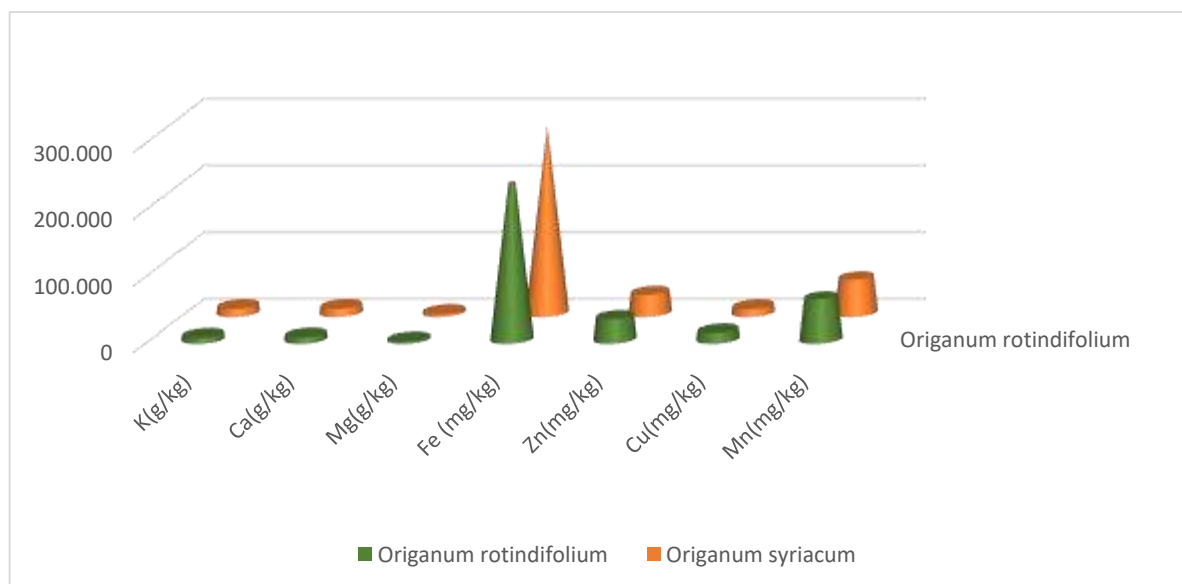


Figure 1. Macro and microelement contents of *O. rotundifolium* Boiss. and *O. syriacum* L. species.

Şekil 1. *O. rotundifolium* Boiss. ve *O. syriacum* L. türlerinin makro ve mikro element içerikleri.

Essential oil yield and the chemical composition of *O. rotundifolium* Boiss. and *O. syriacum* L. species are given in Tables 3 and 4. The essential oil yield was

determined at 2.39% in *O. rotundifolium* Boiss. At GC/MS analyses, a total of 13 compounds were determined in *O. rotundifolium* Boiss. The identified

components in the essential oil are given in Table 3. The major chemical composition of the essential oil in *O. rotundifolium* Boiss. were carvacrol (76.68%), gamma-terpinene (7.97%), and *p*-cymene (7.68%). The essential oil yield of *O. syriacum* L. was found

5.29%. Essention oil analysis has shown that *O. syriacum* L. species contained fifteen compounds. Carvacrol was the dominant essential oil (76.25%) in *O. syriacum* L. species, followed by gamma-terpinene (11.79%) and *p*-cymene (2.94%) (Table 4).

Table 3. Essential oil yield and the chemical composition of *O. rotundifolium* Boiss.

Çizelge 3. *O. rotundifolium* Boiss'in uçucu yağ verimi ve kimyasal bileşimi.

RI	Essential oil compounds	Amount of Essential oil compounds (%)
1016	α -pinene	0.71
1019	α -thujene	0.91
1155	Myrcene	1.99
1173	α -terpinene	1.57
1204	1,8-cineole	0.41
1239	γ -terpinene	7.97
1264	<i>p</i> -cymene	7.68
1437	1-octen-3-ol	0.26
1457	<i>trans</i> -sabinene hydrate	0.22
1539	<i>cis</i> -sabinene hydrate	0.22
1597	β -caryophyllene	1.11
2182	Thymol	0.28
2223	Carvacrol	76.67
Essential oil yield (% of dry weight): 2.39%		Total: 100

RI: Retention index

Table 4. Essential oil yield and the chemical composition of *O. syriacum* L.

Çizelge 4. *O. syriacum* L. un uçucu yağ verimi ve kimyasal bileşimi.

RI	Essential oil compounds	Amount of Essential oil compounds (%)
1016	α -pinene	0.38
1019	α -thujene	0.57
1155	Myrcene	1.97
1156	α -phellandrene	0.28
1173	α -terpinene	1.98
1192	Limonene	0.21
1239	γ -terpinene	11.79
1264	<i>p</i> -cymene	2.94
1382	3-octanol	0.31
1437	1-octen-3-ol	0.19
1457	<i>trans</i> -sabinene hydrate	0.87
1539	<i>cis</i> -sabinene hydrate	0.26
1597	β -caryophyllene	1.75
2182	Thymol	0.25
2223	Carvacrol	76.25
Essential oil yield (% of dry weight): 5.29%		Total:100

RI: Retention index

The ash of a food is the inorganic residue left after the combustion of organic matter. With ash determination, the quality of foodstuffs can be determined. For example, the high amount of ash in foodstuffs such as spices is an undesirable feature in terms of quality (Anonymous, 2022). Momin and Kadam (2011) found that the total ash of bark ranges from 11.80% to 12.10% in *Sesbania grandiflora*. The study is in good harmony with the results (ranging from 6.67–15.33%) that were recorded by researchers on several other edible plant species from Anatolia (Tuncurk et al.

2015). Ibrahim et al (2012) obtained the dry matter from wild and cultivated *O. syriacum* was 65.2% and 22.1%, respectively. The highest content of dry matter is higher than the maximum data reported by Tunçtürk et al. (2007) for the same Lamiaceae species (11.23 and 20.80%).

As a general definition, antioxidants are substances that protect against oxidation and prevent further reactions with oxygen or peroxides. Most of these substances are used as preservatives in various products (Bakır, 2010). Phenolics contribute to the

mechanical strength of the cell wall and play a regulatory role in plant growth (Naczki and Shahidi, 2004). Flavonoids and other plant phenolics are important antioxidants with their high redox potential. The antioxidant effects of phenolic compounds are very important because they bind free radicals, form chelates with metals, and inactivate some enzymes (Yang and Tsao, 2003). Oke-Altuntas et al. (2018), determined the total phenolic contents in the between 21.34 and 231.55 $\mu\text{g mg}^{-1}$. These values are directly proportional to the values obtained in this study (Table 1). It has been reported that the total phenolic contents in *Thymbra spicata* var. *spicata* and *Origanum onites* plants vary between 31.02 and 60.6 mg GAE g^{-1} , respectively (Yılmaz et al., 2019). In the study conducted by Bener in 2019, the total phenolic contents of the *Thymbra spicata* var. *spicata* plant were stated as 67.30 mg GAE g^{-1} dry plant (Bener, 2019). Antioxidant compounds exert their effects through various mechanisms, including the chelation of transition metal ions, inhibition of hydrogen abstraction, decomposition of peroxides, and scavenging of free radicals (Saadart et al. 2017; Ozkan and Ozcan, 2017). Yılmaz et al., (2019) determined the FRAP value in *O. onites* as 289.51 ± 9.59 mg TE g^{-1} . According to other studies from the literature, was observed FRAP from 38.16 ± 0.47 to 132.71 ± 1.86 $\mu\text{M TE g}^{-1}$ dw in *Achilla collina*. In this study, it differed in the range of 102.12-172.03 $\mu\text{mol TE g}^{-1}$. The highest total flavonoid contents (8.50 ± 0.43 mg QE g^{-1}) were found in the leaf parts of *A. collina* (Yılmaz et al., 2021). Lee et al. (2003) found the total flavonoid content of green and black tea to be 47 and 34 mg QE g^{-1} , respectively. It was observed that the total flavonoid content in *O. syriacum* species was higher than the value found in the literature. Differences in terms of total flavonoids, antioxidants, and phenolics content are due to genetic derivation because all plants were of the same grown and age under the same ecological conditions (Ercisli and Orhan, 2007).

In the study, chlorophyll, NBI, flavonol, and anthocyanin were measured in fresh material with a Dualex device. Dualex values are a commonly used method for determining plant health and plant development. Altuner et al. (2022) reported that the NBI value differed between 42.500 and 100.767 dualex indexes in wheat landraces and cereals. In the study, this rate was found to be lower. It is thought that different species and climatic conditions may be effective in this situation. It is known that the amount of chlorophyll varies according to the development status of the plant, ecological conditions, and plant species. Studies have shown that the amount of chlorophyll is 39.6 SPAD in *Calendula officinalis* (Selem et al., 2021), 32.53-36.63 SPAD in *Salvia officinalis* (Aytekin et al., 2021), and 32.9-37.2 SPAD in *O. vulgare* ssp. *hirtum* (Dordas, 2009). It is observed

that the species in this study have lower SPAD chlorophyll measurement values. It is thought that this result is due to ecological differences and the changes in the morphological and physiological structure of the plants growing at the time of cutting and their interaction with each other (Oğuz, 2014). Altuner et al. (2022) reported that the flavonol value differed between 0.427 and 0.607 dualex indexes in wheat landraces and cereals. In the study, this rate was found to be higher.

Mineral nutrition plays a crucial role in maintaining good health, and thus, the determination of elements such as Ca, Fe, Mg, Na, K, Zn, etc., is imperative. The utilization of mineral elements has been widely developed and utilized to address various health concerns (Momin and Kadam, 2011). In recent years, there has been a growing interest in the elemental content of herbs. These elements are integral to enzymatic activities and their activation influences biochemical processes within living cells. While some are required in significant quantities, such as Ca, K, Mg, and Na, others are needed in trace amounts. Microelements like Cu, Fe, Ni, Zn, and Mn play crucial roles in biological systems (Martínez-Ballesta et al., 2010; Moghaddam et al., 2020). Many plant species are abundant in essential minerals including K, Ca, Mn, Zn, Fe, and Cu. These minerals are fundamental components of tissues due to their multifaceted roles, which include facilitating nerve conduction, muscle function, and enzyme systems, aiding in the transportation of nutrients into cells, providing structural support for tissues, and regulating organ functions (Bhat et al., 2010; Tunçtürk et al., 2017). Dogan et al. (2021) showed that in *Achillea collina*, from high to low, macro elements are $\text{K} > \text{Ca} > \text{P} > \text{Mg} > \text{Na}$, and microelements are $\text{Mn} > \text{Cu} > \text{Fe} > \text{B}$. This study found a similar conclusion in *O. rotundifolium* Boiss. and *O. syriacum* L. $\text{Ca} > \text{K} > \text{Mg}$ for macroelements and $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu}$ for microelements. As seen in Table 2, iron (Fe), calcium (Ca), potassium (K), and magnesium (Mg) ratios, which are essential elements for healthy development, are high in both species. Considering the results obtained, *O. rotundifolium* Boiss. and *O. syriacum* L. species as tea, spice, or nutrient will have a positive effect on human health.

Many substances obtained from essential oils are used primarily in the production of pharmaceutical raw materials or fragrance substances by semi-synthesis (Çalikoğlu et al., 2006). Kaçar et al. (2006) found that the essential oil ratio in flowers was between 2.85-4.53% and in leaves between 1.88%-3.06% in their study of *O. onites* species. In the same study, they determined that the main component of the essential oil is carvacrol. Bayar and Çınar (2020) determined the rate of carvacrol as 70.59% in the first year and 69.20% in the second year in *O. onites*. Similar to results from

previous studies, the main component of essential oil of *O. rotundifolium* Boiss. and *O. syriacum* L. species was determined as carvacrol. Ibrahim et al (2012) found that cultivated and wild leaves of *O. syriacum* yielded 0.97% and 1.3% w/w oil, respectively. In the study, this rate was determined as 5.29%. Baser et al. (1992) determined that the essential oil yield of *O. sipyleum* is between 0.1-1.7%. They also reported that the components with the highest ratio detected in essential oil were gamma-terpinene (10.80-26.60%) and *p*-cymen (3.76-36.60%). In study, it was determined that they were the components with the highest amount after carvacrol. Karik et al. (2018) reported that different components may occur in the essential oils of samples collected from different places. Karik and Tınmaz (2007) reported in their study that many different chemotypes emerged based on the essential oils of other subspecies belonging to the *O. vulgare* species.

CONCLUSION

In conclusion of this study, it can be said that *Origanum* species are a valuable medicinal and aromatic product, based on their rich and beneficial nutrient composition. It was determined that the essential oil content of the plants was higher with 5.29% in *O. syriacum* L. species. In the study, *O. rotundifolium* Boiss. and *O. syriacum* L. species essential oil is carvacrol, followed by gamma-terpinene and *p*-cymene. As a result of the study, it was determined that the order of the two species in element contents was for macroelements Ca > K > Mg and microelements Fe > Mn > Zn > Cu. When biochemical and bioactive compound parameters were examined, it was observed that total ash, flavanol, anthocyanin, chlorophyll, total antioxidant, and total flavonoid contents were higher in *O. syriacum* L. species. The results of the conducted study indicate that the species are rich in terms of the examined parameters and that their cultivation is of great importance in preventing intensive harvesting from nature.

Author's Contribution

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

Conflict of interests/Competing interests

The authors declare that there is no conflict of interest.

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Effect of Preservation Methods on Fat-Soluble Vitamins and Stress Biomarkers in *Rhus coriaria* L. (Sumac) of Different Regions

Haval Mohammed ALI¹, Fikret KARATAŞ^{2*}, Dursun ÖZER³, Sinan SAYDAM⁴

¹Chemistry Department, College of Science, University of Duhok, Iraq, ^{2,4}Firat University, Faculty of Science, Department of Chemistry, 23200 Elazığ, Türkiye, ³Firat University, Engineering Faculty, Department of Chemical Engineering, 23200 Elazığ, Türkiye
¹<https://orcid.org/0000-0002-2500-9760>, ²<https://orcid.org/0000-0002-0884-027X>, ³<https://orcid.org/0000-0002-7225-8903>
⁴<https://orcid.org/0000-0003-1531-5454>,

✉: fkaratas@firat.edu.tr

ABSTRACT

The number of fat-soluble vitamins and stress biomarkers in *Rhus coriaria* L. samples taken from different regions was determined by HPLC before and after being subjected to different preservation methods. For this purpose, one group of the samples was analyzed immediately, while the other two groups one of which oiled, and the other group is kept as is for six months. It was determined that the amounts of vitamin A, E, β -carotene and lycopene in fresh sumac samples varied between 1.12 - 2.77, 84.40 - 230.65, 2.48 - 5.31 and 8.10 - 26.90 $\mu\text{g (g dw)}^{-1}$, respectively. The highest loss of vitamins was observed in an unoiled group of samples. The amounts of GSH, GSSG, MDA, 4-HNE, and GSH/GSSG in the same samples varied between 1004.12 - 2550.42, 422.54 - 1375.38, 13.95 - 31.30, 7.12 - 15.40 $\mu\text{g (g dw)}^{-1}$, and 1.16 - 3.49, respectively. While the amount of GSH and GSH/GSSG ratio in the stored sumac samples for six months decreased, on the other hand amount of MDA, GSSG, and 4-HNE increased. Differences in all examined parameters in fresh, unoiled, and oiled sumac samples are statistically significant ($P<0.05$). It was observed that the changes of the studied parameter in all sumac samples were lower in stored oiled samples.

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Muhafaza Yöntemlerinin Farklı Bölge Sumaklarındaki (*Rhus coriaria* L.) Yağda Çözünen Vitaminler ve Stres Biyomarkırları Üzerine Etkisi

ÖZET

Farklı bölgelerde yetişen ve farklı muhafaza yöntemleri uygulanan sumak örneklerindeki yağda çözünen vitaminler ile stress biyomarkırlarının miktarı HPLC ile belirlendi. Bu amaçla öğütülen örneklerden bir grup hemen analizlenirken, diğerleri ise iki kısma ayrılıp, bir kısmı yağlanırken diğer kısım olduğu gibi altı ay bekletildikten sonra analiz edildi. Taze sumak örneklerindeki A ve E vitamini, β -karoten ve likopen miktarlarının sırasıyla 1.12 - 2.77, 84.40 - 230.65, 2.48 - 5.31 ve 8.10 - 26.90 $\mu\text{g (g dw)}^{-1}$, arasında değiştiği gözlemlendi. Sonuçlardan, vitamin A, E, β -karoten ve likopen kaybının yağlanmadan bekletilen grupta fazla olduğu gözlenmiştir ($P<0.05$). Örneklerdeki GSH, GSSG, MDA, 4-HNE ve GSH/GSSG miktarları sırasıyla 1004.12 - 2550.42, 422.54 - 1375.38, 13.95 - 31.30, 7.12 - 15.40 $\mu\text{g (g dw)}^{-1}$ ve 1.16 - 3.49 arasında değiştiği görülmüştür. Bekletilen sumak örneklerindeki GSH ve GSH/GSSG miktarı azalırken, MDA, GSSG ve 4-HNE miktarlarının arttığı tespit edilmiştir. Taze, yağlanmış ve yağlanmamış sumak örneklerindeki incelenen parametrelerdeki değişimlerin istatistiksel olarak anlamlı ($P<0.05$) olduğu görülmüştür. Yağlanmış örneklerdeki değişimlerin yağlanmamış örneklere göre daha az olduğu görülmektedir.

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INTRODUCTION

Rhus coriaria (Sumac), which can grow all over the world, especially in subtropical and temperate climates, is a medicinal plant that is also used as a spice (Shabbir, 2012). It is reported that in the traditional medicine of the Middle East and Iran, sumac has been used for centuries in the treatment of diseases such as dysentery, diarrhea, hemorrhoids, and gout, as well as for healing wounds and lowering blood sugar, cholesterol, and uric acid levels. It is also stated that sumac contains antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, hepatoprotective, xanthine oxidase inhibition, hypoglycemia, and cardiovascular protective activities (Morshedloo et al., 2018). The fruits and leaves of the sumac plant, which has great economic value, are used in the kitchen, medicine, leather, and dye industries (Abu-Reidah et al., 2015; Güvenç et al., 2017). Studies have reported that it contains many physiologically active components such as tannins, anthocyanins, organic acids including malic and citric acid, fatty acids, vitamins, flavonoids, and terpenoid derivatives (Shabbir, 2012; Khalil et al., 2021; Özcan et al., 2021).

Vitamins are organic molecules that have regulatory functions in the living system, act as catalysts in metabolic events, and help the efficient use of nutrients in energy production. Vitamins are divided into two groups: water-soluble and fat-soluble (Kennedy, 2016). Fat-soluble vitamins are essential for living systems due to their different physiological functions in metabolism. Carotenoids have antioxidant functions in plants and animals (Yuan et al., 2020). It is stated that carotenoid compounds are of great importance for human health in protecting against many diseases, and in the treatment of some diseases, and are also necessary for the continuation of normal life functions (Eggersdorfer and Wyss, 2018). Vitamin A, which has important roles in vision, gene expression, reproduction, embryonic development, growth, and immune function, is provided by foods of animal and plant origin (Karaağaç and Pınarlı, 2023). Vitamin E regulates heart, vascular, nerve, and brain functions, helps heal wounds, and increases the durability of DNA molecules. It also has the ability to protect cells from damage caused by free radicals occurring in the body (Stevens, 2021). It is reported that lycopene not only helps repair damaged cells in the body but also has a protective effect against types of cancer and chronic diseases because antioxidant properties (Zengin and Kurt, 2018).

Conversion of Oxidized Glutathione (GSSG) to Reduced glutathione (GSH) is important in terms of preventing free radical damage (Gill et al., 2013).

While GSSG is an indicator of oxidative stress, it also inhibits protein synthesis, GSH has many physiological functions like preventing the harmful effects of drugs (Mendoza-Cózatl et al., 2005). GSH and GSSG are important indicators of cellular redox status and organismal health and are in balance in the cell, and disruption of the balance against GSH causes negative effects. Therefore, reduced glutathione to oxidized glutathione ratio is also known as a stress indicator (Cnubben et al., 2001). Radical compounds cause lipid peroxidation of fatty acids in cell membranes. Lipid peroxides transform into compounds such as Malondialdehyde (MDA) and 4-Hydroxyneoneal (4-HNE), which are indicative of lipid peroxidation (Gawel et al., 2004).

Foods are sensitive to various environmental factors such as moisture, light, oxygen, and microorganisms, and these factors can cause spoilage (Redfearn et al., 2023). He et al. (2023) report that ginger oil is turned into a film and used to preserve foods such as bread, meat, fish, and fruit. Some biochemical parameters in foods change depending on shelf life. Sumac in many cultures generally consumed in ground form together with food.

Aimed of this study is to determine the fat-soluble vitamins and stress biomarkers in sumac samples grown in different regions according to time and storage characteristics (vegetable oil/fat-free). In addition to comparing the effect of storage conditions, ground sumac samples were divided into three portions, fresh; analyzed immediately, and oiled and unoled samples analyzed after 6 months.

MATERIAL AND METHODS

Materials:

All sumac samples from Türkiye and Iraq were obtained fresh from public markets. 500 grams of fresh sumac samples from each region were homogenized and 3 different portions were taken and mixed thoroughly. Then, samples were dried in an oven at 60 °C for 10 hours. From each group of samples, 25 grams were taken from 3 different portions of the sample, ground in a mixer, sieved, and separated from their seeds, then samples were sieved in a 100-mesh sieve (Retsch AS 200). These samples were then divided into three groups one of the groups was oiled by spraying sunflower oil, the second group of samples kept as is and the third group of samples (fresh) was analyzed immediately. On the other hand, the other two groups, oiled and unoled, were packaged and stored in the refrigerator for six months. At the end of six months, sumac samples were analyzed.

Methods:

Determination of vitamin A, E, β -Carotene, Lycopene, and 4-HNE 1.0-gram sumac sample was taken, 6.0 mL of ethanol was added and vortexed, then sonicated in an ice water bath (Wise Clean, WUC-AO3H, 170 W) 10 times for 30 seconds for each sample. Sonicated samples were centrifuged at 8000 rpm for 10 min, then 1.0 mL n-hexane was added to each tube and centrifuged again at 4000 rpm for 6 min. The n-hexane phase was transferred to a glass tube and this process was repeated twice. Hexane was removed under vacuum at 30 °C, and then 1.0 mL of methanol was added to the residue in the tube and transferred to HPLC vials. In HPLC, analyses were carried out on an Inertsil ODS-3 column (25.0 cm x 4.6 mm x 5.0 μ m) using a mixture of methanol and water (95:5) as the mobile phase (İbrahim et al. 2017).

Determination of GSH, GSSG, and MDA The amounts of GSH, GSSG, and MDA in sumac samples were determined by HPLC on the SGE Walkosil II 5C18 RS (15cm x 4.6 mm x 5 μ m) column, using 50 mM NaClO₄ solution containing 0.1% H₃PO₄ as the mobile phase (İbrahim et al. 2017).

Statistical Analysis:

All analyses were repeated three times. Findings were subjected to One-Way ANOVA using SPSS 26.0 for MS Windows, and the results are given mean \pm error. Power analysis was conducted using G*Power version 3.1.9.7 (Faul et al., 2007) to determine the minimum sample size required to test the study hypothesis. Results indicated the required sample size to achieve 0.80 power (1- β) for detecting a medium effect, at a significance criterion of $\alpha = 0.05$, with the effect size of 0.45 was $n = 99$ for One-way ANOVA. Differences between group means were analyzed for significance using the Tukey HSD test and statistical significance was expressed as $p < 0.05$. Significant differences in table rows are indicated by superscript capital letters (A-C) while the same letter indicates there is no

statistical difference between groups. Similarly, the same small letters in the table column indicate that there is no significant difference ($p > 0.05$) within the regions.

RESULTS and DISCUSSIONS

Vitamins are micronutrients necessary for the growth and development of living things, and fat-soluble vitamins are stored in the body and play a role in maintaining homeostasis (Yuan et al., 2020). Some biochemical parameters in foods change depending on shelf life. Sumacs are generally offered for consumption in ground form with foods.

The amounts of vitamins A and E, β -carotene, lycopene, GSH, GSSG, GSH/GSSG, MDA, and 4-HNE found as a result of different treatments applied to sumac grown in different regions are given in Tables 1-9.

Vitamin A is necessary for epithelial tissue, health, and general growth and is effective in reproduction and bone growth (Stevens, 2021). The amount of vitamin A in fresh sumac samples from different regions varies between $1.30 \pm 0.05 - 2.77 \pm 0.06 \mu\text{g (g dw)}^{-1}$. It was observed that the amount of vitamin A in sumac samples oiled and unoled varied between $0.97 \pm 0.03 - 2.34 \pm 0.07$, and $0.85 \pm 0.04 - 2.00 \pm 0.06 \mu\text{g (g dw)}^{-1}$, respectively. The difference between fresh, unoled, and oiled groups is statistically significant ($p < 0.05$). The difference between the amounts of vitamin A in sumac samples grown in Maraş and Sheladize regions is statistically insignificant ($p > 0.05$) (Table 1). Okonkwo and Ogu (2014) reported that the vitamin A contents in Myristica fragrans, Piper guineense, Monodora myristica, and Rosmarinus officinalis samples were 14.57, 7.08, 13.71, and 14.87 $\mu\text{g (100 g)}^{-1}$, respectively. Pereira et al. (2011) found the vitamin A content in yellow guava, guabiroba, and uvaia to be 0.718, 6.838, and 37.834 μg equivalent to retinol (g dry matter)⁻¹, respectively.

Table 1 Amount of vitamin A in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n= 33 each group)

Tablo 1. Sumak örneklerindeki A vitamini miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (her grupta n=33)

Region	Fresh group	Unoled group	Oiled group
Maraş	d 1.93 \pm 0.05 ^A	c 1.51 \pm 0.04 ^B	e 1.75 \pm 0.04 ^C
Elazığ	d 1.89 \pm 0.06 ^A	c 1.46 \pm 0.05 ^B	e 1.70 \pm 0.04 ^C
Shelaza	c 1.69 \pm 0.07 ^A	b 1.15 \pm 0.04 ^B	d 1.45 \pm 0.04 ^C
Trawanish	b 1.30 \pm 0.05 ^A	a 0.92 \pm 0.04 ^B	b 1.17 \pm 0.04 ^C
Shahi	d 1.88 \pm 0.05 ^A	c 1.45 \pm 0.04 ^B	e 1.70 \pm 0.05 ^C
Charput	g 2.77 \pm 0.06 ^A	e 2.00 \pm 0.06 ^B	g 2.34 \pm 0.07 ^C
Süleymaniye	f 2.56 \pm 0.07 ^A	e 1.90 \pm 0.05 ^B	g 2.20 \pm 0.07 ^C
Kadana	c 1.73 \pm 0.05 ^A	b 1.23 \pm 0.04 ^B	d 1.46 \pm 0.04 ^C
Derişke	a 1.12 \pm 0.05 ^A	a 0.85 \pm 0.04 ^B	a 0.97 \pm 0.03 ^C
Ranya	e 2.16 \pm 0.09 ^A	d 1.72 \pm 0.05 ^B	f 1.96 \pm 0.06 ^C
Shalidize	c 1.69 \pm 0.06 ^A	b 1.19 \pm 0.04 ^B	c 1.35 \pm 0.04 ^C

Letters with different superscripts (a-g) within the same column and capital letters (A-C) within the row differ significantly $P < 0.05$

Table 2. Amount of β -carotene in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n=33 each group)

Tablo 2. Sumak örneklerindeki β -karoten miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (her grupta n=33)

Region	Fresh	Unoiled group	Oiled group
Maraş	^d 4.46 ± 0.12 ^A	^f 3.70 ± 0.09 ^B	^d 3.98 ± 0.09 ^C
Elazığ	^c 4.21 ± 0.10 ^A	^d 3.52 ± 0.08 ^B	^d 3.85 ± 0.09 ^C
Shelaza	^b 3.61 ± 0.09 ^A	^b 2.82 ± 0.07 ^B	^b 3.06 ± 0.08 ^C
Trawanish	^c 4.12 ± 0.09 ^A	^d 3.47 ± 0.08 ^B	^d 3.90 ± 0.09 ^C
Shahi	^c 4.11 ± 0.11 ^A	^d 3.45 ± 0.10 ^B	^d 3.92 ± 0.10 ^C
Charput	^c 4.02 ± 0.08 ^A	^c 3.04 ± 0.07 ^B	^c 3.51 ± 0.07 ^C
Süleymaniye	^e 4.73 ± 0.10 ^A	^{e, f} 3.67 ± 0.07 ^B	^f 4.10 ± 0.09 ^C
Kadana	^f 5.31 ± 0.12 ^A	^g 4.30 ± 0.10 ^B	^g 4.90 ± 0.10 ^C
Derişke	^b 3.53 ± 0.09 ^A	^b 2.73 ± 0.07 ^B	^b 3.05 ± 0.08 ^C
Ranya	^e 4.86 ± 0.11 ^A	^f 3.77 ± 0.09 ^B	^f 4.15 ± 0.09 ^C
Shalidize	^a 2.48 ± 0.07 ^A	^a 1.85 ± 0.06 ^B	^a 2.15 ± 0.06 ^C

Letters with different superscripts (a-g) within the same column and capital letters (A-C) within the row differ significantly P<0.05

Table 3. Amount of lycopene in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n=33)

Tablo 3. Sumak örneklerindeki likopen miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (n=33)

Region	Fresh	Unoiled group	Oiled group
Maraş	^e 11.13 ± 0.35 ^A	^d 8.41 ± 0.31 ^B	^d 9.76 ± 0.26 ^C
Elazığ	^b 8.10 ± 0.31 ^A	^b 5.38 ± 0.20 ^B	^b 7.00 ± 0.21 ^C
Shelaza	^d 10.38 ± 0.35 ^A	^c 7.55 ± 0.25 ^B	^c 8.80 ± 0.27 ^C
Trawanish	^g 22.16 ± 0.73 ^A	^f 16.17 ± 0.52 ^B	^h 18.26 ± 0.45 ^C
Shahi	^c 8.87 ± 0.32 ^A	^b 5.58 ± 0.15 ^B	^b 7.10 ± 0.22 ^C
Charput	^h 26.90 ± 0.80 ^A	^g 18.85 ± 0.53 ^B	^g 15.16 ± 0.47 ^C
Süleymaniye	^f 12.57 ± 0.43 ^A	^e 9.38 ± 0.33 ^B	^e 10.70 ± 0.36 ^C
Kadana	^a 7.08 ± 0.24 ^A	^a 4.30 ± 0.13 ^B	^a 5.98 ± 0.17 ^C
Derişke	^h 25.63 ± 0.77 ^A	^g 18.22 ± 0.50 ^B	ⁱ 21.91 ± 0.70 ^C
Ranya	^f 13.30 ± 0.44 ^A	^e 9.65 ± 0.37 ^B	^f 11.30 ± 0.39 ^C
Shalidize	^e 11.63 ± 0.37 ^A	^d 8.55 ± 0.30 ^B	^d 9.84 ± 0.31 ^C

Letters with different superscripts (a-i) within the same column and capital letters (A-C) within the row differ significantly P<0.05

Table 4. Amount of Vitamin E in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n=33)

Tablo 4. Sumak örneklerindeki E vitamini miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (n=33)

Region	Fresh	Unoiled group	Oiled group
Maraş	^e 155.53 ± 3.84 ^A	^e 118.86 ± 3.92 ^B	^e 139.18 ± 4.06 ^C
Elazığ	^b 105.42 ± 3.69 ^A	^b 85.12 ± 3.08 ^B	^b 94.22 ± 3.19 ^B
Shelaza	^a 84.40 ± 3.22 ^A	^a 64.07 ± 2.58 ^B	^a 76.30 ± 2.91 ^C
Trawanish	^c 120.40 ± 4.22 ^A	^c 92.93 ± 3.36 ^B	^c 107.80 ± 3.50 ^C
Shahi	^g 212.51 ± 5.86 ^A	^h 180.67 ± 3.89 ^B	^g 197.29 ± 3.68 ^C
Charput	^f 173.36 ± 4.94 ^A	^g 141.40 ± 3.84 ^B	^f 158.20 ± 3.89 ^B
Süleymaniye	^d 137.17 ± 3.99 ^A	^d 105.54 ± 3.34 ^B	^d 120.68 ± 3.40 ^C
Kadana	^h 230.65 ± 6.55 ^A	ⁱ 190.40 ± 5.16 ^B	^h 207.07 ± 5.29 ^C
Derişke	^f 164.74 ± 4.89 ^A	^f 127.87 ± 4.18 ^B	^e 145.22 ± 3.92 ^C
Ranya	^g 216.95 ± 6.34 ^A	^h 175.45 ± 4.99 ^B	^g 193.08 ± 5.17 ^C
Shalidize	^a 89.26 ± 3.04 ^A	^a 61.86 ± 2.59 ^B	^a 77.18 ± 2.81 ^C

Letters with different superscripts (a-i) within the same column and capital letters (A-C) within the row differ significantly P<0.05

Carotenoids, which protect living systems from free radicals, react with peroxide radicals and molecular oxygen. Carotenoids such as β -carotene and lycopene exhibit antioxidant properties by blocking free radicals (Pereira et al., 2011). It was determined that the amount of β -carotene in fresh sumac samples

varied between 2.48 ± 0.07 - 5.31 ± 0.12, on the other hand, unoiled and oiled groups varied in between 1.85 ± 0.06 - 4.30 ± 0.10, 2.15 ± 0.06 - 4.90 ± 0.10 $\mu\text{g (g dw)}^{-1}$, respectively. β -carotene loss in the unoiled group is higher than in the oiled group. While the lowest amount of β -carotene was found in Shalidize sumac,

the highest amount was found in Kadana region sumac. It can be said that there is no significant difference between Derişke and Shelaza, Elazığ, Trawanish, Shahi, and Charput regions, as well as between Ranya and Süleymania regions. In terms of β -carotene, the difference between all groups is statistically significant ($p < 0.05$) (Table 2). Aremu and Nweze (2017) found the amounts of vitamin A in

Guava, Pawpaw, and Mango fruits as 504.10, 683.93, and 301.61 μg (100 g^{-1}), and the amounts of β -carotene as 3015.27, 4043.45 and 1797.21 μg (100 g^{-1}), respectively. The amount of β -carotene in *A. sativum*, *Z. officinale*, *A. melegueta*, and *E. caryophyllata* samples was reported to be 109.5, 226.8, 308.5 and 98.1 mg (100 g^{-1}), respectively (Omotayo and Adepoju, 2013).

