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SÜTÇÜ İMAM ÜNİVERSİTESİ

# TARIM ve DOĞA DERGİSİ

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## Investigation of Chemical Composition and Biological Activity of *Salix aegyptiaca* L. Roots

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### ABSTRACT

The root of *Salix aegyptiaca* L. was extracted using their yield percentage, total condensed tannin concentration, antimicrobial, antioxidant activity and to determine chemical composition by LC-MS/MS. The root extraction was carried out together with water, ethanol and methanol. Accelerated solvent extraction (ASE), conventional extraction (CE), and microwave extraction (ME) were the extraction methods applied during the investigation. The LC-MS/MS methanol extract was used to detect phenolics. The antioxidant activities and total condensed tannin concentrations of root extracts have been done by UV-visible spectroscopy from 517 to 580 nm, severally. The disk diffusion method was used for antimicrobial activity. The maximum extraction yield (17.2%) was obtained in methanol by the ASE technique whereas, the conventional extraction technique obtained the minimum extraction efficiency (9.1%). By triplicate measurement, the total condensed tannin analysis result was found 35.14 mg/L. Using the ASE technique, the methanol extract was the maximum inhibitory zone (26 mm) against *Candida albicans* ATCC 10231. However, in water extract by conventional extraction, a minimum inhibitory zone (11 mm) was obtained against *Staphylococcus aureus* Cowan 1. The highest and lowest DPPH scavenging activity was determined in methanol (ASE) (98.8%) and ethanol (97.5%) extract respectively. The maximum amounts of quinic acid (63895 µg/g) were discovered using LC-MS/MS.

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## *Salix aegyptiaca* L. Köklerinin Kimyasal Bileşimi ve Biyolojik Aktivitesinin Araştırılması

### ÖZET

*Salix aegyptiaca*'nın kök ekstraktı, verim yüzdesi, toplam kondense tanen konsantrasyonu, antimikrobiyal, antioksidan aktivite ve LC-MS/MS kullanılarak kimyasal bileşiminin belirlenmesi için kullanılmıştır. Kök ekstraksiyonu su, etanol ve metanol ile gerçekleştirildi. Hızlandırılmış solvent ekstraksiyonu (ASE), konvansiyonel ekstraksiyon (CE) ve mikrodalga ekstraksiyonu (ME), araştırma sırasında uygulanan ekstraksiyon yöntemleriydi. Fenoliklerin LC-MS/MS ile tespit edilmesi için metanol ekstraktı kullanıldı. Kök ekstraktlarının antioksidan aktiviteleri ve toplam kondanse tanen miktarları UV-Vis spektrofotometre cihazında sırasıyla 517 nm ve 580 nm olarak ölçülmüştür. Antimikrobiyal aktivite için disk difüzyon yöntemi kullanıldı. Maksimum ekstraksiyon verimi (%17.2) metanolde ASE tekniği ile elde edilirken, minimum ekstraksiyon verimliliği (%9.1) konvansiyonel ekstraksiyon tekniği ile elde edildi. Üçlü ölçümle toplam kondense tanen analizi sonucu 35.14 mg/L bulundu. ASE tekniği kullanıldığında metanol ekstraktı *Candida albicans* ATCC 10231'e karşı maksimum inhibitör bölge (26 mm) olmuştur. Bununla birlikte, su ekstraktında geleneksel ekstrasitasyonla *Staphylococcus aureus* Cowan 1'e karşı minimum inhibitör bölge (11 mm) elde edilmiştir. En yüksek ve

### Biyokimya

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### Anahtar Kelimeler

Antioksidant  
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Kimyasal bileşim  
Kondense tanen  
*Salix aegyptiaca*

en düşük DPPH giderme aktivitesi sırasıyla metanol (ASE) (%98.8) ve etanol (%97.5) ekstraktında belirlendi. LC-MS/MS kullanılarak maksimum kinik asit miktarları (63895 µg/g) keşfedildi.

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## INTRODUCTION

The curative antimicrobial substances were used like medicinal plants. Nowadays, there are many kinds of compounds that can be found in nature. The biologically active compounds that can be isolated from plants have always attracted attention (Prince et al., 2011). Aromatic compounds, including flavonoids, tannins, quinones, phenolic acids, and amines, as well as alkaloid compounds with terpenoids, vitamins, and other endogenous metabolites, can be produced by medicinal aromatic plants (Gulcin, 2020). These compounds have insect and herbivore defense properties (Bharathi et al., 2011). There are nearly 330–500 types (genus *Salix*) and ground plants, 200 hybrids, bush, and trees that are extensively spread to many areas of the world such as Africa, America, Europe, and Asia. The types of *Salix* are conventionally used in folk remedies and include a useful source of effective compounds such as salicin, a prodrug for salicylic acid (Savcı et al., 2020; Tawfeek N et al., 2021; Karageçili et al., 2023). The family of *Salicaceae* conventionally contains the genus *Populus* and *Salix* (willow), which are widespread in the northern mild zone (Isebrands et al., 2014). Nowadays, the *Salicaceae* now includes the most effective tropical fruit-consuming plants that do not produce catkins (Thadeo et al., 2014). Therefore, the genus *Salicaceae* currently encloses nearly 56 genera and 1,220 types (Christenhusz et al., 2016). The family of *Salicaceae* are quick-developing trees or bushes (Isebrands et al., 2014). These families are used for many economic objectives such as the manufacture of wood, paper, fences, housing, shoes, arrow shafts, fish traps, pipe, rope, fancy, and gardening like biomasses (renewable energy). Besides, it was used purpose of ecological enrichment owing to the earth erosion control (Kuzovkina et al., 2014). *Salix aegyptiaca* L. plants were noted as one of the first samples of advanced herbal medicine a long time ago. Salicin is one of the chemical compounds of bark willow trees that can be obtained by chemical processes. Salicylic acid is a new material, and acetylated variants of this compound have become famous drugs in the past, including aspirin. Famous salicylate compounds, which include salicylic acid and acetylsalicylic acid, are produced from the *Salix* plant. This class of substances has anti-inflammatory properties that can suppress the

production of prostaglandins by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Yu et al., 2002; Mahdi et al., 2006). The leaves of *S. aegyptiaca* contain significant levels of phenolics such as *p*-coumaric acid, gallic acid, caffeic acid, catechin, myricetin, vanillin, and epigallocatechin gallate, as well as flavonoids like rutin, quercetin, and salicin (Enayat et al., 2009). Several *Salix* species and their active ingredients, including salicin and salicylic acid, were used in traditional healing to cure a variety of conditions, including fever, chronic and acute inflammation, epilepsy, diabetes, piles, swelling, wounds, earaches, colds, back pain, toothaches, headaches, and cramps. Additionally, they have narcotic, antioxidant, anticancer, cytotoxic, anti-inflammatory, antibacterial, antidiabetic, antiobese, neuroprotective, and hepatoprotective properties. Salicylic acid serves cyclooxygenases (COX I, II) as its primary function since these enzymes are crucial in the production of prostaglandins, which are responsible for regulating pain and inflammation (Tawfeek et al., 2021). *Salix* species have the potential to be important antimicrobials, as demonstrated by the MIC zones and percentages throughout, which indicate their impact on functional foods (Mostafa et al., 2020). Numerous earlier academic works have demonstrated the antibacterial activity of *Salix* plant species and their effective component extracts against a variety of microorganisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Streptococcus mutans*, that form dental biofilms. Meanwhile, *Salix viminalis*, L., and *Salix atrocinerea* studies have shown that the extracts obtained from the species have a strong antioxidant effect thanks to the flavonoids and phenolic compounds in the content (Tawfeek et al., 2021). *Salix* plant is now recognized by the German Pharmacopoeia, and plans are underway for a monograph to be included in the European Pharmacopoeia. The European Scientific Cooperative on Phytotherapy (ES COP, 1997) advocated taking salicin at a dose of up to 240 mg per day. The current article's purpose is to present *S. aegyptiaca* as a medicinal plant by outlining both its historical uses and the most recent research on its innovative pharmacological and therapeutic uses.



## MATERIALS AND METHODS

### Plant sample collection

The Sarsang-Dohuk province in northern Iraq is where the *S. aegyptiaca* L. roots were collected in February 2014. Additionally, identification and confirmation were carried out by Dr. Zeravan Abdulkaliq Sadeeq from the University of Dohuk's Faculty of Agriculture/Dohuk-Iraq

### Sample Preparation

*S. aegyptiaca* L. root was stored and dried under normal room conditions and powdered by using an electric steel blender. The powder sample was put through a series of sieves of varying sizes and refrigerated until examination. All studies were carried out in Kahramanmaraş Sütçü İmam University, Faculty of Forestry laboratory.

### Extract Process

#### Conventional extraction

For conventional extraction, 80 mL water, ethanol (95%), methanol (95%), and 8 g of *Salix* extract were combined separately. The proportion was extracted to solvent 1:10. The extraction took place for three hours at 40°C. Liquid extract was separated by filtration, and the evaporation process was done (Dhanani et al., 2013).

#### Microwave extraction

A volume of 80 mL water, methanol (95%), and ethanol (95%) were combined in beakers with 8 g of extract separately. At standard temperatures extraction performed for 25 minutes after filtration extracts were evaporated (Laghari et al., 2011).

#### Accelerated solvent extraction

Dried 8 g extract was added to a certain flask. The extraction took place at 60 °C for 40 minutes, under pressure and then the sample was evaporated (Comlekcioglu et al., 2013).

### Yield determination

The extraction yield, which was defined as the amount of extract residue in mass compared to the initial amount of dry sample, is an evaluation of the solvent's ability to remove particular components from the main material (Zhang et al., 2007). 50 mL each of water, ethanol, and methanol were used to extract 0.5 g of root extract using various extraction techniques. The yield percentage was calculated by using the following Formula given below: (Karaogul et al., 2016b):

$$\text{Yield percentage (\%)} = \frac{x}{y} \times 100$$

where, X is the oven

- dry weight of the extract (in g). Y is the oven
- dry weight of the sample (in g).

### Calculating the amount of total condensed tannin

The experiment was conducted UV-Vis spectrophotometer. Extract liquor was ready by stirring 0.05 g of Fe<sub>2</sub>SO<sub>4</sub>, 95 mL N-butanol, and 5 mL HCl (35%). Condensed tannin calculation, 0.01 g of both plant extract and mimosa tannin was put separately in a test tube and then 10 mL of extract solution was added and placed in a water bath for heating one hour. The absorbance was measured at 580 nm wavelength (Karaogul et al., 2016c; Yavuz et al., 2023).