Table 5. Amount of GSH in sumac samples (μg (g dw^{-1})) ($n=33$)

Tablo 5. Sumak örneklerindeki GSH miktarı (μg ($\text{g kuru ağırlık}^{-1}$)) ($n=33$)

Region	Fresh	Unoiled group	Oiled group
Maraş	^f 1912.23 \pm 21.60 ^A	^f 1706.40 \pm 19.63 ^B	^f 1810.07 \pm 17.27 ^C
Elazığ	^d 1588.63 \pm 18.77 ^A	^d 1375.14 \pm 16.17 ^B	^d 1441.14 \pm 14.48 ^C
Shelaza	^k 2550.42 \pm 18.48 ^A	^j 2314.90 \pm 17.65 ^B	^j 2426.56 \pm 18.11 ^C
Trawanish	^b 1285.10 \pm 13.89 ^A	^b 1125.44 \pm 14.48 ^B	^b 1178.72 \pm 13.68 ^C
Shahi	^a 1004.12 \pm 12.57 ^A	^a 877.53 \pm 12.25 ^B	^a 913.53 \pm 11.68 ^C
Charput	^e 1759.33 \pm 15.80 ^A	^e 1408.14 \pm 15.11 ^B	^e 1559.14 \pm 14.56 ^C
Süleymaniye	^j 2390.10 \pm 16.56 ^A	ⁱ 2098.39 \pm 17.05 ^B	ⁱ 2187.39 \pm 17.57 ^C
Kadana	^c 1362.07 \pm 12.76 ^A	^c 1169.70 \pm 13.43 ^B	^c 1215.70 \pm 13.43 ^C
Derişke	ⁱ 2152.10 \pm 16.02 ^A	^h 1943.06 \pm 17.13 ^B	^h 2014.06 \pm 16.63 ^C
Ranya	^h 2109.69 \pm 15.86 ^A	^h 1915.95 \pm 17.56 ^B	^h 2015.95 \pm 17.04 ^C
Shalidize	^g 2050.29 \pm 14.99 ^A	^g 1883.45 \pm 16.72 ^B	^g 1969.12 \pm 16.13 ^C

Letters with different superscripts (a-j) within the same column and capital letters (A-C) within the row differ significantly $P < 0.05$

Table 6. Amount of GSSG in sumac samples (μg (g dw^{-1})) ($n=33$)

Tablo 6. Sumak örneklerindeki GSSG miktarı (μg ($\text{g kuru ağırlık}^{-1}$)) ($n=33$)

Region	Fresh	Unoiled group	Oiled group
Maraş	^f 895.06 \pm 13.92 ^A	^f 1147.68 \pm 17.63 ^B	^f 1014.35 \pm 15.45 ^C
Elazığ	^k 1375.38 \pm 18.74 ^A	^h 1492.55 \pm 19.43 ^B	ⁱ 1272.55 \pm 15.95 ^C
Shelaza	ⁱ 992.85 \pm 12.16 ^A	^f 1126.31 \pm 14.32 ^B	^f 1027.31 \pm 13.82 ^C
Trawanish	^d 806.53 \pm 11.06 ^A	^d 1016.97 \pm 12.79 ^B	^d 901.34 \pm 11.46 ^C
Shahi	^a 422.54 \pm 7.61 ^A	^a 519.87 \pm 8.23 ^B	^a 488.21 \pm 7.62 ^C
Charput	^b 503.85 \pm 8.99 ^A	^b 606.96 \pm 9.06 ^B	^b 560.62 \pm 9.05 ^C
Süleymaniye	^g 921.06 \pm 11.07 ^A	^f 1117.22 \pm 13.04 ^B	^g 1052.22 \pm 13.04 ^C
Kadana	^c 693.56 \pm 9.51 ^A	^c 745.23 \pm 9.98 ^B	^c 715.90 \pm 9.35 ^C
Derişke	^j 1075.40 \pm 13.86 ^A	^g 1227.24 \pm 14.77 ^B	^h 1153.91 \pm 13.89 ^C
Ranya	^e 859.07 \pm 11.27 ^A	^d 1015.73 \pm 12.84 ^B	^e 975.73 \pm 12.26 ^C
Shalidize	^h 949.92 \pm 11.84 ^A	^e 1084.80 \pm 12.38 ^B	^f 1005.06 \pm 12.81 ^C

Letters with different superscripts (a-k) within the same column and capital letters (A-C) within the row differ significantly $P < 0.05$

Table 7. GSH/GSSG ratios in sumac samples

Tablo 7. Sumak örneklerindeki GSH/GSSG oranı

Region	Fresh	Unoiled group	Oiled group
Maraş	2.14	1.49	1.78
Elazığ	1.16	0.92	1.13
Shelaza	2.57	2.06	2.36
Trawanish	1.59	1.11	1.31
Shahi	2.38	1.69	1.87
Charput	3.49	2.32	2.78
Süleymaniye	2.59	1.88	2.08
Kadana	1.96	1.57	1.70
Derişke	2.00	1.58	1.75
Ranya	2.46	1.89	2.07
Shalidize	2.16	1.74	1.96

Table 8. Amount of MDA in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n=33)

Tablo 8. Sumak örneklerindeki MDA miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (n=33)

Region	Fresh	Unoiled group	Oiled group
Maraş	^b 18.66 ± 0.67 ^A	^c 22.79 ± 0.45 ^B	^c 20.53 ± 0.38 ^C
Elazığ	^a 13.95 ± 0.53 ^A	^a 16.46 ± 0.36 ^B	^a 15.12 ± 0.23 ^C
Shelaza	^c 22.80 ± 0.81 ^A	^d 26.57 ± 0.75 ^B	^d 24.90 ± 0.31 ^C
Trawanish	^e 28.37 ± 0.87 ^A	^f 32.09 ± 1.00 ^B	ⁱ 30.52 ± 0.35 ^C
Shahi	^{d, e} 27.25 ± 0.96 ^A	^f 32.34 ± 0.95 ^B	^h 29.47 ± 0.38 ^C
Charput	^d 26.19 ± 0.92 ^A	^e 30.39 ± 1.05 ^B	^g 28.46 ± 0.35 ^C
Süleymaniye	^{c, d} 25.38 ± 0.87 ^A	^e 29.38 ± 1.05 ^B	^f 27.33 ± 0.37 ^C
Kadana	^{c, d} 24.97 ± 1.00 ^A	^e 28.62 ± 1.00 ^B	^e 26.37 ± 0.29 ^C
Derişke	^c 24.14 ± 0.96 ^A	^e 29.73 ± 1.01 ^B	^e 26.06 ± 0.34 ^C
Ranya	^b 17.83 ± 0.61 ^A	^b 21.18 ± 0.63 ^B	^b 19.21 ± 0.26 ^C
Shalidize	^f 31.30 ± 1.02 ^A	^g 35.63 ± 1.02 ^B	^j 33.16 ± 0.45 ^C

Letters with different superscripts (a-j) within the same column and capital letters (A-C) within the row differ significantly P<0.05

Table 9. Amount of 4-HNE in sumac samples ($\mu\text{g (g dw)}^{-1}$) (n=33)

Tablo 9. Sumak örneklerindeki 4-HNE miktarı ($\mu\text{g (g kuru ağırlık)}^{-1}$) (n=33)

Region	Fresh	Unoiled group	Oiled group
Maraş	^d 11.69 ± 0.23 ^A	^{b, c} 17.96 ± 0.62 ^B	^d 14.83 ± 0.61 ^C
Elazığ	^a 7.12 ± 0.19 ^A	^a 12.08 ± 0.46 ^B	^a 9.26 ± 0.35 ^C
Shelaza	^e 12.80 ± 0.35 ^A	^b 17.73 ± 0.64 ^B	^d 14.50 ± 0.48 ^C
Trawanish	^e 13.37 ± 0.40 ^A	^c 18.34 ± 0.74 ^B	^{d, e} 15.20 ± 0.50 ^C
Shahi	^c 9.94 ± 0.32 ^A	^b 16.77 ± 0.58 ^B	^c 13.10 ± 0.42 ^C
Charput	^e 13.19 ± 0.40 ^A	^c 19.00 ± 0.73 ^B	^e 16.11 ± 0.49 ^C
Süleymaniye	^f 14.61 ± 0.47 ^A	^{c, d} 20.07 ± 0.72 ^B	^f 17.51 ± 0.60 ^C
Kadana	^a 7.26 ± 0.21 ^A	^a 11.63 ± 0.46 ^B	^a 9.45 ± 0.37 ^C
Derişke	^b 8.14 ± 0.28 ^A	^a 12.14 ± 0.45 ^B	^b 10.48 ± 0.42 ^C
Ranya	^f 15.40 ± 0.47 ^A	^d 21.05 ± 0.77 ^B	^f 17.55 ± 0.50 ^C
Shalidize	^e 13.23 ± 0.39 ^A	^c 18.73 ± 0.66 ^B	^e 16.45 ± 0.54 ^C

Letters with different superscripts (a-f) within the same column and capital letters (A-C) within the row differ significantly P<0.05

The amount of lycopene in the fresh, unoiled and oiled group sumac samples varies between 7.08 ± 0.24 - 26.90 ± 0.80 , 4.30 ± 0.13 - 18.85 ± 0.53 , 7.00 ± 0.21 - $21.91 \pm 0.70 \mu\text{g (g dw)}^{-1}$, respectively. While the highest amount of lycopene was found in the sumac of Charput region, the least amount was found in the sumac of Kadana region. The difference between the lycopene content of fresh sumac samples regarding different regions together with fresh, unoiled, and oiled groups is statistically significant ($p < 0.05$). It was observed that the loss of lycopene in the non-oiled group was greater than the loss in the oiled group (Table 4). It has been reported that the amount of lycopene in guava, papaya, rosehip, and red pepper varies between 52.3 - 55.0 , 1.1 - 53.0 , 6.8 - 7.1 , and 10.8 - $26.2 \mu\text{g g}^{-1}$, respectively (Zengin and Kurt, 2018).

Vitamin E has strong antioxidant properties and helps prevent cell membranes and lipoproteins from being damaged by oxidative stress. Vitamin E has a role in several physiological processes, including immunological function, inflammation control, gene expression regulation, and cognitive functioning (Dror and Allen, 2011). The amount of vitamin E in fresh

sumac samples from different regions was found to vary in between 84.40 ± 3.22 - $230.65 \pm 6.55 \mu\text{g (g dw)}^{-1}$. The highest vitamin E was determined in Kadana region, while the lowest was determined in Shelaza region. While there is no statistical difference between the fresh sample of the Shelaza and Shalidize regions ($p > 0.05$), all other regions are statistically different from each other ($p < 0.05$). It was determined that vitamin E in the unoiled group sumac samples ranged between 61.86 ± 2.59 - 190.40 ± 5.16 , while in the oiled group samples, it varied between 76.30 ± 2.91 - $207.07 \pm 5.29 \mu\text{g (g dw)}^{-1}$. Vitamin E in the oiled group of sumac samples was found to be higher than in the unoiled groups. In other words, vitamin E loss is less in the oiled group. The amounts of vitamin E in the fresh, unoiled, and oiled groups are statistically different ($p < 0.05$) (Table 4). It was reported that the amount of β -carotene in the ginger, garlic, turmeric, black pepper, and clove samples was 56.11 , 68.17 , 151.74 , 92.14 , and 83.43 , and vitamin E was 10.23 , 13.13 , 11.24 , 15.32 and $22.51 \text{ mg (100 g)}^{-1}$, respectively (Ayoade et al., 2023). Uhegbu et al. (2011) reported that the amount of vitamin E in *P. Guineense* and *M. Myristica* was 1.64 and $12.0 \text{ U (100 g)}^{-1}$, respectively.

It has been found that the amount of vitamin E in apricots grown under different conditions varies between 27.10 - 85.10 μg (100 g^{-1}) (Kan et al., 2014). Çakmak et al. (2020) reported that the amounts of vitamin A, E, β -carotene and lycopene in wild white *Myrtus communis* L. fruit were 1.85, 206.57, 5.89 and 9.79 μg (g dw^{-1}), respectively. It was reported that the amounts of vitamins A, E, β -carotene and lycopene in fresh fruits of *Crataegus laevigata* samples grown in the Elazığ region were 0.78, 0.83, 2.88 and 2.34 $\mu\text{g g}^{-1}$, respectively (İbrahim et al. 2017). It was observed that the loss in the amounts of vitamins A, E, β -carotene, and lycopene in sumac samples oiled less than in unoiled samples. It has been noted that parameters such as temperature and shelf life are important in the degradation of vitamins (Kala and Prakash, 2006). It has been reported that the loss of vitamins in chili pepper samples kept for a certain period in unoiled form is greater than in oiled samples (Karatat et al., 2017). Konfo et al. (2023) reported that essential oils, as natural antioxidants, are used in the preservation of foodstuffs. Falowo et al. (2019) reported that 2% and 4% basil essential oil applied to ground beef increased oxidative stability and preserved color during storage. Glutathione, an essential component for cellular immune system function, has a peptide structure and serves as the primary intracellular antioxidant. Additionally, it plays a role in amino acid transport in metabolism and the reduction of sulfhydryl groups in proteins (Mendoza-Cózatl et al., 2005).

It was determined that the amount of GSH in fresh sumac samples varied between $1004.12 \pm 12.57 - 2550.42 \pm 18.48$, while GSSG varied between $422.54 \pm 7.61 - 1375.38 \pm 18.74$ μg (g dw^{-1}). The highest amounts of GSH and GSSG were in Shelaza and Elazığ regions, respectively, on the other hand, the lowest amounts were observed in the Shahi region (Tables 5 and 6). The amounts of both GSH and GSSG in fresh sumac samples are statistically different according to every region ($p < 0.05$). The amount of GSH in the unoiled and oiled group sumac samples was found to vary between $877.53 \pm 12.25 - 2314.90 \pm 17.65$ and $913.53 \pm 11.68 - 2426.56 \pm 18.11$ μg (g dw^{-1}) respectively. It was determined that the amount of GSSG in the same samples varied between $519.87 \pm 8.23 - 1492.55 \pm 19.43$ and $488.21 \pm 7.62 - 1272.55 \pm 15.95$ μg (g dw^{-1}). It was observed that while the amount of GSH in the stored sumac samples decreased, on the other hand, GSSG increased. This might be the result of oxidation during the waiting period of the samples. While the loss of GSH in unoiled samples was higher than in the oiled samples, the increase in the amount of GSSG was found to be greater in the unoiled samples. From these results, it can be said that oiling prevents oxidation of the samples forming a thin film in between air and the sumac surface. The differences between the sumac samples of all three groups in terms of both GSH and

GSSG amounts are statistically significant ($p < 0.05$) (Tables 5 and 6).

Cerit et al. (2020) reported that the amount of GSH in red pepper, turmeric, cardamom, and ginger was 42, 41, 112, and 1076 nM (g dw^{-1}), respectively. Tesoriere et al. (2005) discovered that the levels of GSH in three distinct cultures of prickly pears ranged from 3.40 to 8.10 mg (100 g^{-1}). The GSH/GSSG ratio is higher under normal conditions but decreases under stress (Kocsy et al., 2001). As seen in Table 7, the highest GSH/GSSG ratio was found in fresh sumac samples, while the lowest ratio was observed in unoiled sumac samples. These results confirm that oiling the samples partially reduces oxidation. It was reported that when hydrogen peroxide was applied to spinach, green banana, and red pepper for the disinfection process, the amount of GSSG increased compared to the control group, while the GSH and GSH/GSSG ratio decreased (Qiang et al., 2005). MDA and 4-HNE, which are formed as a result of the peroxidation of polyunsaturated fatty acids, are used as stress indicators (Barrera et al., 2018). As seen in table 8, the amount of MDA in fresh, unoiled and oiled sumac samples varied between $(13.95 \pm 0.53 - 31.30 \pm 1.02, 16.46 \pm 0.36 - 35.63 \pm 1.02$ and $15.12 \pm 0.23 - 33.16 \pm 0.45$ μg (g dw^{-1}), respectively. The lowest amount of MDA was observed in fresh sumac samples, while the highest amount was observed in an unoiled sample. The difference between MDA in the fresh, unoiled, and oiled groups is statistically significant ($p < 0.05$). It has been reported that the MDA concentration in mature ber fruit (*Ziziphus mauritiana* Lam) is 4.498 nmol/g (Kumar et al., 2011). Çakmak et al. (2023) reported that the amount of MDA in fresh and sun-dried black *Myrtus communis* L. fruit were 5.32 and 6.80 μg (g dw^{-1}). It was determined that the amount of 4-HNE in fresh, unoiled and oiled sumac samples from different regions varied between $7.12 \pm 0.19 - 15.40 \pm 0.47, 11.63 \pm 0.46 - 21.05 \pm 0.77, 9.26 \pm 0.35 - 17.55 \pm 0.50$ μg (g dw^{-1}), respectively. The lowest amount of 4-HNE in the fresh sumac sample was observed in the sumac of the Elazığ region, while the highest was observed in the Ranya region sumacs. In terms of the amount of 4-HNE, it can be said that there is no significant difference between the Elazığ and Kadana regions, Shelaza, Trawanish and Sheladize regions, and Ranya and Süleymania regions. The amount of 4-HNE in fresh, unoiled, and oiled groups of sumacs in the same regions is statistically different ($p < 0.05$) (Table 9).

The highest amounts of GSSG, MDA, and 4-HNE were found in unoiled sumac samples. This might be explained that the oiling partially reduces oxidative stress. Muktar et al. (2023) found that the amounts of GSH, GSSG, MDA, and 4-HNE in bitter tomatoes as 364, 225, 1.50, and 24.57, respectively, and the same parameters in White Garden Egg were 1930, 962, 8.40 and 38.25 μg (g dw^{-1}). Çakmak et al. (2020) found the

amounts of GSH, GSSG, MDA and GSH/GSSG ratios in wild white *Myrtus communis* L. fruits as 609.90, 184.24, 5.73 $\mu\text{g (g dw)}^{-1}$) and 3.31, respectively, while the same parameters in cultivated white *Myrtus communis* L. fruits were 571.80, 115.50, 4.50 $\mu\text{g (g dw)}^{-1}$ and 4.95, respectively.

CONCLUSION

Charput and Suleymaniye are richer in vitamin A, Kadana and Ranya are richer in β -carotene, Charput, Derişke and Trawanish are richer in lycopene. Kadana, Ranya, and Shahi sumacs are richer in vitamin E than other regions. Derişke and Trawanish are poorer in vitamin A, Sheladize is poorer in beta carotene, Kadana is poorer in lycopene, and Shelaza and Shalidize sumacs are poorer in vitamin E. While Shelaza is the richest in terms of GSH, Shahi region sumac has the lowest in terms of GSSG. Elaziğ region sumac has the lowest amounts of MDA and 4-HNE. It was found that the changes in all the measured parameters of oiled sumac samples were lower than the unoled samples. It can be concluded from these results that, to protect the sumac sample from degradation it should be oiled to preserve it for longer shelf life. The difference between regions in the amounts of fat-soluble vitamins, glutathione, and stress biomarkers might be due to geographical and ecological conditions.

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Author Contribution

Authors declare that they all contributed equally to the article.

Conflicting of Interest

All authors declare that there is no conflict of interest.

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Kesirli-Frekanslı Fourier Yöntemlerle Tarımsal İstihdamın Mekanizasyon ve Krediyile İlişkisi Üzerine Bir Zaman Serisi Analizi

Ömer KESKİN¹

¹Van Yüzüncü Yıl Üniversitesi, Özalp Meslek Yüksekokulu, Dış Ticaret Bölümü, Van, Türkiye

¹<https://orcid.org/0000-0002-1939-2791>

✉: omerkeskin@yyu.edu.tr

ÖZET

Bu çalışma, Türkiye'deki tarımsal istihdamın mekanizasyon ve krediyile ilişkisini belirlemeyi amaçlamaktadır. 1981-2022 dönemine ait yıllık zaman serilerinin kullanıldığı çalışmada ilk olarak değişkenlerin durağanlıkları, sırasıyla kesirli-frekanslı Fourier ADF ve geleneksel ADF birim kök testleriyle sınanmıştır. Daha sonra değişkenler arasında eşbütünleşme ilişkisi olup olmadığını belirlemek için kesirli-frekanslı Fourier ADL eşbütünleşme testi uygulanmıştır. Eşbütünleşme test sonucu, tarımsal istihdamla mekanizasyon arasında negatif, kredi arasında ise pozitif ilişki olduğunu göstermiştir. Buna göre tarımsal mekanizasyon düzeyinde yaşanan %1 yükseliş istihdam sayısında uzun ve kısa dönemde sırasıyla %3.74 ve %0.42 düşüşe, tarımsal kredi bakiyesinde yaşanan %1 yükseliş ise istihdam sayısında uzun ve kısa dönemde sırasıyla %0.14 ve %0.02 yükselişe neden olmaktadır. Analizde son olarak kesirli-frekanslı Fourier TY nedensellik testi uygulanıp eşbütünleşme testine ait sonucu destekler nitelikte sonuçlara ulaşılmıştır.

Tarım Ekonomisi

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Kesirli-frekanslı Fourier
Tarımsal istihdam
Tarımsal mekanizasyon
Tarımsal kredi
Zaman serisi analizi

A Time Series Analysis on the Relationship of Agricultural Employment with Mechanization and Credit Using Fractional-Frequency Fourier Methods

ABSTRACT

The present study investigates the relationship between agricultural employment with mechanization and credit in Türkiye. Firstly, the stationarities of the variables are examined by applying the fractional-frequency Fourier ADF and conventional ADF unit root tests in the study, which covers the annual time series from 1981 to 2022, respectively. Then, the fractional-frequency Fourier ADL cointegration test was applied to assess the existence of the cointegration relationship between the variables. The findings of the fractional-frequency Fourier bootstrap ADL procedure revealed that there is a negative relationship between agricultural employment and mechanization and a positive relationship between credit. According to this finding, a 1% increase in the agricultural mechanization level causes a 3.74% and 0.42% decrease in the number of employments in the long and short run, respectively, and a 1% increase in the agricultural credit balance causes a 0.14% and 0.02% increase in the number of employments in the long and short run, respectively. Finally, the fractional-frequency Fourier TY causality test was applied, with the findings supporting the cointegration test finding.

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GİRİŞ

Tarım sektörü, ekonomik olmanın yanı sıra stratejik bir üretim sektörü mahiyetindedir. Çünkü bir yandan

nüfusa beslenmesi için gerekli besini diğer yandan birçok sanayiye üretimde bulunulması için gerekli hammaddeyi sağlamaktadır (Doğan ve ark., 2015). Sektör, ülke ekonomilerindeki birincil sektör olup

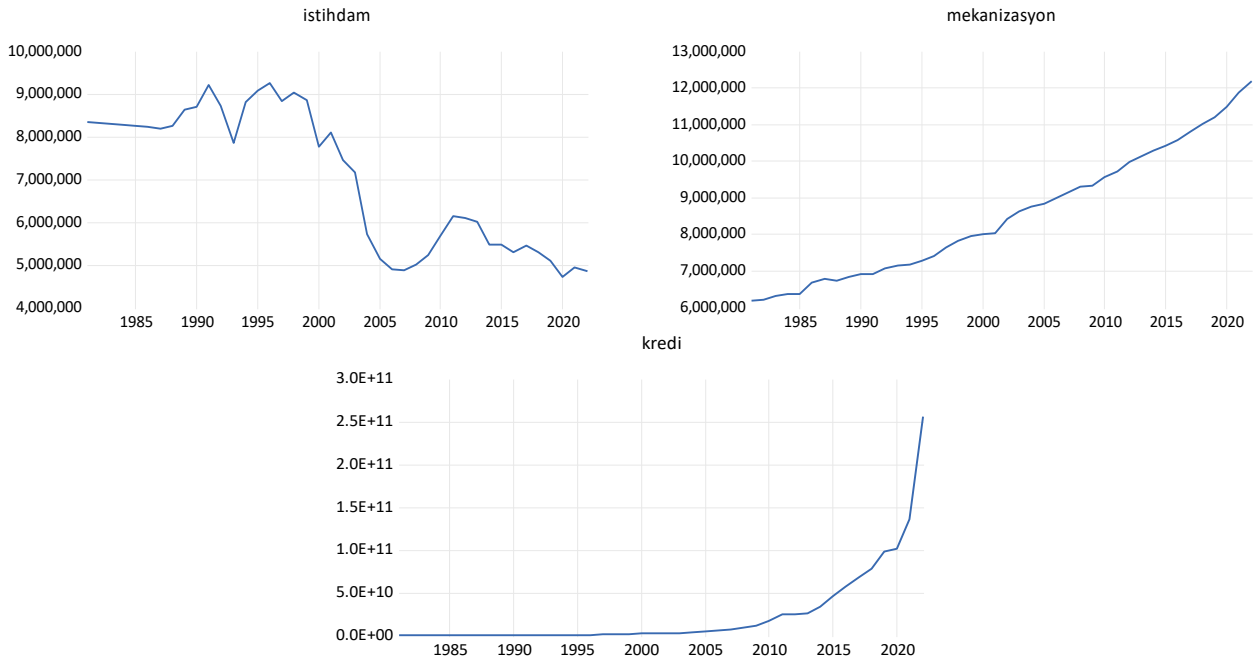
nüfusun önemli istihdam alanlarından biridir. Ancak tarımsal istihdam, ülke ekonomileri geliştikçe çözülmektedir. İktisat literatüründe ekonomik gelişme olgusu sanayileşmeyle bir tutulmaktadır. Bu doğrultuda, söz konusu çözüme, sanayi sektöründeki üretimi artıracak gerekli iş gücünü karşılayabilmek amacıyla, tıpkı gelişmiş ülkelerin gelişme sürecinde yaptıkları gibi, gelişmiş bir ülke olma yolundaki her ülke tarafından model olarak benimsenip uygulanmaktadır (Durgun, 2012).

Dolayısıyla bir ülke ekonomisi geliştikçe tarım sektörünün ekonomideki görece ağırlığının azalacağı bilinen ve beklenen bir olgudur. Nitekim, Türkiye’de özellikle 1950’lerden sonra sektörün milli gelir, ihracat ve istihdam içindeki nispi payları azalış göstermiştir. Örneğin, sektörün milli gelir içindeki payı, 1950’lerde %40’in üzerinde seyredirken 2000’lerin başında %10’un altına inmiştir. Tarımsal istihdamda da benzer nitelikte ancak daha yavaş bir azalma gerçekleşmiş ve sektörün toplam istihdam içindeki payı 1980’lerin sonlarında %50’nin biraz altında, 2000’lerin ortasında ise %25’in altında seyretmiştir (Aydoğuş, 2011). 2023’ün üçüncü çeyreği itibarıyla bu oran %14.8’e düşmüştür (TÜİK, 2023b).

Türkiye’de uygulanan kalkınma politikalarının sanayi sektörünü öne çıkarmasının yanı sıra tarımsal mekanizasyonun iş gücüne olan talebi azaltması,

miras yoluyla tarım arazilerinin parçalanması, tarımsal girdi maliyetlerinin yükselmesi, kırsalda elde edilen gelirin yeterli olmaması ve kırsaldan göçün artması tarımsal istihdamdaki çözülmeyi açıklayan temel nedenler arasında sayılabilir (Işın ve ark., 2010; Şahin, 2023). Diğer bir neden ise özel sektör sermaye kaynaklarının/kredilerin tarım sektörüne yeterince aktarılmaması, başka bir deyişle, tarımsal altyapıyı iyileştirmeye yönelik yeterince kullanılmıyor olmasıdır (Tarım ve Orman Bakanlığı, 2019). Oysa sermaye aktarımının teşvik edilmesiyle kırsalın tarım sektörüne nitelikli (etkin ve verimli) iş gücü konusunda katkı vermesi sağlanırsa Türkiye’de süregelen istihdam sorununun ağırlığı ekonominin genel etkinliği de artırılmak suretiyle hafifletilebilir (Hekimoğlu & Baş, 2018; Üçler, 2022).

Yukarıdaki açıklamalardan hareketle bu çalışmanın amacı, Türkiye örneğinde tarımsal istihdam sayısının mekanizasyon düzeyi (tarımsal makine-alet miktarı) ve kredi bakiyesiyle ilişkisini ortaya koymaktır. Söz konusu değişkenler arasındaki ilişkinin çalışma konusu olarak ele alınmasının nedeni, Türkiye’de tarımsal mekanizasyon varlığı ve kredi bakiyesi noktasında önemli gelişmeler yaşanırken istihdamdaki çözülmeye hızlı bir şekilde devam ediyor olmasıdır (bkz. Şekil 1).



Şekil 1. Serilerin izlediği seyir
Figure 1. Course of the series

Şekil 1 incelendiğinde her ne kadar tarımsal istihdam serisiyle mekanizasyon ve kredi serileri özellikle 2000’lerin başından itibaren ters yönde hareket etmiş gibi görünse de aralarındaki etkileşimin uzun ve kısa dönemde nasıl olduğunu açık bir şekilde ortaya koymak için detaylandırılmış bir analize ihtiyaç

vardır.

Tarım sektöründeki teknolojik ve finansal gelişimin iş gücü kullanımına etkisini yakın geçmişte Fourier fonksiyonlarıyla güçlendirilip literatüre kazandırılmış olan ekonometrik testleri kullanarak analiz etmesi ve konu çerçevesinde özgün politika

önerileri geliştirmesi itibarıyla bu çalışmanın önemli olduğu söylenebilir.

Türkiye’de tarımsal istihdamda yaşanan gelişmelerle ilgilenen araştırmacıların, politika yapımcıların, özel sektör kuruluşlarının ve diğer tüm tarım sektörü paydaşlarının dikkatini geliştirilen özgün politika önerilerine çekmek suretiyle bu çalışmanın fayda sağlaması ve ilgili literatüre katkı vermesi umulmaktadır.

Çalışmanın bundan sonraki kısımlarında ilk olarak literatürdeki çalışmalar, materyal ve yöntem ile ilgili

açıklamalara yer verilmiştir. Daha sonra analizlerden elde edilen bulgular ortaya konulup değerlendirilmiş ve bulgulardan hareketle sonuç kısmında bazı özgün politika önerileri geliştirilmiştir.

LİTERATÜR TARAMASI

Literatürde tarımsal istihdamı etkileyen faktörler konusunu doğrudan ele almış birçok çalışma bulunmaktadır. Söz konusu çalışmalardan bir kısmı, literatür özeti olarak Çizelge 1’de Türkiye ve yabancı ülke örneklemi şeklinde ayrı ayrı yer almaktadır.

Çizelge 1. Literatür özeti

Table 1. Summary of literature

Yazar(lar) ve yıl	İncelenen dönem	Kullanılan yöntem	Amaç	Bulgular
Türkiye örnekleminde yapılmış çalışmalar				
Prilliadi (2023)	1990-2019	Autoregressive Distributed Lag (ARDL) Sınır Testi	Türkiye’de yenilenebilir enerji, döviz kuru ve enflasyonun tarımsal istihdama etkilerine ilişkin ampirik kanıt sağlamak	Yenilebilir enerji, sadece kısa vadede tarımsal istihdamı teşvik eden itici bir güçtür.
Yücel & Çalışkan (2020)	2009Q1-2018Q2	ARDL Sınır Testi	Türkiye’de tarım sektöründeki verimlilik ve makineleşme düzeyinin tarımsal istihdama etkisini araştırmak	Tarım sektöründe verimlilik ve makineleşme düzeyi yükseldikçe tarımsal istihdam oranı düşmektedir.
Şimşir (2012)	1970-2008	Regresyon Analizi, Granger Nedensellik Testi	Türkiye’de tarımsal kredilerin tarımsal istihdama etkisini araştırmak	Tarımsal kredilerin tarımsal gelir ve istihdam üzerinde doğrudan olumlu etkisi bulunmaktadır.
Doğan (2012)	1953-2001	Basit Veri Analizi	Türkiye’de traktör ve biçerdöverin etkilerini değerlendirmek	Traktör ve biçerdöver kullanımı, hem zamandan hem emek gücünden tasarruf sağlamakta ve tarımsal üretimde verimliliği artırmaktadır.
Yabancı ülkeler örneklemlerinde yapılmış çalışmalar				
Shani & Musa (2021)	2018-2019	Anket	Nijerya’da tarımsal mekanizasyonun tarımsal istihdama ve işlenen arazi büyüklüğüne etkisini araştırmak	Tarımsal mekanizasyon, hektar başına iş gücü kullanımını azaltıp işlenen arazi büyüklüğünü ciddi bir ölçüde artırmaktadır.
Barman & Deka (2019)	2014-2015	Mülakat	Hindistan’da tarımsal mekanizasyonun tarımsal istihdama etkisini belirlemek	Tarımsal mekanizasyon düzeyindeki yükseliş ve çiftlik grubu büyüklüğündeki artış tarımsal istihdam miktarında azalışa neden olmaktadır.
Toyin ve ark. (2017)	1975-2015	ARDL Sınır Testi, Toda-Yamamoto-Dolado-Lütkepohl Nedensellik Testi	Güney Afrika’da tarımsal işleme alt sektörü üretimiyle tarımsal istihdam arasındaki ilişkiyi incelemek	Tarımsal işleme alt sektöründeki çıktı, tarım sektöründeki istihdam miktarı üzerinde olumsuz etkiye sahiptir.
Behera (2016)	1972-73; 2009-10	Sıradan En Küçük Kareler Regresyonu	Hindistan’da tarımsal istihdamın belirleyicilerini ortaya koymak	Tarım dışı üretim ve verimlilik gibi değişkenler tarımsal istihdamı olumsuz, kamu yatırımları ve dış ticaret hadleri gibi değişkenler ise olumlu etkilemektedir.
Pablo (2016)	1900-2012	Pearson'un Doğrusal	Ekvator’da tarımsal katma	Tarım sektörünün ulusal

		Korelasyon Katsayısı	değer oranıyla tarımsal istihdam oranı arasındaki ilişkiyi tespit etmek	zenginliğe katkısı ne kadar az ise tarımsal istihdam bir o kadar fazla olmaktadır. Yani söz konusu iki değişken arasında negatif ilişki bulunmaktadır.
Keikha ve ark. (2014)	1981-2011	Eşik Değerli Otoregresif Model	İran'da tarımsal kredilerin tarımsal istihdama etkisini incelemek	Tarımsal kredilerin tahmini eşik değeri %44 olarak hesaplanmış olup bu noktanın aşılması halinin tarımsal istihdam üzerindeki etkisi çok olumsuzdur. Kişi başına düşen gelir, sanayi katma değeri, yurt içinde kullanılan krediler ve doğrudan yabancı yatırımlar gibi değişkenlerde yaşanan artış tarımsal istihdamı çözücü bir etkiye sahiptir. Mekanize çiftliklerde mekanize olmayan çiftçiliklere göre hektar başına daha az iş gücü kullanılmaktadır.
Felipe ve ark. (2014)	1987-2012	ARDL Sınır Testi	Çin'de tarımsal istihdamın çözümlenmesine etki eden faktörleri tahmin etmek	
Lingard & Bagyo (1983)	1979-1980	T Testi	Endonezya'da tarımsal mekanizasyonun tarımsal üretime ve istihdama etkisini belirlemek	

Kaynak: Yazar tarafından oluşturulmuştur.

Ülkelerde tarımsal kredilerin tarımsal istihdamı olumlu etkilediği (Keikha ve ark., 2014; Şimşir, 2012), tarımsal mekanizasyonun ise olumsuz etkilediği (Shani & Musa, 2021; Yücel & Çalışkan, 2020; Berman & Deka, 2019; Lingard & Bagyo, 1983) sayılı çalışmayla belirlenmiştir.

Literatür özetinde görüldüğü üzere tarımsal istihdam konusu, tarım sektörüyle ilişkili değişkenlerle ilişkisi temelinde ve geleneksel hale gelmiş anket, mülakat, eşbütünleşme ve nedensellik test yöntemleri kullanılarak ele alınmıştır. Çizelge 1'deki ilgili çalışmalar arasında Yücel ve Çalışkan (2020) ve Şimşir (2012) tarafından yapılmış çalışmalar, bu çalışmadaki konuya benzer bir konuyu ele almış olan ancak çeşitli eksiklikler barındıran Türkiye örneklemindeki çalışmalardır. Şöyle ki Yücel ve Çalışkan (2020), tarımsal mekanizasyon değişkeni olarak sadece hektar başına düşen traktör sayısını kullanmışlardır. Oysa TÜİK, tarımsal üretimde kullanılan 85 farklı makine ve alet sayısı ile ilgili uzun yıllardan beri veri yayınlamaktadır. Dolayısıyla bu çalışma, tarımsal mekanizasyon değişkeni olarak TÜİK'in bu daha kapsamlı verisini kullanması yönüyle söz konusu çalışmadan farklılaşmaktadır.

Ayrıca yazarların çalışması, sadece 2009-2018 dönemini (10 yıllık) çeyreklik veriler kullanarak ele alması ve bu verileri sadece yapısal değişimleri dikkate almayan geleneksel ARDL sınır testini uygulayarak analiz etmesi (nedensellik testiyle desteklememesi) nedeniyle teknik yönden zayıftır. Aynı zayıflık, Şimşir (2012) tarafından regresyon analizi ve Granger nedensellik testi kullanılarak yapılmış çalışma için de geçerlidir. Bu çalışmanın tekniksel olarak söz konusu çalışmalardan farkı ise

kalıcı ve kalıcı olmayan yapısal değişimleri dikkate alan güncel nitelikteki eşbütünleşme ve nedensellik testlerini birlikte uygulaması ve 1981-2022 dönemini (42 yıllık) yıllık veriler kullanarak ele almasıdır.

Literatürde yapılmış çalışmalardan da anlaşılacağı üzere tarımsal istihdamın mekanizasyonla ilişkisinin negatif, krediyle ilişkisinin ise pozitif olması beklenmektedir. Çünkü tarımsal mekanizasyon, zamandan ve insan gücünden tasarruf sağlarken tarımsal üretimdeki verimliliğin artmasını beraberinde getirmektedir (Doğan, 2012). Diğer taraftan tarımsal krediler ise kırsal kesimlerdeki üretkenliği ve istihdam düzeyini iyileştirici yönde bir etkiye sahiptir (Senanayake, 2002).

MATERYAL ve METOD

Daha önce belirtildiği üzere bu çalışma, Türkiye'de tarımsal istihdamın mekanizasyon ve krediyle ilişkisini araştırmaktadır. Bu kapsamda tarımsal istihdam sayısı, toplam tarımsal makine-alet miktarı (mekanizasyon düzeyi) ve tarımsal kredi bakiyesi verileri kullanılmıştır. Veri setleri, ulaşılabilen verilerden hareketle, dönem olarak 1981-2022 yıllarını kapsamaktadır (Çizelge 2).

Normal şartlarda zaman serilerinde mevsimsel etkilerle karşılaşmak olasıdır. Ancak bu çalışmada yıllık veriler kullanıldığı için mevsimsel ayrıştırma yapmaya gerek kalmamıştır. Çalışma kapsamında ilk olarak birim kök testleriyle değişkenlerin durağanlıkları, daha sonra eşbütünleşme ve nedensellik testleriyle değişkenler arasındaki ilişkiler analiz edilmiştir. Analiz yöntemleri olarak Bozoklu ve ark. (2020) tarafından önerilmiş Kesirli-Frekanslı

Fourier Augmented Dickey-Fuller (KFFADF) birim kök, İlkay ve ark. (2021) tarafından önerilmiş Kesirli-Frekanslı Fourier Autoregressive Distributive Lag (KFFADL) eşbütünleşme ve Pata & Yılcı (2020)

tarafından önerilmiş Kesirli-Frekanslı Fourier Toda-Yamamoto (KFFTY) nedensellik testleri benimsenmiştir.

Çizelge 2. Veriler ve toplandığı kaynaklar
Table 2. Data and data sources

Veri	Modellerde kullanılan kısaltma	Veri kaynağı/kaynakları	Veri ile ilgili bilgi
Tarımsal istihdam	ist	(TÜİK, 2023a)	Bin kişi olarak yayınlanan ve 15+ yaş grubunu temsil eden veridir.
Tarımsal mekanizasyon	mek	(TÜİK, 2023d), (TÜİK, 2023c)	85 farklı makine ve alet sayısının toplamını temsil etmekte olan ve iki farklı veri kaynağından derlenen veridir.
Tarımsal kredi	kre	(TBB, 2023)	Ticari bankaların tarımsal ihtisas kredilerinin bakiyesini temsil etmekte olan ve TL cinsinden yayınlanan veridir.

Geleneksel ADF birim kök testi literatürde popüler olmasına rağmen ciddi bir eksiklik barındırmakta olup söz konusu eksiklik yapısal değişimi dikkate almamasıdır. Şöyle ki ele alınan değişken yapısal bir değişimden etkileniyorsa ve durağanlık test edilirken bu değişimi dikkate almayan bir birim kök testi kullanılırsa gerçekte değişken durağan olsa bile kullanılan test değişkenin durağan olmadığını ortaya koyacaktır. Diğer taraftan yapısal değişimi dikkate alan birim kök testlerinin birçoğunda değişim genellikle kukla değişken marifetiyle modele dahil edilmekte ve değişimin ne zaman gerçekleştiğinin, yani sayısının önceden bilindiği varsayılmaktadır.

Ancak kukla değişken kullanıldığında sadece keskin yapısal değişimlerin gerçekleştiği kabul edilmektedir. Gerçek hayatta ise yapısal değişimler, keskin değil, genellikle daha yumuşak bir şekilde gerçekleşmektedir. İşte bu yapısal değişimleri modellemek amacıyla Fourier fonksiyonları kullanılabilir (Bozoklu ve ark., 2020).

KFFADF testi, bir serideki yapısal değişimleri dikkate aldığı için geleneksel ADF testine göre, Fourier fonksiyonlarını kullandığı için de yapısal değişimleri kukla değişken marifetiyle dikkate alan birim kök testlerine göre çok daha güçlü bir testtir.

$$\Delta ist_t = \alpha_0 + \alpha_1 \sin\left(\frac{2\pi kt}{T}\right) + \alpha_2 \cos\left(\frac{2\pi kt}{T}\right) + \alpha_3 ist_{t-1} + \sum_{i=1}^P \phi_i \Delta ist_{t-1} + \varepsilon_t \quad (1)$$

$$\Delta mek_t = \beta_0 + \beta_1 \sin\left(\frac{2\pi kt}{T}\right) + \beta_2 \cos\left(\frac{2\pi kt}{T}\right) + \beta_3 mek_{t-1} + \sum_{i=1}^P \phi_i \Delta mek_{t-1} + \mu_t \quad (2)$$

$$\Delta kre_t = \delta_0 + \delta_1 \sin\left(\frac{2\pi kt}{T}\right) + \delta_2 \cos\left(\frac{2\pi kt}{T}\right) + \delta_3 kre_{t-1} + \sum_{i=1}^P \gamma_i \Delta kre_{t-1} + v_t \quad (3)$$

KFFADF testinde 1, 2 ve 3 numaralı eşitliklerdeki modeller tahmin edilmektedir. Bu modeller içinde *ist* tarımsal istihdam, *mek* tarımsal mekanizasyon, *kre* tarımsal kredi, Δ birinci fark operatörü, π 3.1416, k frekans değeri, t trend terimi, T gözlem sayısı, P uygun gecikme uzunluğu ve ε_t , μ_t ve v_t ise hata terimleri anlamına gelmektedir (Bozoklu ve ark., 2020).

Tahmin aşamasında ilk olarak frekans değerinin (kesirli veya tamsayı) tespiti yapılmaktadır. Daha sonra F testi kullanılarak *sin*, *cos* trigonometrik terimlerinin içinde bulunduğu fonksiyonların anlamsız olduğu temel hipotezi (H_0) sınanmaktadır. Bu hipotezin reddedilememesi durumunda geleneksel ADF testine başvurulmaktadır. Fonksiyonların anlamlı çıkması durumunda ise Fourier ADF test istatistiği kullanılarak ilgili değişkenin durağanlığına

bakılmaktadır. F testi için kritik değerleri Enders ve Lee (2012), durağanlık için kritik değerleri ise Bozoklu ve ark. (2020) tabloştırmışlardır.

Banerjee ve ark. (2017) tarafından önerilmiş olan Fourier ADL eşbütünleşme testi, Fourier fonksiyonlarını kullanarak sayısı belli olmayan keskin değil, yumuşak yapısal değişimleri dikkate almaktadır. Dolayısıyla bu test, yapısal değişimleri kukla değişken marifetiyle dikkate alan eşbütünleşme testlerine göre daha etkilidir. Ancak bu testte frekans değeri tamsayı hesaplandığı için kalıcı yapısal değişimler yakalanamamaktadır. Söz konusu eksikliği gidermek için İlkay ve ark. (2021), frekans değerinin 0.1 ile 5 arasında, yani kesirli bir sayı olmasına izin verecek şekilde Fourier ADL eşbütünleşme testini genişletmişler ve kalıcı yapısal değişimleri de yakalayabilecek hale getirmişlerdir.

$$\Delta ist_t = \alpha_0 + \beta_1 \sin\left(\frac{2\pi kt}{T}\right) + \beta_2 \cos\left(\frac{2\pi kt}{T}\right) + \delta_1 ist_{t-1} + \delta_2 mek_{t-1} + \delta_3 kre_{t-1} + \delta_4 \Delta ist_{t-1} + \delta_5 \Delta mek_{t-1} + \delta_6 \Delta kre_{t-1} + \varepsilon_t \quad (4)$$

KFFADL eşbütünleşme testinde 4 numaralı eşitlikteki model tahmin edilmektedir. Bu model içindeki *ist* bağımlı değişken olan tarımsal istihdamı, *mek* ve *kre* ise bağımsız değişkenler olarak belirlenen mekanizasyonu ve krediyi temsil etmektedir. Testin uygulanabilmesi, değişkenlerin I (1) olmasını gerektirmektedir (İlkay ve ark., 2021).

Tahmin aşamasında ilk olarak frekans değeri ve test istatistiği hesaplanmakta, daha sonra test istatistiğiyle tablo kritik değeri karşılaştırılarak değişkenler arasında eşbütünleşme ilişkisi olmadığı temel hipotezi sınanmaktadır. Sınamada test istatistiği tablo kritik değerinden (mutlak değer olarak) büyük çıkarsa değişkenler arasında eşbütünleşme ilişkisi var anlamı çıkmaktadır. İlgili

$$Y_t = \alpha_0 + \alpha_1 \sin\left(\frac{2\pi kt}{T}\right) + \alpha_2 \cos\left(\frac{2\pi kt}{T}\right) + \sum_{i=1}^{l+d \max} \theta_i Y_{t-i} + \sum_{i=1}^{l+d \max} f_i X_{t-i} + \varepsilon_t \quad (5)$$

KFFTY testinde 5 numaralı eşitlikteki model tahmin edilmektedir. Bu model içinde *l* optimal gecikme uzunluğunu, *d* max değişkenlerin maksimum entegrasyon derecesini, ε_t ise hata terimini ifade etmektedir (Pata & Yılanıcı, 2020). Çalışmada tarımsal istihdamın, mekanizasyonun ve kredinin sırasıyla bağımlı değişken (Y_t) olarak atandığı 5 numaralı eşitlikteki model kullanılarak KFFTY testi gerçekleştirilmektedir.

Tahmin aşamasında ilk olarak değişkenlerin en çok I (1) olup olmadığı belirlenmektedir². Daha sonra değişkenler arasında nedensellik ilişkisi olmadığı temel hipotezi sınanmaktadır. Bu noktada Wald istatistiği, kritik değerleri elde etmek için ise bootstrap simülasyonları kullanılmaktadır. Test sonucunda doğrudan bootstrap olasılık değerlerine bakılarak nedensellik ilişkisi olup olmadığına karar verilmektedir (Pata & Yılanıcı, 2020).

Çalışmada belirlenen hipotezler şu şekildedir;

H₁: Tarımsal mekanizasyonla tarımsal istihdam arasında negatif ve istatistiksel olarak anlamlı ilişki vardır.

H₂: Tarımsal kredilerle tarımsal istihdam arasında pozitif ve istatistiksel olarak anlamlı ilişki vardır.

BULGULAR ve TARTIŞMA

Bu kısımda EViews 13 ve Gauss 24 programları kullanılarak uygulanan testlerden elde edilen bulgular ortaya konulup değerlendirilmektedir.

Testlerin uygulanışı, değişkenlerin doğal logaritmaları (*log*) üzerinden (*logist*, *logmek*, *logkre*)

² İlgili Gauss 24 programı, değişkenlerin en fazla I (1) olduğu varsayımı altında çalışmaktadır.

kritik değerler, İlkay ve ark. (2021) tarafından tablolaştırılmıştır. Son aşamada ise *sin*, *cos* trigonometrik terimleri 4 numaralı eşitlikteki modele eklenerek Full Modified Ordinary Least Square (FMOLS) tahmincisiyle hem uzun dönem hem hata düzeltme katsayısının tahmini yapılmaktadır.

KFFTY testi, değişkenlerin farkları alınmadan uygulanabilmesi itibarıyla VAR modeline dayalı geleneksel nedensellik testlerine göre (Değişken farkının alınması, uzun vadeli bilgi kaybına neden olur.), nedensellik ilişkisini etkileyebilecek kalıcı olmayan yapısal değişimlerin yanı sıra kalıcı yapısal değişimleri de dikkate alması itibarıyla da geleneksel Fourier TY testine göre çok daha güçlüdür (Pata & Yılanıcı, 2020).

gerçekleştirilmiştir³.

Bağımlı ve bağımsız değişkenler dahil edilerek kurulmuş olan tam logaritmik model eşitlik 6 içindedir.

$$\log ist_t = \alpha + \beta \log mek_t + \delta \log kre_t + \varepsilon_t \quad (6)$$

Modeldeki *t* zamanın, α sabit terimin, β ve δ katsayıların ve ε hata teriminin karşılığıdır.

Aşağıda tanımlayıcı istatistiklerin yanında birim kök, eşbütünleşme ve nedensellik testlerinin sonuçları yer almaktadır (Çizelge 3, 4, 5, 6 ve 9).

Çizelge 3'e göre tarımsal istihdam değişkeni normal dağılım göstermemekte, mekanizasyon ve kredi değişkenleri ise normal dağılım göstermektedir. Ayrıca tarımsal kredi değişkeninde oynaklık, diğer değişkenlere göre daha yüksektir.

Çizelge 4'te görüldüğü üzere tüm değişkenler temelinde trigonometrik terimlerin içinde bulunduğu fonksiyonlar anlamsız çıkmıştır. Ayrıca *k*, mekanizasyon ve kredi değişkenleri temelinde kesirli hesaplanmıştır. Yani bu değişkenlerde görülen yapısal değişimlerin etkileri kalıcıdır. Tüm değişkenler temelinde Fourier fonksiyonları anlamsız çıktığı için geleneksel ADF testi yapılmıştır.

Çizelge 5'te görüldüğü üzere tüm değişkenler, birinci farkları alındığı durumda durağan nitelik sergilemektedir. Tüm değişkenlerin I (1) olduğu belirlendiği için KFFADL eşbütünleşme ve KFFTY nedensellik testlerini yapmak için şart sağlanmıştır.

³ Serilerin doğrusal olması, katsayılarının esneklik şeklinde yorumlanması, ölçüm birimlerinden bağımsızlaştırılması ve analiz sonucunda değişen varyans sorununun ortaya çıkmasını önlemek için değişkenlerin doğal logaritmaları alınmıştır.

Çizelge 3. Tanımlayıcı istatistikler
Table 3. Descriptive statistics

	logist	logmek	logkre
Ortalama	15.737701	15.940246	20.498990
Medyan	15.845224	15.922657	21.806669
Maksimum	16.041106	16.316305	26.268254
Minimum	15.366471	15.639306	12.754194
Standart Sapma	0.240633	0.201905	4.193752
Çarpıklık	-0.216520	0.172871	-0.567951
Basıklık	1.334092	1.790492	1.982967
Jarque-Bera	5.184851 (p=0.074)	2.769278 (p=0.250)	4.068108 (p=0.130)
Gözlem	42	42	42

Çizelge 4. KFFADF birim kök testi sonucu
Table 4. KFFADF unit root test result

Değişkenler	Frekans değeri (k)	En küçük kareler toplamı	F kısıt test istatistiği	Uygun gecikme uzunluğu	FADF test istatistiği
logist	2	0.110120	4.848784#	2	-3.637515
logmek	2.3	0.003357	5.490992#	9	-3.083123
logkre	0.8	1.728028	6.111900#	1	-2.678131

trigonometrik terimlerin içinde bulunduğu fonksiyonların anlamsız olduğu temel hipotezinin %10 anlamlılık düzeyinde kabul edildiğini ifade etmektedir. F kısıt test istatistiğinin karşılaştırıldığı %10 anlamlılık düzeyindeki tablo kritik değeri 7.78'dir.

Çizelge 5. Geleneksel ADF birim kök testi sonucu
Table 5. Conventional ADF unit root test result

Değişkenler	Test istatistikleri		
	Sabitli	Trendli ve sabitli	Trendsiz ve sabitsiz
logist	-0.426564	-2.607517	-1.345978
logmek	1.806171	-1.613209	9.766719
logkre	-2.152899	-0.742197	2.873805
logist (1)	-5.073423*	-5.042590*	-4.951964*
logmek (1)	-6.041613*	-6.245015*	0.397760
logkre (1)	-4.541925*	-5.096855*	-0.836133

* ilgili değişkenin durağan olmadığı temel hipotezinin %1 anlamlılık düzeyinde reddedildiğini ifade etmektedir.

Not: Bir değişkene durağan demek için her üç modelde de durağan nitelik sergilemesi şart değildir. Önemli olan, modellerden en az birinin t istatistik değerinin anlamlı olmasıdır. Yani bir modelde ilgili parametrenin (trendli ve sabitli modelde @trend gibi) t istatistik değerinin koşullu hipotez testindeki ilgili tablo kritik değerinden büyük olması yeterlidir.

Çizelge 6. KFFADL eşbütünleşme testi sonucu
Table 6. KFFADL cointegration test result

Tahmin edilen model	Gecikme uzunluğu	Frekans değeri (k)	Test istatistiği	Tablo kritik değeri	Eşbütünleşme ilişkisi
logist = f(logmek, logkre)	3	1.2	-5.472092	-5.371*	Var

* k = 1.2, n (bağımsız değişken sayısı) = 2 ve %1 anlamlılık düzeyine karşılık gelen tablo kritik değerini ifade etmektedir.

Çizelge 6'da görüldüğü üzere -5.47 değeri, mutlak değer olarak %1 anlamlılık düzeyindeki -5.37 değerinden büyüktür. Dolayısıyla değişkenler arasında bir eşbütünleşme ilişkisi bulunmakta olup temel hipotez (H_0 : Değişkenler arasında eşbütünleşme ilişkisi yoktur.) reddedilmiştir. Ayrıca k değerinin kesirli olması, eşbütünleşme ilişkisini etkileyen yapısal değişimlerin kalıcılığını göstermektedir. Bundan sonra uzun dönem katsayıları ve hata düzeltme mekanizmasının çalışıp

çalışmadığı ortaya konulmuştur (Çizelge 7 ve 8).

Çizelge 7'de görüldüğü üzere tarımsal mekanizasyon ve kredi değişkenleri ile sabit terim ve sin trigonometrik terimi için olasılık değerleri %1 anlamlılık düzeyinde, cos trigonometrik terimi için olasılık değeri ise %10 anlamlılık düzeyinde anlamlı çıkmıştır. Bu bağlamda, tarımsal mekanizasyon değişkeniyle tarımsal istihdam değişkeni arasında negatif, tarımsal kredi değişkeniyle tarımsal istihdam

değişkeni arasında ise pozitif bir ilişki vardır. Sonuca göre uzun dönemde mekanizasyon düzeyindeki %1 yükseliş istihdam sayısında %3.74 oranında düşüşe,

kredi bakiyesindeki %1 yükseliş ise istihdam sayısında %0.14 oranında yükselişe neden olmaktadır.

Çizelge 7. FMOLS uzun dönem katsayıları tahmin sonucu
Table 7. FMOLS long run coefficients estimation result

Bağımlı değişken: logist				
Bağımsız değişkenler	Katsayı	Standart hata	t-istatistik	Olasılık değeri (p)
logmek	-3.736109	0.907679	-4.116114	0.0002*
logkre	0.137099	0.043599	3.144543	0.0033*
c	72.455849	13.575167	5.337381	0.0000*
sin	0.147568	0.043017	3.430407	0.0015*
cos	0.135499	0.075308	1.799262	0.0804**

* ve ** sırasıyla %1 ve %10 anlamlılık düzeylerinde anlamlı olduğunu ifade etmektedir. FMOLS tahmincisi için Jarque-Bera olasılık değeri 0.341 olarak belirlenmiştir. Bu durum, tahminci temelindeki hata teriminin normal dağılıma sahip olduğunu göstermektedir.

Çizelge 8. Hata düzeltme katsayısı tahmin sonucu
Table 8. Error correction coefficient estimation result

Değişken	Katsayı	Standart hata	t-istatistik	Olasılık değeri
Hata düzeltme katsayısı	-0.472656	0.004713	-100.286400	0.0000*
logmek	-0.419186	0.130387	-3.214937	0.0028*
logkre	0.022801	0.005617	4.058906	0.0003*
c	-0.017630	0.004636	-3.802314	0.0005*

* %1 anlamlılık düzeyinde anlamlı olduğunu ifade etmektedir.

Çizelge 8'de görüldüğü üzere hata düzeltme katsayısı, %1 anlamlılık düzeyinde anlamlı olup negatif işaretlidir. Yani hata düzeltme mekanizması işlemektedir. Şöyle ki kısa dönemde dengeden uzaklaşan değişkenler, uzun dönemde denge noktasına tekrardan yakınlık sergilemektedir. Diğer taraftan değişkenler arasındaki kısa dönem ilişkisi de

uzun dönem ilişkisinin yönüyle aynıdır. Buna göre kısa dönemde tarımsal mekanizasyon düzeyinde yaşanan %1 yükseliş tarımsal istihdam sayısında yaklaşık %0.42 düşüşe, tarımsal kredi bakiyesinde yaşanan %1 yükseliş ise tarımsal istihdam sayısında yaklaşık %0.02 yükselişe neden olmaktadır.

Çizelge 9. KFFTY nedensellik testi sonucu
Table 9. KFFTY causality test result

Bağımlı değişken	Bağımsız değişken	Bootstrap olasılık değeri	Uygun gecikme uzunluğu ve frekans değeri (k)	Karar
logist	logmek	0.0393**	2 ve 1	logmek → logist
logist	logkre	0.0071*	2 ve 1	logkre → logist
logmek	logist	0.5429	2 ve 1	logist ⇌ logmek
logmek	logkre	0.9076	2 ve 1	logkre ⇌ logmek
logkre	logist	0.2457	2 ve 1	logist ⇌ logkre
logkre	logmek	0.5463	2 ve 1	logmek ⇌ logkre

* ve ** sırasıyla %1 ve %5 anlamlılık düzeylerinde anlamlı olduğunu ifade etmektedir. Simülasyon sayısı 10.000, bilgi kriteri Akaike ve maksimum gecikme uzunluğu 3 kullanılmıştır.

Çizelge 9'a göre tarımsal mekanizasyon ve tarımsal kredi değişkenlerinden tarımsal istihdam değişkenine doğru tek yönlü nedensellik ilişkisi vardır ($p < 0.01$, $p < 0.05$). Bu sonuç, KFFADL eşbütünlük testinin sonucunu desteklemektedir. Diğer değişkenler arasında nedensellik ilişkisi bulunmamaktadır. Ayrıca k değeri kesirli olmadığı için değişkenler arasındaki nedensellik ilişkisini etkileyen yapısal

değişimler kalıcı değil demektir.

Yapılan analiz sonucunda çalışmada belirlenen iki hipotez de doğrulanmıştır.

SONUÇ ve ÖNERİLER

Tarımsal mekanizasyonun istihdama etkisini Türkiye örneğinde sadece Yücel ve Çalışkan (2020) araştırmışlardır. Ancak bu çalışma, tarımsal

mekanizasyon değişkenini temsilen sadece hektar başına düşen traktör sayısını dikkate aldığı için çok kısıtlı ve teknik yönden zayıftır. Bu zayıflık, tarımsal kredilerin istihdama etkisini Türkiye örnekleminde analiz etmiş tek çalışma olan Şimşir'in (2012) çalışması için de geçerlidir. Dolayısıyla Türkçe literatürdeki çalışmaların arasında tarımsal mekanizasyonun ve kredinin istihdama etkisi ile ilgili güçlü bir bulgu/sonuç bulunmamaktadır. Mevcut çalışmada güncel zaman serisi analizi yöntemleri kullanılarak söz konusu değişkenler arasındaki ilişkiyi net bir şekilde ortaya koymak amaçlanmıştır.

Çalışmada kullanılan gözlem sayısını olabildiğince yüksek tutabilmek için çeşitli veri kaynaklarından veriler derlenmiştir. Veri dönemi, derlenebilen verilerden hareketle, 1981-2022 olarak kararlaştırılmıştır. Analiz, KFFADF, KFFADL ve KFFTY testleri tercih edilerek gerçekleştirilmiştir.

KFFADL eşbütünleşme testinin sonuçlarına göre; hem uzun hem kısa dönemde tarımsal mekanizasyon değişkeniyle istihdam değişkeni arasında negatif, tarımsal kredi değişkeniyle istihdam değişkeni arasında ise pozitif ilişki vardır. Tarımsal mekanizasyon düzeyindeki %1'lik yükseliş istihdam sayısında uzun ve kısa dönemde sırasıyla %3.74 ve %0.42 oranında düşüşe, tarımsal kredi bakiyesindeki %1'lik yükseliş ise istihdam sayısında uzun ve kısa dönemde sırasıyla %0.14 ve %0.02 oranında yükselişe neden olmaktadır. Ayrıca çalışmadaki hata düzeltme mekanizması çalışmaktadır. Buna göre şokların etkileriyle kısa dönemde ortaya çıkabilecek uzun dönem dengesinden sapmalar hızlı bir şekilde ortadan kalkmaktadır. Diğer taraftan tarımsal mekanizasyon ve kredi değişkenlerinden istihdam değişkenine doğru var olan tek yönlü nedensellik ilişkisi eşbütünleşme testine ilişkin sonuçları doğrulamaktadır.

Tarımsal mekanizasyonun ve kredinin istihdama etkisinin analizi çerçevesinde varılan "Türkiye'de mekanizasyon düzeyinde yaşanan yükseliş istihdam sayısını düşürmektedir." şeklindeki sonuç, Doğan (2012), Houssou & Chapoto (2015), Kirui (2019), Adu-Baffour ve ark. (2019), Afridi ve ark. (2020), Daum ve ark. (2020), Yücel & Çalışkan (2020), Tandoğan (2022) ve Caunedo & Kala (2022) tarafından yapılmış olan çalışmaların sonuçlarını destekleyip literatürdeki "uyarılmış yenilik teorisi"ni (Hayami & Ruttan, 1970; Binswanger & Pingali, 1984) doğrulamaktadır. Ancak Mano ve ark. (2017), Rajkhowa & Kubik (2021) ve Hamilton ve ark. (2022) tarafından yapılmış olan çalışmaların sonuçlarını ise desteklememektedir.

Bununla birlikte tarımsal mekanizasyonun ve kredinin istihdama etkisinin analizi çerçevesinde ulaşılan "Türkiye'de kredi bakiyesinde yaşanan yükseliş istihdam sayısını yükseltmektedir." şeklindeki diğer sonuç ise Şimşir (2012), Fink ve ark.

(2014), Seven & Tümen (2020) ve Orji ark. (2021) tarafından ulaşılan çalışma sonuçlarını desteklerken Keikha ve ark. (2014) tarafından ulaşılan çalışma sonucunu ise desteklememektedir.

Araştırmanın bu bulgularından hareketle Türkiye'de tarımsal istihdam sayısındaki değişimin temel dinamiklerinden ikisinin mekanizasyon düzeyindeki ve kredi bakiyesindeki yükseliş olduğu açıkça söylenebilir. Tarımsal mekanizasyon istihdamı negatif, kredi ise pozitif etkilemektedir. Bilindiği üzere tarım sektörü, nüfusun besin ihtiyacını karşılaması, millî gelirin yükselişine katkı vermesi ve tarım dışı sektörlerle üretim girdisi sağlaması itibarıyla stratejik bir sektör olma niteliğini gelişmişlik düzeyi ne olursa olsun her bir ülkede korumayı sürdürmektedir. Özellikle yakın zamanda yaşanan COVID-19 salgını ve hemen sonrasında başlayan Rusya-Ukrayna savaşı bu gerçeği tekrar tekrar göstermiştir. Tarım sektörünün sayılan işlevlerinin sağlıklı bir şekilde sürmesi noktasında ise sektörde yer alan iş gücünün varlığı ve niteliği oldukça önemlidir. Buradan hareketle;

-Tarım sektörüyle tarıma dayalı sanayi özellikle organizasyon yapısı Türkiye'yi kapsayan Tarım Kredi Kooperatifleri başta olmak üzere diğer tüm tarımsal kooperatiflerle iş birliği içerisinde daha fazla ilişkilendirilmeli ve sektörün uluslararası pazarlardaki rekabet gücünü artırıcı çeşitli yapısal düzenlemeler yapılarak sektördeki mevcut ve potansiyel iş gücü "yerinde" istihdam edilmelidir.

-Günümüzdeki Tarıma Dayalı İhtisas Organize Sanayi Bölgesi oluşumunun Türkiye geneline yayılması ve böylelikle nitelikli iş gücünün beraberinde getireceği tarımsal üretim artışının sağlanması için hazine destekli sunulan Tarım ve Orman Bakanlığı kredilerinin kapsamı genişletilip bakiyesi artırılmalı ve reel sektörün finanse edilmesini amaçlayan katılım bankaları tarafından faizsiz kredi hizmetleri geliştirilip sunulmalıdır.

-Türkiye'deki tarımsal mekanizasyon düzeyinin yükseltilebilmesi ve mevcut mekanizasyon kaynaklarının daha rasyonel ve verimli kullanılabilmesi için ticari bankalar tarafından çok düşük faizli ve/veya faizsiz kredi mekanizmaları işletilmeli ve kullanım ömrünü tamamlamış mekanizasyon varlığı hurda indirimi uygulamasıyla cazip tutarlarda değerlendirilmelidir.

-Türkiye'de tarımsal mekanizasyon düzeyinin dengeli ve yeterli gelişimi için coğrafi bölgelerdeki üretim deseni göz önünde tutularak tarımsal makine ve alet planlamasının gerçekleştirilmesi, doğru makine ve alet kullanımına yönelik eğitim(ler)in Tarım ve Orman Bakanlığı ve tarımsal kooperatifler tarafından düzenlenmesi ve tarım bankacılığı hizmeti veren kredi kaynaklarının çeşitlendirilmesi önem arz etmektedir.

-Devlet desteklemeleriyle ve hibe programlarıyla özellikle emek-yoğun üretim gerektiren bitkisel ürünlerin (kesme çiçek, tıbbi ve aromatik bitkiler ve keten gibi) yetiştiriciliğinde gençler başta olmak üzere kırsaldaki kadınlar için istihdam sağlamaya yönelik teşvik edici mekanizmalar oluşturulmalıdır.

-Kırsaldaki nüfusa tarımsal kooperatiflerin çatısı altında örgütlenme bilinci aşlamak amacıyla her ilçede eğitim verecek sürekli eğitim merkez(ler)i ve ülke genelinde kesintisiz bir şekilde yayın yapacak televizyon ve radyo kanal(lar)ı kurulmalıdır. Eğitimler ve yayınlar, tarımda kooperatifleşmenin ilkelerini ve tarıma ilişkin teknik bilgiyi nüfusa benimsetecek şekilde planlanmalıdır. Bu doğrultuda belediyeler, Türkiye Radyo ve Televizyon Kurumu, Tarım ve Orman Bakanlığı ve üniversiteler arasında sıkı bir iş birliği yapılmalıdır.

Bu çalışmadan hareketle başka çalışmalarda Türkiye’de hava durumunun, işlenen tarım arazisi büyüklüğünün ve döviz kurunun tarımsal istihdama etkisi güncel nitelikteki zaman serisi analizi yöntemleri kullanılarak incelenebilir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazar, bu çalışmanın tamamını kendisinin hazırladığını beyan eder.

Çıkar Çatışması Beyanı

Yazar, bu çalışmada herhangi bir çıkar çatışması olmadığını beyan eder.

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Comparative Analysis of CNN Algorithms for Mushroom Classification with Proposed Lightweight CNN Model

Ahmet NAMLI^{1,2}, Didem ÖLÇER²

^{1,2}Department of Computer Engineering, Faculty of Engineering, Ankara, Türkiye

¹<https://orcid.org/0000-0002-4649-3299>, ²<https://orcid.org/0000-0001-7736-1021>

✉: ahmetnamlics@gmail.com

ABSTRACT

The classification of mushroom species presents significant ecologic and health-related challenges; advancement in classification techniques is required to gain reliable identifications. This study aims to explain a methodology that was devised and evaluated in the development of a novel, lightweight Convolutional Neural Network (CNN) designed specifically for the task of mushroom classification. The paper provides a custom CNN model that is computationally cost-effective and capable of high-precision classification, fit for real-time usage. Hence, the proposed model was evaluated on this dataset of curated mushroom images with traditional classifiers and state-of-the-art CNN architectures, such as EfficientNet-B7, ResNet50, InceptionV3, and MobileNetV2. The custom model is depth-wise separations engineered in such a way that while they reduce the computational load, they don't compromise the effectiveness of the model. The custom model achieved a test score of 0.68, which is moderate compared to more established models such as EfficientNet-B7 or ResNet50. This approach helps the model function effectively even on platforms having low computational resources. A comprehensive evaluation reveals that a custom CNN has reasonable accuracy in the identification of different mushroom species vis-à-vis existing models, but also significantly lightens the classification process.