### Quantitative phenolic and flavonoid compound analysis by LC-MS/MS

A total of 27 active substances were measured in *S. aegyptiaca* L. during the study. The method contains the linearity ranges of the researched standard compounds as well as the rectilinear regression equations. It was discovered that correlation coefficients were higher than 0.99. The reported analytical method's limits of detection (LOD) and quantitation (LOQ) were displayed. LOD and LOQ for the substances under study varied from 0.05 to 25.8 and 0.17 to 85.9 g/L, respectively. Additionally, the phenolic compound recoveries ranged from 96.9% to 106.2%. The equation below was used to calculate the conclusion (Ertas et al., 2014a; Ismael, B.Q et al., 2019).

$$\text{Quantification of compound (\mu g/g)} = RxU^f \div 100$$

Where,

R is the result from LC-MS/MS (μg), U<sup>f</sup> is the percent relative uncertainty at 95% confidence level (%)

### DPPH radical scavenging activity

Blois (1958) used the DPPH method to examine root extracts of *S. aegyptiaca* L. that had been dissolved in ethanol and methanol. For the study's ethanol and methanol extractions, 0.1 mM DPPH was prepared. Following that, methanol and ethanol were combined with 0.1, 0.2, and 0.3 mL of the sample solvents until a total of 3 mL had been used. One mL of DPPH was then added to each after that. The mixture was vigorously mixed before being left at room temperature for 30 minutes. A Shimadzu UV-Vis 1240 spectrophotometer was used to detect the absorbance at 517 nm. As a reference, butylated hydroxytoluene (BHT) was used. The following equation was used to quantify radical scavenging activity as the sample's free radical inhibition percentage (Gülçın et al., 2003; Göçeri A et al., 2022).

Inhibition of DPPH radical scavenging activity

$$(\%) = \frac{A-B}{A} \times 100$$

Where

A is the absorbance of DPPH,

B is the absorbance in the presence of the sample and BHT.

## Anti-microbial activity Microorganisms

The experiment utilized four fungi and seven bacteria. Microorganisms were supplied by the Biology Department's Microbiology Lab at Kahramanmaraş Sütçü University in Turkey. Microorganism information is given in Table 1. (Karaogul et al., 2016a; Göçeri et al., 2020).

## Disc diffusion assay

Antimicrobial activity was performed using seven bacteria and four fungi (Table 1). By using the disc diffusion method, *Salix* root extracts in ethanol, methanol, and water were tested individually against each type of organism (CLSI, 2022/M100). The dextrose agar from Mueller-Hinton and Sabouraud was sterilized using an autoclave at 121°C for 15

minutes. One each of the 29 sanitized petri plates, the medium was introduced aseptically. Each disc was soaked in 100 µl of plant extract before being permitted to dry. Discs are then placed on an MHA medium, which contains 10 µl of bacteria. A 20 µl suspension of fungus was used to inoculate the SDA medium. 10 µl gentamicin and ampicillin were used as the positive controls (6 mm in diameter), whereas ethanol, methanol, and water were used as the negative controls. Additionally, 100 µl of a 10 mm-diameter Nystatin unit/disc was employed for antifungal activity. For each test, plates were incubated for 24 hours at 37 degrees and 48 hours at 25 to 27 degrees. The bacteria and fungi activities were assessed by measuring the inhibition zones (Karaogul et al., 2016a; Göçeri et al., 2020). All tests were performed in triplicate.

Table 1. Microorganism information  
 Çizelge 1. Mikroorganizma bilgileri

| Organism code | Organisms                       | Type             | Source     |
|---------------|---------------------------------|------------------|------------|
| B2            | <i>Bacillus megaterium</i>      | Gram(+)bacteria  | DSM32      |
| B11           | <i>Klebsiella pneumoniae</i>    | Gram(-)bacteria  | FMC5       |
| B13           | <i>Escherichia coli</i>         | Gram(-)bacteria  | DM         |
| B19           | <i>Pseudomonas aeruginosa</i>   | Gram(-)bacteria  | DSM50071   |
| B20           | <i>Staphylococcus aureus</i>    | Gram(+) bacteria | Cowan1     |
| B25           | <i>Micrococcus luteus</i>       | Gram(+) bacteria | LA2971     |
| B29           | <i>Bacillus subtilis</i>        | Gram(+) bacteria | IMG22      |
| M1            | <i>Candida albicans</i>         | Fungi            | ATCC10231  |
| M2            | <i>Candida utilis</i>           | Fungi            | NRRL-Y-900 |
| M3            | <i>Saccharomyces cerevisiae</i> | Fungi            | WET136     |
| M4            | <i>Yarrowia lipolytica</i>      | Fungi            | NCIM3589   |

## Statistical analyses

Descriptive statistical methods, such as mean and standard deviation, were used to analyze each result. The statistical program used is Anova SPSS statistical software version 18 (SPSS Inc. Chicago, USA). The mean differences were either found to be significant ( $p < 0.05$ ) or non-significant ( $p > 0.05$ ).

## RESULTS and DISCUSSION

### Calculation of yield

Table 2 lists the yield percentage results for *S. aegyptiaca* L. The ASE process was used in methanol to provide the highest yield (17.2%). While using the traditional method, water had the lowest yield (9.1%). The findings were confirmed by earlier research published by Anokwuru (2011), which found that methanolic extract produced the maximum yield (17.23%). It was determined that the best method among the extraction methods studied was the ASE.

### Total condensed tannin

Table 3 shows the total condensed tannin content of *S. aegyptiaca* roots. A standard calibration curve was used to determine the tannin concentration ( $R^2=0.999$ ),

with values ranging from 6.25 mg/L to 50 mg/L and listed in Table 4. According to the triple measurements, 35.14 mg/L was the average total condensed tannin concentration. The water-soluble antioxidant tannins have a molecular weight of 500-3000 g/mol. Tannins are naturally occurring phenolic compounds found in vegetables, seeds, and fruits. Tannins are frequently used in the production of wine as stabilizers to do things like balance wine color, stop certain enzymes from contaminating grapes, and work as wine-finishing substances (Sanz et al., 2008). According to their findings, *Salix sachalinensis* leaves had a condensed tannin concentration of  $8.2 \pm 0.79$  mg  $g^{-1}$ . According to Otthudi (2005), tannins exhibit antifungal properties. Its ability to connect with the cell walls of fungus or interact with extracellular and soluble proteins may be the cause of its activity. These chemicals have the potential to sever fungus membranes (Tsuchiya et al., 1996).

### Identification of *S. aegyptiaca* root methanol extract compounds by LC-MS/MS

Methanol was used to create the *S. aegyptiaca* root extract, and 27 components (three non-phenolic acids,

ten phenolic acids, and fourteen flavonoids) were analyzed by LC-MS-MS. The chemicals were examined using negative ionization modes in this investigation. The analysis results are shown in Table 5 below. These results were different from previous studies. Because in other studies, salicylic acid was obtained as the

main compound (Karawya et al., 2010; Rabbani et al., 2010; Cooper et al., 2014). However, these results conformed with the results of Zhang (Zhang et al., 2014). The findings also revealed increased concentrations of tr-aconitic and malic acids (Table 5).

Table 2. Yield percentage of *S. aegyptiaca* L.  
 Çizelge 2. *S. aegyptiaca* L.'nin verim yüzdesi

| Methods                        | Solvents | Yield (%)         |                 |
|--------------------------------|----------|-------------------|-----------------|
|                                |          | Mean <sup>1</sup> | SD <sup>2</sup> |
| Conventional extraction        | Water    | 9.1               | ±0.1            |
|                                | EtOH     | 10.6              | ±0.15           |
|                                | MeOH     | 11.7              | ±0.6            |
| Microwave extraction           | Water    | 10.4              | ±0.1            |
|                                | EtOH     | 12.03             | ±0.6            |
|                                | MeOH     | 12.6              | ±0.15           |
| Accelerated solvent extraction | EtOH     | 15                | ±0.1            |
|                                | MeOH     | 17.2              | ±0.15           |

EtOH: ethanol, MeOH: methanol, <sup>1</sup>Values presented as mean ± SD of three measurements, <sup>2</sup>SD: Standart deviation

Table 3. Condensed tannin of *S. aegyptiaca* L. Roots  
 Çizelge 3. *S. aegyptiaca* L. kökü'nün kondense taneni

| <i>Salix aegyptiaca</i> L. | Condensed Tannin (mg/L) |       |       | Average | Standard deviation | Variation (V) |
|----------------------------|-------------------------|-------|-------|---------|--------------------|---------------|
|                            |                         |       |       |         |                    |               |
|                            | 35.32                   | 34.85 | 35.25 | 35.25   | 35.14              | 0.71          |

Table 4. Standards for mimosa tannin calibration  
 Çizelge 4. *Mimosa tanen kalibrasyonu için standartlar*

| Mimosa tanin calibration |            |
|--------------------------|------------|
| Concentration            | Absorbance |
| 6.25                     | 0.094      |
| 12.5                     | 0.179      |
| 25                       | 0.365      |
| 50                       | 0.75       |

The time of collection, plant parts, genesis, extract methods, and solvents could all have an impact on the outcomes (Huang et al., 2005). A high amount of tannic acid (555 µg/g) and a low amount of tr-caffeic acid (8.7 µg/g) were detected in the roots of plants respectively. A substantial amount of salicylic acid (204.9 µg/g) was found in the methanol extract, but no rosmarinic acid was detected. The outcome is shown in Table 5. The research of Bravo (1998), Enayat (2009), Sonboli (2010), and Enayat (2013) supports these findings. The highest hyperoside (275.3 µg g<sup>-1</sup>) and the lowest coumarin (0.63 µg g<sup>-1</sup>) compounds were detected respectively. Chrysin, however, was not discovered in the roots of *S. aegyptiaca* L. These substances exist and their results are consistent with other experiments (Qin et al., 2005; Nahrstedt et al., 2007).

### Radical scavenging activity in DPPH

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which absorbs characteristically at 517 nm

and is purple, is significantly reduced upon exposure to radical scavengers by the contribution of hydrogen atoms or electrons. The extract's best ability to scavenge free radicals can be seen by its absorbance at 517 nm (Taslimi et al., 2017; Gulcin et al., 2023). The best antioxidant mechanism by which lipid oxidation is inhibited is known as free radical scavenging. This work provides a means for serially assessing a compound's or extract's ability to scavenge free radicals and is a popular examination of antioxidant activity (Amarowicz et al., 2004). Table 6 shows the BHT values along with the DPPH radical scavenging activity of the *S. aegyptiaca* L. root extracts made using various extraction methods. The two extracts with the highest and lowest DPPH radical scavenging activity were found to be the ethanol extract by microwave extraction method at 0.3 µl concentration (0.0162 mg mL<sup>-1</sup>) and the methanol extract by rapid solvent extraction method at 0.1 µl concentration (0.0264 mg/mL). As shown in Table 6, all extracts produce butylated hydroxytoluene (BHT) in DPPH tests. The presence of polyphenolic chemicals may be the cause of *S. aegyptiaca* L.'s DPPH radical scavenging activity. Because they donate hydrogen atoms, phenolic substances are known to have antioxidant properties (Ho et al., 1994). Due to their scavenging or antioxidative properties (Hatano et al., 1989), phenols are important components of herbs (Duh et al., 1999).