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ÖZET

Mantar türlerinin sınıflandırılması, ekolojik ve sağlıkla ilgili önemli zorluklar ortaya koymaktadır; güvenilir tanımlamalar elde etmek için sınıflandırma tekniklerinde ilerleme kaydedilmesi gerekmektedir. Bu çalışma, mantar sınıflandırma görevi için özel olarak tasarlanmış yeni, hafif bir Evrişimsel Sinir Ağının (CNN) geliştirilmesi üzere tasarlanan ve değerlendirilen bir metodolojiyi açıklamayı amaçlamaktadır. Makale, hesaplama açısından uygun maliyetli ve yüksek hassasiyetli sınıflandırma yapabilen, gerçek zamanlı kullanıma uygun özel bir CNN modeli sunmaktadır. Bu nedenle, önerilen model, geleneksel sınıflandırıcılar ve EfficientNet-B7, ResNet50, InceptionV3 ve MobileNetV2 gibi son teknoloji CNN mimarileri ile mantar görüntülerinden oluşan bu veri kümesi üzerinde değerlendirilmiştir. Özel modelin, hesaplama karmaşıklığını azaltırken modelin etkinliğinden ve yeteneğinden ödün vermeyecek şekilde tasarlanmasına özen gösterilmiştir. Özel model, EfficientNet-B7 veya ResNet50 gibi daha yerleşik modellerle karşılaştırıldığında orta düzeyde bir değer olan 0,68'lik bir test puanı elde etti. Bu yaklaşım, modelin düşük hesaplama kaynaklarına sahip platformlarda bile etkili bir şekilde çalışmasına yardımcı olur. Kapsamlı bir değerlendirme, tasarlanan CNN'in yalnızca mevcut modellere kıyasla farklı mantar türlerinin tanımlanmasında makul bir doğruluğa sahip olduğunu değil, aynı zamanda sınıflandırma sürecini de önemli ölçüde hafiflettiğini ortaya koymaktadır.

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INTRODUCTION

A mushroom is the edible reproductive structure of various types of fungi that are attached to basidiomycetes and grow on the surface of the ground. Fungi are a diverse and integral part of ecological systems, closely linked to living beings (Hibbett et al., 2007; Liu et al., 2016; Wang, 2022; Zahan et al., 2021; Zhang et al., 2021). The presence of diverse fungi is essential for comprehending these organisms' complex nature and importance in our ecosystems. Although there have been recent advancements in the identification of fungal species, only a small fraction, specifically 5%, of the estimated 3.8 million fungal species have been identified (Hawksworth & Lücking, 2017). Approximately 14,000 (Zahan et al., 2021) - 19,000 (Hawksworth & Lücking, 2017) mushroom species have been identified, while experts concur that there are still several undiscovered species. The delicate balance between edible and poisonous mushrooms emphasizes the significance of meticulous species identification. While certain edible mushrooms may not possess a pleasant taste, there exist compelling reasons to consume them. These reasons include the fact that edible mushrooms possess notable advantages, such as their ability to eradicate cancer cells, combat infections, and enhance the human immune system. In addition, mushrooms are exceptionally nourishing, serving as a valuable protein source, containing few calories and unsaturated fats, and offering a substantial supply of vitamins and iron (Ria et al., 2021). Edible mushrooms are favored for their delectable flavor and abundant nutritional content. Mushrooms are a common dietary source that is rich in protein and low in fat (Dan, 2020; Yan et al., 2023; Yu et al., 2020). Therefore, mushrooms provide a more nutritious substitute for conventional sources of protein. The therapeutic potential of mushrooms highlights their usefulness beyond their nutritional composition. Moreover, due to a lack of awareness regarding toxic mushrooms, a significant number of individuals perish as a result of consuming them. This highlights the necessity for enhanced public knowledge and education regarding mushroom species. Presumably, mushrooms with flawless cellular structure, vibrant colors, and no interaction with birds and insects are toxic (Zahan et al., 2021). When discussing the hazards of eating mushrooms, it is crucial to emphasize the problems linked to poisonous species. The distinctive morphological characteristics of poisonous mushrooms include vibrant and colorful scales on the cap and a ring-shaped structure beneath

the top. Novice gatherers are prone to committing errors. Toxic mushrooms have a detrimental impact on the neurological system, perhaps resulting in fatalities when ingested in large amounts (Ketwongsa et al., 2022; Zahan et al., 2021). Therefore, precise identification is essential for ensuring safety. Performing biochemical tests and interpreting morphological traits might be challenging for non-specialists in everyday life. These issues require the creation of identifying systems that are easier for users to understand and utilize. Consequently, numerous researchers have dedicated their efforts to developing various models and methodologies (Tutuncu et al., 2022). These endeavors are a reaction to the increasing demand for easily accessible resources for identifying mushrooms.

The majority of the existing articles on the categorization of wild mushrooms employ machine learning (ML) techniques. These strategies offer a more easily understood and attainable method for identifying mushrooms. The literature employs many ML techniques, such as decision trees, simple Bayesian, AdaBoost, and support vector machine (SVM) methods (Tutuncu et al., 2022). Each of these strategies possesses unique advantages and constraints. Nevertheless, these techniques depend on manually labeled features, and the algorithm acquires knowledge about the labeled wild mushroom data rather than the gathered visual data for classification (Peng et al., 2023). The reliance on manual labeling is a constraint that could be overcome by employing more sophisticated methods, such as deep learning DL. The wide-ranging applications exemplify the extensive influence of DL. DL techniques offer notable benefits in the field of pattern recognition because they can extract the most useful information from complex and multidimensional data. For this reason, it is especially pertinent when it comes to identifying mushrooms. Possessing this skill is essential for precisely discerning the species of mushrooms.

Several studies have utilized artificial intelligence to analyze the features of mushrooms and create models that aid consumers in identifying various mushroom species and preventing mushroom poisoning. Tarawneh et al. (Tarawneh et al., 2023) developed an innovative model that integrates Decision Trees, Naive Bayes, and SVM to distinguish between edible and poisonous mushrooms, achieving an impressive accuracy of 94%. This integrated approach leverages the strengths of individual ML algorithms to synthesize a more reliable decision framework,

utilizing the UCI Mushroom Dataset to validate its efficacy. Similarly, Tutuncu et al. (2022) employed a combination of Decision Tree, Naïve Bayes, SVM, and AdaBoost algorithms, with AdaBoost achieving %100 classification accuracy. This study underscores the potential of ensemble methods in enhancing the predictive capabilities of ML models, particularly in applications where public health is at stake. The study of Ria et al. (2021) further illustrates the role of neural networks in mushroom classification. By reviewing various supervised learning approaches (Ma et al., 2020), including Random Forest and K-Nearest Neighbour alongside artificial neural network (ANN), the study highlights ANN's superior ability to manage complex data patterns, thereby enhancing classification processes.

In an innovative shift towards deep learning, Peng et al. (2023) proposed a multidimensional feature fusion attention network that combines the strengths of CNNs and Vision Transformers. This approach, validated on two comprehensive datasets, showcases superior accuracy and model robustness, setting a new benchmark in the field. Complementing these studies, Zhao et al. (2021) explored the use of an ensemble of CNNs through a bagging algorithm to improve classification accuracy. This research highlights a paradigm shift in biological classifications, emphasizing the transition towards DL models that are capable of handling complex image data and achieving high levels of accuracy. Wang (2022) utilized a Vision Transformer model for mushroom classification, achieving a remarkable 95.97% accuracy. This study not only marks a significant advancement in the application of transformer-based models but also demonstrates their effectiveness in reducing intraclass variability and enhancing feature discrimination. Additionally, Zahan et al. (2021) demonstrated the efficacy of CNNs in recognizing complex patterns inherent in biological entities, including fungi. They employed InceptionV3, VGG16, and ResNet50 models to classify mushrooms, showcasing InceptionV3's superior performance on a contrast-enhanced dataset, thus highlighting the importance of preprocessing techniques and architectural choices. Long et al. (2023) developed an advanced ML model using the MobileViT architecture, optimized for efficiency and accuracy in mobile applications. This research addresses the challenge of large variance within mushroom classes by implementing an innovative separable self-attention mechanism, resulting in improved computational efficiency and classification accuracy.

These collective efforts in applying ML and DL models to mushroom classification not only advance our understanding of fungal biodiversity but also significantly contribute to public health by improving the accuracy and reliability of identifying edible and

poisonous mushrooms. The ongoing development of these technologies promises further enhancements in food safety and ecological research, underpinning the critical role of interdisciplinary approaches in modern scientific inquiries.

The widespread adoption of ML techniques has led to the development of numerous applications across various domains. ML algorithms can independently acquire real-world knowledge by emulating human learning processes, making them useful in various fields (Portugal et al., 2018). In agriculture, ML approaches have been used for different areas (Boyacı et al., 2023; Karadaş&Bulut, 2024). ML approaches have been primarily used to categorize mushrooms (Kamilaris & Prenafeta-Boldú, 2018), but many methods require extensive training and testing, leading to limited precision. Previous studies have explored ML strategies like Decision Trees, Naïve Bayes, AdaBoost, and SVMs, but these often rely on manually labeled features, which limits their generalizability to new datasets (Zhao et al., 2021). Additionally, complex models in CNNs require high processing power and hardware requirements, and there is a lack of research comparing the efficacy of commonly used models in mushroom classification. This study aims to achieve similar results using a more lightweight model to address these challenges and improve the effectiveness of ML in mushroom classification.

The study presents a significant advancement in the use of CNNs for classifying mushrooms, focusing on the application of artificial intelligence (AI) in addressing biological classification issues. It creates a specialized CNN model to identify 11 specific mushroom species, enhancing the accuracy of species identification. The methodology used in the study provides a comprehensive framework for future research in related domains, offering a repeatable model for researchers interested in using ML techniques for mushroom classification. One of the contributions of the study is the comparison study between the custom CNN model and well-established models like EfficientNet-B7, ResNet50, InceptionV3, and MobileNetV2 enhances discussions on the efficacy of different neural network architectures in dealing with highly specialized classification tasks. The smaller size of the custom model results in a decreased computational load, which can significantly speed up the training and inference processes, especially on less powerful hardware. The study also addresses the problem of data imbalance when training ML models, emphasizing the importance of constructing a robust dataset and proposing techniques to improve data representation. The paper offers a basis for creating lightweight systems that can assist mycologists, ecologists, and the public in rapidly and precisely identifying mushroom species, promoting biodiversity

monitoring, and public safety.

MATERIAL and METHOD

The dataset (Zhecheng, 2023) used in this study consists of 7,767 photos, with each image representing one of 11 different mushroom species. The species are classified into the following categories: Lactarius, Russula, Boletus, Cortinarius, Amanita, Inocybe, Exidia, Entoloma, Agaricus, Hygrocybe, and Suillus. The dataset was thoroughly examined to identify authentic photos, excluding seven unsuitable files. 74 exact duplicates and eight nearly identical pairs were identified. Quality control included removing blurry, dark, black-and-white, bright, and low-informational images. The dataset was filtered to 7,671 photos, a valuable resource for training machine learning models. Table 1 shows the class distributions of the images.

Table 1. Number of samples from each mushroom species

Çizelge 1. Her mantar türünden örnek sayısı

Mushroom Species	Number of Samples
Lactarius	1498
Russula	1141
Boletus	1069
Cortinarius	834
Amanita	748
Inocybe	611
Exidia	432
Entoloma	363
Agaricus	351
Hygrocybe	314
Suillus	310

Dataset Splits

One often-used approach to assess the performance of these models is to divide the dataset into several subsets that are used for training, validation, and testing. The test set is not used throughout the model training process but is instead kept aside for a final evaluation. The approach was to assign 10% of the dataset for the testing phase which corresponds to 768 images. The remaining 90% of the data is utilized for training and validating the model. However, employing a random data split into training and validation sets might result in notable issues, particularly when dealing with imbalanced datasets characterized by the underrepresentation of certain classes. To tackle this problem, the technique of Stratified K-Fold Cross-Validation is utilized.

Dataset Pre-processing

In that study, a methodological approach was adopted where image pre-processing steps were integral, utilizing specific functions associated with several

well-established CNN models provided by the TensorFlow Keras applications module (Chollet, 2015; Abadi et al., 2015). These models included ResNet50, InceptionV3, EfficientNetB7, and MobileNetV2, each requiring tailored pre-processing to conform input images to the conditions that optimally match each model's training environment. Additionally, the study introduced a novel CNN architecture for which, rather than developing a new pre-processing function, the 'preprocessing_input' function which is meant to adequate your image to the format the model requires from 'tensorflow.keras.applications.resnet50' (Chollet, 2015; Abadi et al., 2015) was adopted. This choice not only facilitated consistency in handling input data but also strategically leveraged established pre-processing norms to ensure the robustness and reliability of the new model under test conditions.

Methods

In the realm of mushroom classification using CNNs, several well-known models have demonstrated substantial efficacy, including ResNet50 (He et al., 2016), InceptionV3 (Szegedy et al., 2016), EfficientNetB7 (Tan & Le, 2019), and MobileNetV2 (Sandler et al., 2018). These models, utilizing DL architectures, will be tested one by one in this study for their ability to handle the inherent complexity of identifying and classifying various mushroom species from images, and will be compared with the model subject to the study.

In the process of fine-tuning various deep learning architectures such as ResNet50, InceptionV3, EfficientNetB7, and MobileNetV2 for a specific classification task with 11 output classes, several strategic modifications are implemented to adapt these models more effectively to the task. Initially, a 2D Global Average Pooling layer is introduced after the final convolutional layer of each model. This layer aggregates the features into a single 2D map per channel, effectively reducing the spatial dimensions and the complexity of the model while retaining essential spatial information.

Following this dimensionality reduction, two fully connected Dense layers with 128 and 256 neurons are added respectively. Each of these layers employs a Rectified Linear Unit (ReLU) activation function to introduce non-linearity, enhancing the model's ability to capture and learn complex patterns from the data. To further improve the training dynamics and stabilize the learning process, batch normalization layers follow each dense layer. These layers normalize the inputs for each layer, centering the mean output close to zero and the standard deviation close to one, which helps in mitigating issues related to input variation sensitivity. To combat the risk of overfitting, a dropout layer follows each batch normalization step. During training, these dropout layers randomly nullify a

fraction of the input units, ensuring that the model does not overly depend on any specific neuron and thereby promoting a more generalized learning outcome. The culmination of this fine-tuning process is a final dense layer with 11 units, corresponding to the number of classification classes, which utilizes a softmax activation function to yield the probability distribution across the classes.

These enhancements collectively tailor the original architectures of ResNet50, InceptionV3, EfficientNetB7, and MobileNetV2, optimizing them for improved performance on the dataset in question by effectively balancing complexity, computational efficiency, and generalization capability.

Regarding the model constructed which is the focus of the study, in constructing the CNN described, the architecture is meticulously organized into sequential layers, each designed to handle specific transformations of the input data for effective feature learning and classification. The model adopts 10 convolutional layers arranged in increasing complexity with filters ranging from 16 to 256. Each convolutional layer utilizes a (3, 3) kernel size and employs 'same' padding to ensure that the spatial dimensions of the output feature maps remain unchanged, thus preserving edge information across the network's depth.

The activation function selected for this model is the Exponential Linear Unit (ELU), which presents several advantages over the commonly used ReLU. According to research by Clevert et al. (2016), ELU can help reduce the vanishing gradient problem common in deep neural networks by maintaining mean activations closer to zero, which facilitates a faster and more effective learning process. The ELU function is particularly effective in DL architectures as it allows for negative outputs, contributing to a more robust learning mechanism by adding slight perturbations to the activation map, thereby enhancing generalization.

Weight initialization in this model is performed using the "He uniform" method, which is particularly suited to networks employing ELU activation functions. He et al. (2015) demonstrated that this initializer could significantly impact the network's ability to learn efficiently by maintaining the variance of activations throughout the layers. By initializing weights from a uniform distribution within a range derived from the number of input units, the "He uniform" initializer ensures that the gradient magnitudes are neither too small (causing vanishing gradients) nor too large (leading to exploding gradients), thus facilitating stable and rapid convergence during training.

Batch normalization is applied consistently after each convolutional operation. This technique, as expounded by Ioffe and Szegedy (2015), normalizes the outputs of the previous layers by recalibrating the mean and

variance. Such normalization stabilizes the learning process and allows for higher learning rates, reducing the model's training time significantly. Additionally, it provides a form of regularization, albeit indirectly, by smoothing the optimization landscape.

Dropout is strategically incorporated at various stages within the network with rates determined through hyper-parameter tuning, which helps in identifying optimal values that prevent overfitting while allowing the network to retain a significant capacity for learning patterns in the data. Srivastava et al. (2014) first introduced this technique, demonstrating its effectiveness in reducing overfitting by randomly omitting subsets of features at each training stage, thereby compelling the network to learn more robust features. The varying dropout rates from 0.1 to 0.7 reflect a targeted approach, with higher rates likely used in layers that are more prone to overfitting due to their complexity and capacity.

The inclusion of pooling layers, specifically max pooling, serves to reduce the dimensionality of the feature maps, thus decreasing the computational load and the number of parameters in the network. This reduction not only speeds up the training process but also minimizes overfitting by abstracting the highest-valued features from the preceding feature maps.

Towards the end of the model, global average pooling is utilized to convert each feature map to a single value, effectively summarizing the spatial information, which is critical for maintaining the most relevant features for classification tasks. Following this, dense layers with ELU activation and additional dropout are employed to finalize the feature processing and lead to a classification decision made by a softmax-activated output layer. This layer distributes the probability across the various classes, facilitating a multi-class classification.

All CNN configurations are compiled with the Adam optimizer, known for its efficiency in handling sparse gradients and adapting the learning rate during training, and it employs categorical cross-entropy as a loss function, ideal for multi-class problems where each class is mutually exclusive.

In sum, as indicated in Figure 1, the detailed architectural choices and parameter settings in mentioned CNN are aligned with current best practices in DL for image classification, emphasizing stability, efficiency, and robustness in learning. The integration of advanced techniques like ELU, He initialization, and batch normalization alongside strategic dropout application underpins the model's capability to perform effectively in complex visual recognition tasks.

When creating the model, care was taken to make it lightweight. The custom model created for this study consists of 1,450,523 parameters, occupying

approximately 5.53 MB of memory. The parameter counts and memory requirements of other models, such as EfficientNet-B7 (64,462,882 parameters, 245.91 MB), ResNet50 (23,887,371 parameters, 91.12 MB), InceptionV3 (22,102,443 parameters, 84.31 MB), and MobileNetV2 (2,459,339 parameters, 9.38 MB),

are significantly higher than this. The smaller size of the custom model results in a decreased computational load, which can significantly speed up the training and inference processes, especially on less powerful hardware.

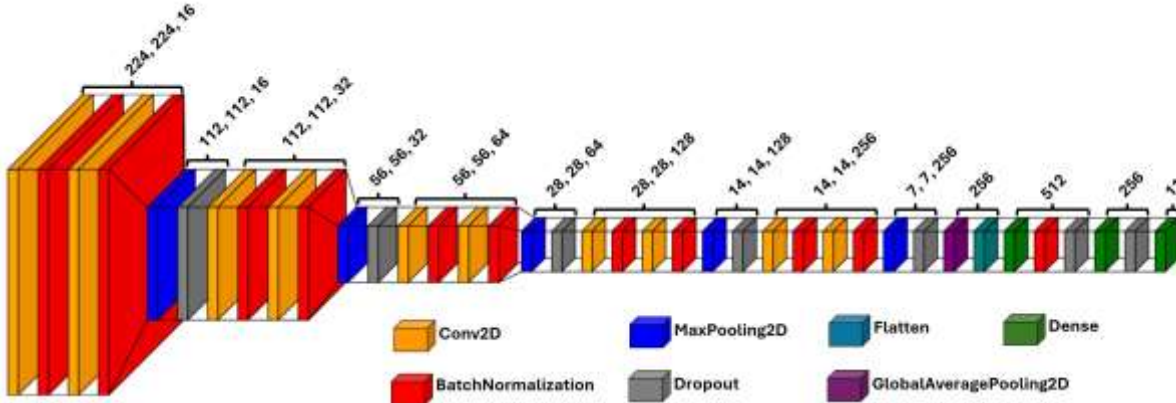


Figure 1. Custom CNN model architecture
 Şekil 1. Tasarlanan CNN model mimarisi

```

Algorithm 1: Training algorithm
batchSize ← 32
numberOfFolds ← 10 // To create a stratified 10-fold cross
validation.
Initialize the dataset
Reserve test set // %10 of whole dataset reserved for test set
Initialize the stratified k-fold cross validation // initializes a stratified
K-fold cross-validator with 10 folds, shuffling the data
to randomize the fold assignments.
for i ← 1 to numberOfFolds do
    Create and compile new model
    Arrange train and validation sets // From the remaining images after
    separating the test set, according to stratified
    10-fold cross validation.
    for e ← 1 to epochNumber do
        Train the model
    end
    if bestValidationAccuracy < foldsValidationAccuracy then
        bestCandidate ← model
    end
    Plot training and validation accuracies for fold i
    Plot training and validation losses for fold i
end
bestFoldsModel ← bestCandidate // Stored for use in the test step.
Calculate average and best validation accuracies // Calculated on the final
validation accuracies of all folds.
    
```

Figure 2. Pseudocode of training process
 Şekil 2. Eğitim sürecinin sözde kodu

In this study, 10-fold cross-validation was employed, considering the specific attributes of the dataset. The batch size was set to 32 for training iterations, balancing computational efficiency and maintaining a sufficiently stochastic gradient estimation. The training was conducted over 75 epochs to allow the network sufficient iterations to adequately learn and adapt to the dataset without overfitting. During each fold of cross-validation, models were monitored for validation accuracy improvements, and the best-performing weights were saved. These methodological choices ensure the training process is both efficient and robust, leading to a CNN well-tuned for generalization beyond the training dataset. The same methodology, as described in Figure 2, was used

for all CNN models used, only the models themselves were changed. By keeping the other variables constant, the aim was to observe the performance of the models under identical situations. After successfully training and identifying the best-performing model across various validation folds, the research focus shifts towards evaluating the model's practical efficacy on unseen data and ensuring the robustness of its predictive capabilities. The model is systematically evaluated against the test data to ascertain its performance metrics, notably accuracy and loss. During the test phase, similar to the training phase, all the models were treated uniformly and exposed to identical testing procedures.

RESULTS and DISCUSSION

This chapter presents all the outcomes of the process of model training, validation, and testing. The results of the setup are thoroughly examined, and detailed comparisons and illustrations are provided.

Figure 3 displays the training and validation metrics of the most successful fold of the model, which is retained for the testing phase. Upon analysis, it is evident that the training and validation accuracies exhibit a consistent upward trend. However, despite the inability to reach the same level of continuity, it is evident that the training and validation losses are decreasing. However, there is a gradual widening of the gap between them. With this information, the validation accuracies of each fold of the model and the average validation accuracy are shown in Table 2 below.

Table 2. Validation accuracies of the model in folds

Çizelge 2. Modelin katlamalardaki validasyon doğrulukları

Fold Number:	1	2	3	4	5	6	7	8	9	10	Average
Validation Accuracy:	0.67	0.70	0.69	0.62	0.68	0.65	0.67	0.67	0.68	0.70	0.67

As a result of the cross-validation, the model trained on the most successful fold was passed to the test step in which the images (%10 of the dataset) that were never seen in the training phase were used. The tests yielded a prediction accuracy rate of 0.68.

Table 3 displays the precision, recall, and F1-score values for each mushroom species, together with the corresponding number of samples representing these species in the test dataset. Precision, measuring the accuracy of positive predictions, shows substantial variability across classes. For instance, *Hygrocybe* achieves perfect precision, indicating precise predictions, but has low recall. In contrast, *Agaricus* and *Suillus* show lower precision, highlighting issues with false positives. Recall evaluates the model's ability to detect all relevant examples in a class. *Boletus* excels in recall, capturing most instances, whereas *Hygrocybe* performs poorly, missing many true instances. The F1 score combines precision and recall, offering a unified measure of performance. *Boletus* has a high F1 score, indicating balanced precision and recall, while *Suillus* has a notably low score, suggesting areas for improvement. According to Table 3 and Table 4, the model exhibits varied proficiency across classes, with strong results in some and underperformance in others. These findings could inform targeted improvements or training adjustments to boost accuracy and recall across all categories, ensuring robust performance irrespective of class distribution.

The confusion matrix which is given in Figure 4 offers a comprehensive evaluation of a model's performance for each class by classifying predictions into true positives, true negatives, false positives, and false



Figure 3. Training and validation metrics graph

Şekil 3. Eğitim ve doğrulama metrikleri grafiği

negatives, and this is an important key to understanding the precision, recall, and F1-score metrics mentioned earlier. This comprehensive data is essential for discerning the model's distinct advantages and disadvantages, demonstrating its effectiveness in other domains, and revealing any inclinations to inaccurately classify one category as another.

Stratified k-fold cross-validation ensures that there is minimal variation in the validation accuracies, but results showed that some classes did not achieve the desired level of success in fulfilling the model's purpose due to the distribution of the dataset. When the dataset is analyzed, as indicated in Table 1, it is seen that there is a linear proportionality between recall scores and image distributions. In the rigorous assessment of CNNs applied to an 11-class mushroom classification task, various established and custom models were evaluated using stratified 10-fold cross-validation to gauge their performance during the training phase and subsequently tested with independent test data to verify their generalization capabilities.

During the training phase, as indicated in Table 5, the EfficientNet-B7 model demonstrated the highest efficacy, in best fold validation accuracy and average accuracy across folds. This superior performance suggests that EfficientNet-B7's methodical approach to scaling network dimensions systematically is highly effective for this task. In contrast, the InceptionV3 model, despite its sophisticated architecture that allows for complex feature extraction through varied convolutional filter sizes, lagged in performance, in best fold accuracy and an average of 63.78%. Test

scores of each model are shown in Table 5. EfficientNet-B7 continued to outperform the other models in test scores. ResNet50, known for its deep residual learning framework that facilitates the training of deeper networks by addressing the

vanishing gradient problem, also performed commendably in both the validation and testing phases. This indicates its effectiveness in capturing and generalizing the underlying patterns of the dataset.

Table 3. Custom model's test scores by species
Çizelge 3. Tasarlanan modelin türlere göre test sonuçları

Mushroom species	Precision	Recall	F1-Score	Sample
Agaricus	0.49	0.49	0.489	39
Amanita	0.84	0.61	0.71	77
Boletus	0.77	0.92	0.84	104
Cortinarius	0.55	0.68	0.61	81
Entoloma	0.43	0.69	0.53	32
Exidia	0.87	0.75	0.81	36
Hygrocybe	1.0	0.40	0.57	30
Inocybe	0.66	0.61	0.63	67
Lactarius	0.67	0.64	0.65	146
Russula	0.74	0.76	0.75	132
Suillus	0.47	0.33	0.39	24

Table 4. Metrics of custom CNN model
Çizelge 4. Tasarlanan CNN modelinin ölçümleri

Metrics	Precision	Recall	F1-Score	Sample
Macro average	0.68	0.62	0.63	768
Weighted average	0.70	0.68	0.68	768

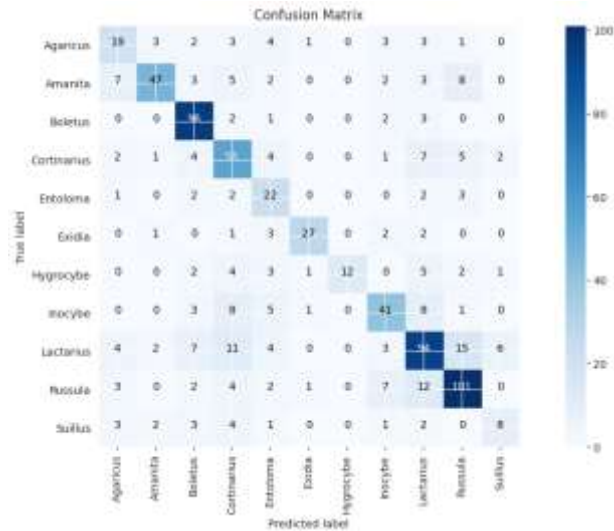


Figure 4. Confusion matrix of custom CNN model
Şekil 4. Tasarlanan CNN modelinin karmaşıklık matrisi

Conversely, the Custom Model and InceptionV3 showed moderate to low performance in both phases. These outcomes may signal the need for further model refinement or a re-evaluation of their network architectures and training parameters to better suit the classification task at hand. MobileNetV2, designed for efficiency in mobile environments, achieved reasonable success. Its performance indicates a balanced trade-off between computational efficiency and predictive accuracy, making it a viable option for applications where deployment constraints are a factor.

For macro averages, which are given in Table 6, which treat all classes equally, the EfficientNet-B7 model demonstrates superior performance in F1-score, precision, and recall. This suggests that EfficientNet-B7 is exceptionally consistent across all classes, balancing accuracy and coverage effectively. ResNet50 follows with a solid F1-score, indicating reliable performance, though slightly less consistent across classes compared to EfficientNet-B7.

Table 5. Comparison of models by validation accuracies and test scores
Çizelge 5. Modellerin validasyon doğrulukları ve test puanlarına göre karşılaştırılması

Model	Best Validation Accuracy	Fold's Average Accuracy	Validation Accuracy	Test Score
Custom model	0.70	0.67	0.68	0.68
ResNet50	0.82	0.79	0.75	0.75
EfficientNet-B7	0.82	0.80	0.81	0.81
InceptionV3	0.68	0.64	0.64	0.64
MobileNetV2	0.75	0.72	0.72	0.72

Table 6. Comparison of models by macro average
Çizelge 6. Modellerin makro ortalamaya göre karşılaştırılması

Model	Precision	Recall	F1-Score
Custom model	0.68	0.63	0.64
ResNet50	0.78	0.74	0.75
EfficientNet-B7	0.81	0.80	0.80
InceptionV3	0.65	0.60	0.61
MobileNetV2	0.70	0.72	0.70

The Custom Model, InceptionV3, and MobileNetV2 show lower effectiveness, in macro average F1-scores. These scores indicate challenges in either precision or recall, which could be due to various factors such as model architecture limitations, insufficient training, or the intrinsic difficulty of some classes that could not be effectively learned by these models.

When considering the weighted averages, as indicated in Table 7, which account for the prevalence of each class, a similar pattern emerges. EfficientNet-B7 maintains its lead in F1-score, highlighting its robustness and ability to generalize well across the varied sizes of the classes. It is followed by ResNet50 and MobileNetV2, which also show competent performance with weighted F1 scores. This indicates that while these models perform well on more populous classes, there might be room for improvement in handling less represented classes.

Table 7. Comparison of models by weighted average
Çizelge 7. Modellerin ağırlıklı ortalamaya göre karşılaştırılması

Model	Precision	Recall	F1-Score
Custom model	0.70	0.68	0.68
ResNet50	0.77	0.75	0.75
EfficientNet-B7	0.82	0.82	0.81
InceptionV3	0.66	0.65	0.64
MobileNetV2	0.73	0.72	0.72

The Custom Model and InceptionV3 exhibit less optimal results with weighted F1 scores. These outcomes suggest that these models, while reasonably effective for some classes, struggle with achieving high accuracy and coverage across all classes, particularly those that are less frequent in the dataset. The data suggests that while some models like EfficientNet-B7 and ResNet50 are capable of delivering robust and balanced performances across diverse class distributions, others such as the Custom Model and InceptionV3 may benefit from further tuning and training to enhance their precision and recall capabilities, ensuring more consistent performance

across all classes. In addition, it was observed that the custom model showed more accurate values than InceptionV3, although it showed lower accuracies than the other 3 models. Considering the number of model variables and their complexity, it can be concluded that the results are promising, although not completely satisfactory.

Finally, EfficientNet-B7's performance across various fungal classes, which is observed to be the most successful in the light of the results mentioned, as evidenced by the detailed metrics for precision, recall, and F1-score, showcases a complex landscape of effectiveness that varies significantly from one class to another. These results provide a more granular insight into the model's capacity to accurately identify and classify instances within a multi-class framework.

CONCLUSION

The study explores the application of CNNs for mushroom classification, focusing on developing a customized model capable of accurately categorizing 11 different mushroom classes. The custom model demonstrates a feasible approach to mushroom classification, especially suitable for scenarios where computational efficiency is a priority given its relatively simpler architecture compared to deeper, more computationally expensive models. The model's performance, particularly in the testing phase, reveals significant insights for both academic and practical applications in mycological classification. The custom model achieved a test score of 0.68, which is moderate compared to more established models like EfficientNet-B7 or ResNet50, known for their complex architecture and ability to achieve higher accuracy in image-based tasks. However, the model faced difficulties with the Suillus class, resulting in a lower F1-score of 0.39. The uneven distribution of training data contributed to the variation in findings, suggesting that the model struggles with under-represented categories. The results indicate a direct relationship between class representation in the training data and the model's performance. Classes with fewer examples tended to yield poorer recall and precision, suggesting that the model struggles with under-represented categories. The testing phase also highlighted a consistent average validation accuracy of about 0.67, pointing to the model's stability but highlighting its limited capability to transcend its training when faced with new, unseen data. Advanced models like EfficientNet-B7, ResNet50, and MobileNetV2 have the potential to significantly improve performance, incorporating advanced methods like compound scaling and residual learning. However, the model's applicability is limited by its moderate accuracy and the need for enhanced training strategies to improve its generalization capabilities across all mushroom classes. Despite all this

considering the number of model variables and their complexity, it can be concluded that the results are promising, although not completely satisfactory. The model was found to achieve better results than the much more complex InceptionV3. This is promising for future studies. The results highlight the importance of a well-prepared dataset in training a successful ML model. At the same time, dataset augmentation strategies to increase the robustness of the model are planned for future work. In conclusion, the process of refining and optimizing CNNs for mushroom classification is still in progress, offering a strong foundation for further research. This study provides a good foundation and a strong starting point for further research that aims to expand the capabilities of these advanced ML models in mushroom classification. The ultimate goal is to develop a model that not only achieves high accuracy across all mushroom classes but also serves as a reliable tool for mycologists and enthusiasts in the field, marrying the technical prowess of CNNs with the intricate beauty of mushroom species.

Contribution of the Authors

- 1- Ahmet Namlı executed the experiment with the help of Assist. Prof. Didem Ölçer.
- 2- Ahmet Namlı wrote the article and critically reviewed it by Assist. Prof. Didem Ölçer.

Conflict of Interest

The authors declare that there is no conflict of interest

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Determining the Meat Consumption Preferences of Tourists in the Kars Province

Adem AKSOY¹, Ahmet Semih UZUNDUMLU²

¹Department of Agricultural Economics, Faculty of Agriculture, University of Ataturk, 25240, Erzurum-Türkiye, ²Department of Agricultural Economics, Faculty of Agriculture, University of Ataturk, 25240, Erzurum-Türkiye

¹<https://orcid.org/0000-0003-4342-9272>, ²<https://orcid.org/0000-0003-4342-9272>

✉: aaksoy@atauni.edu.tr

ABSTRACT

This study aimed to understand the meat consumption preferences of local people and tourists in Kars, which is known for its high-goose production and cultural and historical tourism. The AHP method was used to analyze the data, with 250 consumers interviewed through proportional sampling. Fish was the top preferred meat type for 29.18% of visitors, followed by chicken meat (26.41%), red meat (24.64%), and goose meat (19.77%). The most important criteria for meat consumption in Kars were taste (27.08%), price (23.97%), ease of transportation (19.47%), nutritional content (18.18%), and smell (12.20%). In terms of taste, consumers prefer fish as their first choice, followed by goose meat as their second choice. In terms of affordability, chicken and fish are the first choices, followed by fish. They prefer fish and goose meat for their nutritional and safety value. In terms of smell, fish and goose meat are among the most important choices. The demand for organic fish with higher nutritional value from Çıldır Lake is higher than other types of meat. The affordability of chicken and fish also influences consumers' preferences for these meat types. However, loyalty to red meat and chicken remains low in terms of nutrition and safety. To enhance the economic conditions for producers, consumers, and restaurants, it is crucial to streamline the availability of fish, enhance trust and perception of the nutritional value of red and white meat, and make goose meat more cost-effective. This will lead to increased consumption of meat products and improved service opportunities.

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Kars İlinde Turistlerin Et Tüketim Tercihlerinin Belirlenmesi

ÖZET

Bu çalışma, çok sayıda kaz üretimi, kültür ve tarih turizmi ile tanınan Kars'ta yerel halkın ve turistlerin et tüketim tercihlerini anlamayı amaçlamıştır. Orantılı örnekleme yoluyla 250 tüketiciyle görüşülen verileri analiz etmek için AHP yöntemi kullanılmıştır. Ziyaretçilerin en çok tercih ettiği et türü %29,18 ile balık olurken, bunu %26,41 ile tavuk eti, %24,64 ile kırmızı et ve %19,77 ile kaz eti takip etmiştir. Kars'ta et tüketiminde en önemli kriterlerin lezzet (%27,08), fiyat (%23,97), ulaşım kolaylığı (%19,47), besin içeriği (%18,18) ve koku (%12,20) olduğu tespit edilmiştir. Lezzet açısından tüketiciler ilk tercih olarak balığı, ikinci tercih olarak ise kaz etini tercih ettiğini, uygun fiyat açısından ise tavuk ilk tercih olurken, onu balık takip etmektedir. Besin değeri ve güvenme kriter olunca balık ve kaz eti tercihte ilk iki sırayı oluşturmaktadır. Koku açısından balık ve kaz eti en önemli tercihler arasındadır. Çıldır Gölü'nden besin değeri yüksek olan organik balıklara olan talep diğer et türlerine göre daha fazladır. Tavuk ve balığın uygun fiyatlı olması da tüketicilerin bu et türlerine yönelik tercihlerini etkiliyor. Ancak kırmızı et ve tavuğa bağlılık, beslenme ve güvenlik endişeleri açısından düşük kalmaktadır. Üreticilerin, tüketicilerin ve restoranların ekonomik durumlarının iyileştirilmesi için balığa erişimin kolaylaştırılması, kırmızı ve beyaz ete olan güvenin ve beslenme algısının artırılması, kaz eti fiyatlarının daha uygun hale getirilmesi gerekiyor. Bu, et ürünleri tüketiminin artmasına ve hizmet fırsatlarının iyileşmesine yol açacaktır.

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Anahtar Kelimeler

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INTRODUCTION

A balanced and healthy diet includes all kinds of nutrients in required amounts (Ercan & Irmak, 2018). It is important for one to take 75-80 g of protein of vegetable and animal origin daily (İlgü & Güneş, 2002). Red and white meat, eggs, and milk are sources of animal protein (Akın & Çelen, 2020). Meat has an important share in animal-based proteins and is important for the growth and development of all living creatures (Karacan, 2017). Regardless of the type of meat obtained from any animal, meat is consumed by humans almost every day due to some of its features (Taşkın et al., 2020).

Meat is classified as red meat (beef, veal, sheep, lamb, goat, deer), white meat (poultry and seafood), and processed meat (products obtained with processed forms of red and white meat) (Boada et al., 2016). Products obtained from red and white meat such as salami, sausage, fermented sausage, bacon, ham, hamburger, canned meat, and cold cuts are categorized as processed meat (Wolk, 2017; Taşçı, 2019). When it comes to red meat; beef, veal, buffalo, lamb, and sheep come to mind in Turkey, but pork is also included in this group across the world. In addition, red meat contains important fatty acids, several vitamins such as B₃, B₁₂, and D, and minerals such as selenium, iron, and zinc (Taşçı, 2019).

Meats are an important food source that is becoming increasingly significant in international commerce. Based on 2021 figures, the global production of chicken, pig, cattle, sheep, goat, turkey, buffalo, and goose meat amounts to approximately 358 million tons. The distribution of this quantity was as follows: chicken, 34.5%; pig, 34.2%; beef, 19.4%; sheep, 2.9%; goat, 1.8%; turkey, 1.4%; buffalo, 1.9%; geese, 1.2%; and other animals, 2.7%. Turkey produces approximately 4.6 million tons of meat from various animals, such as chickens, cows, sheep, goats, turkeys, geese, and buffalo. The distribution of this quantity is as follows: chicken, 51.8%; beef, 33.7%; sheep, 10.5%; goat, 2.5%; turkey, 1.1%; goose, 0.1%; buffalo, 0.3% (FAOSTAT, 2024). There are several factors affecting the meat consumption. The most important two factors affecting red meat consumption are household income (Agcakale, 2018) and expensive red meat prices compared to its substitutes (Akçay, 2013). For the types of red meat, the softness of mutton is an advantageous aspect, but its smell and oily nature are considered a negative aspect in general consumer preferences (Özyürek et al., 2019). In addition, liver, heart, kidney, tongue, head, and tail fat, which are by-products of red meat that have a lower price compared

to red meat, are in the red offal group, while tripe, brain, and trotter are included in the white offal group (MEGEP, 2011).

Consumers who consider red meat more delicious than other types of meat generally prefer beef and lamb, and eat kebab, pita (Turkish pizza with ground meat), lahmacun (very thin Turkish pizza covered with seasoned minced meat and onions), and grilled meat in restaurants (Süren & Küçükkömürler, 2018). In addition, 70% of consumers prefer offal in restaurants (Küçükkömürler & Koluman, 2021). In the provinces of Kars, Ardahan, and Iğdır, red meat (beef and lamb) ranks first and second place in people's preferences for meat consumption in restaurants, followed by fast food (Gündüz et al., 2019). In restaurants in Kars province, doner kebab, kebab, and boiled meat are preferred more than pita, lahmacun, and offal.

Chicken meat consumption has increased in recent years due to the increase in the price of red meat. Due to the high price of red meat compared to chicken, consumers can meet their protein and nutritional needs at a lower price (Çelik, 2012). Due to high feed efficiency and fast-growing of poultry and the increased number of broiler chicken enterprises along with recent technological advances, chicken has become more economical than other meats (Keskin & Demirbaş, 2012; Uzundumlu and Dilli, 2023). There has been a significant increase in the consumption of poultry across the world in recent years as it meets people's animal protein needs for a healthy diet. Poultry is a low-fat and high-protein source, rich in vitamins and minerals, and affordable compared to red meat (Adamski et al., 2017; Kozák, 2021). In particular, the rate of undesirable saturated fatty acids is lower in poultry than in red meat (Adamski & Wencek, 2012; Nowak & Trziszka, 2010). In the provinces of Kars, Ardahan, and Iğdır, chicken is listed as third in people's preferences for meat consumption in restaurants (Gündüz et al., 2019). In general, chicken is served as a doner, grilled, or fried chicken in restaurants, and consumers mostly prefer chicken doner (Kara et al., 2020).

Among poultry, goose meat is a high-quality protein source with a sufficient number of amino acids necessary for human life (Liu et al., 2011). Goose meat has very high nutritional value and very low calories (Oral & Ak, 2020). Although goose meat contains beneficial fat for health, its consumption, and supplementary production are low compared to other poultry due to its high price and low consumer awareness about its nutritional values. In addition, since goose meat is produced seasonally, it is always

possible to find it fresh in markets (Buzala et al., 2014). The most important quality characteristics of poultry meat for consumers are appearance, texture, juiciness, flavor, and consistency (Becker, 2000). The physical activity of the animal is another important factor in the sensory properties of the meat. Active animals such as geese have more muscle density and toughness than inactive poultry (Geldenhuis et al., 2014). Goose breeding in the world and Turkey has lagged due to low breeding levels compared to other poultry. Turkey's goose breeding has seen increased importance due to its nutritional value, high protein, low cholesterol, and valuable feathers. Despite being consumed in many countries, it is consumed in specific regions as part of local culture (Gündüz et al., 2019). TURKSTAT (2024) indicated that 37% of the tourists visiting Kars specifically came for the purpose of consuming gas meat. Nutritional values, organic production, and smell are also considered important criteria in meat consumption preferences. Thanks to the increase in cultural and historical tourism activities in recent years, local and foreign tourists of Kars province try goose meat, one of the local dishes, while visiting the city. Consequently, a significant number of individuals were unaware of the presence of goose flesh. Thus, when the demand for these challenging-to-raise animals as a source of consumption grows, their production will also expand, thereby encouraging the growth of more producers.

Fish is one of the sources of high-quality protein and is rich in several vitamins, minerals, and essential fatty acids (Uzundumlu, 2017). In addition, fish is an important source of iodine (Kearney, 2010). Seafood is the main source of animal protein for subsistence households of many developing countries with water resources. In these households, seafood constitutes more than half of the animal protein consumption and 20% of the total food expenditure (Ashitey, 2019). Fish price, and health benefits of fish in terms of nutrition, taste, food safety, and appearance are important factors in fish consumption (Zhang, 2004), but the most determining factor in fish consumption compared to other meat types is its effect on healthy nutrition (Uzundumlu, 2017). In the provinces of Kars, Ardahan, and Iğdır, seafood ranks fourth in people's preferences for meat consumption in restaurants (Gündüz et al., 2019). Fish consumption in restaurants increases as income increases across Turkey. While the consumption of trout and anchovy is common in restaurants in Kars province, those who go on a trip to the Çıldır Lake can consume mirror (yellow) carp.

The research conducted in Kars province focused on consumers' meat preference rankings while selecting meat in restaurants. However, the specific criteria and options that influenced these preferences were not studied. This study holds unique significance in addressing this gap. The objective of this study was to

ascertain the meat consumption preferences of tourists in restaurants located in the Kars region. The suitability of consumers' meat consumption preferences was assessed in this context based on specific criteria.

MATERIAL and METHOD

Material

Both primary and secondary data sources were used in the study. The primary data source consisted of face-to-face surveys with domestic and foreign tourists in Kars. The secondary data sources included the written results of studies on similar subjects and statistical records about the study area obtained from Kars Municipality and Kars Provincial Directorate of Culture and Tourism.

Method

The Method Applied to Determine the Number of Questionnaires

In 2021, face-to-face surveys were conducted with tourists who had goose meat in eateries in Kars to gather a foundational dataset for the study. The sample size for the tourist survey was determined using a proportional sampling approach (Newbold, 1995; Miran, 2007).

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

where

n: Sample size,

N: Population (219,200),

σ^2p : Variance of the ratio (0.000651),

p: Ratio of tourists who prefer goose meat in restaurants (it was determined as 0.80).

The p-value was determined considering the data obtained from the pre-surveys. Based on this sample size calculation (95% confidence interval and 5% deviation), the sample was determined to include 246 people. Considering the possibility of missing data and information in some questionnaires, a total of 250 questionnaires were applied to the study.

Method Used in the Analysis

The Analytical Hierarchy Process (AHP) method is an analytical approach that formulates complex decisions based on several sciences and uses them in the analysis. It was first proposed by Thomas L. Saaty in the 1970s and has been extensively developed since then. This method helps decision-makers to make the most appropriate selection decision with the numerical values they have given to relevant criteria and options (Kuber et al., 2017).

In this study, the AHP method was used to determine the order of meat consumption preferences of visitors to Kars. As shown in several studies, economic, social,

environmental, and health-related factors are effective in consumers' decision to consume different types of meat in restaurants. Many consumers can choose the best option among alternatives in line with their previous experiences and friend recommendations, thus reducing the opportunity cost in economic terms. For this reason, scientific methods such as AHP are used by consumers to decide on the most appropriate option with a low opportunity cost. For the AHP, the weights of alternatives and criteria are determined according to pairwise comparisons, and the most appropriate choice for consumers is determined by making calculations such as Consistency Ratio (CR) and Consistency Index (CI) (Uzundumlu et al., 2019).

$$A = [a_{ij}]_{n \times n} = \begin{bmatrix} a_{11} & a_{12} & \dots & a_{1n} \\ a_{21} & a_{22} & \dots & a_{2n} \\ \dots & \dots & \dots & \dots \\ a_{n1} & \dots & \dots & a_{nn} \end{bmatrix}_{n \times n} \quad \text{ve} \quad W = [w_{i1}]_{n \times 1} = \begin{bmatrix} w_{11} \\ w_{21} \\ w_{31} \\ w_{41} \end{bmatrix}_{1 \times n}$$

$$D=A*W \quad \text{ve} \quad D = [d_{i1}]_{n \times 1} = \begin{bmatrix} d_{11} \\ d_{21} \\ d_{31} \\ d_{41} \end{bmatrix}_{1 \times n} \quad \text{ve} \quad e_{i1} = \frac{d_{i1}}{w_{i1}} \quad (i=1,2,\dots,n) \quad E = [e_{i1}]_{n \times 1} = \begin{bmatrix} e_{11} \\ e_{21} \\ e_{31} \\ e_{41} \end{bmatrix}_{1 \times n}$$

λ value is found by taking the arithmetic mean of the sum of e values. $\lambda = \frac{\sum_{i=1}^n e_{i1}}{n}$

In the next stage, the consistency indicator is calculated.

$$\text{Consistency Indicator (CI)} = \frac{\lambda - n}{n - 1}$$

The Random Index (RI) is 0 when n is equal to 1 or 2, 0.52 when n is equal to 3, 0.89 when n is equal to 4, 1.11 when n is equal to 5, and 1.25 when n is equal to 6.

$$\text{CR} = \text{CI} / \text{RI}$$

In traditional AHP, even numbers (2-8) are intermediate values according to pairwise comparisons of customer targets, and matrices are formed by considering both options and criteria by using odd numbers (1-9) (Kwong, & Bai, 2002). Considering the numbers for making sense of the hierarchical structure, if one (1) is assigned, two factors are equally preferred. If three (3) is preferred for one factor, this factor is preferred over others at a moderate rate (51-60%) and the value of 1/3=0.33 is written for the opposite factor. If five (5) is preferred for one factor, this factor is strongly preferred over others (61-70%) and the value of 1/5=0.20 is written for the opposite factor. If seven (7) is preferred for one factor, this factor is strongly preferred over others (71-90%) and the value of 1/7=0.14 is written for the opposite factor. If nine (9) is preferred for one factor, this factor is almost certainly preferred over others and the value of 1/9=0.11 is written for the opposite factor (Yaraloğlu, 2001).

In AHP, the best criteria are created in line with the

Inconsistency ratio

The greater the inconsistency ratio, the more inconsistent the judgments. In general, a value less than 0.1 (i.e., CR ≤ 0.1) confirms that the assessment within the matrix is acceptable or indicates a good level of consistency in the comparative judgments represented in this matrix. However, a value greater than 0.1 (i.e., CR ≥ 0.1) indicates inconsistency of judgments within this matrix. D column matrix is obtained by multiplying the A pairwise comparison matrix with the W column matrix showing the weights of the criteria. Additionally, e_{i1} values are calculated by dividing the D column matrix and W column matrix by their mutual elements (Uzundumlu, 2011).

options, the pairwise comparison scores of both the option and the criteria are determined, the pairwise comparison matrices of both the option and the criteria are created, the weight scores of both the option and the criteria are calculated, and the most suitable option is determined by calculating the consistency ratios of the given scores.

RESULTS AND DISCUSSION

When examining where the tourists reside, 34.8% come from Kars and surrounding provinces, 28.8% reside in big cities and the rest reside in other provinces of Turkey. Table 1 shows that the average age of the tourists is 34.75, 57% are married and 61.2% are university graduates.

Figure 1 presents the most suitable meat preference decision tree for consumers in Kars according to some criteria.

Determination of the consumers' meat consumption preferences, as shown in Figure 1, there are four meat options, namely goose, chicken, red meat, and fish, and five (5) criteria for each option, namely price, taste, accessibility, nutrient, and smell.

Priorities of the options

Table 2 presents the explanatory statistics of meat consumption preferences of visitors to Kars using the AHP.

Table 1. Descriptive Statistics of Tourist
 Çizelge 1. Turistlere ait tanımlayıcı istatistikler

Characteristics	Min.	Max.	Mean	Std. Deviation
Age	16	74	34.75	12.174
Gender (Famele=0, Male=1)	0	1	0.56	0.498
Tall (cm)	153	190	171.242	8.264
Weight kg)	45	115	73.16	14.269
Marital status (Sing=0, Married=1)	0	1	0.57	0.512
Total family income (TL/month)*	3000	45000	1053.84	5321.058
Educational background	Primary School	Middle School	High School	University
(%)	2.4	6.4	30.0	61.2

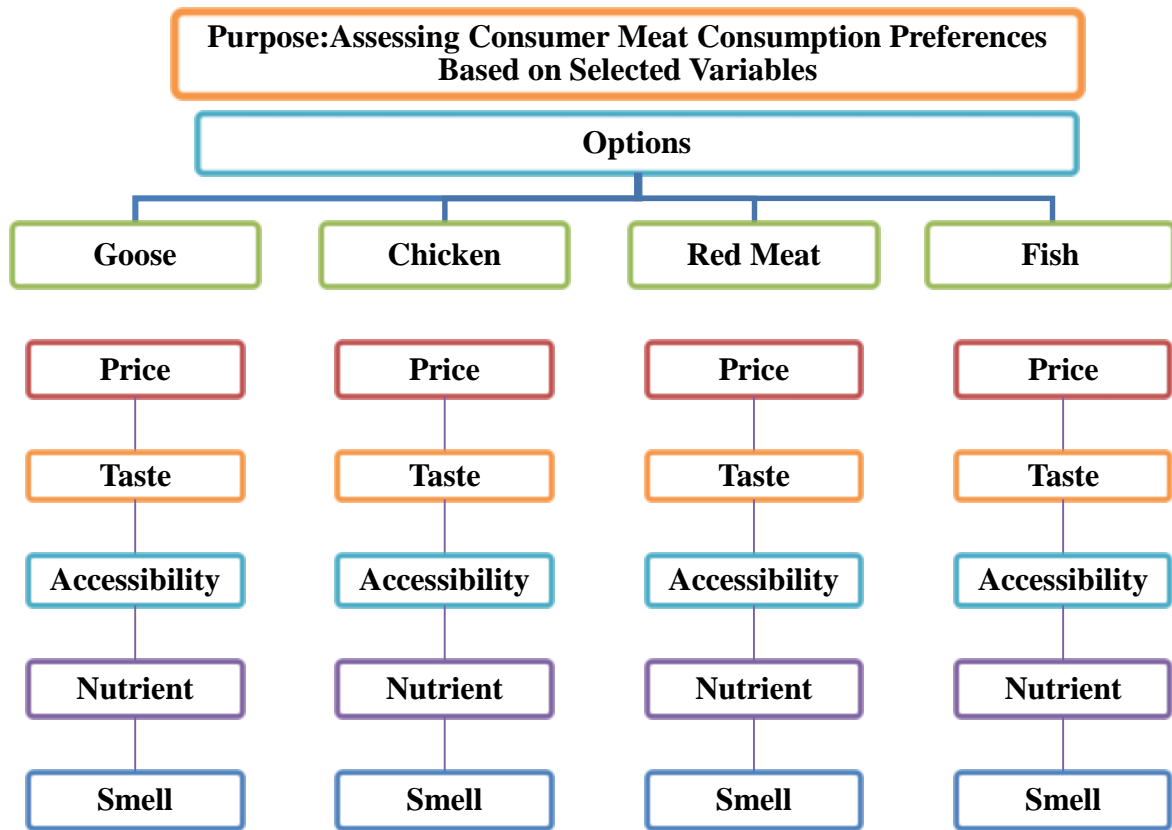


Figure 1. Decision tree for choosing the most suitable type of meat
 Şekil 1. En uygun et tipi tercihindeki karar ağacı

Of the visitors, 29.18% preferred fish, 26.41% chicken, 24.64% red meat and 19.77% goose. As a result of the Kruskal-Wallis test, the difference between the means of at least two groups was statistically significant even at the significance level of 1%. Onurlubaş et al. (2015) conducted a study in İstanbul, Ankara, İzmir, Antalya, Samsun, Erzurum, and Gaziantep provinces in Turkey about food consumed in restaurants, and found that 66% of consumers preferred meat, of whom 48.2% preferred red meat and 17.8% preferred white meat. Süren and Küçükkömürler (2018) found that 41.0% of consumers preferred beef-veal, 35.6% mutton-lamb, 16.7% chicken, and 6.7% fish in restaurants in Ankara.

By emphasizing the importance of out-of-home consumption in meat consumption, Biermann and Rau (2020) stated that 15% of consumers in Germany consumed meat more frequently at home, 42% equally frequently both at and outside the home, and 43% more frequently outside the home (42). On the other hand, they determined that German people consumed meat more frequently when eating outside the home. Table 3 presents the explanatory statistics regarding the criteria considered for meat consumption in restaurants in Kars according to the AHP method.

Table 2. Explanatory statistics of out-of-home meat consumption options in the AHP method
Çizelge 2. AHS yönteminde ev dışı et tüketim seçeneklerinin açıklayıcı istatistikleri

Options	X̄	Se	Min	Max	X _{mean}
Goose	0.1977	0.1099	0.0373	0.5460	0.1855
Red meat	0.2464	0.1089	0.0415	0.5520	0.2227
Chicken	0.2641	0.1204	0.0375	0.5490	0.2529
Fish	0.2918	0.1160	0.0493	0.5835	0.2764

X̄: Mean, Se: Standard error, Min: Minimum, Max: Maximum, X_{mean}: Median

Kruskal-Wallis test, Chi square (0.05,3): 7.81473

H: 81.7358393 (***) p<0.01)

Table 3. Descriptive statistics of AHP criteria

Çizelge 3. AHS kriterlerinin açıklayıcı istatistikleri

Criteria	X̄	Se	Min	Max	X _{mean}
Smell	0.1220	0.1105	0.0252	0.5141	0.0838
Nutrient	0.1818	0.1283	0.0272	0.5744	0.1359
Accessibility	0.1947	0.1305	0.0279	0.5159	0.1903
Price	0.2307	0.1777	0.0228	0.5465	0.1542
Taste	0.2708	0.1585	0.0328	0.5722	0.2591

X̄: Mean, Se: Standard error, Min: Minimum, Max: Maximum, X_{mean}: Median

Kruskal-Wallis test, Chi square (0.05,4): 9.48773

H: 138.087246 (***) p<0.01)

The most important criteria for visitors to Kars in consuming meat in restaurants are listed as taste (27.08%), price (23.07%), ease of accessibility (19.47%), nutritional content (18.18%), and smell (12.20%). As a result of the Kruskal-Wallis test, the difference between the means of at least two groups was statistically significant even at the significance level of 1%. Akçay et al. (2018) determined the most important criteria affecting meat consumption preferences as health (52.46%), nutritional value (24.04%), taste (18.08%), and price (5.45%). In addition, Uzundumlu et

al. (2011) found that for people living in Istanbul, the most important criteria affecting meat consumption preferences were taste (29%), nutritional content (28%), hygiene (24%), and price (19%), respectively.

Criteria and options matrix

Table 4 presents the proportional status of meat consumption preferences of individuals who came from outside of Kars province and consumed meat in restaurants in Kars according to AHP criteria and options.

Table 4. Comparative averages of meat consumption preferences according to AHP criteria and options

Çizelge 4. Et tüketim tercihlerinin AHS kriter ve seçeneklerine göre karşılaştırmalı ortalamaları

Factors	Goose	Chicken	Red meat	Fish	Total
Price	0.12254	0.35930	0.25773	0.26043	1
Taste	0.26600	0.17174	0.24496	0.31730	1
Accessibility	0.10569	0.40991	0.25432	0.23009	1
Nutrient	0.27393	0.14011	0.23546	0.35050	1
Smell	0.25359	0.21002	0.20106	0.33533	1
Total	1.02180	1.29110	1.19350	1.49360	5
Ratio	20.4350	25.8215	23.8706	29.8729	100

Mean consistency ration: 0.060394591

Total number of observations: 250

Number of consistent observations: 229 (%91.6)

Since the consistency ratio in this study was below 0.10% (0.06%), the comparison matrix was consistent, and the percentage of consistent observations was 91.6%. As seen in Table 4, the most important variables for goose meat consumption are its nutritional value, taste, and smell, and the factors that negatively affect goose consumption are high price and

easy access. The most important factors in choosing chicken are ease of accessibility and its cheaper price compared to other meat prices. In addition, its taste, nutritional value, and smell characteristics are less appreciated by many consumers compared to other meat varieties. There is no significant difference between the variables of preferring red meat, but the

variable of smell has the least effect on its consumption. Moreover, taste, smell, and nutritional value are the most important factors in preferring fish, and the factors that negatively affect fish consumption are high price and difficulty of accessibility. Considering the criteria, among the out-of-home meat consumption preferences of visitors to Kars, fish ranks first (29.87%), followed by chicken (25.82%), red meat (23.87%) and goose (20.44%). Akçay et al. (2018) examined the academicians' meat consumption preferences and found that fish ranked first (38.84%), followed by lamb (20.23%), beef (15.78%), chicken (15.10%) and turkey (10.07%) (43). They also examined the criteria of each option and determined the important criteria for fish as health, nutrition, and taste and the less important ones as price. The important criteria for lamb and beef consumption were taste and nutritional value and the less important ones were health and price. The most important criterion for chicken and turkey was price, while other criteria were less important compared to price. Uzundumlu et al. (2011) determined that 30% of the households in Istanbul preferred beef, 27% fish, 25% chicken, and 18% mutton (44). In their study, taste and hygiene were reported as the most important criteria for beef and mutton, and price and nutrient content for chicken and fish.

CONCLUSION

Among the tourists to Kars, 29.18% expressed a preference for fish, 26.41% for chicken, 24.64% for red meat, and 19.77% for goose when dining at restaurants. The most important criteria for visitors to Kars in consuming meat in restaurants are listed as taste (27.08%), price (23.07%), easy access (19.47%), nutritional content (18.18%), and smell (12.20%), respectively. The most important reason why the visitor's least preferred meat is goose, a local delicacy of Kars, is its high price. Those who ate goose meat reported to prefer it because of its high nutritional value and taste. Most of the visitors preferred fish in the first place in their meat preferences due to its nutritional value, taste, and smell. They preferred chicken in the second place due to its ease of accessibility and price.

The reasons why geese are very low in number compared to chickens in Türkiye are low domestic demand for goose products, their high prices, and consumers' little knowledge about them. However, goose meat, which offers various advantages over substitute products in terms of nutrition, is intensively produced and consumed only in certain provinces of Türkiye. Even in these provinces, it lags alternatives in terms of consumption. Goose is a type of poultry that can be grown in pastures like sheep and can withstand adverse weather conditions. There is a potential for goose production and consumption in Türkiye. Since

goose production in Turkey is mostly carried out by small family farms, production costs are quite high. Therefore, consumer interest is low due to high consumer prices. To increase consumers' consumption of goose meat, which is a different meat, it is necessary for production to be carried out in large enterprises at low costs. The number of professional enterprises producing goose should be increased in provinces with a suitable climate. The results of this study provide some information to the producers who produce geese and the consumers who come to Kars, especially restaurants that serve meat dishes. In line with this information, producers and consumers will be informed and will also contribute to restaurants that cook meat dishes to develop more effective marketing strategies by taking into account the factors affecting their preferences.

Author Contributions

Preparation of survey and collection of data, A.A., A.S.U; methodology, A.S.U and A.A.; econometric analysis, A.S.U.; writing, A.A., and A.S.U; writing—review and editing, A.A.

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İklim Değişikliğinin Türkiye’de Tarımsal Üretime Etkisi

İsmail Cem ÖZKURT¹✉

¹Kafkas Üniversitesi, İktisadi ve İdari Bilimler Fakültesi, İktisat Bölümü, Merkez KARS.

¹<https://orcid.org/0000-0003-0871-9215>

✉: icozkurt@kafkas.edu.tr

ÖZET

Tarım sektörü insanlık tarihi kadar eski bir geçmişe sahiptir. Hayatın devamı için yeme-içme faaliyetlerinin yapılması zorunluluğu bu sektöre stratejik bir önem kazandırmaktadır. İklim değişikliği ise sanayi devriminin bir sonucu olarak başta sera gazı emisyonlarının sebep olduğu olumsuzluklar olmak üzere tüm Dünya’yı etkileyen bir durumdur. Sanayi devrimi ile birlikte tarımsal üretimin ekonomi içindeki payı azalsa da yakın zamanda gerçekleşen Covid-19 pandemi dönemi tarımsal üretim ve arzının göz ardı edilemeyecek kadar önemli olduğunu bir kez daha göstermiştir. Tarımsal üretimin sahip olduğu bu önem aynı zamanda iklim değişikliklerinin tarımsal üretimi ne kadar ve nasıl etkilediği sorusunu ortaya çıkarmıştır. İklim değişikliği göstergelerinin olumsuz etkilerinin hemen ortadan kalkmayacağı gerçeği hem sorunların hem de çözüm yollarının tespitini önemli hale getirmektedir. Çalışmanın amacı, Türkiye’de tarımsal üretim miktarının iklim değişikliklerinin göstergesi olarak kabul edilen değişkenlerden nasıl ve ne yönde etkilendiğini ortaya koymaktır. Bu amaçla birim kök testlerinden sonra Varyans Ayrıştırması, Etki-Tepki Fonksiyonu ve Toda Yamamoto Nedensellik Analizi yapılmıştır. Çalışma sonuçları, Türkiye’de tarımsal üretim ile sera gazı emisyonu arasında bir nedensellik ilişkisinin yanı sıra kuraklık, ortalama sıcaklık ve yağış değişkenleri ile sera gazı emisyonu arasında da nedensellik ilişkisi olduğunu, yani sera gazı emisyonunun doğrudan ve dolaylı olarak iklim değişikliğinin nedeni olduğunu göstermektedir. Buna göre öncelikli olarak sera gazı emisyonlarını kontrol altına alacak çalışmalara ihtiyaç olduğu değerlendirilmektedir.

Tarım Ekonomisi

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Nedensellik Analizi.

Impact of Climate Change on Agricultural Production in Türkiye

ABSTRACT

The agriculture sector has a history as old as human history. The necessity of eating and drinking activities for the continuation of life gives this sector strategic importance. Climate change is a situation that affects the whole world, especially the negativities caused by greenhouse gas emissions as a result of the industrial revolution. Although the share of agricultural production in the economy decreased with the industrial revolution, the recent COVID-19 pandemic period has once again shown that agricultural production and supply are too important to be ignored. This importance of agricultural production has also raised the question of how much and how climate changes affect agricultural production. The fact that the negative effects of climate change indicators will not disappear immediately makes it important to identify both problems and solutions. The aim of the study is to reveal how and in what direction the amount of Türkiye’s agricultural production is affected by the variables accepted as indicators of climate change. For this purpose, Variance Decomposition, Impulse-Response Function, and Toda-Yamamoto Causality Analysis were performed after unit root tests. According to the results obtained, there is a causality relationship between agricultural production and greenhouse gas emissions in Türkiye, while there is a causality relationship between drought, change in average temperatures, and change in precipitation variables and greenhouse gas emissions

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variables. In light of the finding that greenhouse gas emissions are directly and indirectly the cause of climate change in Türkiye, studies to control greenhouse gas emissions should be urgently implemented.

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GİRİŞ

İklim değişikliği Birleşmiş Milletler İklim Değişikliği Çerçeve Sözleşmesi’nde, bir zaman periyodu içinde görülen doğal iklim değişikliklerine ilave olarak küresel atmosferik yapıyı doğrudan ya da dolaylı olarak bozan insan faktörünün iklimlerde sebep olduğu değişiklikler olarak tanımlanmıştır (Anonymous, 2014). Bir diğer tanımlamaya göre ise iklim değişikliği, iklimin ortalama durumunda ya da onun değişkenliğinde onlarca ya da daha uzun yıllar boyunca süren istatistiksel olarak anlamlı değişimlerdir (Türkeş, 2008).

İklim değişiklikleri çok değişik sebeplerden dolayı ortaya çıkmaktadır. Küresel iklim değişikliğinin temel kaynakları, insan kaynaklı sera gazı salınımı, fosil yakıt kullanımı, sanayi, ulaştırma, arazi kullanımında meydana gelen değişimler, katı atık yönetimi ve tarımsal nedenlerdir (Çakmak & Gökalp, 2011). Tarımsal üretimin bizzat kendisinden kaynaklanan sebepler de iklim değişikliğine yol açmaktadır. Bu sebepler arasında, gübreleme, ilaçlama, tarımsal üretim ile arz esnasında fosil yakıt kullanımı ve yetiştirilen hayvanların ürettikleri gübreler sayılabilir (Bayraç & Doğan, 2016).

İklim değişikliklerinin etkilerini değerlendirebilmek amacıyla mahsul modelleri kullanılmaktadır. Bu modellerden birincisi sürece dayalı ürün modelidir. Bu model, belirli ürünlerin davranışlarını yakından gösterme avantajına sahiptir. Olumsuz yönü ise çok fazla veri gerektirmesi ve kalibrasyon için özel verilere ihtiyaç duyulmasıdır. Diğer iki model ise, düz istatistiksel ilişkiler ve hedonik modellerdir. İki yöntem de sürece dayalı modelleri tamamlayan güçlü yönleri sahiptirler. Daha az zorlayıcı verilere ihtiyaç duyulması bu modellerin **avantajı** olarak ifade edilebilir. Özellikle hedonik yöntem gerçek koşulların değerlendirilmesinde ön plana çıkmaktadır. Bu modeller, dar bir coğrafi alanda belirli bir ürün çerçevesinde daha kolay uygulanabilmektedir (Robertson ve ark. 2013).

İklim değişikliklerine bağlı olarak tarımsal üretiminin azalması ekonomi üzerinde çeşitli yönlerden olumsuz etkilerde bulunmaktadır. Bu olumsuz etkilerin başında mevsimsel değişimler nedeniyle tarımsal üretimde dalgalanmaların artmasına bağlı olarak belirsizliğin artması gelmektedir. Üretim miktarındaki belirsizlikler ürün fiyatlarında oynaklıkların artmasına neden olabilmektedir. Nitekim Busnita ve ark. (2017), çalışmalarında

Endonezya’da pirinç fiyatlarında meydana gelen oynaklıkta iklim değişikliğinin rolünü incelemişlerdir. Çalışma sonucunda, sıcaklıklarda meydana gelen değişimlerin kısa ve uzun vadede pirinç üretimini olumsuz etkilediği ancak pirinç fiyatlarındaki dalgalanmayı olumlu etkilediği tespit edilmiştir. Takle ve ark. (2013), konuya gıda arz ve talebi açısından yaklaşmışlardır. Çalışmada, iklim değişikliklerinin bu şekilde devam etmesi halinde 2050 yılından sonra küresel gıda arzının talebi karşılayamayacağını ortaya koymuşlardır. Hasegawa ve ark. (2015), çalışmalarında iklim değişikliğinin önemli bir göstergesi olan sıcaklık değişimleri üzerinde durmuşlar, değişimin 2°C’de sınırlı tutulması durumunda iklim değişikliklerinin olumsuz etkilerinin sınırlandırılabilirliğini savunmuşlardır. Ancak, azaltım maliyetlerinin yüksek olmasının düşük gelirli ülkelerde büyük olumsuzluklara neden olabileceğini ve gıda tüketimindeki değişimin daha çok gelirdeki değişime bağlı olduğunu ortaya koymuşlardır. Nsabimana & Habimana (2017), bir diğer önemli değişken olan yağış miktarındaki değişimlerin Ruanda’da gıda ürünleri fiyatlarına etkisini incelemişlerdir. Gıda fiyatlarının, yağış miktarında meydana gelecek şoklara karşı aşırı kırılgan olduğunu ortaya koymuşlardır.