Table 5. *S. aegyptiaca* L. roots' methanol-extracted quantitation by LC-MS/MS  
Çizelge 5. *S. aegyptiaca* L. Köklerinin methanol ekstresinin LC-MS/MS miktarı

| No | Chemical Compounds  | RT <sup>a</sup> | Parent ion (m/z) <sup>b</sup> | Limit of detection/Limit of quantification (µg/L) <sup>e</sup> | If  | Quantitation µg/g extract |
|----|---------------------|-----------------|-------------------------------|--|-----|---------------------------|
| 1  | Quinic acid         | 3.3             | 191                           | 22.3/74.5  | 4.8 | 63895                     |
| 2  | Malic acid          | 3.5             | 133                           | 19.2/64.1  | 5.3 | 38020                     |
| 3  | tr-Aconitic acid    | 4.1             | 173                           | 16/52  | 4.9 | 816                       |
| 4  | Gallic acid         | 4.2             | 170                           | 5/16   | 5.1 | 183.91                    |
| 5  | Chlorogenic acid    | 5.4             | 353                           | 7.3/24.3   | 4.9 | 29.93                     |
| 6  | Protocatechuic acid | 5.6             | 153                           | 26/86  | 5.1 | 287                       |
| 7  | Tannic acid         | 6.4             | 183                           | 10.2/34.2  | 5.1 | 555                       |
| 8  | tr- caffeic acid    | 7.3             | 179                           | 4.4/15   | 5.2 | 8.7                       |
| 9  | Vanillin            | 8.7             | 151                           | 10.1/34  | 4.9 | 441                       |
| 10 | p-Coumaric acid     | 9.5             | 163                           | 15.2/51  | 5.1 | 221                       |
| 11 | Rosmarinic acid     | 9.57            | 359                           | 10.4/35  | 4.9 | N.D                       |
| 12 | Rutin               | 10.1            | 610                           | 17/57  | 5.0 | 52.4                      |
| 13 | Hesperidin          | 9.6             | 611.1                         | 22/72  | 4.9 | 67.94                     |
| 14 | Hyperoside          | 10.4            | 463.1                         | 12.4/41.4  | 4.9 | 275.3                     |
| 15 | 4-OH Benzoic acid   | 11.7            | 137                           | 3/10   | 5.2 | 201.3                     |
| 16 | Salicylic acid      | 11.7            | 137                           | 4/13.3   | 5.0 | 204.9                     |
| 17 | Myricetin           | 11.9            | 317                           | 10/33  | 5.9 | 28.4                      |
| 18 | Fisetin             | 12.6            | 285                           | 11/36  | 5.5 | 4.83                      |
| 19 | Coumarin            | 12.5            | 147                           | 9.1/30.4   | 4.9 | 0.63                      |
| 20 | Quercetin           | 14.4            | 301                           | 2/7  | 7.1 | 19                        |
| 21 | Naringenin          | 14.6            | 271                           | 3/9  | 5.5 | 105.2                     |
| 22 | Hesperetin          | 15.2            | 301                           | 3.3/11   | 5.3 | 0.83                      |
| 23 | Luteolin            | 15.4            | 285                           | 6/19.4   | 6.9 | 4.4                       |
| 24 | Kaempferol          | 15.4            | 285                           | 2/7  | 5.2 | 8.6                       |
| 25 | Apigenin            | 17.3            | 269                           | 0.1/0.3  | 5.3 | 1.12                      |
| 26 | Rhamnetin           | 18.9            | 315                           | 0.2/1  | 6.1 | 0.7                       |
| 27 | Chrysin             | 21.1            | 253                           | 0.05/0.17  | 5.3 | ND                        |

RT<sup>a</sup>: Retention time, Parent ion (m/z)<sup>b</sup>: Molecular ions of the standard compounds, R<sup>2c</sup>: Coefficient of determination, RSD<sup>d</sup>: relative standard deviation, U<sup>f</sup> (%): Percent relative uncertainty at 95% confidence level, Values in µg/g (w/w)<sup>g</sup> of plant methanol extract, ND: not detected.

Table 6. DPPH scavenging activities in root extracts of *S. aegyptiaca* L.  
Çizelge 6. *S. aegyptiaca* L.'nin kök ekstraktlarının DPPH aktiviteleri

| Methods                   | Solvents <sup>1</sup> | DPPH radical scavenging activity (%) |      |      |                              |     |      |
|---------------------------|-----------------------|--------------------------------------|------|------|------------------------------|-----|------|
|                           |                       | Extract volume (mL)                  |      |      | BHT volume (mL) <sup>2</sup> |     |      |
|                           |                       | 0.1                                  | 0.2  | 0.3  | 0.1                          | 0.2 | 0.3  |
| Conventional <sup>3</sup> | MeOH                  | 98.5                                 | 97.9 | 97.5 | 90.4                         | 92  | 90   |
| Microwave <sup>4</sup>    | MeOH                  | 98.7                                 | 98.3 | 98.5 | 90.4                         | 92  | 90   |
| ASE <sup>5</sup>          | MeOH                  | 98.8                                 | 98.3 | 97.6 | 90.4                         | 92  | 90   |
| Conventional <sup>6</sup> | EtOH                  | 98.5                                 | 98.2 | 97.5 | 67.4                         | 66  | 78.3 |
| Microweve <sup>7</sup>    | EtOH                  | 98.4                                 | 97.9 | 97.5 | 67.4                         | 66  | 78.3 |
| ASE <sup>8</sup>          | EtOH                  | 98.5                                 | 98.5 | 98.1 | 67.4                         | 66  | 78.3 |

<sup>1</sup>MeOH: methanol, EtOH: ethanol, ASE: Accelerated solvent extraction, <sup>2</sup>53 mg/L, <sup>3</sup>0.0183 mg/ml, <sup>4</sup>0.0188 mg/ml, <sup>5</sup>0.0264 mg/ml, <sup>6</sup>0.0162 mg/ml, <sup>7</sup>0.0183 mg/ml, <sup>8</sup>0.0231 mg/ml

With ethanol and methanol extracts and increasing DPPH radical concentrations, the DPPH radical scavenging activity of *S. aegyptiaca* L. roots increased. The experiment by Sulaiman et al. (2013) obtained the same results. The results showed that in methanol extract with microwave extraction, DPPH radical scavenging at 0.2 µl was greater than 0.3 µl but less

than 0.1 µl. This was supported by earlier research (Enayat et al., 2009; Sonboli., 2010). The presence of significant levels of phenolic chemicals such gallic acid, quinic acid, malic acid, chlorogenic acid, caffeic acid and salicylic acid may be the cause of the *S. aegyptiaca* L. root's considerable antioxidant activity in methanol and ethanol extracts (Li et al., 2000; Zheng et al., 2001;



Mokbel et al., 2005; Proestos et al., 2005; Farah et al., 2006; Gorzalczyk et al., 2008; Shabir et al., 2011; Zhang et al., 2013).

### *Salix aegyptiaca's* antimicrobial activities

Antimicrobial capacity is the applicable reason for controlling microorganisms in contamination treatments and food degradation. There are plenty of experiments to indicate plant extracts' antimicrobial and biological activities. To assess the antibacterial properties of *S. aegyptiaca* L. roots extract, the disc diffusion method was used with ethanol, methanol, and water as solvents. The tests were carried out against 11 microorganisms, which included 3 Gram(-), 4 Gram(+), and 4 fungi (disc concentration: 25 mg/disc). The results are shown in Table 7. Additionally, Table 8 provides a summary of the impacts of synthetic antibiotic activity against bacteria. This study is the first to document the root extracts' antibacterial properties. The varying sizes of the inhibition zones for all of the actions against the organisms made the *S. aegyptiaca* L. extract statistically significant ( $p < 0.05$ ). Test results are shown in Table 7. The *S. aegyptiaca* L. inhibitory zones range from 11-24 and 12-26 mm for bacteria and fungi, respectively. Using the ASE technique, the highest inhibitory zone against *C. albicans* ATCC10231 was 26 mm. While *S. aureus* Cowan1 had a minimum inhibitory zone of 11 mm in a water extract using a traditional extraction method. Additionally, neither *C. utilis* NRRL-Y-900 nor *S. cerevisiae* WET136 had an inhibitory zone. The outcomes demonstrated that the *B. megaterium* DSM32 (24 mm) zone of inhibition was significantly decreased by the methanol extract of the roots made using the ASE method (Table 7). These results are better than those of Hussain et al. (2011) but lower than those of Dıĝrak et al. (2001). The experiment's utilization of different plant species, locations, heights, and plant parts may be the reason for this dissimilarity. The inhibitory zones against *S. aureus*, *E. coli*, *K. pneumonia*, and *M. luteus* that were produced by conventional and microwave water extracts were also validated by earlier research (Bonjar et al., 2004). The outcomes demonstrated that *M. luteus* LA2971 was impacted by all extracts, except water extract employing conventional extraction, with zone inhibition varying from 12 to 21 mm (Table 7). This outcome was superior to that of Ateş et al. (2003). Additionally, ethanol and methanol extracts utilizing ASE and conventional extractions had higher inhibitory zones against *B. subtilis*, ranging from 16.3 to 22.3 mm. This outcome was better than Ayepola et al. (2008). According to Angioni (2006), who reported that the antimicrobial activity of plant extract varies from different searches conducted in various zones, the antibacterial activity of a plant extract can vary depending on the age of the plant, collection time,

freshness, physical factors (water, temperature) and extraction process. This could be due to several things, including the influence of the temperature, the quality, quantity, and content of the extracted product, as well as different bacterial strain sources. However, with microwave extraction in methanol, and ethanol as well as in water extract with traditional extraction, the inhibitory zone values against *B. subtilis* were 13–15 mm (Ayepola et al., 2008). The antibacterial properties of *S. aegyptiaca* L. roots in methanol extracts were comparable to those found by Ali et al. (2010), against *S. Aureus*, *K. Pneumonia*, *E. coli*, and *P. aeruginosa*. The outcomes against *K. Pneumonia*, *E. coli*, and *S. aureus* in ethanol extracts using all extraction techniques were also comparable to those of Hussein et al. (2011). Whereas, the outcomes were better than that of Al-Kadum et al. (2008). The dissimilarity among results could be owing to solvents, plant species, and plant parts used in this investigation. Using organic solvents is better than using water for antimicrobial activity. Methanol and ethanol root extracts have shown powerful antimicrobial effects against all strains. According to some research, water is not as effective as other solvents, like methanol or ethanol, for the extraction of antibacterials (Parekh et al., 2005). The G(-) bacteria were shown to be more resistant than the G(+) bacteria in all of the outcomes (Palombo et al., 2011). That could be differences between bacteria's cell structures. Because of their exterior membrane, G(-) bacteria are thought to be more resistant (Kaye et al., 2004). When compared to Gentamicin 10 g/ml, *S. aegyptiaca* L. displayed poor antibacterial activity, however, it was effective against practically all bacteria when compared to Ampicillin 10 g/ml (refer to Table 8). The roots' antibacterial properties may be explained by the presence of more phenolic compounds such as salicylic acid, chlorogenic acid, 4-hydroxybenzoic and coumaric acid (Proestos et al., 2005; Shabir et al., 2011), rutin and caffeic acid (Coneac et al., 2008), quinic acid (Farah et al., 2006), gallic acid (Akiyama et al., 2001), malic acid (Mokbel et al., 2005). According to methanol extracts and the ASE technique, the maximum inhibition zone against *C. albicans* was 26 mm (Table 7).