İklim değişikliğine bağlı olarak tarımsal üretimin azalması diğer makroekonomik değişkenleri de etkilemektedir. Üretimin azalmasına ve belirsizliğin artmasına bağlı olarak fiyat artışlarının yaşanması enflasyona yol açmaktadır. Bunun yanı sıra üretim açığının ithalat yoluyla kapatılması durumunda cari açığın artması söz konusu olacaktır. Tarım sektörü büyük oranda emek gücüne bağlıdır. Bu durumda tarımsal üretimin azalmasına bağlı olarak sektörde meydana gelecek küçülme işsizliğin artmasına neden olmaktadır. İklim değişikliğinin tarımsal üretimi olumsuz etkilemesi sonucu zarar eden üreticilerin ya da aşırı hava olayları nedeniyle mahsulleri zarar gören üreticilerin bu zararları devlet tarafından karşılanmak zorunda kalınacağından bütçe dengesi üzerinde olumsuz etkilere neden olmaktadır.

Diğer yandan iklim değişikliğine neden olan faktörlerin bazı olumlu katkıları da bulunmaktadır. Karbondioksit gazının atmosfer içerisindeki payının artması bazı mahsullerin daha hızlı büyümesine yol açarken, sıcaklık artışı bitkilerin daha kısa sürede büyümelerini sağlayarak erken ekim ve hasat imkânlarını ortaya çıkarmaktadır (Bayraç & Doğan,

2016; Kara & Yereli, 2022). C₃ olarak ifade edilen ve içerisinde pirinç ile buğday gibi önemli tahılları barındıran bu ürün grubu yüksek karbondioksit konsantrasyonuna ihtiyaç duymaktadır. Artan karbondioksit konsantrasyonu bu ürün grubunda üretimin artmasını sağlayacaktır (Doğan & Tüzer, 2011). Aynı zamanda sıcaklık artışı ile özellikle soğuk kuzey bölgelerde hem ısının artması hem de yetiştirme döneminin uzaması sayesinde tarımsal üretimde artışlar beklenmektedir. Daha önceki yıllarda soğuk nedeniyle tarıma elverişli olmayan bölgelerin küresel sıcaklık artışına bağlı olarak tarımsal üretime açılması söz konusu olmuştur. Bu konuda Olesen & Bindi (2002)'nin, iklim değişikliğinin Avrupa'da tarımsal üretkenliğe olan etkisini inceleyen çalışması ön plana çıkmaktadır. Çalışma sonuçlarına göre, sıcaklık artışı ekim alanlarının Avrupa'nın kuzey bölgelerine doğru genişlemesine ve örneğin tahılların yetiştirme süresinin azalmasına, köklü bitkilerin ise yetiştirme sürelerinin uzamasına neden olmaktadır. Karbondioksit konsantrasyonunun artması bitki verimliliğinin artmasına neden olmaktadır. Bu olumlu etkiler tarım üzerinde olumlu sonuçlar yaratabilir. Ancak Avrupa'nın güney bölgeleri dezavantajlı konumdadır. Yağışların azalmasına bağlı olarak ortaya çıkacak su kıtlığı, daha düşük hasat verimine, verim değişkenliğinin artmasına ve geleneksel ürünlerin ekilme alanlarının azalmasına neden olabilecektir.

Küresel iklim değişiklikleri tarımsal üretim teknolojilerinde de değişikliklere neden olmuştur. Tarımsal üretimin azalmasına bağlı olarak üretimde daha fazla teknoloji kullanılmaya başlanmış böylelikle tarım sektörü emek yoğun sektörden teknoloji yoğun sektöre doğru evrim geçirmiştir. Sanayi devrimi ile birlikte ekonomik faaliyetlerin artması iklim değişikliklerinin bir numaralı sebebi olarak görülse de ekonomik büyümenin sağlanması beraberinde iklim değişiklikleri ile mücadelede kullanılacak finansal kaynakların da artmasını sağlamıştır. Bu durumda ekonomik büyümenin sağlanması ile büyümenin sonucu olan sera gazlarının etkilerinin artması arasında insan hayatına olan olumsuz etkileri yönünden zıt bir ilişki ortaya çıkmaktadır. Bu zıt ilişki nedeniyle zihinlerde ekonomik faaliyetleri azaltmak suretiyle refahtan ödün verilmesi ile iklim değişikliklerin yarattığı etkileri göze alarak ekonomik faaliyetlere aynen devam edilmesi şeklinde bir ikilem oluşmaktadır (Alper & Anbar, 2008).

Literatürde yer alan Bayraç & Doğan (2016), Dumrul & Kılıçarslan (2017), Akcan ve ark. (2022) ve El-Khalifa ve ark. (2022) çalışmalarında, iklim değişikliğinin belirli bir coğrafi alan üzerindeki etkilerini gösteren mahsul verimi yerine, mahsulün getirilerini dikkate alan ve ölçüm birimi olarak da tarımsal gelirin GSYH içindeki payını kullanan Ricardocu yaklaşımı izlemişlerdir. Bu çalışmada ise

tarımsal üretim miktarı milyon ton cinsinden bağımlı değişken olarak kullanılmıştır. GSYH'daki değişimlere karşın tarımsal ürün değerlerinde aynı oranda değişimler yaşanmaması durumunda tarımsal gelirin GSYH içindeki payı azalacaktır. Bu gerçekleşme iklim değişikliklerinin tarımsal ürünlerin değerinde meydana getirdiği değişimleri ölçmede hatalı sonuçlar vereceği kanaatiyle çalışmada tarımsal üretim miktarı bağımlı değişken olarak kullanılmıştır. Böylelikle doğrudan iklim değişikliklerinin tarımsal üretim miktarı üzerindeki etkileri seçilen bağımsız değişkenler vasıtasıyla analiz edilmeye çalışılmıştır. Çalışmanın literatüre katkıda bulunması beklenen ikinci güçlü yönü ise sera gazı emisyonlarının bağımsız değişken olarak analize dâhil edilmesidir. Diğer çalışmalarda çoğunlukla karbondioksit emisyonu gibi sera gazı emisyonunu oluşturan gazlardan sadece biri analize dâhil edilmiş iken bu çalışmada diğer gazları (metan, diazot monoksit, kloroflorokarbon ve ozon) da içeren sera gazı emisyonları analize dâhil edilmiştir. Sera gazları emisyonlarının iklim değişikliğinde oynadığı role literatürdeki çalışmalarda önemle yer verilmiş olmasına rağmen bu emisyonları analize dâhil eden çalışma sayısı çok azdır. Çalışmanın üçüncü güçlü yönü kuraklık ve yağış değişkenlerinin yüzdelik değişim olarak analize dâhil edilmesidir. Kuraklık değişkeninin analize dâhil edildiği çalışma sayısı çok azdır. Oysa kuraklık hem iklim değişikliğinin bir sonucu olması nedeniyle önemli bir ölçüt iken aynı zamanda tarımsal üretimi doğrudan etkilediği de bilinen bir gerçektir. Yağış değişkeni ise literatürde genellikle yıllık yağış miktarı olarak ele alınmıştır. Çalışmada ise yağış miktarlarında bir önceki yıla göre yüzdelik değişim ele alınmıştır. Bu şekildeki bir yaklaşım ile yağış değişkeninde meydana gelen değişimlerin analize daha iyi yansıtılacağı düşünülmektedir.

Bu zamana kadar yapılan çalışmalarda genellikle başta Birleşmiş Milletler olmak üzere uluslararası kuruluşlar tarafından hazırlanan iklim değişikliği raporları ve geleceğe yönelik projeksiyonlar kullanılmıştır. İklim değişikliğinin tarımsal üretim üzerindeki etkilerini ekonometrik yöntemlerle araştırmaya çalışan çalışmalarda genellikle Gecikmesi Dağıtılmış Otoregresif Sınır Testi (Autoregressive Distributed Lag Bound Test, ARDL) kullanılmıştır. Bu çalışmada ise konu farklı bir açıdan ele alınmış ve alternatif bir yöntem olarak Varyans Ayrıştırması kullanılmıştır. Bunun yanı sıra Etki-Tepki Fonksiyonu yapılarak tepkinin şiddeti ve yönü hakkında çıkarımlarda bulunulmuş ve Toda-Yamamoto Nedensellik Analizi ile de nedensellik ilişkisi incelenmiştir. Bu yöntemleri kullanan çalışmaların bulunmaması çalışmanın önemini ve literatüre katkısını göstermektedir.

MATERYAL ve METOD

Çalışmada kuraklık, sera gazı emisyonları, ortalama sıcaklık dağılımı ve yağış miktarındaki değişiklikler iklim değişikliğinin göstergeleri olarak dikkate alınmıştır (Olesen ve ark. 2000; Southworth ve ark. 2000; Zaied 2013; Belloumi 2014; Loum & Fogarassy 2015; Bayraç & Doğan 2016; Eruygur & Özokçu 2016; Dumrul & Kılıçarslan 2017; Akyüz 2018; Chandio ve ark. 2020; Onurlu & Ulaş 2021; Akcan ve ark. 2022; El-Khalifa ve ark. 2022; Eştürk & Mert 2022; Özgür & Demirtaş 2022; Taha ve ark. 2022; Chopra 2023).

Serilerde yer alan değerler eğer düzey değerler ise yani

Çizelge 1. Analizde kullanılan değişkenler ve açıklamaları

Table 1. Variables used in the analysis and their explanations

Değişkenler <i>Variables</i>	Simgesel Gösterimi <i>Symbolic Display</i>	Açıklama <i>Description</i>
Tarımsal Üretim	lnurt	Toplam tarımsal üretim miktarı, milyon ton cinsinden
Sera	lnsera	Sera gazı emisyonları, CO ₂ eşdeğeri, milyon ton cinsinden
Kuraklık	kurak	Yıllık kuraklık değerlerinde görülen yüzde değişim
Sıcaklık	lnsıcak	Yıllık ortalama sıcaklık değerleri, santigrat cinsinden
Yağış	yag	Metrekareye düşen yıllık yağış miktarlarında görülen yüzde değişim

Kuraklık değişkeni yıllık ölçülen kuraklık değerlerindeki yüzdelik değişimi ifade etmektedir. Bir önceki yıla göre kuraklık değişimlerinde bir azalma meydana gelmesi negatif değerler almasına neden olmaktadır. Değişken, negatif değerler ve yüzdelik değişim içerdiğinden doğal logaritması alınmamıştır. Yağış değişkeni, bir önceki yıla göre yağış miktarlarında meydana gelen yüzdelik değişimleri göstermektedir. Bir önceki yıla göre daha az yağış alınması yüzdelik olarak negatif değerlere neden olduğundan doğal logaritması alınmamıştır. Diğer değişkenler doğal logaritmaları alınarak analiz edilmişlerdir. Tarımsal üretim, sera gazı emisyonları, kuraklık ve yağış değişkeni verileri Ekonomik İşbirliği ve Kalkınma Örgütü istatistik veri tabanından (OECD, 2023), ortalama sıcaklık değişkeni verileri ise Devlet Meteoroloji İşleri Genel Müdürlüğü'nden (MGM, 2023) elde edilmiştir.

Zaman serilerine dayalı ekonometrik analizlerde değişkenlerin durağanlığı önemli rol oynamaktadır. Serilerin birim kök içerip içermediğini tespit edebilmek amacıyla Augmented Dickey-Fuller (ADF) ve Philips-Perron (PP) Birim Kök Testleri yapılmıştır (Dickey & Fuller, 1981; Philips & Perron, 1988). Augmented Dickey-Fuller ve Philips-Perron Birim Kök Testi sonuçları bir değişkenin zaman serisinin durağan olup olmadığını değerlendirmek amacıyla kullanılmaktadır. Dickey & Fuller (1981), çalışmalarında birim kökün varlığını tespit edebilmek amacıyla rassal ve durağan olmayan bir seri ve bu serideki δ parametre değerini tahmine yönelik bir yöntem geliştirmişlerdir. Ancak δ parametre tahmini normal dağılmadığından t istatistikleri yerine t kritik değerlerini oluşturmuşlardır (Enders, 2009). Phillips-

yüzdelik bir değişimi ifade etmiyorlarsa doğal logaritmaları alınarak analize dâhil edilirler. Bir serinin doğal logaritmasının alınması ile verilerdeki çarpıklığın azaltılması veya ortadan kaldırılması amaçlanır. Aynı zamanda regresyon analizlerinde bağımlı ve bağımsız değişken arasındaki ilişkinin doğrusal olması gerekliliği vardır. Logaritma alma işlemi ile üstel veriler doğrusala çevrilmiş olur.

Çizelge 1'de ekonometrik analizde kullanılan değişkenlerin neler olduğuna ve bu değişkenlerin neleri ifade ettiğine yer verilmiştir.

Perron Testi, hata terimini otokorelasyonsuz olmasına bağlı olarak ele alır. Hatalarla ilgili varsayımlardan yola çıkılarak Augmented Dickey-Fuller Testi daha kapsamlı hale getirilmiştir. Hata teriminin varyansının zamana bağlı olarak değiştiği ve varyans değerlerinde meydana gelen sürekli değişimin heteroskedasitiye işaret ettiği savunulur. Değişkenlerin analiz dönemi boyunca meydana gelen yapısal kırılmalardan etkilenmesi söz konusu olmadığından yapısal kırılmaları gösteren birim kök testlerine yer verilmemiştir.

Değişkenler arasındaki ilişkileri tespit etmeye yönelik çalışmalarda kullanılan testlerin gecikme uzunluğuna karşı duyarlı olduğu belirtilmektedir. Değişkenlerin gecikme uzunluğunu gösteren k katsayısını doğru tespit etmek modelin güvenilirliği açısından önemlidir. Uygun gecikme uzunluğunun tespit amacıyla yapılan testte Ardışık Modifiye Edilmiş LR Kriteri, Nihai Tahmin Hatası (Final Prediction Error, FPE), Akaike Bilgi Kriteri (Akaike Information Criteria, AIC), Schwarz Bilgi Kriteri (Schwarz Informatin Criteria, SC) ve Hannan-Quinn Bilgi Kriteri (Hannan-Quinn Information Criteria, HQ) yer almaktadır. Bu kriterlerden AIC, SC ve HQ kriterleri diğer bilgi kriterlerine göre daha güçlüdür. Uygun gecikme uzunluğu tespit edilirken gecikme uzunluğunun yüksek değerli olarak belirlenmesi seriler arasındaki uzun dönemli ilişkiyi açıklama gücünü zayıflattığından test anında elde edilen verilerden en küçük değerler hangi bilgi kriterinden elde edilmişse bu gecikme uzunluğu uygun gecikme uzunluğu olarak kabul edilmektedir (Kaya ve ark., 2017).

Durağanlık testlerinden sonra kurulan modelin

güvenilirliğini belirlemek amacıyla bazı tanısal testler yapılmaktadır. Bu testlerden otokorelasyon LM testi modelde yer alan hataların zaman içinde ve kendi aralarında birbirine bağımlı olup olmadıklarını tespit etmek amacıyla yapılmaktadır. Eğer hatalar arasında bu şekilde bir bağımlılık yok ise buna otokorelasyon veya serisel korelasyon bulunmaması varsayımı adı verilir. Otokorelasyon sorunu, hata terimleri arasında ilişki olmadığı ($E(u_i, u_j)=0, i \neq j$) varsayımının geçerli olmamasıdır. Diğer bir deyişle hata terimleri arasında ilişki vardır. $E(u_i, u_j) \neq 0, i \neq j$. u_t ile u_{t-1} arasında otokorelasyon; kovaryansların veya beklenen değerlerin sıfıra eşitliği demektir. Normallik testinin amacı, bir veri dizisinin normal dağılıma uygunluğunun incelenmesidir. Jarque-Bera sınaması normal dağılımdan ayrılmayı ölçmek için kullanılan bir ölçüdür. Bu sınama çoklu doğrusal regresyon sonuçları elde edildikten sonra ele geçen hataların normal dağılım gösterip göstermediğini araştırmak için kullanılır. Değişen varyans White testi seride değişen varyans sorununun varlığını tespit etmek amacıyla kullanılan bir testtir. Bu testte, asıl denklemin hata tahmin karelerinin bağımlı, açıklayıcı değişkenlerin kendileri, kareleri ve çarpımlarının açıklayıcı değişken olduğu denklem tahmin edilir. Elde edilen test istatistiğinin olasılık değeri eğer ele alınan anlamlılık düzeylerinden (0,1, 0,05 veya 0,10) büyük ise H_0 hipotezi red edilir yani değişen varyans sorunu yoktur (Gemicioğlu, 2019).

Literatürde yer alan çalışmalarda genellikle ARDL analizi kullanılmıştır. Çalışmada ise alternatif bir yöntem olarak Varyans Ayrıştırması kullanılmıştır. ARDL analizi değişkenler arasında kısa ve uzun dönemli eşbütünleşme ilişkisinin varlığını incelemektedir. Elde edilen eşbütünleşme katsayısı bağımsız değişkenlerin bağımlı değişkeni etkileme yönü ve şiddetini gösteren tek bir katsayıdır. Varyans Ayrıştırması ise bir değişkendeki değişimin yüzde kaçının kendisinden ve yüzde kaçının da diğer değişkenlerden kaynaklandığını göstermektedir. Böylelikle belirlenen dönem boyunca değişkenlerin birbirlerini ve bağımlı değişkeni açıklamada meydana gelen değişimleri görmek mümkün olmaktadır (Mert & Çağlar, 2019). Varyans Ayrıştırması analizinde değişkenlerin sıralaması önemli bir konudur. Dışsaldan içsele doğru bir sıralama yapılmaktadır (Tarı, 2006). Varyans Ayrıştırması'nda değişkenlerin etkileme derecesini ve etkilemenin sabitlendiği yani istikrara kavuştuğu dönem ya da dönemleri tespit etmek mümkündür. Bu özelliklerinden dolayı Varyans Ayrıştırması'nın ARDL analizine göre daha ayrıntılı bilgiler vereceği kanaatiyle bu yöntem kullanılmıştır.

Etki-Tepki Fonksiyonları, rassal hata terimlerinden birinde meydana gelen bir birimlik standart sapmalı şokun, içsel değişkenlerin şimdiki ve gelecekteki değerlerine olan etkisini yansıtan fonksiyonlardır. Etki-tepki fonksiyonları, çalışılan değişkenler

arasındaki dinamik etkileşimin belirlenmesinde, simetrik ilişkilerin saptanmasında ve VAR analizinde büyük rol oynamaktadır. (Sarı, 2008). Bir diğer açıdan bakıldığında Etki-Tepki Fonksiyonları, VAR modellerinde şokların ve etkilerin tepkilerini incelemek için kullanılmaktadır. Diğer tüm değişkenler ve şoklar sabit iken içsel değişkene yönelik bir birimlik şokun ve bunun VAR modelindeki tüm içsel değişkenler üzerindeki etkilerini ifade etmektedir (Anonymous, 2022). Grafiklerde yer alan düz yatay sıfır çizgisi tepkinin söndüğünü yani yok olduğunu göstermektedir. Kırmızı renkli görünen iki kesikli çizgi ise 0,95 güven aralığının alt ve üst sınırlarını göstermektedir.

Toda-Yamamoto Nedensellik Analizi ise Granger Nedensellik Analizi'nden yola çıkılarak 1995 yılında literatüre kazandırılmış bir nedensellik testidir. Analiz, birim kök ve eşbütünleşme gibi analizler olmadan da yapılabilmektedir. Analizde önemli olan gecikme uzunluğunun doğru tespit edilmesi ve tüm bileşenlerin birlikte kullanılmasıdır (Toda & Yamamoto, 1995).

İklim değişikliklerinin tarımsal üretime olan etkisini analiz etmek amacıyla 1 numaralı denklem kullanılmıştır.

$lnurt_t = \beta_0 + \beta_1 lnsera_t + \beta_2 lnsıcak_t + \beta_3 kurak_t + \beta_4 yağ_t + \epsilon_t$ (1)
Denklem 1'de yer alan $lnurt$ logaritması alınmış tarımsal üretim miktarını gösteren bağımlı değişken, $lnsera$ logaritması alınmış sera gazı emisyonunu, $lnsıcak$ logaritması alınmış ortalama sıcaklık dağılımlarını, $kurak$, yıllık kuraklık değerlerinde görülen yüzde değişimi, $yağ$ yıllık milimetre yağış miktarlarında meydana gelen yüzde değişimi, ϵ_t ise hata terimi temsil etmektedir.

BULGULAR ve TARTIŞMA

Çalışmanın bu bölümünde birim kök testleri, uygun gecikme uzunluğunun belirlenmesi, yapısal testler, Varyans Ayrıştırması, Toda-Yamamoto Nedensellik Testi ve Etki-Tepki Analizi yapılmış ve elde edilen sonuçlara yer verilmiştir.

Çizelge 2'de yer alan Augmented Dickey Fuller (ADF) testi sonuçları incelendiğinde sabitli ve trendli modelde $lnurt$ değişkeni farkı alınmadan 0.05 önem düzeyinde, $lnsera$ değişkeni birinci farkı alındığında 0.01 önem düzeyinde, $lnsıcak$ değişkeni farkı alınmadan 0.01 önem düzeyinde, $kurak$ değişkeni farkı alınmadan 0.05 önem düzeyinde ve $yağ$ değişkeni farkı alınmadan 0.01 önem seviyesinde durağandır.

Yine Çizelge 2'de yer alan Philips Perron (PP) testi sonuçları incelendiğinde, $lnurt$ değişkeni farkı alınmadan 0.01 önem düzeyinde, $lnsera$ değişkeni birinci farkı alındığında 0.01 önem düzeyinde, $lnsıcak$ değişkeni farkı alınmadan 0.01 önem düzeyinde, $kurak$ değişkeni farkı alınmadan 0.05 önem düzeyinde ve $yağ$ değişkeni de farkı alınmadan 0.01 önem düzeyinde durağandır.

Çizelge 2. Birim kök testleri

Table 2. Unit root tests

ADF Birim Kök Testi ADF Unit Root Test				
Değişkenler Variables	Düzyey Level	Olasılık Probability	Birinci Fark First Difference	Olasılık Probability
Lnurt	-4.245613** (-3.562882)	0.0110		
Lnsera	-2.863106 (-4.284580)	0.1874	-5.410610* (-4.296729)	0.0007
Lnsıcak	-5.975197* (-4.284580)	0.0001	-	
Kurak	-3.747979** (-3.562882)	0.0337		
Yag	-6.033807* (-4.284580)	0.0001		
PP Birim Kök Testi PP Unit Root Test				
Lnurt	-4.285627* (-4.284580)	0.0100		
Lnsera	-2.789145 (-4.284580)	0.2115	-7.105295* (-4.296729)	0.0000
Lnsıcak	-6.061611* (-4.284580)	0.0001		
Kurak	-3.582321** (-3.562882)	0.0480		
Yag	-6.771837* (-4.284580)	0.0000		

*, ** ve *** sırasıyla 0,01, 0,05 ve 0,10 anlamlılık düzeylerini ifade etmektedir. Parantez içindeki değerler kritik değerlerdir.
*, **, and *** indicate significance levels of 0.01, 0.05 and 0.10, respectively. Values in parentheses are critical values.

Her iki durağanlık testinde de değişkenlerden sadece Lnsera değişkeni birinci farkı alındığında yani I(1)'de durağan hale gelirken diğer değişkenler farkı alınmadan yani I(0)'da durağandır. VAR analizi yapılırken öncelikle kurulan VAR modeli için uygun

gecikme uzunluğunun tespit edilmesi gereklidir.

Çizelge 3'de yer alan uygun gecikme uzunluğu testi sonuçlarına göre tüm kriterlerde uygun gecikme uzunluğu 1 olarak bulunmuştur. Analiz 1 gecikmeye göre yapılmıştır.

Çizelge 3. Uygun gecikme uzunluğunun belirlenmesi

Table 3. Determining the appropriate lag length

Lag	LogL	LR	FPE	AIC	SC	HQ
0	-132.2350	NA	0.006472	9.149003	9.382536	9.223712
1	-55.00505	123.5680*	0.000204*	5.667003*	7.068201*	6.115258*
2	-31.44469	29.84312	0.000262	5.762979	8.331841	6.584780

Ekonometrik çalışmalarda kullanılan modellerin bazı ön koşulları ya da varsayımları karşılaması gerekmektedir. Bu nedenle öncelikle modelin bu ön koşulları sağlayıp sağlamadığı, çalışmanın amacına uygun ve güçlü bir model olup olmadığı test edilmiş ve sonuçlar Çizelge 4'te verilmiştir.

Otokorelasyon LM Testi modeldeki hataların kendi aralarında birbirine bağımlı olup olmadıklarını belirlemek için kullanılır. Normallik testinde veri dizisinin normal dağılıp dağılmadığı incelenmekte bu amaçla Jarque-Bera sınaması kullanılmaktadır.

Çizelge 4. Yapısal testler

White testi seride değişen varyans sorununun tespiti amacıyla kullanılmaktadır. Testlerin olasılık değerlerinin her üç anlamlılık düzeyinden büyük olması modelde değişen varyans ve otokorelasyon sorununun olmadığını göstermektedir. Normallik Testi'nde Jarque-Bera sınamasının olasılık değerinin yine her üç anlamlılık düzeyinden büyük olması serinin normal dağıldığını göstermektedir (Teyyare, 2018).

Table 4. Structural tests

Testler	Test İstatistik Değeri	Olasılık
Otokorelasyon LM Testi	24.902	0.4812
Normallik Testi	12.71680	0.2399
White Değişen Varyans Testi	164.7697	0.1937

Varyans ayrıştırması analizi yoluyla bağımlı değişken olan lnurt değişkenine bağımsız değişkenlerin ne ölçüde etki ettiği belirlenmeye çalışılmaktadır. Çizelge 5'de yer alan Varyans ayrıştırması sonuçlarına göre, ilk dönemde (yılda) lnurt değişkeni tamamen kendisinden etkilenmektedir. İlerleyen dönemlerde bu etkinin derecesinin azaldığı görülmektedir. lnsera değişkeni, bağımlı değişkeni en çok etkileyen bağımsız değişkendir. İlk dönemde (yılda) sadece %4.4 etki oranına sahip iken ilerleyen dönemlerde etkileme derecesinin giderek arttığı ve onuncu dönemde %33.08 etki oranına ulaşıldığı görülmektedir. Bir diğer tespit ise etki oranının devamlı artması ve istikrara kavuşmamasıdır. İkinci bağımsız değişken olan lnsıcak değişkeni ikinci dönemde %2,07 etki oranına sahip iken etki oranındaki artışın sınırlı kaldığı ve onuncu dönem sonunda sadece %5'lik bir etki oranında

ulaşıldığı görülmektedir. Bu değişkenin etki oranındaki artış da devamlı bir seyir izlemekte ve istikrara kavuşmamaktadır. Üçüncü bağımsız değişken olan kurak değişkeni ikinci dönemde %2,77 etki oranına sahip iken üç ile altıncı dönemler arasında %3'lük ve yedi ile onuncu dönemler arasında ise %4'lük bir etki oranına sahiptir. Bu durum kurak değişkeninin yedinci dönem (yıl) ile birlikte istikrara kavuştuğunu göstermektedir. Son bağımsız değişken olan yağış değişkeni ise ikinci dönemde %0,23 gibi oldukça düşük bir etki oranına sahip iken üç ile beşinci dönemlerde %1'lik ve altı ile onuncu dönemler arasında ise %2'lik bir etki oranına sahip olduğu görülmektedir. Yağış değişkeni, altıncı dönem itibariyle istikrara kavuşmaktadır. Bu özelliği ile kurak ve yağış değişkenlerinin benzer yapıya sahip olduğu söylenebilir.

Çizelge 5. Varyans ayrıştırması

Table 5. Variance decomposition

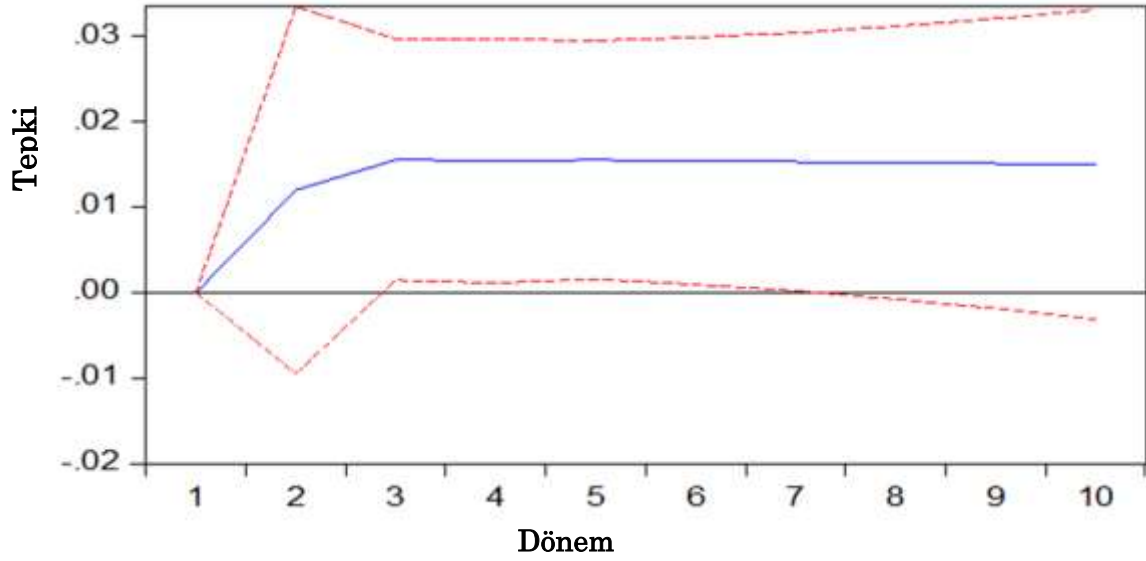
Periyot Period	S.E. S.E.	lnurt Lnurt	lnsera lnsera	lnsıcak lnsıcak	Kurak Drought	Yag Yag
1	0.052918	100.0000	0.000000	0.000000	0.000000	0.000000
2	0.056935	90.49201	4.429949	2.070854	2.773176	0.234010
3	0.060335	82.91112	10.57135	2.426729	3.016033	1.074765
4	0.063268	76.46752	15.50584	3.134741	3.487319	1.404581
5	0.066058	71.18229	19.72833	3.563828	3.714959	1.810587
6	0.068671	66.75329	23.25362	3.958858	3.926506	2.107728
7	0.071158	62.99607	26.26391	4.278359	4.089890	2.371766
8	0.073525	59.76269	28.85427	4.556535	4.232093	2.594417
9	0.075789	56.95139	31.10879	4.796738	4.353966	2.789118
10	0.077958	54.48388	33.08770	5.007860	4.461036	2.959521

Etki-Tepki fonksiyonları, bağımsız değişkene verilen bir birimlik şokun bağımlı değişkene olan etkisini göstermektedir. Şekillerde yatay eksen dönemleri dikey eksen ise bağımsız değişkene verilen bir birimlik şoka bağımlı değişkenin tepkisini göstermektedir. Çalışma yıllık verilere dayandığından dönem ifadesini yıl olarak ele almak mümkündür. Bu durumda grafikte on yıllık bir periyot görülmektedir. Bunun yanı sıra, şekillerde yer alan düz yatay sıfır çizgisi tepkinin sönümlendiğini yani yok olduğunu göstermektedir. Kırmızı renkli görünen iki kesikli çizgi ise 0,95 güven aralığının alt ve üst sınırlarını göstermektedir.

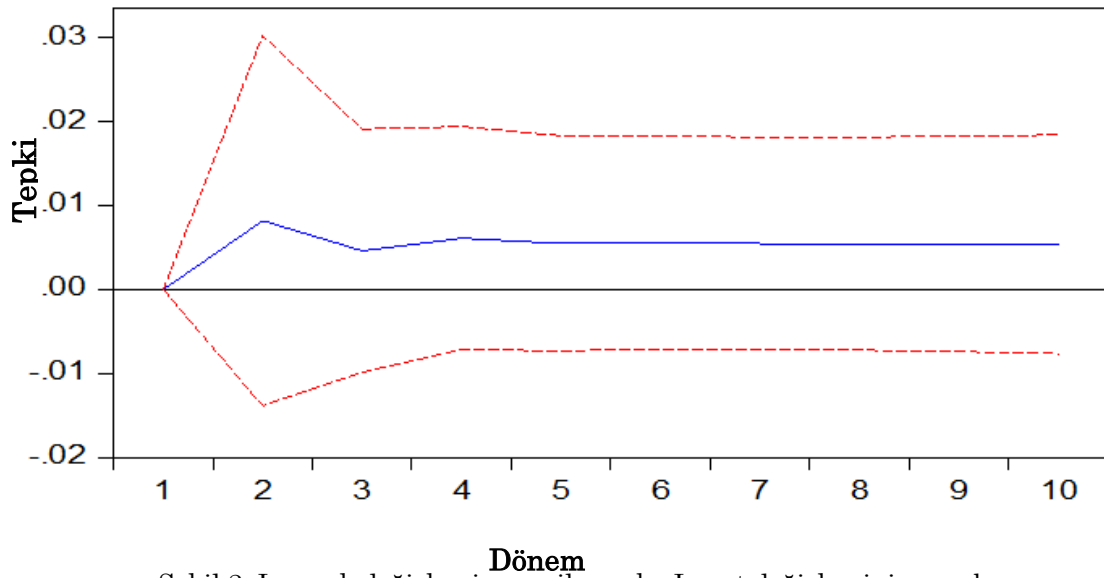
lnsera değişkenine verilen bir birimlik şoka lnurt değişkeninin tepkisi Şekil 1'de görülmektedir. Buna göre, lnsera değişkenine verilen şoka lnurt değişkeninin cevabı pozitif yönde olmakta birinci dönemden üçüncü döneme kadar artış göstermektedir. Tepkinin şiddetinin üçüncü dönem itibariyle istikrar

kazandığı ve dönem sonuna kadar aynı düzeyde kaldığı görülmektedir. Ancak tepkinin şiddeti sıfır eksenine yaklaşmamaktadır. Bu durum tepkinin sönümlenmediğini (ortadan kalkmadığını) göstermektedir. Bu gerçekleşme tepkinin uzun dönemler boyunca devam ettiğini göstermektedir.

Şekil 2'de görüldüğü üzere lnsıcak değişkenine verilen bir birimlik şoka lnurt değişkeninin cevabı dönem (yıllar) boyunca pozitif yönde olmaktadır. İkinci döneme kadar artış trendi izleyen tepkinin yönü üçüncü döneme doğru azalış sergilemektedir. Üçüncü dönemden dördüncü döneme doğru hafif bir yükseliş trendi izledikten sonra tepkinin şiddetinin dördüncü dönem ile birlikte istikrar kazandığı görülmektedir. lnsıcak değişkenine lnurt değişkeninin tepkisi tıpkı lnsera değişkenine verdiği tepki gibi sönümlenmemekte dönem boyunca pozitif ve istikrarlı bir seyir izlemektedir.