Additionally, employing methanol and ethanol extracts for traditional and microwave extraction procedures, respectively, 12 mm of the *Y. lipolytic* inhibition zone was obtained. Furthermore, it was shown that *S. cerevisiae* and *C. utilis* had higher resistance to the plant extracts. Antifungal activity results are compiled in Table 7. The roots of *S. aegyptiaca* L. displayed varying levels of antifungal activity. As a result of employing the ASE approach, methanol extracts provided the maximum inhibition zone (26 mm) against *C. albicans*, according to the data.

Table 7. Root extract inhibition zones (mm) against microorganisms

Çizelge 7. Mikroorganizmalara karşı kök ekstraktı inhibisyon bölgeleri (mm)

| Microorganisms |       | Inhibition zone (mm) <sup>1</sup> |      |       |           |       |      |                     |       |
|----------------|-------|-----------------------------------|------|-------|-----------|-------|------|---------------------|-------|
|                |       | Extraction                        |      |       |           |       |      | Accelerated solvent |       |
|                |       | Conventional                      |      |       | Microwave |       |      | EtOH                | MeOH  |
|                | Water | EtOH                              | MeOH | Water | EtOH      | MeOH  | EtOH | MeOH                |       |
| B2             | Mean  | 11.6                              | 18   | 19    | ND        | 16.3  | 17   | 19                  | 24    |
|                | SD    | ±0.57                             | ±0.6 | ±0.6  | ND        | ±0.6  | 1    | ±0.6                | 1     |
| B11            | Mean  | ND                                | 15   | 14.6  | 11.3      | 15.3  | 15.3 | 16.3                | 17.3  |
|                | SD    | ND                                | 1    | ±0.6  | ±0.6      | ±0.6  | ±0.6 | ±1.2                | ±1.2  |
| B13            | Mean  | ND                                | 14.3 | 17.0  | ND        | 14    | 13   | 16                  | 18.6  |
|                | SD    | ND                                | ±0.6 | ±1.0  | ND        | ±1.52 | 1    | 1                   | ±0.6  |
| B19            | Mean  | ND                                | 17   | 16    | ND        | 13.6  | 15.3 | 18                  | 17.3  |
|                | SD    | ND                                | 1    | 1     | ND        | ±0.6  | ±1.2 | 1                   | ±0.6  |
| B20            | Mean  | 11                                | 16   | 14.6  | ND        | 14    | 17.3 | 15                  | 19.3  |
|                | SD    | 0.0                               | ±0.6 | ±0.6  | ND        | 1     | ±0.6 | ±0.6                | ±1.52 |
| B25            | Mean  | ND                                | 18.3 | 16.0  | 12        | 14.3  | 12   | 19.3                | 21    |
|                | SD    | ND                                | ±0.6 | 1     | 1         | ±0.6  | ±0.6 | ±0.6                | ±1.73 |
| B29            | Mean  | 13                                | 16.3 | 19.3  | ND        | 15    | 15   | 19                  | 22.3  |
|                | SD    | 0.0                               | ±0.6 | ±0.6  | ND        | ±0.6  | 1    | ±1.73               | ±1.52 |
| M1             | Mean  | 15.3                              | 23.3 | 23.3  | 18        | 20.3  | 23.3 | 24.3                | 26    |
|                | SD    | ±0.57                             | ±1.5 | ±0.6  | ±0.6      | ±0.6  | ±1.5 | ±1.5                | ±4.04 |
| M2             | Mean  | ND                                | ND   | ND    | ND        | ND    | ND   | ND                  | ND    |
|                | SD    | ND                                | ND   | ND    | ND        | ND    | ND   | ND                  | ND    |
| M3             | Mean  | ND                                | ND   | ND    | ND        | ND    | ND   | ND                  | ND    |
|                | SD    | ND                                | ND   | ND    | ND        | ND    | ND   | ND                  | ND    |
| M4             | Mean  | ND                                | 14   | 12    | 13        | 12    | 14.3 | 17                  | 20    |
|                | SD    | ND                                | ±0.6 | ±0.57 | ±0.6      | ±0.6  | ±0.6 | ±0.6                | ±0.6  |

B2: *B. Megaterium*, B11: *K. Pneumoniae*, B13: *E. Coli*, B19: *P. Aeruginosa*, B20: *S. Aureus*, B25: *M. Luteus*, B29: *B. Subtilis*, M1: *C. Albicans*, M2: *C. Utilis*, M3: *S. Cerevisiae*, M4: *Y. Lipolytica*, ASE: A. solvent extraction, ND: not detected, EtOH: ethanol, MeOH: methanol, <sup>1</sup>The values presented as mean ± SD of three replications: Mean differences were statistically classified as significant ( $p < 0.05$ ). P- value (0.00)

Table 8. Synthetic antibiotics' effects on microorganisms

Çizelge 8. Sentetik antibiyotiklerin mikroorganizmalar üzerindeki etkileri

| Microorganism        | Inhibition zone (mm) <sup>1</sup> |                        |                    |
|----------------------|-----------------------------------|------------------------|--------------------|
|                      | Ampicillin<br>10 µg/ml            | Gentamicin<br>10 µg/ml | Nystatin/<br>units |
| <i>B. megaterium</i> | 7±0.6                             | 35±0.6                 | -                  |
| <i>K. pneumoniae</i> | -                                 | 44±2                   | -                  |
| <i>E. coli</i>       | -                                 | 34.3±1.52              | -                  |
| <i>P. aeruginosa</i> | 6±0.0                             | 34.3±1.2               | -                  |
| <i>S. aureus</i>     | 6.3±0.6                           | 36.3±1.52              | -                  |
| <i>M. luteus</i>     | 9.3±0.6                           | 35±1.15                | -                  |
| <i>B. subtilis</i>   | 6.3±0.6                           | 39±1.15                | -                  |
| <i>C. albicans</i>   | -                                 | -                      | 15±0.6             |
| <i>C. utilis</i>     | -                                 | -                      | 18±0.6             |
| <i>S. cerevisiae</i> | -                                 | -                      | 14±0.6             |
| <i>Y. lipolytica</i> | -                                 | -                      | 13±0.0             |

(-): no inhibition zone. <sup>1</sup>The values presented as mean ± SD of three replication

This did not line up with the findings of the Bonjar et al. (2004) study, which showed no inhibition of *C. albicans* growth. *Laurus nobilis* extract according to Dıġrak et al. (2001), was ineffective against *C. albicans*. The plant species, extract type, and concentration all affect the extracts' antifungal activity. The inhibition zones for *C. albicans* with all investigated techniques for methanol and ethanol extracts ranged from 20.3 to 26 mm. According to Aneja et al. (2009) and Sulaiman et al. (2013), this was accurate. According to their findings, *Amomum subulatum* fruit extracts inhibited *C. albicans* (29.3 mm) growth. *E. arborea* and *M. piperita* extracts also demonstrated antifungal efficacy against *C. albicans* mm and *A. Niger* 18-23 mm, according to Ertürk (2006). The *C. albicans* and *Y. lipolytic* demonstrated moderate antifungal activity in the water extracts with traditional and microwave extractions 13–18 mm respectively. This outcome was somewhat comparable to that of Ertürk et al. (2006), who noted that *M. officinalis*, *P. nigrum*, *C. annual*, and *C. cyminum* extracts had antifungal activity against *C. albicans* with inhibition zones ranging from 10 to 16 mm.

Additionally, these findings showed that *C. utilize* and *S. cerevisiae* were more resistant to all extracts carried out using different techniques. Similarly, Bonjar et al. (2004) also supported this finding. Tested methanol and ethanol extracts had stronger antifungal effects than the common antifungal nystatin against *C. albicans* and *Y. lipolytica*. The outcomes of the water extract, however, were comparable to those of the synthetic medicine nystatin (Table 7). This could be explained by the presence of gallic acids (Akiyama et al., 2001; Panizzi et al., 2002) and quinic acid (Zhang et al., 2013) or phenolic and flavonoids compounds (Zidon et al., 2005). It could be the presence of chlorogenic, salicylic, caffeic, and 4-hydroxybenzoic acids. The antibacterial capabilities of each of the phenolic compounds found have been confirmed (Proestos et al., 2005).

## CONCLUSIONS

*Salix aegyptiaca* L. Roots were examined for their chemical composition and biological properties. According to the latest information, it seems obvious that *S. aegyptiaca* has good chemical effects as well as (especially) antioxidant, and antimicrobial activity. It is also recommended that isolated chemicals discovered in this study be put to use in the creation of fresh antibacterial and antioxidant medications. These plant chemicals could possess anti-inflammatory and antioxidant properties, their potential to prevent oxidative stress and inflammation, which are major causes of disease, including cancer, needs further investigation. Because of these features, considerable biological and chemical investigations should be conducted on human metabolism and must be focused on future studies. Eventually, this research shows that *S. aegyptiaca* could be utilized in new medicinal drugs and supply the main information for further research on therapeutic plants. It would be recommended that *S. aegyptiaca* farming must be available place.

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## Author's Contributions

The contribution of the authors is equal.

## Statement of Conflict of Interest

The authors have declared no conflict of interest.

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## Evaluation of Biological Activities of Various Extracts of *Glaucium alakirensis*, *Marrubium bourgaei*, and *Peucedanum alpinum* from Türkiye

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### ABSTRACT

Plant species contain many secondary metabolites, and these compounds differ from species to species. These differences in the concentrations of these compounds have many health implications. Today, studies on plants' antioxidant and antibacterial effects are gaining importance. In particular, the adverse effects of some existing antibiotics and the constant development of bacterial resistance are leading to the search for new natural antimicrobial agents. In this study, methanol, ethanol, ethyl acetate, acetone, and chloroform extracts were obtained from the aerial parts of *Marrubium bourgaei* Boiss and *Glaucium alakirensis* Aykurt, K.Yıldız & A.Özçandır, and *Peucedanum alpinum* B.L.Burt & Davis, species which are naturally distributed in Türkiye. The antioxidant activity of the extracts was determined by the DPPH (2,2 Difenil-1-pikrihidrazil) and ABTS (2,2' azino-bis(3-ethylbenz-thiazoline-6-sulfonic-acid)) methods, the total phenolic content by Folin-Ciocalteu method, the total flavonoid content by aluminium chloride colorimetric method, and the antibacterial activity against ten bacteria by the disc diffusion method. According to the results, methanol, ethanol, and acetone extracts had higher antioxidant activity, total phenolic, and total flavonoid contents than other extracts. However, the total flavonoid content of *M. bourgaei* was higher in the ethyl acetate extract. When evaluated for their antibacterial activity, ethanol, chloroform, and ethyl acetate in *P. alpinum*, chloroform in *M. bourgaei*, and methanol, chloroform, and ethyl acetate in *G. alakirensis* extracts showed antibacterial activity against more bacteria than others. This is the first study to evaluate and compare the total phenolic and flavonoid content, and antioxidant and antibacterial activities of 5 different extracts of these plants.

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Antibacterial-antioxidant activity  
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## Türkiye'den *Glaucium alakirensis*, *Marrubium bourgaei*, and *Peucedanum alpinum*'ün Çeşitli Ekstraktlarının Biyolojik Aktivitelerinin Değerlendirilmesi

### ÖZET

Bitki türleri birçok ikincil metabolit içerir ve içermiş oldukları bu bileşikler türden türe farklılık göstermektedir. Bitkilerin ikincil metabolitlerinin konsantrasyonlarındaki bu farklılıkların sağlık üzerinde farklı etkileri vardır. Bitkilerin antioksidan ve antibakteriyel etkileri üzerine yapılan çalışmalar günümüzde yeniden önem kazanmaktadır. Özellikle mevcut bazı antibiyotiklerin olumsuz etkileri ve sürekli olarak artan bakteriyel direnç, yeni ve doğal antimikrobiyal ajan arayışına neden olmaktadır. Bu çalışmada, Türkiye'de doğal olarak yayılış gösteren *Marrubium bourgaei* Boiss, *Glaucium alakirensis* Aykurt, K.Yıldız & A.Özçandır ve *Peucedanum alpinum* B.L.Burt & Davis türlerinin toprak üstü kısımlarından metanol, etanol, etil asetat, aseton ve kloroform kullanılarak ekstraktlar elde edilmiştir. Bu ekstraktların antioksidan aktiviteleri DPPH (2,2 Difenil-1-pikrihidrazil) ve ABTS (2,2' azino-bis(3-etilbenz-tiazolin-6-sülfonik-asit)) yöntemleriyle, toplam fenolik madde içerikleri Folin-Ciocalteu yöntemiyle, toplam flavonoid madde içerikleri ise alüminyum klorür

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Antibakteriyel-antioksidan aktivite  
*Glaucium alakirensis*  
*Marrubium bourgaei*  
*Peucedanum alpinum*  
Fenolik-Flavonoid içerik

kolorimetrik yöntemi ile belirlenmiştir. Ayrıca elde edilen bu bitkisel ekstraktların antibakteriyel aktiviteleri disk difüzyon yöntemi kullanılarak on bakteri için değerlendirilmiştir. Elde edilen sonuçlara göre, bitkilerin metanol, etanol ve aseton ekstraktları diğer ekstraktlarından daha yüksek antioksidan aktivite, toplam fenolik ve flavonoid madde içeriğine sahiptir. Ancak, *M. bourgaei*'nin toplam flavonoid madde içeriği etil asetat ekstraktında daha yüksek bulunmuştur. Ekstraktların antibakteriyel aktiviteleri değerlendirildiğinde, *P. alpinum*'da etanol, kloroform ve etil asetat, *M. bourgaei*'de kloroform, *G. alakirensis*'de ise metanol, kloroform ve etil asetat ekstraktları diğer ekstraktlara göre daha fazla bakteriye karşı etkilidir. Gerçekleştirilen bu çalışma, kullanılan bu üç bitkinin beş farklı ekstraktının toplam fenolik madde içeriğini, toplam flavonoid madde içeriğini, antioksidan aktivitelerini ve antibakteriyel aktivitelerini değerlendiren ve karşılaştıran ilk çalışmadır.

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## INTRODUCTION

Since the existence of humanity, plants have been used both as food and for medicinal purposes (Dubick, 1986; Johns, 1990; Balick & Cox, 1996). In recent years, bioactive compounds from plants have been increasingly preferred, mainly because of the potential side effects of synthetic compounds (Naveed et al., 2018). The unnecessary and unconscious use of antibiotics has increased bacterial resistance to antibiotics and led to numerous side effects and various clinical problems in patients (Hughes & Andersson, 2017). Therefore, in recent years, the search for new antimicrobial agents from plants and the elaboration of information on the possibilities of using phytochemicals in this context has gained renewed attention (Savoia, 2012; Vaou et al., 2021).

One of the plants used in this study, *Glaucium alakirensis* was recently discovered in the Alakır Valley (Kumluca, Antalya) and has a minimal distribution range (Aykurt et al., 2017). Moreover, there is very limited information on the pharmacological and phytochemical properties of *G. alakirensis*. However, numerous studies have been conducted on closely related species. For example, *G. flavum* Crantz is one of the most intensively researched species within this genus. Various medicinal properties are attributed to this plant, such as antioxidant, bronchodilator, antitussive, and hypoglycemic effects (Arafa et al., 2016).

*Marrubium bourgaei*, which is native to Türkiye, is only found in high mountainous regions. The species of this genus are recognized for their various health-promoting effects and are used in traditional medicine to treat ailments such as asthma, hypotension, and pain relief (Bardai et al., 2001). Furthermore, several

species of this genus contain various secondary metabolites of biological importance, including diterpenes, polyphenols, steroids, phenylpropanoids, and flavonoids (Hamedeyazdan et al., 2014).

*Peucedanum alpinum* B.L.Burt & Davis is only known from the regions of Antalya (Türkiye) and Crete (Greece). The specialised roots of the genus *Peucedanum* L. have been used in traditional Chinese medicine for over 1500 years to cure heat and congestion in the lungs (Skalicka-Woźniak et al., 2010). The leaves of some species are also said to have healing properties for tissue injuries (Danna et al., 2022). Information on *P. alpinum* species is very limited; there are no studies on their phytochemical or pharmacological properties.

In this study, methanol, ethanol, ethyl acetate, acetone, and chloroform extracts were obtained from *P. alpinum* and the species *G. alakirensis* and *M. bourgaei* endemic to Türkiye. In selecting the plant species for this study, certain factors were considered that make these plants particularly interesting. First of all, these plants are endemic to Türkiye indicates that they have a potential uniqueness in terms of their chemical composition and bioactive compounds. Secondly, these species belong to plant families known for their medicinal properties: Lamiaceae (Uritu et al., 2018), Apiaceae (Sayed-Ahmad et al., 2017), Papaveraceae (Zielińska et al., 2021) and literature studies have shown that close relatives of these plants also show good results in terms of their health properties. Finally, the antioxidant activity, the total content of flavonoids and phenols, and the antibacterial activity of the five different extracts of these three species were analyzed and compared for the first time in this study.



## MATERIALS and METHODS

### Collection of Plant Materials

The plant materials used in this study were collected in the alpine and sub-alpine zones of the Western Taurus Mountains. *P. alpinum* (M. Gülben 1040 & E. Delik, AKDU 6365) and *G. alakirensis* (M. Gülben 1042 & E. Delik, AKDU 6367) were collected from screes and slopes between the Dibek Nature Reserve and the Kırkmuar Plateau at an altitude of 1330 m in Kumluca (Antalya/Türkiye), which is also the type location of *G. alakirensis*. Meanwhile, *M. bourgaei* (M. Gülben 1041 & E. Delik, AKDU 6366) was collected at the entrance of the high mountain plain of the Kırkmuar Plateau (2000 m), 6-7 km southwest of the other two species' location. The identities of the plants were determined by the plant systematists C. Aykurt and M. Gülben.

### Plant Extraction

The protocols used by Berber et al. (2013) and Gonelimali et al. (2018) were used to prepare the plant extracts with minor modifications. Fresh plant samples brought from the field to the laboratory were allowed to dry at room temperature (RT) in the shade. The samples dried for about 15-20 days were pulverised using a mechanical grinder. The pulverised plant samples (10 g) were placed in 100 mL of solvent (Merck, Germany) (ethanol, methanol, acetone, ethyl acetate, chloroform) and shaken gently overnight in a shaker. Then each solvent was filtered separately with filter paper (Whatmann No:1), and the samples were placed in a fume hood to evaporate the solvents. After drying, the obtained residues were stored at +4°C to be used for the experiments. In each experimental study, the extracts were prepared at concentrations of 1 mg mL<sup>-1</sup> by dissolving in dimethyl sulphoxide (DMSO) (Merck, Germany).

### Antioxidant Measurements with ABTS Free Radical Scavenging Method

The plant extracts were subjected to the ABTS radical scavenging assay according to the protocol described by Xiao et al. (2014). The ABTS reagent was prepared by reacting 7 mM aqueous ABTS solution (Sigma Aldrich, USA) and 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (Sigma Aldrich, USA) for 16 h at RT in the dark. Then the ABTS<sup>+</sup> stock solution was diluted with pure ethanol until it reached an OD of 0.70 at λ<sub>734</sub>. 4 mL of the ABTS<sup>+</sup> stock solution was added to 1 mL of the sample and incubated at RT for 6 min. Then the absorbance value of the samples was measured at 734 nm and the radical scavenging capacity (%) was calculated using Equation (1).

$$\%Inhibitor = \frac{(Absorbance_{Control} - Absorbance_{Sample})}{Absorbance_{Control}} \times 100(1)$$

### Antioxidant Measurements with DPPH Free Radical Scavenging Method

The plant extracts were subjected to the DPPH radical scavenging assay according to the protocol described by Subhasree et al. (2009). Initially, a 6x10<sup>-5</sup> M methanol solution of DPPH (Sigma Aldrich, USA) was prepared. Subsequently, 3 mL of the methanol (Merck, Germany) of DPPH was added to 100 μL of the samples and mixed well. The prepared samples were stored at RT in a dark environment for 15 min. Then, the absorbance values of the samples were measured at 516 nm and the % DPPH radical scavenging capacity was calculated using Equation (1).

### Evaluation of Total Phenolic Substance Content

The total phenolic substance content in the plant extracts was analyzed by a colourimetric method based on the Folin-Ciocalteu (FC) reagent (Škerget et al., 2005). The FC reagent was formulated according to the procedure described by Singleton & Rossi, (1965) outlined. 2.5 mL of the FC reagent (diluted 1:10) was added to 500 μL of the samples and incubated for 2 min. Then, 2 mL of Na<sub>2</sub>CO<sub>3</sub> (7.5%) (Sigma Aldrich, USA) was added to the samples, and the samples were vortexed for 30 s. The vortexed samples were kept at 50°C for 5 min. The absorbance values of the samples were measured at λ<sub>760</sub>, and the gallic acid equivalents of the samples (mg mL<sup>-1</sup> GAE) were calculated.

### Evaluation of Total Flavonoid Substance Content

The total flavonoid content in the plant extracts was assessed using the aluminum chloride colourimetric method (Ghasemi et al., 2009). 1.5 mL of methanol (Merck, Germany), 100 μL AlCl<sub>3</sub> (10%) (Sigma Aldrich, USA), 100 μL CH<sub>3</sub>CO<sub>2</sub>K (1 M) (Sigma Aldrich, USA), and 2.8 mL distilled water were added to 500 μL of each of the plant extracts, and the samples were incubated at RT for 30 min. After incubation, the absorbance values of the samples were measured at λ<sub>415</sub>, and the quercetin equivalents (mg QE mL<sup>-1</sup>) were calculated.