Şekil 1. Lnsera değişkenine verilen şoka Lnurt değişkeninin cevabı
Figure 1. Response of the Lnurt variable to the shock given to the Lnsera variable



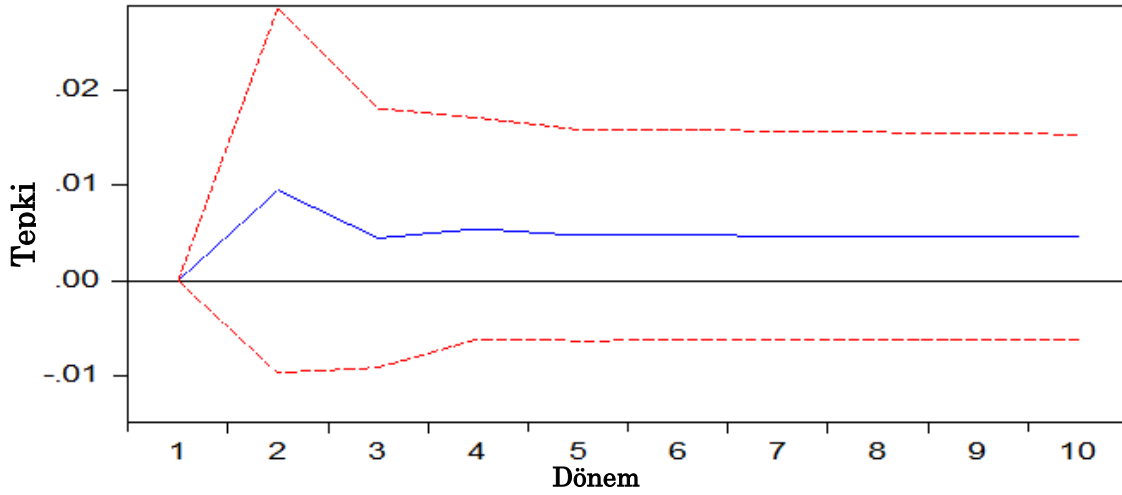
Şekil 2. Lnsıcak değişkenine verilen şoka Lnurt değişkeninin cevabı
Figure 2. The response of the variable Lnurt to the shock given to the variable Lnsıcak

Kurak değişkenine verilen bir birimlik şoka lnurt değişkeninin cevabını gösteren Şekil 3'e göre, birinci dönemden ikinci döneme doğru tepkinin pozitif olduğu ve şiddetinin arttığı görülmektedir. Tepkinin şiddeti ikinci dönemden üçüncü dönem doğru azalış trendi izlerken tepkinin şiddetinin dördüncü dönem itibariyle istikrar kazandığı ancak dönem sonuna kadar hep pozitif kaldığı ve sönümlenmediği görülmektedir.

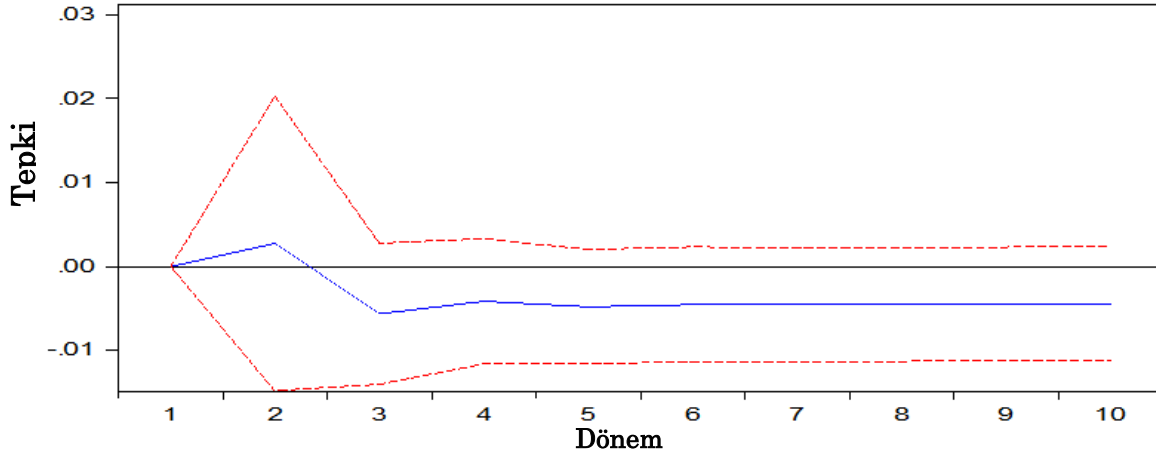
Şekil 4, yağış değişkenine verilen bir birimlik şoka tarımsal üretimin tepkisini göstermektedir. Yağış değişkenine verilen bir birimlik şoka tarımsal üretim miktarı ikinci döneme kadar pozitif ancak az şiddette cevap vermektedir. Tepkinin yönü üçüncü dönemde negatife dönmektedir. Tepkinin seyri dördüncü dönemden itibaren istikrar kazanmakla birlikte

onuncu dönemin sonunda daha negatif olmakta ve ortadan kalkmamaktadır.

Etki-Tepki Fonksiyonu ile tepkinin şiddeti ve yönü hakkında fikir sahibi olunmaktadır. Buna göre yapılan analizden elde edilen en önemli sonuç tepkinin onuncu dönem sonunda dahi ortadan kalkmamasıdır. Bu durum, iklim değişikliğini temsil eden bağımsız değişkenlerin tarımsal üretim miktarını temsil eden bağımlı değişken üzerindeki etkilerinin kalıcı olduğunu göstermektedir. Buna dayanarak iklim değişikliklerinin etkilerinin kolay kolay ortadan kalkmayacağını bugünden alınacak tedbirlerin sonuçlarının hemen görülmesinin imkânsız olduğu belirtilebilir.



Şekil 3. Kurak değişkenine verilen şoka Lnurt değişkeninin cevabı
Figure 3. Response of the Lnurt variable to the shock given to the Drought variable



Şekil 4. Yag değişkenine verilen şoka Lnurt değişkeninin cevabı
Figure 4. The response of the Lnurt variable to the shock to the Yag variable

Toda-Yamamoto Nedensellik Testi değişkenler arasındaki karmaşık nedensellik ilişkilerini anlamak amacıyla kullanılan bir yöntemdir. Bu yöntemeye dayanan analiz Toda-Yamamoto Nedensellik Testi sonuçlarına dayanmaktadır. Çizelge 6'da yer alan sonuçlar incelendiğinde, Türkiye'de analiz dönemi içerisinde tarımsal üretim miktarındaki değişimlerin tek nedeninin sera gazı emisyonları olduğu görülmektedir. Nedensellik ilişkisinin 0.01 anlamlılık düzeyinde gerçekleşmesi bu ilişkinin istatistiksel anlamda güçlü olduğunu göstermektedir. Diğer üç değişken olan kuraklık, ortalama sıcaklıklarda yaşanan değişim ve yağış değişimi sera gazı emisyonlarındaki değişimin nedeni olmaktadır. Bu ilişkiler 0.10 anlamlılık düzeyinde gerçekleşmektedir. Bu durumda değişkenlerde meydana gelecek değişimler sera gazı emisyonlarını, sera gazı emisyonlarındaki değişimler de tarımsal üretim miktarını etkilemektedir. Dolayısıyla bu üç değişkenin

tarımsal üretim miktarı üzerinde dolaylı etkilerde bulunduğu söylenebilir.

Literatürde yer alan çalışmalarda konuya değişik açılardan yaklaşmıştır. Bunlardan birincisi iklim değişikliğinin tarımsal GSYH'ye etkisini araştıran çalışmalardır. Kumar & Parikh (2001), Dumrul & Kılıçarslan (2017), El-Khalifa ve ark. (2022), çalışmalarında iklim değişikliği göstergelerinin tarımsal GSYH'nın azalmasına neden olduğu sonucuna ulaşmıştır. Bir diğer yaklaşım ürün verimliliği üzerinedir. Genellikle tahıl üretim miktarı ve verimliliğini ele alan; Olesen ve ark. (2000), Southworth ve ark. (2000), Olesen & Bindi (2002), Guiteras (2009), Zaied (2013), Hasegawa ve ark. (2015), Loum & Fogarassy (2015), Eruygur & Özokçu (2016), Chandio ve ark. (2020), Eştürk & Mert (2022), Taha ve ark. (2022) çalışmalarında iklim değişikliği göstergelerinden sıcaklık ve kuraklık değişkenlerinin

Çizelge 6. Toda-Yamamoto nedensellik testi

Table 6. Toda-Yamamoto causality test

Nedenselliğin yönü <i>Direction of causation</i>	Olasılık <i>Probability</i>
Insera → Inurt	0.0004*
Insıcak → Inurt	0,4488
kurak → Inurt	0,6630
yag → Inurt	0.7265
Inurt → Insera	0,3153
Insıcak → Insera	0.0587***
kurak → Insera	0.0707***
yag → Insera	0.0869***
Inurt → Insıcak	0.7423
Insera → Insıcak	0.1910
kurak → Insıcak	0.5715
yag → Insıcak	0.9910
Inurt → kurak	0,6971
Insera → kurak	0,3236
Insıcak → kurak	0,1712
yag → kurak	0,1799
Inurt → yag	0.2866
Insera → yag	0.3472
Insıcak → yag	0.8011
kurak → yag	0,1935

*, ** ve *** sırasıyla 0,01, 0,05 ve 0,10 anlamlılık düzeylerini ifade etmektedir.

*, ** and *** indicate significance levels of 0.01, 0.05 and 0.10, respectively.

tahıl ya da mahsul verimliliği üzerinde farklı etkilerde bulunduğu bazı çalışmalarda verimi arttırdığı (Olesen & Bindi, 2002; Zaied, 2013; Chandio ve ark. 2020) bazı çalışmalarda ise (Olesen ve ark. 2000; Southworth ve ark. 2000; Guiteras, 2009; Eruygur & Özokçu, 2016; Taha ve ark. 2022) azalmaya neden olduğu tespit edilmiştir. Çalışmada yer alan Etki-Tepki fonksiyonunda yağış değişkenine verilen bir birimlik şoka tarımsal üretimin üçüncü dönemden itibaren negatif tepki verdiği tespit edilmiştir. Bu sonuç, daha önce yürütülen benzer çalışmalar ile uyumluluk göstermektedir. Çalışmada sera gazı emisyonları açısından elde edilen sonuçlar ile benzerlik gösteren çalışmalardan El-Khalifa ve ark. (2022)'ye göre karbondioksit emisyonlarında meydana gelen artış iklim değişikliğinin bir numaralı nedeni iken mevcut çalışmada sera gazı emisyonundaki artış hem tarımsal üretimdeki hem de iklimdeki değişikliğin nedeni olarak tespit edilmiştir. Bu sonuç, yağış ve karbondioksit konsantrasyonlarındaki artışın tarım sektörünü etkileyeceğini bildiren Mendelsohn & Williams (2004) ile de benzerlik göstermektedir. Diğer yandan, Onurlu & Ulaş (2021), Avrupa Birliği ülkelerinde sera gazı emisyonları ile ekili tarım arazileri arasında çift yönlü nedensellik ilişkisi olduğunu belirtmesine rağmen mevcut çalışma sonuçları sera gazlarından tarımsal üretime doğru tek yönlü nedensellik ilişkisi olduğunu göstermektedir.

Yine konu ile ilgili olarak yürütülen Özgür & Demirtaş (2022) çalışmasının sonucu olan, iklim değişikliğinden şeker pancarı üretimine doğru tek yönlü nedensellik ilişkisi ile sıcaklık ve yağışın şeker pancarı üretimi üzerindeki nedensellik ilişkisi çalışmada sıcaklık ve yağış tarımsal üretimin dolaylı nedenidir şeklinde elde edilmiş bu yönüyle elde edilen sonuçlar ile benzerlik göstermektedir.

SONUÇ ve ÖNERİLER

İnsanlık tarihi kadar uzun bir geçmişe sahip olan tarım sektörü, sanayi devrimi ile birlikte ülkelerin ekonomik büyümelerindeki önemini kaybetse de geçen yıllarda yaşanan Covid-19 dönemi tarımsal üretim ve arzının önemini bir kez daha ortaya koymuştur. Sanayileşme ile birlikte çok sayıda ülke ekonomik açıdan gelişmiş ülkeler seviyesine yükselmiş olsa da bu gelişmenin maliyeti iklim değişikliği olmuştur. Az gelişmiş ve gelişmekte olan ülkelerin ekonomik büyümelerini sağlayabilmek için doğal kaynakları geleceklerini hiç düşünmeden fütursuzca harcamaları ve kurdukları sanayi tesislerinde çevreye verilen zararların minimize edilmesine yönelik gerekli tedbirleri almamaları bu grupta yer alan ülkelerde çevre sorunlarının daha büyük boyutlara ulaşmasına neden olmaktadır.

İklim değişikliği sadece doğa olaylarının oluş sıklığı ve oluş şeklinde bir değişiklik olarak ifade edilemez. İklim değişikliği çok sayıda makroekonomik sorunun temelini teşkil etmektedir. İklim değişikliklerine bağlı olarak ortaya çıkacak olan tarımsal üretimdeki azalmalar ve buna bağlı olarak artan fiyatlar yoksul kesimin gıdaya ulaşabilmesini zorlaştıracaktır. Bu durum küresel gıda güvenliğinin tehlike altına girmesine neden olacaktır. Tarımsal ürünler aynı zamanda gıda sektöründe üretim yapan firmaların da girdisini oluşturmaktadır. Tarımsal üretimde meydana gelen azalmalar bu sektörde yer alan firmaların hammadde bulmakta zorlanmasına, üretimin azalmasına ve buna bağlı olarak da fiyatların yükselmesine neden olacaktır. Dünya nüfusunun giderek artması tarımsal ürünlere olan talebi de arttırmaktadır. Bu durum tarımda yüksek verimli modern yöntemlerin daha fazla kullanılmasının yanı sıra bu zamana kadar kullanılmayan başta orman arazileri olmak üzere tarımsal olmayan arazilerin tarıma açılması arazi kullanımında değişikliklere neden olacaktır. Bu unsur gelecekte iklim değişikliği ile beraber en büyük tehlikelerden birisidir. İklim değişikliklerinin gelecek nesillerin yaşam standartlarını olumsuz etkileyerek sürdürülebilir kalkınmanın devamlılığını tehlikeye atması beklenmektedir.

Tarım sektörü istihdam piyasasında önemli bir role sahiptir. Özellikle az gelişmiş ülkelerde tarımsal üretimin daha çok emek yoğun teknolojiye dayanması, üretimde meydana gelecek azalmaların bu gruptaki

ülkelerde var olan işsizlik sorununun daha da derinleşmesine neden olacaktır. Tarım sektörünün önemli rol oynadığı bir diğer makroekonomik değişken ekonomik büyümedir. Sektördeki büyümenin yoksul ülkelerde büyüme oranlarını diğer sektörlere göre iki ila dört kat daha fazla etkilediği akademik çalışmalarla ortaya konulmuştur. Bu durumda tarım sektörünün küçülmesi özellikle yoksul ülkelerin ekonomik büyümelerini oldukça olumsuz etkileyecektir.

İklim değişikliğinin etkilerine sadece ülke bazlı yaklaşmak yetersiz bir yaklaşım olacaktır. İklim değişikliği ve yarattığı olumsuz etkileri küresel bazda ele almak gereklidir. Bir ülkenin sera gazı emisyonlarını azaltmaya yönelik aldığı tedbirlerden komşu ülkelerin benzer yönde çabalar sarf etmemeleri halinde beklenen olumlu sonuçlar alınmayacaktır. Bu nedenle sorunun kaynağına inilerek en önce başta sera gazı emisyonları olmak üzere iklim değişikliğine en fazla neden olan ülkelerde emisyon azaltıcı tedbirlere başvurularak bunun küresel yansımaları takip edilmelidir.

Sera gazlarının atmosferde kalma süreleri çok uzundur. Dolayısıyla bugün sera gazı salınımı tamamen durdurulsa dahi atmosferde uzun yıllar kalmaya devam edeceğinden olumsuz etkilerinden hemen kurtulmak mümkün olmayacaktır. İklim değişikliğine bağlı olarak ortaya çıkan yeni durum tarımsal üretim yöntemlerinde değişikliğe gidilmesine neden olmaktadır. Yağış miktarlarında meydana gelen azalmalara karşın önlem olarak sulama sistemlerinde değişikliğe gitmek gereklidir. Damlama sulama tekniği bu durumda başvurulabilecek bir önlemdir. Aynı zamanda ülkede yetiştirilen tarım ürünlerinin kompozisyonunda da değişikliğe gidilerek daha az su isteyen ürünlere yönelmek bir diğer önlem olacaktır. İklim değişikliklerine bağlı olarak Türkiye’de kurak ve yarı kurak alanlara yenilerinin eklenmesi beklenmektedir. Kuraklığın artması beraberinde üretilen tarımsal ürünlerin de değişmesine neden olarak sıcak, kurak ve mevsimsel özelliklere uygun ürünlere yönelmek zorunda kalınacaktır. Bu nedenle sorunların giderilmesine yönelik olarak Ar-Ge faaliyetlerine başta kamu olmak üzere tüm paydaşların daha fazla kaynak aktarmaları gerekmektedir.

Ekonometrik analizden elde edilen sonuçlara göre Türkiye’de tarımsal üretimi en çok etkileyen etmen sera gazı emisyonlarıdır. Hem Varyans Ayırıştırması’nda hem de Toda-Yamamoto Nedensellik Analizi’nde bu sonuca ulaşılmıştır. Sera gazı emisyonlarında meydana gelecek artışlar başta tahıl olmak üzere bazı tarımsal üretim miktarlarının artmasını sağlasa da sıcaklık artışına bağlı olarak bu ürünlerin protein ve besin içeriği açısından verimsiz olmasına neden olmaktadır. Temel olarak sera gazı emisyonlarında meydana gelen artışlar iklim

değişikliğinin diğer göstergelerini de doğrudan etkilemektedir. Sera gazı emisyonları küresel ısınmayı arttırarak sıcaklık değerlerinin artmasına, buna bağlı olarak da tarımsal üretimde önemli yer tutan yağış miktarının azalmasına neden olmaktadır.

Etki-tepki fonksiyonundan iki önemli sonuç çıkarmak mümkündür. Bunlardan birincisi, yağış değişkenine verilen bir birimlik şoka tarımsal üretimin üçüncü dönemden itibaren negatif tepki vermesidir. Bu sonuca göre, yağış miktarlarında meydana gelecek değişiklikler tarımsal üretimin azalmasına neden olacaktır. Yağışların azalmasına paralel olarak kuraklık sorununun daha da derinleşmesi beklenbilir. İkinci önemli sonuç ise, etkilere verilen tepkinin incelenen dönemler boyunca sönümlenmediği yani ortadan kalkmadığı sonucudur. Bu sonuç bize iklim değişikliğine neden olan etkilerin uzun dönemler boyunca devam edeceğini bugün önleyici tedbirler alınsa dahi bu tedbirlerin sonuçlarının uzun vadede ortaya çıkacağını göstermektedir.

Çalışmanın temel amacı; Türkiye’de iklim değişikliğinin tarımsal üretime olan etkisini tespit etmektir. Çalışma bağımlı değişken olarak tarımsal üretim miktarını ele almasıyla literatürde yer alan diğer çalışmalardan ayrılmakta ve bu yönüyle literatüre katkıda bulunmaktadır. Bu yaklaşım farklılığının ileride yapılacak çalışmalara da ilham vermesi beklenmektedir. Elde edilen sonuçlar çalışmanın amacını açıklar ve doğrular niteliktedir. Özellikle kuraklık, yağış ve sıcaklık değişkenlerinin sera gazı emisyonlarındaki değişimlerin nedeni olduğu sonucu sera gazı emisyonlarının iklim değişikliğinde ne kadar önemli bir rol oynadığını göstermektedir. Sera gazının bu önemi ilgili tüm taraflarca öncelikle ele alınması gereken bir sorun olduğunu göstermektedir. Bu bağlamda iklim değişikliğinin temel sebebi olarak da kabul edilebilecek olan sera gazı emisyonlarını kontrol altına almaya yönelik olarak bu gazları atmosfere en fazla bırakan ülkelere başlamak üzere emisyonları azaltmaya yönelik çabalar iklim değişikliğine yönelik kısa vadede etkilerinin azaltılmasına uzun vadede ise tamamen ortadan kaldırılmasına katkıda bulunacaktır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazar makalenin tamamına katkı sağlamış olduğunu beyan eder.

Çıkar Çatışması Beyanı

Makalede herhangi bir çıkar çatışması bulunmamaktadır.

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Siyah Alaca Sığırlarda Pik Süt Verimine Bazı Çevre Faktörlerin Etkisi

Naci TÜZEMEN¹, Mustafa TANKAL²

¹Kastamonu Üniversitesi, Mühendislik ve Mimarlık Fakültesi, Genetik ve Biyomühendislik Bölümü, 37150, Kastamonu, Türkiye. ²Gökkale Tarım İşletmesi, Devrekani- Kastamonu, Türkiye²

¹<https://orcid.org/0000-0001-8804-5323>, ²<https://orcid.org/0000-0003-4696-2048>,

✉: nacituzemen@kastamonu.edu.tr

ÖZET

Bu araştırma, Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırlarda pik süt verimine pike ulaşma süresi, laktasyon sırası, buzağılama mevsimi, buzağılama yılı, servis periyodu ve kuruda kalma süresi gibi bazı çevre faktörlerin etkisini tespit etmek amacıyla yapılmıştır. Yüksek süt verimi ve bunun sürdürülebilir olması sığırlarda çevre faktörlerinin pik süt verimi üzerine etkisinin incelenmesi, ayrıca ileri dönemler için yapılacak seleksiyon, planlama ve işletmede yapılan yetiştiricilik uygulamaları için çok önemlidir. Sığır yetiştiriciliğinde kontrol edilebilen çevre faktörleri olarak servis periyodu ve kuruda kalma süresi yüksek süt verimi ve bunun devamlılığı açısından ayrıca dikkate alınması gereken uygulamalardır. Bu çalışmada, incelenen çevre faktörlerinin, pik süt verimine etkileri çok önemli bulunmuştur ($P<0.01$). İncelenen veriler sonucu Gökkale Tarım İşletmesinde pik süt verimi genel ortalaması $40,36 \pm 0,141$ kg'dır. Pik süt verimi ile pike ulaşma süresi arasında $r = -0.281$ gibi negatif bir korelasyon bulunmuştur. İlgili korelasyonlar istatistiksel olarak çok önemlidir ($P<0.01$).

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The Effect of Some Environmental Factors on Peak Milk Yield in Holstein Cattle

ABSTRACT

This research was conducted to determine the effect of some environmental factors such as time to peak milk yield, lactation order, calving season, calving year, service period, and dry period on peak milk yield in Holstein cattle raised in Gökkale Agricultural Enterprise. High milk yield and its sustainability are very important for examining the effects of environmental factors on peak yield in cattle, as well as for future selection, planning and breeding practices in the enterprise. As controllable environmental factors in cattle breeding, service period and dry period are practices that should also be taken into consideration in terms of high milk yield and its continuity. In this study, the effects of the environmental factors examined on milk yield in the peak period were found to be very important ($P<0.01$). As a result of the data examined, the general average peak milk yield in Gökkale Agricultural Enterprise is 40.36 ± 0.141 kg. A negative correlation of $r = -0.281$ was found between peak milk yield and time to peak. The relevant correlations are statistically significant ($P<0.01$).

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GİRİŞ

Süt sığırcılığının temel amacı, işletmede yetiştirilen hayvanların sürdürülebilir biçimde süt ve döl verimlerini hem nitelik hem de nicelik olarak artırmaktır (Sehar & Özbeyaz, 2005). Sığırlarda süt

üretimi, hem kalıtsal hem de çevresel faktörler tarafından etkilenir ve bu faktörlerin birbirinden ayrı düşünülmesi mümkün değildir (Tekerli, 1996). Bu nedenle, hem genetik hem de çevresel faktörlerin birlikte ele alınması ve iyileştirilmesi gerekmektedir

(Toksoy, 2007). Süt üretimi, genetik ve çevre faktörlerinin etkisiyle şekillenen kompleks bir olgudur (Tüzemen ve ark., 2013). Çevreye uygun genotiplerin seçimi için, genotiplerin verim performanslarının yanı sıra, verimi etkileyen çevre faktörlerinin de belirlenmesi gerekmektedir (Sehar & Özbeyaz, 2005). Çevresel faktörler çeşitlidir ve bazıları günlük değişimlere neden olan kısa süreli etkilere sahipken, diğerleri bütün laktasyon süresince verimi etkileyebilir (Tekerli, 1996). Bir sağım sırasında memedeki sütün tamamen sağılmaması kısa süreli bir varyasyon iken, ineğin buzağılamadaki yaşı ise, o buzağılamayı izleyen bütün laktasyon süresince etkisini sürdüren uzun süreli bir varyasyon kaynağını ifade eder (Toksoy, 2007).

Süt sığırcılığı işletmelerinde, ekonomik gelirin sağlanması adına ürünün talep ile dengelenmesi ve üretimin kontrol altında tutulması önemlidir. Bu dengeyi sağlamanın ve üretimin sürdürülebilirliğini sağlamanın önemli yollarından biri düzenli yapılan ıslah ve seleksiyondur. Bu süreç, hem bireyin hem de sürünün elde ettiği süt miktarlarının düzenli olarak kaydedilmesiyle desteklenir, böylece üretim sürekli olarak izlenir (Toksoy, 2007; Özhan ve ark., 2015).

Pik süt verimi, laktasyon verimi ve laktasyon persistensi gibi önemli performans ölçütlerini iyileştirmek için erken laktasyonda bir seçim kriteri olarak kullanılabilir. Yapılan araştırmalar, güç doğum yapan ineklerin pik süt verimine daha uzun sürede ulaştığını ortaya koymaktadır. Ayrıca, pik süt verimi ile laktasyon persistensi arasında belirgin bir fenotipik korelasyon olduğu belirlenmiştir (Albarrán-Portillo ve Pollott, 2013). Bu nedenle, pike erken ulaşmanın laktasyon persistensini olumsuz yönde etkilediği gözlemlenmektedir.

Yapılan bir çalışma, ineklerin pik süt verimine ulaşma sürelerinin <41 gün, 41-57 gün ve >57 gün olmak üzere üç gruba ayrıldığını ve laktasyon persistensinin en yüksek oranda 41-57 günlerde pike ulaşanlarda gözlemlendiğini ortaya koymuştur (Sharma ve ark., 2018; Güler ve Akmaz, 2020).

Takip eden laktasyonlarda ideal verimlilik için sağılan ineklere yeterli kuruda kalma süresi planlanmalıdır. Yeterli kuruda kalma süresi ineklerde 6 ile 8 haftadır. İneklere yeterli süre kuruda kalma fırsatı verilmemesi, gelecek laktasyondaki süt verimine olumsuz yönde etkileyecek ve yüksek süt veriminin sürdürülebilirliği ortadan kalkacaktır. Pik süt verimi ile kuruda kalma süresi arasında nasıl bir bağlantı olduğu üzerine yapılmış daha fazla çalışmaya ihtiyaç bulunmaktadır. Atashi ve ark., (2013), daha kısa kuru dönemler (0 ila 35 gün ve 36 ila 50 gün) daha düşük başlangıç süt verimi ile ilişkilidir ve 0 ila 35 günlük ve 36 ila 50 günlük kuru dönem uzunluğuna sahip ineklerde 51 ila 60 günlük kuru dönem uzunluğuna sahip ineklerde pik laktasyona daha geç ulaşılmıştır. Kuru dönemleri olmayan veya kısa kuru dönemleri

olan ineklerin günde 7,0 kg yağ ve proteinle düzeltilmiş süt (FPCM) verimi 305 günlük süt veriminden daha düşük olurken, aynı şekilde geleneksel kuru dönemli ineklerin günde 2,3 kg FPCM verimi 305 günlük süt veriminden daha düşük olduğu tespit edilmiştir (Kok ve ark., 2016).

Esasen lüzumundan fazla kuruda kalma süresinin de süt üretimine pek fazla olumlu etkisi bulunmamaktadır. Kuruda kalma periyodunun 60 günden fazla olmasının süt üretimine pek fazla etkisi olmazken, 30 günden daha az dinlenme periyodu, süt üretiminde % 5-10 oranında düşmeye neden olmaktadır (Özhan ve ark., 2015).

Chen ve ark., (2016), 0 veya 30 günlük kuru periyodu olan ineklerde, 60 günlük DP'si olan ineklere göre daha düşük pik verimi, daha geç pik verimi ve yağ ve protein açısından düzeltilmiş süt (FPCM305) daha düşük olduğu belirlenmiştir. Hoeij ve ark (2017), 0 günlük kuru dönem, 30 günlük kuru dönemle karşılaştırıldığında erken laktasyondaki ineklerin süt verimini azalttığı ve enerji dengesini ve metabolik durumunu iyileştirdiğini bildirmişlerdir.

Kuruda kalma süresi esas olarak ineklerin dinlenme, yenilenme ve gelecek laktasyona hazırlanması sürecidir. Yeterli miktarda kuruda kalma süresi, hayvanın mineral ve vitamin depolamasına ve aynı zamanda fazla verimden dolayı yıpranmış veya tükenmiş olan dokuların tamamlanmasına imkân sağlar ve ineklerin verimlerinin sürdürülebilir olmasında rol oynar (Tüzemen & Yanar, 2013).

Servis periyodunu etkileyen faktörlerden biri, involüsyon süresidir. İnvölüsyon, doğumdan sonra üreme organlarının gebelik öncesindeki ölçü ve formuna dönüşmesi olayına verilen isimdir. Bu olayın gerçekleşmesi için geçen süreye involüsyon süresi denir ve sığırlarda genellikle ortalama 30-35 gün arasındadır (Akman ve ark., 2001; Uygur, 2004; Özhan ve ark., 2015; Middleton ve ark., 2019).

Buzağılamayı takiben üreme organları bir dinlenme ve yenilenme periyodu sonunda yeni bir gebeliğe girmektedir. Laktasyon içindeki bu yeni gebelikle birlikte endokrin sisteminde bazı değişiklikler meydana gelir. Aynı zamanda, fetus gelişirken ilerleyen gebelik sürecinde besin madde ihtiyacı artar ve bu durum süt verimini azaltır. Oluşan yeni gebelik sonucunda laktasyon süresi kısalmakta ve böylece süt verimi etkilenmektedir. Stodola ve ark, (1979), yaptıkları bir çalışmada, maksimum süt üretiminin, servis periyodunun 121-130 gün arasında olduğunu ve en düşük süt üretiminin ise 40-50 günlük servis periyodunda olduğunu bildirmişlerdir. Araştırmacılar, servis periyodu ile süt üretimi arasında önemli derecede ($P<0.05$) pozitif bir korelasyon hesaplamışlardır. Pik süt verimi ile servis periyodu arasındaki ilişkiyi daha iyi anlamak için daha fazla çalışmaya ihtiyaç vardır.

Bu araştırma, Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırlarda pik süt verimine; pike ulaşma süresi, laktasyon sırası, buzağılama mevsimi ve buzağılama yılı, servis periyodu ve kuruda kalma süresi gibi bazı çevre faktörlerinin etkilerinin incelenmesi ve bu özellikler arasındaki korelasyonların tespiti amacıyla yapılmıştır.

MATERYAL ve METOD

Materyal

Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırların 2010-2019 yılları arasındaki 2980 adet laktasyon kaydı üzerinde bir araştırma yapılmıştır. 2010 yılındaki veri eksikliği nedeniyle, bu yılın verileri 2011 yılıyla birleştirilerek analiz edilmiştir. Süt verimi özellikleri; buzağılama yılı, buzağılama mevsimi, laktasyon sırası, buzağılama yaşı, buzağılama aralığı, servis periyodu ve kuruda kalma süresi gibi birçok çevre faktöründen etkilenmektedir. Ancak, bu çalışmada, pike ulaşma süresi, laktasyon sırası, buzağılama mevsimi, buzağılama yılı, servis periyodu ve kuruda kalma süresi faktörleri modele dahil edilerek analiz yapılmıştır. Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırlarda, pik süt verimine bazı çevre faktörlerinin etkisi incelenmiştir. Araştırmada kullanılan veriler, işletmede kullanılan tam otomatik sağım sistemi (De Laval Apro 6.93) versiyonundan elde edilmiş ve hesaplanmıştır (Tankal & Tüzemen, 2022).

Metod

Süt verimi özelliklerinin çevre faktörlerinin etkisini belirlemek için varyans analizi ve önemli bulunan değerler için Duncan çoklu karşılaştırma testi uygulanmıştır. Pik süt verimi ile ilgili korelasyonların hesaplanmasında servis periyodu, kuruda kalma süresi ve pike ulaştığı gün verileri kategorize edilmemiş veriler üzerinden hesaplanmıştır. İstatistiksel verilerin hesaplanmasında General Linear Model (GLM) Univariate yöntemi (SPSS, 2020) kullanılmıştır. Deskriptif istatistik bilgilerinde, ortalama, standart hata, minimum ve maksimum değerlerin yanı sıra, hesaplanan ortalamaların %95 güven sınırları da verilmiştir. Ortalamanın %95 güven sınırları, ortalamanın standart hatasının yaklaşık 2 fazlası ve 2 eksisini ifade eder. Araştırmada süt verimi özellikleri ile kuruda kalma çevre faktörünün analizi için aşağıdaki linear model kullanılmıştır (Düzgüneş ve ark, 1987; Efe ve ark, 2000; Genç ve Soysal, 2018).

$$Y_{ijklmno} = \mu + a_i + b_j + c_k + d_l + f_m + g_n + e_{ijklmno}$$

Matematik Modelde,

$Y_{ijkl} = ijklm$. grubundaki n. ineğe ait pik süt verimi değeri,

μ = ilgili süt verim özelliğinin ait beklenen ortalama değeri,

a_i = i. pike ulaşma süresinin etki miktarı (i = 1, ..., 7)

b_j = j. laktasyon sırasının etki miktarı (j = 1, ..., 4)

c_k = k. buzağılama mevsiminin etki miktarı (k = 1=kış, 2=İlkbahar, 3= Yaz, 4= Sonbahar)

d_l = l. buzağılama yılının etki miktarı (l = 2011, ..., 2019)

f_m = m. servis periyodunun etki miktarı (m = 1, ..., 7)

g_n = n. kuruda kalma süresinin etki miktarı (n = 1, ..., 5)

$e_{ijklmno}$ = şansa bağlı hata'nın etki miktarını göstermektedir.

Pike ulaşma süresinin etkisinin incelenmesinde değerlendirme kolaylığı için aşağıdaki sınıflandırma yapılmıştır. Bunlar > 30 gün (1); 31-60 gün (2); 61-90 gün (3); 91-120 gün (4); 121-150 gün (5); 151-180 gün (6); 181 gün < (7) şeklinde 7 sınıfa ayrılmıştır

Servis periyodunun etkisinin incelenmesinde şu şekilde bir sınıflandırma yapılmıştır. Bunlar > 80 gün (1); 81-110 gün (2); 111-140 gün (3); 141-170 gün (4); 171-200 gün (5); 201-230 gün (6); 231 gün < (7) şeklinde 7 sınıfa ayrılmıştır (Schaeffer & Henderson, 1972; Akbulut, 1990; Tüzemen ve ark., 1998).