### Determination of Antibacterial Activity

The antibacterial activity of the plant extracts was determined using the disc diffusion method (Bauer, 1966). In the experiments, *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (K55), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (PY79), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (DSM 22648), *Salmonella enterica* (LT2), and clinical isolates of *Proteus mirabilis* and *Listeria monocytogenes* were used. The clinical bacterial isolates were provided by Prof. Dr. Meral Dilara Ögünç (Akdeniz University, Antalya, Türkiye).

The bacteria were adjusted to 0.5 McFarland in NaCl (0.85%) solution (Merck, Germany) and spread on Mueller-Hinton Agar medium (MHA) (Merck, Germany). 30 µL plant extracts containing antibiogram discs (Bioanalyse, Türkiye) were placed on inoculated MHA. In the experiments, kanamycin discs (30 µg) (Cayman Chemical, USA) were used as positive controls, and antibiogram discs impregnated with 30 µL DMSO as negative controls. The Petri dishes were placed in an incubator at 37°C for 24 h and then the zone diameters were measured.

### Statistical Evaluation

Experiments were conducted in triplicate, and statistical analyses were carried out utilizing one-way analysis of variance (ANOVA) (IBM SPSS 22 software was used (SPSS, USA)). The Tukey test was used to perform multiple comparisons. Data were presented as

mean ± standard deviation and statistical significance was determined at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Antioxidant Measurements with ABTS Free Radical Scavenging Results

The ABTS assay is an antioxidant assay based on the formation of the blue-green ABTS<sup>+</sup> radical and can be applied to both hydrophilic and lipophilic antioxidant systems (Kim et al., 2002). This study investigated the ABTS radical scavenging capacity of the plant extracts obtained with different solvents (Figure 1). When the ABTS activities of the plants were analyzed, the highest ABTS activities were found in the methanol, ethanol, and acetone extracts of the plants. *P. alpinum* had the highest ABTS activity in all 3 extractions.

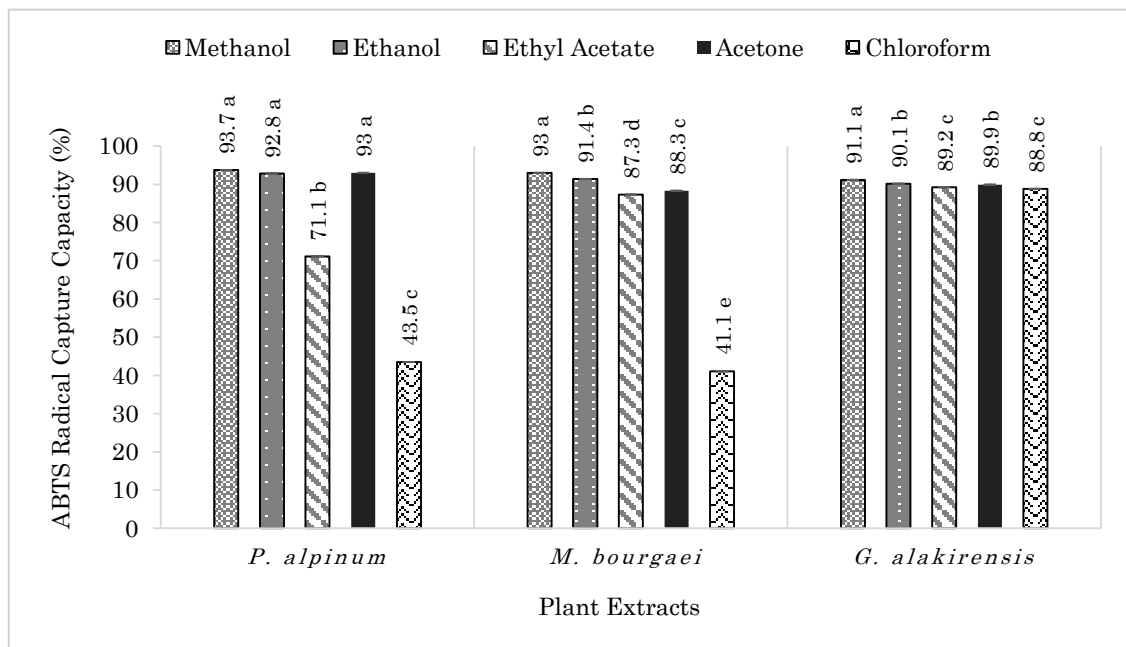


Figure 1. ABTS radical scavenging capacity of plant extracts (%) (different superscripts indicate a statistically significant difference ( $p < 0.05$ ))

Şekil 1. Bitki ekstraktlarının ABTS radikal temizleme kapasitesi (%) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ( $p < 0.05$ ))

It was found that the chloroform extracts of *M. bourgaei* had the lowest value in terms of ABTS scavenging capacity ( $p < 0.05$ ), while the methanol extract of *M. bourgaei* had the highest ABTS radical scavenging capacity ( $p < 0.05$ ). Yumrutaş and Saygıdeğer (2010), in their study with *M. parviflorum* Fisch. & Mey. and *Lamium amplexicaule* L., found that the ABTS activity of methanol extracts of the plants was higher than that of hexane extracts. In another study, Okur et al. (2019) reported that methanol extracts of *M. vulgare* L. had higher ABTS activity compared to trolox. Hayat et al. (2020) reported that ethanol extracts of *M. vulgare* from two

different regions in north-eastern Morocco showed higher ABTS activity. They also stated that extracts prepared with polar solvents such as ethanol and methanol would give better results in ABTS and DPPH experiments than extracts prepared with intermediate or weakly polar solvents.

According to the findings, there was no statistical difference between methanol, ethanol, and acetone extracts of *P. alpinum* ( $p > 0.05$ ); however, the chloroform extract of the plant had the lowest ABTS scavenging capacity compared to the other extracts. The lowest ABTS scavenging capacity of the

chloroform extract may be because chloroform has the lowest polarity of the solvents used. In their study, Matejić et al. (2013) found that the highest ABTS activity was found in water extracts of *P. longifolium* Waldst. & Kit. and *P. aegopodioides* (Boiss.) Vandas, ethyl acetate extract of *P. alsaticum* and acetone extract of *P. officinale*, but there was no ABTS activity in ethyl acetate and acetone extracts of *P. aegopodioides* species. Moreover, Danna et al. (2022) showed that the ethanol extracts of the leaf had higher ABTS activity than the ethanol extracts of the root of *P. ostruthium* (L.) Koch. Furthermore, they stated that this is because the plant's fresh leaves have more phenolic and flavonoid compounds than the roots.

It was found that the samples from methanol extractions of *G. alakirensis* were significantly higher than the other extraction samples ( $p < 0.05$ ). Ozsoy et al. (2018), in their study of the methanolic extract of *G. grandiflorum* Boiss. & A.Huet var. *grandiflorum*, stated that ABTS scavenging activity was close to that of the routine used as a control. Alali et al. (2007)

reported in their study in Jordan that the ABTS activity of water and methanol extracts of *G. aleppicum* Boiss. & Hausskn. showed the highest value among 95 species.

### Antioxidant Measurements with DPPH Free Radical Scavenging Results

When the DPPH activities of the samples were evaluated, a similar pattern was observed as for the ABTS scavenging activity, and the samples from the chloroform extraction showed a lower DPPH scavenging capacity than the other extracts for all plants (Figure 2) ( $p < 0.05$ ). When the DPPH scavenging capacity of the plants was evaluated, the highest DPPH scavenging capacity was found in the methanol, ethanol, and acetone extracts of the plants. *G. alakirensis* had the highest DPPH scavenging capacity in methanol and ethanol extracts. In addition, *P. alpinum* had the highest DPPH scavenging capacity among the acetone extracts of the plants.

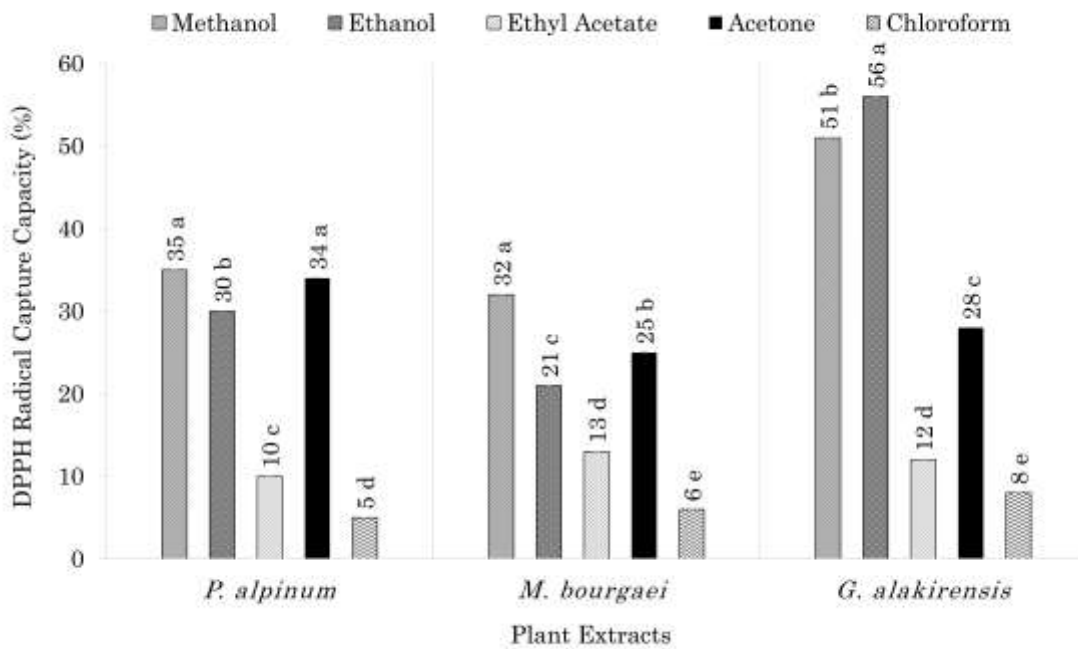


Figure 2. Ability of plant extracts to bind DPPH radicals (%) (different superscripts indicate a statistically significant difference ( $p < 0.05$ ))

Şekil 2. Bitki ekstraktlarının DPPH radikallerini bağlama yeteneği (%) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ( $p < 0.05$ ))

DPPH experiments use a radical that dissolves in organic solvents (such as alcohol). Therefore, DPPH is only applicable to hydrophobic antioxidant systems (Kim et al., 2002). Few studies are addressing the effects of different extraction solvents on the biological activities of the plant samples used in this study. In particular, too much attention has been paid to publications using the essential oils of *Marrubium*. However, studies on the essential oil of *Marrubium* are

widely available and it is found that the methanol extract of *Marrubium* has better biological activities. For example, Sarikurkcu et al. (2008) evaluated the antioxidant properties of the methanol extracts and essential oil of *M. globosum* Montbret & Aucher and reported that the lowest DPPH radical scavenging activity was found in the essential oil and the strongest antioxidant activity was found in the methanol extract. Similarly, in this study, the methanol extract of *M.*



*bourgaei* showed the highest DPPH scavenging activity ( $p < 0.05$ ). Yumrutaş and Saygıdeğer (2010) stated that the antioxidant effect of methanol extracts might be related to the presence of various compounds such as polar thermolabile and/or thermostable phenols. Chemsal et al. (2016) reported in their study with the methanol extracts and essential oil of *M. deserti* (de Noé) Coss. that the methanol extract showed high antioxidant activity in DPPH analysis, while the essential oil showed weak activity. Furthermore, their findings indicated that polar extracts exhibited greater antioxidant activity compared to non-polar extracts. According to Kumoro et al. (2009), the polarities of the solvents used in this study can be listed as methanol, acetone, ethanol, ethyl acetate, and chloroform from strong to weak, and therefore methanol extracts of *P. alpinum* and *M. bourgaei* are thought to have higher antioxidant activity. Sarikurkcu et al. (2020) investigated the enzyme inhibitory and antioxidant activities of water, methanol, and ethyl acetate extracts of *M. lutescens* Boiss. & Heldr. and reported that the water extract exhibited the highest antioxidant capacity, followed by the methanol extract.