Kuruda kalma süresinin etkisinin incelenmesinde değerlendirmede şöyle bir sınıflandırma yapılmıştır. Bu değerler ; > 20 gün (1), 21-40 gün (2), 41-60 gün (3), 61-80 gün (4), 81< gün (5), şeklinde beş sınıfa ayrılmıştır (Tomar & Balaine, 1973; Tüzemen ve ark., 1998; Watters ve ark., 2008).

BULGULAR ve TARTIŞMA

Pik Süt Verimini Etkileyen Bazı Faktörler Arasındaki Korelasyonlar

Pik süt verimi ile pike ulaşma süresi, servis periyodu, kuruda kalma süresi gibi etkili çevre faktörleri arasında (veriler kategorize yapılmadan) hesaplanan korelasyon katsayıları Çizelge 1 'de verilmiştir.

Çizelge 1 incelendiğinde pik süt verimi ile pike ulaşma süresi arasında $r = -0.281$ gibi negatif bir korelasyon bulunmuştur. İlgili korelasyon istatistiksel olarak çok önemli ($P < 0.01$) bulunmuştur.

Pik süt verimi ile incelenen faktörler arasında hesaplanan korelasyon katsayılarının pozitif ve çok önemli olduğu yönündeki sonuçlar (Akbulut ve ark., 1998; Tüzemen, ve ark., 1999; Duru ve Tuncel, 2004; Cura, 2016; Kaya, ve Bardakçioğlu, 2016)' in bulgularına uyumludur.

Ele alınan diğer çevre faktörlerinden kuruda kalma süresinin pik süt verimi ile hesaplanan korelasyon katsayıları pozitif ve çok düşük değerler aldığı Çizelge 1'den görülmektedir. Ayrıca servis periyodunun pik süt verimi ile arasındaki hesaplanan korelasyon katsayısı pozitif ve çok küçük değerde ($r = 0.064$) olmakla beraber istatistiksel olarak çok önemlidir ($P < 0.01$) (Çizelge 1).

Çizelge 1. PİK SÜT VERİMİ ETKİLEYEN FAKTÖRLER ARASINDAKİ KORELASYONLAR VE ÖNEMLİLİK DURUMLARI
Table 1. Correlations and Significance Among Factors Affecting Milk Yield at Peak

	N	PİK SÜT VERİMİ	PIKE ULAŞILAN GÜN	SERVİS PERİYODU
Pike Ulaşılan Gün	2980	-0.281**		
Servis Periyodu	2980	0.064**	0.054**	
Kuruda Kalma Süresi	2980	0.005	-0.054*	-0.138**

* : Önemli (P<0.05) ** : Çok Önemli (P<0.01)

PİK SÜT VERİMİNİ ETKİLEYEN BAZI ÇEVRE FAKTÖRLERİNİN ANALİZİ

PİK SÜT VERİMİNE BİRÇOK ÇEVRE FAKTÖRÜNÜN ETKİSİ BULUNMAKTADIR. BU ÇALIŞMADA PİK SÜT VERİMİNE ETKİ EDEN ÇEVRE FAKTÖRLERİ OLARAK BUZAĞILAMA YILI, BUZAĞILAMA MEVSİMİ, LAKTASYON SIRASI, PIKE ULAŞMA SÜRESİ, SERVİS PERİYODU, KURUDA KALMA SÜRESİ ELE

ALINMIŞTIR. PİKTE SÜT VERİMİNİ ETKİLEYEN ÇEVRE FAKTÖRLERİNE AIT VARYANS ANALİZİ VE ÖNEMLİLİK DURUMU ÇİZELGE 2'DE VERİLMİŞTİR. ÇİZELGE 2'DEN GÖRÜLECEĞİ ÜZERE İNCELENEN ÇEVRE FAKTÖRLERİNİN TAMAMININ PİK SÜT VERİMİNE ETKİSİ ÇOK ÖNEMLİDİR (P<0.001). BU NEDENLE ELE ALINAN ÇEVRE FAKTÖRLERİNİN PİK SÜT VERİMİNE ETKİLERİ DETAYLI OLARAK AŞAĞIDA İNCELENMİŞTİR.

Çizelge 2. PİK SÜT VERİMİNİ ETKİLEYEN ÇEVRE FAKTÖRLERİNE AIT VARYANS ANALİZİ VE ÖNEMLİLİK DURUMU
Table 2. Variance Analysis and Significance Status of Environmental Factors Affecting Milk Yield in Peak

Varyasyon Kaynağı	S.D	Kareler Toplamı	Kareler Ortalaması	F Değeri	Önem Durumu
Genel	2979	175553.958			
Pike Ulaşma Süresi	6	954.261	159.044	4.214	<0.001
Laktasyon Sırası	3	43270.066	14423.355	382.177	<0.001
Buzağılama Mevsimi	3	1078.496	359.499	9.526	<0.001
Buzağılama Yılı	8	2471.804	308.976	8.187	<0.001
Servis Periyodu	6	1962.367	327.061	8.666	<0.001
Kuruda Kalma	4	2833.065	708.266	18.767	<0.001
Şansa Bağlı Hata	2749	111295.369	37.740		

** : Çok Önemli (P<0.001)

PİK SÜT VERİMİNE PIKE ULAŞILAN GÜNLERİN ETKİSİ

PİK DÖNEMİNDE SÜT VERİMİNE BİRÇOK ÇEVRE FAKTÖRÜNÜN ETKİSİ BULUNMAKTADIR. PIKE ULAŞILAN GÜN SINIFLARININ ETKİSİ İLE İLGİLİ OLARAK, PİK SÜT VERİMİ ORTALAMALARI, STANDART HATALARI, VARYANS ANALİZİ VE DUNCAN ÇOKLU KARŞILAŞTIRMA SONUÇLARI VE TANIMLAYICI İSTATİSTİKLER ÇİZELGE 2 VE 3 'TE VERİLMİŞTİR.

ÇİZELGE 2'DEN GÖRÜLECEĞİ ÜZERE PIKE ULAŞILAN GÜN SINIFLARININ PİK SÜT VERİMİNE ETKİSİ ÇOK ÖNEMLİ (P<0.001) BULUNMUŞTUR. HAYVANIN HANGİ DÖNEMDE EN YÜKSEK PİK SÜT VERİMİNE ULAŞTIĞI İRDLENMESİ GEREKEN ÖNEMLİ BİR NOKTADIR. PIKE ULAŞMANIN ERKEN VEYA GEÇ OLMASI VE BUNUN LAKTASYON SÜRECİNİ NASIL ETKİLEDİĞİNİN BİLİNMESİ YÜKSEK SÜRDÜRÜLEBİLİR SÜT VERİMİ İÇİN VE SELEKSİYON İÇİN KRİTER OLABİLECEKTİR. ÇİZELGE 3'TE GÖRÜLECEĞİ ÜZERE PIKE ULAŞILAN GÜNLERE AIT PİK SÜT VERİMİ GENEL ORTALAMASI $40,36 \pm 0,141$ kg hesaplanmıştır. Bulunan en yüksek PİK SÜT VERİMİ DEĞERİ $42,77 \pm 0,240$ kg olmuştur. EN DÜŞÜK PİK SÜT VERİMİ DEĞERİ İSE $35,54 \pm 0,438$ kg'dır. PIKE ULAŞILAN GÜN SINIFLARINA GÖRE, PİKTE SÜT VERİMİ ORTALAMALARI VE DUNCAN ÇOKLU KARŞILAŞTIRMASI SONUCU, PIKE ULAŞMA 2. SINIFINDA (31- 60 GÜN) EN YÜKSEK PİK SÜT VERİMİ DEĞERİ ELDE EDİLMİŞTİR (ÇİZELGE 3).

ŞEKİL 1'DE GÖRÜLECEĞİ ÜZERE PİK SÜT VERİMİ, PIKE ULAŞILAN GÜN SINIFLARINDAN 2. SINIFA KADAR YÜKSELMEKTE VE EN YÜKSEK DEĞERE ULAŞMAKTADIR DAHA SONRAKİ SINIFLARDA

HIZLI BİR DÜŞÜŞ GÖSTERMEKTEDİR. BU DURUM SELEKSİYONDA PİK DEĞERİNE ERKEN ULAŞAN İNEKLERİN TERCİH EDİLMESİ GEREKTİĞİ KANAATI OLUŞTURMAKTADIR.

PİK SÜT VERİMİNE LAKTASYON SIRASININ ETKİSİ

VARYANS ANALİZİ SONUÇLARINA GÖRE, ÇİZELGE 2 İNCELENDİĞİNDE, PİKTE SÜT VERİMİNE LAKTASYON SIRASININ ETKİSİ İSTATİSTİKSEL OLARAK ÇOK ÖNEMLİ (P<0.001) OLDUĞU GÖRÜLMÜŞTÜR. AYRICA FARKLI LAKTASYON SIRASI GÖRE, PİK SÜT VERİMİNE AIT ORTALAMALAR, MINIMUM VE MAKSİMUM DEĞERLER YANINDA, ORTALAMALARIN GÜVEN SINIRLARI ÇİZELGE 4'TE VERİLMİŞTİR. ÜÇÜNCÜ LAKTASYON SIRASI $45,38 \pm 0,324$ kg İLE EN YÜKSEK PİK VERİMİ ORTALAMA DEĞERİNE ULAŞMIŞTIR. ÜÇÜNCÜ LAKTASYON SIRASINA KADAR PİK SÜT VERİMİNDE YÜKSELME OLDUĞU VE 3. LAKTASYON SIRASINDAN SONRA İSE PİK SÜT VERİMİNDE DÜŞÜŞÜN BAŞLADIĞI ŞEKİL 2'DEN GÖRÜLMÜŞTÜR.

TEKERLİ, (1996) PİK SÜT VERİMİNE LAKTASYON SIRASININ VE MEVSİMLERİN ETKİSİNİ YÜKSEK DÜZEYDE ÖNEMLİ (P<0,01) BULMUŞTUR. PİK SÜT VERİMİ İLE İNCELENEN FAKTÖRLER ARASINDA HESAPLANAN KORELASYON KATSAYILARININ POZİTİF VE ÇOK ÖNEMLİ OLDUĞU YÖNÜNDEKİ SONUÇLAR (AKBULUT, 1990; DURU VE TUNCEL, 2004)' ÜN BULGULARINA UYUMLUDUR. ARAŞTIRMADA $45,38 \pm 0,324$ kg BULUNAN PİK SÜT VERİMİ LİTERATÜR (SHANKS VE ARK.,1981; BATRA VE ARK., 1987; TEKERLİ, 2000) DEĞERLERİNDEN DAHA YÜKSEKTİR.

Çizelge 3. Pike Ulaşılan Gün Sınıflarına Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Test Sonuçları ve Tanımlayıcı İstatistikler

Table 3. Peak Milk Yield Means, Standard Errors, Multiple Comparison Test Results and Descriptive Statistics for Peak Day Classes

Pike Ulaşılan Gün Sınıfları	N	X	± Sx	Ortalamanın %95 Güven Sınırları			
				Alt	Üst	Minimum	Maximum
< 30 = 1	147	41.84 ^a	0.663	40.53	43.15	22	59
31-60 = 2	1046	42.77 ^a	0.240	42.30	43.24	19	65
61-90 = 3	756	40.38 ^b	0.278	39.83	40.92	17	67
91-120 = 4	410	38.88 ^c	0.344	38.20	39.55	18	60
121-150 = 5	278	37.78 ^{cd}	0.380	37.04	38.53	12	55
151-180 = 6	137	37.29 ^d	0.502	36.30	38.29	19	53
181 < = 7	206	35.54 ^e	0.438	34.68	36.40	11	50
Toplam Ortalama	2980	40.36	0.141	40.09	40.64	11	67

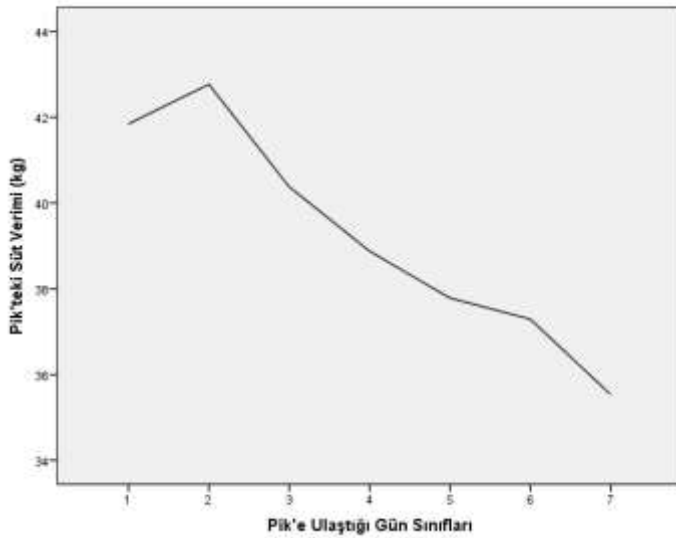
a,b,c,d,e,: Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsiz, farklı harfle gösterilen ortalamalar arasındaki farklar istatistiksel olarak çok önemlidir (P<0.01).

Çizelge 4. Laktasyon Sırasına Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Sonuçları ve Tanımlayıcı İstatistikler

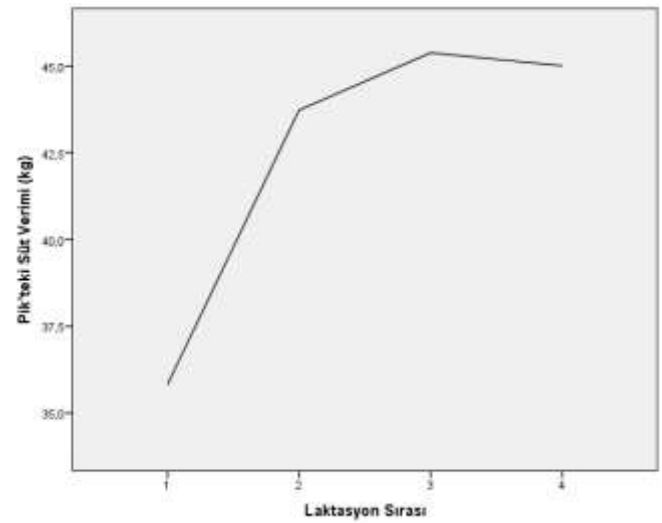
Table 4. Peak Milk Yield Means, Standard Errors, Multiple Comparison Results and Descriptive Statistics of the Lactation Order

Laktasyon Sırası	N	X	± Sx	Ortalamanın %95 Güven Sınırları			
				Alt	Üst	Minimum	Maximum
1	1402	35.82 ^a	0.148	35.53	36.11	11	59
2	891	43.73 ^b	0.231	43.28	44.19	20	63
3	463	45.38 ^c	0.324	44.75	46.02	18	64
4	224	45.01 ^c	0.495	44.03	45.98	19	67
Toplam/Ortalama	2980	40.36	0.141	40.09	40.64	11	67

a,b,c,: Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsiz, farklı harfle gösterilen ortalamalar arasındaki farklar istatistiksel olarak çok önemlidir (P<0.01).



Şekil 1. Pike Ulaşma Süresine Göre Pik Süt Veriminin Değişimi
Figure 1. Change in Milk Yield at Peak According to Time to Reach Peak



Şekil 2. Laktasyon Sırasına Göre Pik Süt Veriminin Değişimi
Figure 2. Change in Peak Milk Yield According to Lactation Order

Pik Süt Verimine Buzağılama Mevsiminin Etkisi

Çizelge 2 incelendiğinde, pik süt verimine buzağılama mevsiminin etkisi istatistiksel olarak çok önemlidir ($P<0.001$). Farklı buzağılama mevsimine göre, pik süt verimine ait ortalamalar, minimum ve maksimum değerler yanında, ortalamaların güven sınırları çizelge 5'te verilmiştir. Üçüncü buzağılama mevsimi olan yaz döneminde $39,81 \pm 0,293$ kg ile en düşük pik verimi değeri elde edilmiştir. Birinci buzağılama mevsimi olan kış dönemi pik süt veriminde en yüksek değer olmuştur. İlkbahar döneminden sonra ise pik süt veriminde düşüşün başladığı Şekil 3'den görülmektedir.

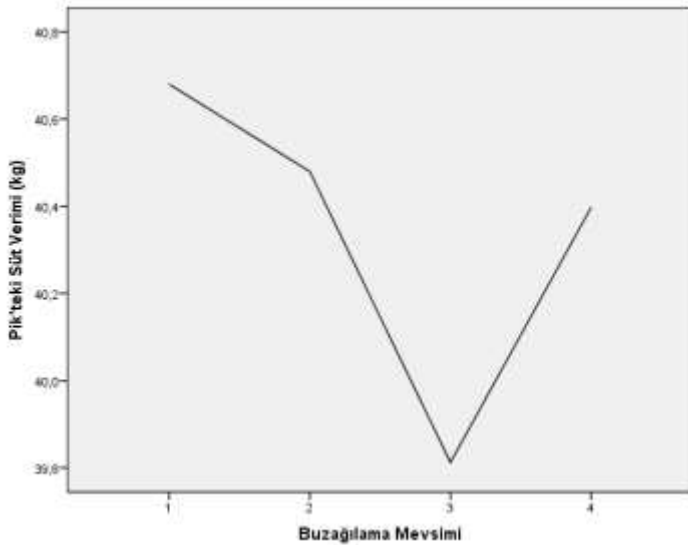
Bu araştırmada en yüksek pik süt veriminin kış ve ilkbahar mevsim grubunda olduğu belirlenmiştir ve Sehar ve Özbeyaz, (2005)'in bulguları ile benzerdir. Aynı araştırmacılar, ilkbaharda buzağılama ineklerin önemli düzeyde yüksek süt verdiklerini bildirmiştir.

Çizelge 5. Buzağılama Mevsimine Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Sonuçları ve Tanımlayıcı İstatistikler

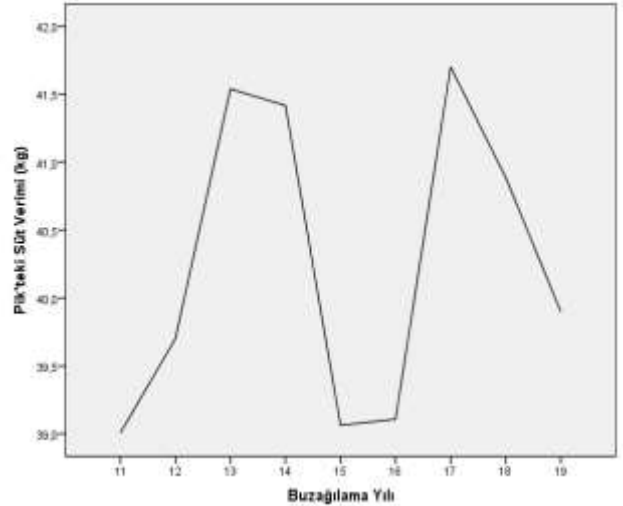
Table 5. Peak Milk Yield Means, Standard Errors, Multiple Comparison Results, and Descriptive Statistics of the Calving Season

Buzağılama Mevsimi	N	X ± Sx	Ortalamanın %95 Güven Sınırları				
			Alt	Üst	Minimum	Maximum	
1 = Kış	703	40.68 ^a	0.302	40.09	41.27	11	65
2 = İlkbahar	886	40.48 ^{ab}	0.257	39.97	40.98	18	67
3 = Yaz	634	39.81 ^b	0.293	39.24	40.39	17	59
4 = Sonbahar	757	40.40 ^{ab}	0.277	39.85	40.94	19	64
Toplam/Ortalama	2980	40.36	0.141	40.09	40.64	11	67

a,b,: Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsiz, farklı harfle gösterilen ortalamalar arasındaki farklar istatistiksel olarak çok önemlidir ($P<0.01$).



Şekil 3. Buzağılama Mevsimine Göre Pik Süt Veriminin Değişimi
Figure 3. Variation of Milk Yield at Peak According to Calving Season



Şekil 4. Buzağılama Yılına Göre Pik Süt Veriminin Değişimi
Figure 4. Change in Milk Yield at Peak According to Calving Year

Pik Süt Verimine Buzağılama Yılıının Etkisi

Çizelge 2 incelendiğinde, pik süt verimine buzağılama yılının etkisi istatistiksel olarak çok önemlidir ($P<0.001$). Farklı buzağılama yılına göre, pik süt verimine ait ortalamalar, minimum ve maksimum değerler yanında, ortalamaların güven sınırları çizelge 6’te verilmiştir.

Araştırmada, 2011, 12, 15 ve 16 buzağılama yıllarında sırası ile 39,01±0,450 kg, 39,70±0,517 kg 39,06±0,431 kg ve 39,11±0,387 kg ile en düşük pik süt verimi değeri elde edilmiştir. 2013 ve 2014 buzağılama yılında pik süt veriminde en yüksek değer olmuştur. İncelenen yıllara göre şekil 4’den, 2015 ve 2016 yıllarında ise pik süt veriminde en düşük değere indiği görülmektedir. Yıllardan yıla pik süt verimi değerlerinde önemli değişim söz konusu olduğu dikkat çekmektedir. Esasen tamamen barınaklarda yetiştirilen bu sığırların

yıllara göre değişiminin dengelenebilmesi gerekmektedir.

Pik Süt Verimine Servis Periyodunun Etkisi

Sığır yetiştiriciliğinde yılda bir yavru elde edilmesi hedeflenir. Bu hedefin gerçekleşebilmesi için 305 günlük laktasyon ve 2 aylık kuruda kalma süresi ve 50-80 gün servis periyodu öngörülmektedir. Doğum öncesi periyod olan kuruda kalma süresinin uzunluğu ineklerde süt verimindeki varyasyonu oluşturan önemli bir çevre faktörüdür. Doğum sonrasında üreme organlarının dinlenmesi için uygun servis periyodu gereklidir, doğum öncesi kuru periyot, ineklerin doğumdan sonra sağlıklı ve üretken olmaları için hayati önem taşır (Akbulut ve ark., 1998; Tapkı ve ark., 2007; Şahin ve Ulutaş, 2010; Tüzemen ve ark., 2013; Keser, 2016.)

Çizelge 6. Buzağılama Yılına Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Sonuçları ve Tanımlayıcı İstatistikler

Table 6. Peak Milk Yield Means, Standard Errors, Multiple Comparison Results, and Descriptive Statistics for the Calving Year

Buzağılama Yılı	N	X ± Sx	Ortalamanın %95 Güven Sınırları				
			Alt	Üst	Minimum	Maximum	
11	161	39.01 ^a	0.450	38.12	39.90	23	52
12	200	39.70 ^a	0.517	38.68	40.72	19	59
13	299	41.54 ^b	0.412	40.73	42.35	23	59
14	336	41.42 ^b	0.438	40.56	42.28	17	63
15	412	39.06 ^a	0.431	38.22	39.91	11	63
16	465	39.11 ^a	0.387	38.35	39.87	18	64
17	438	41.70 ^b	0.349	41.02	42.39	20	67
18	498	40.89 ^b	0.313	40.27	41.50	19	60
19	171	39.90 ^{ac}	0.530	38.85	40.95	26	63
Toplam/Ortalama	2980	40.36	0.141	40.09	40.64	11	67

a,b,c: Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsiz, farklı harfle gösterilen ortalamalar arasındaki farklar istatistiksel olarak çok önemlidir ($P<0.01$).

Pik süt verimine servis periyodunun etkisi istatistiksel olarak çok önemlidir ($P<0.001$) (Çizelge 2). Hayvanın hangi servis periyodu sınıfında en yüksek pik süt verimine ulaştığının irdelenmesi gerekir. Pik süt verimine ulaşmada servis periyodu süresinin uzun veya kısa olduğu durumuna göre, bunun laktasyon sürecini nasıl etkilediğinin bilinmesi ekonomik ve sürdürülebilir süt verimi için iyi bir seleksiyon kriteri olabilecektir. Farklı servis periyodu sınıflarına göre, pik süt verimine ait ortalamalar, minimum ve maksimum değerler yanında, ortalamaların güven sınırları Çizelge 7’te verilmiştir. Üçüncü servis periyodu sınıfına kadar bir düşüş gözlenirken, üçüncü servis periyodundan sonra dikkat çeken bir yükseliş olduğu şekil 5’den görülmektedir. Servis periyodunun pik süt verimi değerlerinde önemli etkisi söz konusu olduğu dikkate alınarak yüksek seviyede süt üretiminde servis periyodu süresinde gerekli hassasiyet gösterilmelidir.

Pik süt verimi ile servis periyodu arasında hesaplanan korelasyon katsayılarının pozitif ve çok önemli olduğu yönündeki sonuçlar (Duru, ve Tuncel, 2004; Erdem ve ark.,2007)' nin bulgularına uyumludur. Buzağılama aralığının kısaltılması büyük ölçüde servis periyodunun kontrolüne bağlıdır. Servis periyodunun süt verimine etkili olduğunu bildiren çok sayıda araştırma bulunmaktadır (Tüzemen ve ark., 1998; Bastin ve ark., 2012; Buckley ve ark., 2014; Keser, 2016; Toledo-Alvarado ve ark., 2017; Güngör, 2019).

Süt Verimine Kuruda Kalma Süresinin Etkisi

Sığırlarda, doğum ve laktasyona girmeden önce yıpranan dokularının onarılması ve yeni laktasyon için besin maddeleri depolanması yönünden kuruda kalma süresi çok önemlidir. Ancak sığır yetiştiriciliğinde kuru dönem hayvanların üretim yapmadığı bir devredir. Dolayısıyla kuru dönemin kısa ve uzun oluşunun pik süt verimine etkileri, her işletmenin ayrı

ayrı incelemesi ve değerlendirmesi gereken bir husustur.

Bu çalışmada, pik süt verimine kuruda kalma süresinin etkisi istatistiksel olarak çok önemlidir ($P<0.001$) (Çizelge 2). Hayvanın hangi kuruda kalma sınıfında en yüksek pik verimine ulaştığı irdelenmesi gereken önemli bir noktadır. Yüksek pik verimine ulaşmada kuruda kalma süresinin uzun veya kısa olduğu, bunun laktasyon sürecini nasıl etkilediğinin bilinmesi ekonomik ve yüksek sürdürülebilir süt

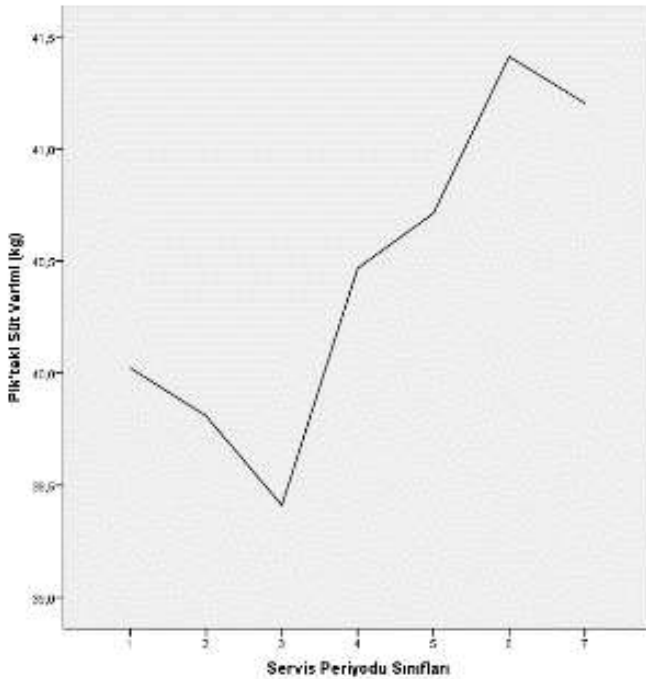
verimi için kriter olabilecektir. Farklı kuruda kalma sınıflarına göre, pik süt verimine ait ortalamalar, minimum ve maksimum değerler yanında, ortalamaların güven sınırları Çizelge 8'de verilmiştir. Pik süt verimi incelendiğinde, 20 kg'ın altında olan değerlerin kuruda kalma süresi ile ilgili bir stabilitesi olmadığı anlaşılmaktadır. Ancak pik süt verimi 20 kg'ın üzerinde olduğu değerlerin ise 3. cü kuruda kalma sınıfları (41- 60 gün) civarında yoğun seyrettiği görülmektedir (Şekil 6).

Çizelge 7. Servis Periyodu Sınıflarına Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Sonuçları ve Tanımlayıcı İstatistikler

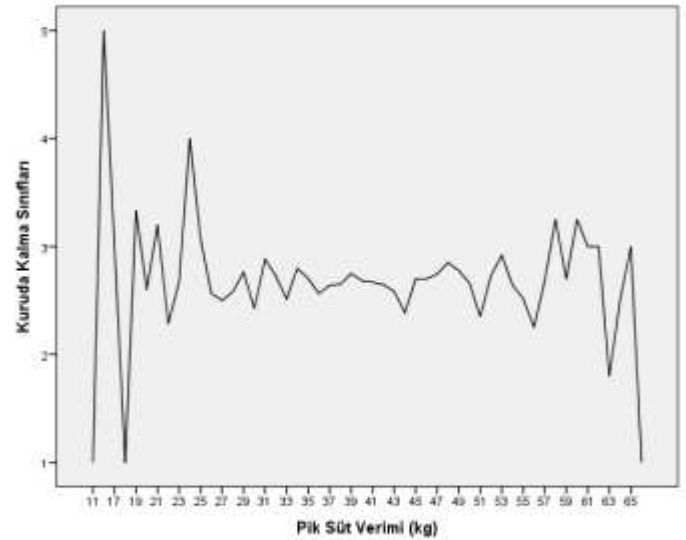
Table 7. Peak Milk Yield Means, Standard Errors, Multiple Comparison Results and Descriptive Statistics for Service Period Classes

Servis Periyodu Sınıfları	N	X ± Sx	Ortalamanın %95 Güven Sınırları				
			Alt	Üst	Minimum	Maximum	
< 80 = 1	571	40.02 ^{ab}	0.316	39.40	40.64	18	65
81-110 = 2	483	39.81 ^{ab}	0.344	39.13	40.49	20	64
111-140 = 3	436	39.41 ^a	0.347	38.73	40.09	17	63
141-170 = 4	387	40.47 ^{bc}	0.408	39.67	41.27	11	64
171-200 = 5	278	40.71 ^{bcd}	0.476	39.78	41.65	12	60
201-231=6	236	41.41 ^d	0.492	40.44	42.38	26	59
231<=7	589	41.20 ^{cd}	0.321	40.57	41.83	22	67
Toplam/Ortalama	2980	40.36	0.141	40.09	40.64	11	67

a,b,c,d; Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsiz, farklı harfle gösterilen ortalamalar arasındaki farklar istatistiksel olarak çok önemlidir ($P<0.01$).



Şekil 5. Servis Periyodu Sınıflarına Göre Pik Süt Veriminin Değişimi
Figure 5. Change in Peak Milk Yield According to Service Period Classes



Şekil 6. Kuruda Kalma Sınıflarına Göre Pik Süt Veriminin Değişimi
Figure 6. Change in Peak Milk Yield According to Drying Classes

Yüksek seviyelerde süt üretiminde kuruda kalma süresinin pik süt verimi değerlerine önemli etkisi dolayısıyla, sürüde kuruda kalma süresine gerekli hassasiyet gösterilmelidir. Kuru periyodun süresi, ineklerde süt verimi üzerinde önemli bir etkiye sahiptir (Chen ve ark.,2016, Kok ve ark., 2016, Hoeij ve ark.,2017). Kısa süreli kuru periyotlar süt verimini düşürürken, daha uzun süreli kuru periyotlar süt verimini artırmaktadır. Bu nedenle, ineklerin

doğumdan önce yeterince dinlenebilmesi ve yenilenebilmesi için ideal bir kuru periyot süresinin belirlenmesi gerekir (Atashi ve ark.,2013, Kok ve ark.,2019). Türkiyede yapılan çalışmalarda kısa kuruda kalma süresi ve uzun kuruda kalma sürelerinde süt verimi bakımından benzer farklılıklar bulunmuştur (Akbulut ve ark., 1992, Bakır ve Çetin., 2003, Erdem ve ark., 2007; Şahin ve Ulutaş, 2010; Keser, 2016).

Çizelge 8. Kuruda Kalma Sınıflarına Ait Pik Süt Verimi Ortalamaları, Standart Hataları, Çoklu Karşılaştırma Sonuçları ve Tanımlayıcı İstatistikler

Table 8. Peak Milk Yield Means, Standard Errors, Multiple Comparison Results, and Descriptive Statistics for Drying Classes

Kuruda Kalma Sınıfları	N	X ± Sx	Ortalamanın %95 Güven Sınırları				
			Alt	Üst	Minimum	Maximum	
< 20 = 1	715	40.51 ^a	0.296	39.93	41.09	11	67
21-40 = 2	195	39.83 ^a	0.526	38.79	40.86	22	64
41-60 = 3	1628	40.33 ^a	0.186	39.97	40.70	17	65
61-80 = 4	263	40.72 ^a	0.473	39.79	41.65	19	60
81 < = 5	179	40.15 ^a	0.637	38.89	41.40	12	60
Toplam/Ortalama	2980	40.36	0.141	40.09	40.64	11	67

a: Aynı sütunda aynı harfle gösterilen ortalamalar arasındaki farklar önemsizdir (P<0.05).

SONUÇ

Sığır yetiştiriciliğinde süt verimini etkileyen birçok çevresel faktör bulunmaktadır ve bu faktörlerin çoğu verim üzerinde belirgin bir etkiye sahiptir. Bu faktörlerin analizi, sürdürülebilir ve yüksek süt verimi için önemli seçim kriterleri olarak kullanılabilir. Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırlarda pik süt verimini etkileyen bazı çevresel faktörler incelenmiştir. Sürdürülebilir yüksek süt verimi için yılda bir yavru elde edilmesi esas alınmalıdır. Bu hedefe ulaşabilmek için özellikle servis periyodu ve kuruda kalma süresi gibi faktörlerin analizine ihtiyaç bulunmaktadır.

Gökkale Tarım İşletmesinde yetiştirilen sığırlarda kuruda kalma süresinin pik süt verimine etkisinin çok önemli olduğu gözlemlenmiştir. Kısa bir kuru periyot yanında uzayan bir servis periyodu ineklerin laktasyon performansını düşürerek ekonomik kayıplara yol açması söz konusudur. Bu nedenle, ineklerin doğumdan önce en az 41- 60 gün dinlenmeleri ve yeni laktasyona hazırlanabilmeleri için gerekli süredir.

Gökkale Tarım İşletmesinde en yüksek pik süt verimi kışın doğan ineklerde, en düşük pik süt verimi ise birinci laktasyon sırasında olan ineklerde belirlenmiştir. Bu nedenle, laktasyon sırası ve buzağılama mevsimi gibi çevresel faktörlerin, seleksiyon çalışmalarında dikkate alınması önerilir. Sonuç olarak, Gökkale Tarım İşletmesinde yetiştirilen Siyah Alaca sığırlarda, sürdürülebilir yüksek süt üretimi için çevresel faktörlerin (bilhassa kuruda

kalma süresi, servis periyodu gibi döl verimi özelliklerinin) dikkate alınması esastır. İşletmede istenilen seviyelerde sürdürülebilir bir süt sığırcılığı programı yapılabilmesi ancak etkili çevre faktörlerin gerektiği biçimde analizi ile gerçekleştirilecektir.

TEŞEKKÜR

Gökkale Tarım işletmesine ve çalışanlarına 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.

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