In this study, there was no significant difference in DPPH activity of methanol and acetone extracts of *P. alpinum* ( $p > 0.05$ ). Kim et al. (2018) evaluated the antioxidant properties of water, hexane, ethyl acetate, and ether extracts of the plants *Saposhnikovia divaricata* (Turcz. ex Ledeb.) Schischk., *P. japonicum* Thunb., and *Glehnia littoralis* (A.Gray) F. Schmidt and found that ether and ethyl acetate extracts of *P. japonicum* had higher DPPH scavenging activity compared to the other extracts. Similarly, Sarkhail et al. (2013) investigated the antioxidant activities of hydroalcoholic extracts of *P. knappii* Bornm. (n-hexane, dichloromethane, ethyl acetate, and water) and reported that ethyl acetate exhibited the highest antioxidant activity. They also isolated two flavonol glycosides, isorhamnetin-3-O- $\beta$ -D-glucopyranoside, and rhamnetin-3-O- $\beta$ -D-glucopyranoside, which are considered to be effective tyrosinase inhibitors and antioxidants from the ethyl acetate extract of *P. knappii*. However, in the study, the antioxidant activity of the methanol and acetone extracts of *P. alpinum* was higher. Al et al. (2012) evaluated the antibacterial and antioxidant activity of the methanol extract of *P. zenkeri* Engl. and reported that the DPPH scavenging capacity of 1 mg mL<sup>-1</sup> methanol extract was 93.39%. They also stated that this high antioxidant activity could be due to phenolic compounds, anthraquinones, flavonoids, tannins, and anthocyanins. However, in this study, the methanol extract of *P. alpinum* was found to have a DPPH scavenging capacity of 35%. The antioxidant activity of the plant varies depending on the developmental stage of the plant, plant tissue, plant species, and

environmental factors such as light, temperature, and water stress (Upadrasta et al., 2011; Zlatić et al., 2019). Therefore, these factors may have caused differences in biological activity between plant species belonging to the same genus.

In this study, the ethanol extract of *G. alakirensis* had the highest DPPH scavenging capacity ( $p < 0.05$ ). Özçandır et al. (2024) showed that the ethanolic extract of *G. alakirensis* had higher antioxidant activity than rosmarinic acid and caffeic acid, which were used as positive controls. They also reported that the ethanolic extract of the plant contained phenolic substances such as chlorogenic acid (32.95 ppm), catechin (39.08 ppm), hydroxybenzoic acid (28.37 ppm), gallic acid (5.57 ppm), and quercetin (0.73 ppm). In their research with *G. grandiflorum*, Ozsoy et al. (2018) showed that the methanol extract of the plant had significant DPPH activity, but this capacity was lower than that of rutin, which they used as a control. In their study with *G. flavum*, Boulaaba et al. (2019) showed that the ethanol extract of the plant had a higher radical scavenging capacity than the ethyl acetate extract. In this study, the ethanol extract of *G. alakirensis* also had a higher antioxidant activity than the ethyl acetate extract. It is assumed that this is due to the the polarity of ethanol, which is higher than that of ethyl acetate (Kumoro et al., 2009).

### Evaluation of Total Phenolic Substance Content Results

Various antioxidant compounds, especially phenolic compounds, are closely associated with the antioxidant activity of a plant (Balasundram et al., 2006). Phenolic compounds are prominent substances with antioxidant functions for human health and are widely distributed in plants (Karaman et al., 2022; Şahin et al., 2022). When the total phenolic content of the plants was evaluated in this study, all extracts of *G. alakirensis* had higher total phenolic content than *P. alpinum* and *M. bourgaei*. There are no studies in the literature investigating the effects of the different extraction methods on the antioxidant activities of the plants used in this study. This study is the first to determine and compare the effects of 5 different extraction methods on the total phenolic content of these plants (Figure 3). However, similar studies on closely related plant species can be found in the literature. In their study, Sarikurkcu et al. (2018) evaluated the antioxidant properties of the essential oil and hexane, dichloromethane, methanol, ethyl acetate, and water extracts of *M. parviflorum* and reported that the water extract had the highest total phenolic content. They also found that there was a significant correlation between the total phenolic content and the DPPH experiments of the extracts and that the antioxidant activities of the extracts were directly dependent on the amount of phenolic compounds. However, in this



study, the methanol extract of *M. bourgaei* had the highest phenolic content ( $p < 0.05$ ). Similar to the study Sarikurkcu et al. (2020) investigated the enzyme inhibitory and antioxidant effects of methanol, ethyl acetate, and water extracts of *M. lutescens* and reported that the methanol extract had the highest

total phenolic content ( $54.80 \pm 0.52$ ). They also stated that different results obtained by using different solvents may be related to the type of solvent used, the analytical techniques, and the characteristics of the samples..

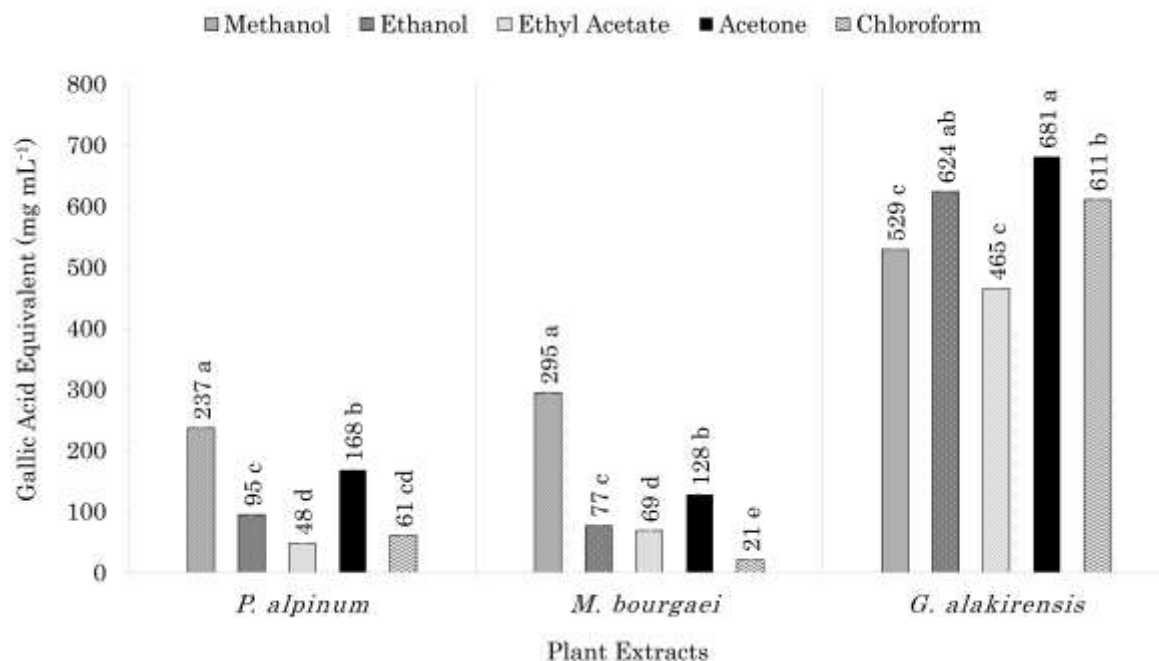


Figure 3. Total phenolic content of plant extracts (mg GAE mL<sup>-1</sup>) (different superscripts indicate a statistically significant difference ( $p < 0.05$ ))

Şekil 3. Bitki ekstraktlarının toplam fenolik madde içerikleri (mg GAE mL<sup>-1</sup>) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ( $p < 0.05$ ))

In this study, the methanol extract of *P. alpinum* had the highest phenolic content ( $p < 0.05$ ). Kim et al. (2018), in their study on *P. japonicum* extracts obtained with different solvents (water, hexane, ethyl acetate, ether), reported that the ether and ethyl acetate extracts of the plant had higher total phenolic content. They also stated that there was a correlation between the total phenolic substance and the antioxidant activities of the extracts. Matejić et al. (2013) compared the acetone, methanol, ethyl acetate, and water extracts of four *Peucedanum* species and reported that the total phenolic content of the extracts ranged from 52.18-118.32 mg GAE g<sup>-1</sup>. In the study, the total phenolic content of *P. alpinum* extracts ranged between 48-237 mg GAE mL<sup>-1</sup>.

It was found that the acetone extract of *G. alakirensis* had the highest phenolic content in the study ( $p < 0.05$ ). In the study by Kocanci et al. (2017), the total phenolic content of methanol extracts of *G. acutidentatum* Hausskn. & Bornm. and *G. corniculatum* (L.) Curtis was higher than that of the water extracts, but it was not statistically different. Boulaaba et al. (2019) reported in their study with *G. flavum* that the total phenolic content of the plant's ethanol extract was

higher than that of the ethyl acetate extract. They also found that the number of phenolic compounds identified in the ethanol extract such as caffeic acid, syringic acid, isoquercitrin, trans-hydroxycinnamic acid, catechin hydrate, and chlorogenic acid was higher than the number of total compounds identified in ethyl acetates such as trans-hydroxycinnamic acid, catechin hydrate, and chlorogenic acid and that catechin hydrate and isoquercitrin were the major components

### Evaluation of Total Flavonoid Substance Content Results

Flavonoids are secondary compounds commonly found in plants with diverse bioactivities and potential health benefits (Fu et al., 2021). This study is the first to evaluate and compare the total flavonoid content of 5 different plant extracts. The data on the total flavonoid content of the herbal extracts are shown in Figure 4. The acetone extract of *G. alakirensis* had the highest total flavonoid content of all plants ( $p < 0.05$ ).

No studies were found that demonstrate the effects of the extracts from the plants used in this study on biological activity. However, similar studies in the literature on closely related plant species may be

useful to compare the results. Interestingly, in this study, the highest values for ABTS, DPPH, and total phenolic content were found in the methanol extract, while the highest value for total flavonoid content was found in the ethyl acetate extract of *M. bourgaei* ( $p < 0.05$ ). Sarikurkcu et al. (2018) evaluated the antioxidant properties of essential oil and solvent extracts (hexane, methanol, ethyl acetate, dichloromethane, and water) of *M. parviflorum* and reported that the methanol extract had the highest content of total flavonoids. Sarikurkcu et al. (2020) investigated the antioxidant and enzyme-inhibitory

effects of water, methanol, and ethyl acetate extracts of *M. lutescens* and reported that the water extract had the highest total flavonoid content ( $27.20 \pm 0.81$ ), while the methanol extract had the highest total phenolic content ( $54.80 \pm 0.52$ ). It is known that these changes in total flavonoid content depend on the climatic conditions and geography of the area where the plant is collected, as well as the polarity of the solvent and the type of extract (Hayat et al., 2020; Bouterfas et al., 2016).

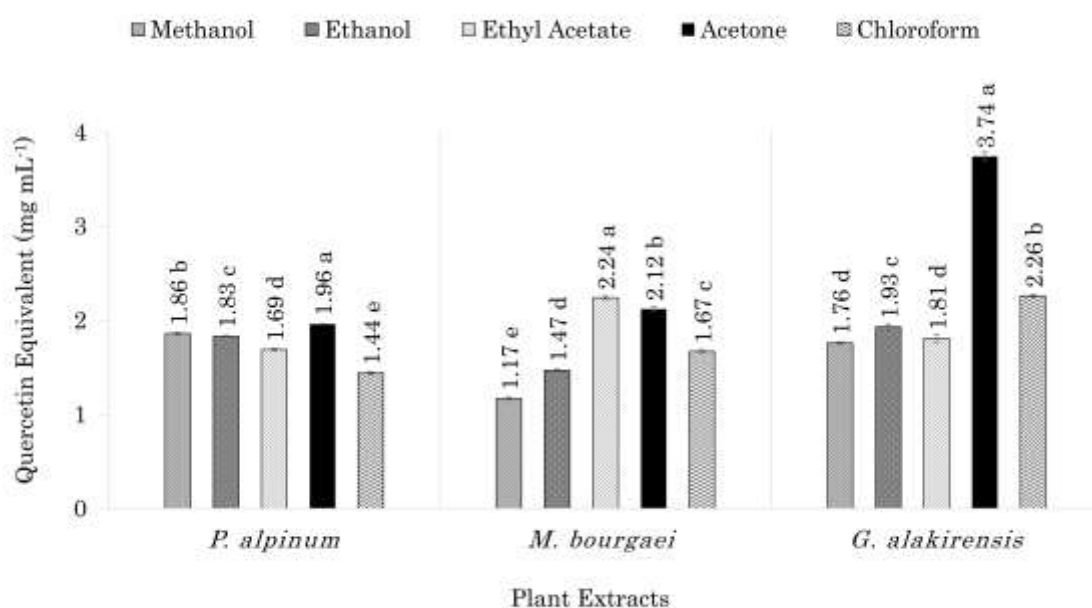


Figure 4. Total flavonoid content of plant extracts ( $\text{mg KE mL}^{-1}$ ) (different superscripts indicate a statistically significant difference ( $p < 0.05$ ))

Şekil 4. Bitki ekstraktlarının toplam flavonoid madde içerikleri ( $\text{mg KE mL}^{-1}$ ) (farklı üst simgeler istatistiksel olarak anlamlı bir farkı gösterir ( $p < 0.05$ ))

The total phenolic content of plant extracts varies depending on the temperature and time during the extraction process, the polarity of the extraction solvents, and the solubility of the phenolic substances in the solvent used for the extraction process (Abozed et al., 2014; Özer et al., 2021). In the present study, the acetone extract of *P. alpinum* had the highest phenolic content ( $p < 0.05$ ). Matejić et al. (2013) evaluated the antioxidant activity of extracts from four *Peucedanum* species (methanol, ethyl acetate, acetone, and water) and reported that the total flavonoid content of the extracts ranged from 4.43 to 234.67 mg quercetin equivalent (Qu)  $\text{g}^{-1}$  extract. Sarkhail et al. (2013) investigated the bioactivity of *P. knappii* extracts (dichloromethane, ethyl acetate, n-hexane, and water) and reported that ethyl acetate had the highest total phenolic and flavonoid content.

Similar to the results of total phenolic content, the

acetone extract of *G. alakirensis* had the highest total flavonoid content ( $p < 0.05$ ). Boulaaba et al. (2019) investigated the antioxidant properties of petroleum ether, ethanol, and ethyl acetate extracts of *G. flavum* and reported that the ethanol extract had the highest total flavonoid content. Shaghaghi et al. (2019) investigated the antioxidant properties of plants of the genera *Papaver* and *Glaucium* from different geographical regions of Iran and reported that the highest total flavonoid content was found in *G. mathiolifolium* Mobayen. Moreover, they reported that the antioxidant activity and total phenolic and flavonoid content varied among species as well as among different organs of the plants.

#### Determination of Antibacterial Activity Results

Certain medicinal plants are promising as potential reservoirs for new antibacterial agents (Koné et al., 2004). In the study to demonstrate the antibacterial

activity of the different plant extracts, kanamycin was used as a positive control and was effective on all bacterial strains; DMSO, which was used as a negative control, was not effective on any bacterial strain (Table 1). In this study, while all extracts of *M. bourgaei* showed antibacterial activity against *B. cereus*, *P. aeruginosa*, and *E. coli*, they were not effective against *P. mirabilis* and *L. monocytogenes* (Table 1). Antibacterial studies on *Marrubium* essential oil are more common in the literature. However, the methanol extract of the plant was found to have better antibacterial activity than the essential oil extracts. For example, Chemsal et al. (2016) evaluated the activities of the essential oil and methanol extract of *M. deserti* in terms of biofilm formation and anticholinesterase and reported that the antibiofilm activity of the methanol extract against six bacteria (*S. epidermidis*, *B. cereus*, *Micrococcus luteus*, *Streptococcus mutans*, *S. aureus*, *B. subtilis*) and *Candida albicans* was higher than that of the essential oil. Laouer et al. (2009), in their study, analysed the chemical composition, antimicrobial and antioxidant activity of the essential oil of *M. deserti* and did not observe any antimicrobial activity against *P. aeruginosa*, *S. aureus*, and *E. coli* strains. In this study, however, the methanol and ethyl acetate extracts of *M. bourgaei* were particularly effective against these three strains. Golmakani et al., (2016) investigated the essential oil composition and antibacterial properties of *M. duabense* Murata essential oils and found that they only observed antibacterial activity against *Clostridium perfringens* and no effect against *S. aureus*, *Salmonella*, and *E. coli*. However, in this study, the methanol extract of *M. bourgaei* was effective against these three strains. Based on the results of this study, it can be said that the extracts of *M. bourgaei* are at least as worthy of research as their essential oil. It is known that small hydrophilic molecules can easily pass through the outer membrane of Gram-negative bacteria, a property required for molecules with antibacterial activity. Methanol is also a small molecule that can penetrate the outer membrane and cause bacterial death (Kang et al., 2011). In our study, we did not find that methanol extracts were more effective against Gram-negative bacteria. This may suggest that the difference may be due to the selective effect of the secondary metabolites extracted from the plant species on the bacterial cells and not to the effect of the extraction method alone. The antimicrobial activity of plant extracts and essential oils varies according to their secondary metabolites (such as alkaloids, polyphenols, phenolic/flavonoid compounds, sulfur-containing compounds, terpenes, and coumarins). The antimicrobial activity exhibits a wide range depending on the alkylation of the glycosidic bonds of the OH groups, the number, structure, and position of the substituent groups, the type of plant, the topography,

and the climate of the country where the plant was collected (Vaou et al., 2021). Similarly, plant species used in this study may have different antimicrobial activities due to secondary metabolites.

In this study, ethanol, ethyl acetate, and chloroform extracts of *P. alpinum* were effective against all bacteria except *S. enterica*. The methanol extract was effective against all bacteria except *S. enterica* and *K. pneumoniae*. Acetone extract had the lowest antibacterial activity and was not effective against *B. cereus*, *S. epidermidis*, *S. enterica*, and *B. subtilis* strains (Table 1). Similarly, Schillaci et al. (2003) evaluated the pharmacological activities of acetone extracts of *P. nebrodense* (Guss.) Nyman and observed no antibacterial activity against the strains they used (*B. subtilis*, *S. aureus*, *Streptococcus agalactiae*, *P. aeruginosa*, *E. coli*, *Candida albicans*, and *Candida tropicalis*). Kim et al. (2018) investigated the antimicrobial activities of *P. japonicum*, *S. divaricata*, and *G. littoralis* extracts (water, ethyl acetate, hexane, ether) and reported that only ether and ethyl acetate extracts of *P. japonicum* showed antimicrobial activity. Madumelu et al. (2013) evaluated methanol extracts of *P. winkleri* H. Wolff. for its phytochemical component and antimicrobial properties and reported that the extract inhibited the growth of pathogens and was comparable to the standard drugs used. However, they were unable to demonstrate antibacterial activity against *S. pyogenes*, *P. aeruginosa*, and *Candida krusei*.

*G. alakirensis* all extracts were effective against *K. pneumoniae*, *E. coli*, *S. aureus*, *P. mirabilis*, and *L. monocytogenes*. In addition, ethyl acetate and acetone extracts of the plant formed the same zone diameter as the positive control for *P. mirabilis*. According to Özçandır et al. (2024), *G. alakirensis* ethanol extracts were effective against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *C. tropicalis* at concentrations of 50-200 µg mL<sup>-1</sup>. Plant extracts were effective against microorganisms at concentrations of 50-200 µg mL<sup>-1</sup>. However, Mojdeh et al. (2017) reported that alkaloid fractions and methanol extracts of *G. aucheri* and *G. vitellinum* were not effective against bacteria and also *Candida albicans*. Morteza-Semnani et al. (2005) investigated the antibacterial activity of extracts from three *Glaucium* species and found that methanol extracts were effective against Gram-negative microorganisms, while chloroform extracts were most effective against all strains tested. They also reported that 10 and 50 µg methanol and chloroform extracts of the plants were not effective against *S. aureus* (except chloroform extract of *G. oxylobum*). However, in this study, methanol and chloroform extracts (30 µg disc<sup>-1</sup>) of *G. alakirensis* were found to be effective against *S. aureus*. Tosun et al. (2006) worked with 14 different plants used in traditional medicine and reported that the methanol

extract of *G. grandiflorum* was not effective against any of the bacteria and fungi (except *C. krusei*) they used. However, in this study, the antibacterial

activities of *G. alakirensis*, especially the chloroform, methanol, and ethyl acetate extracts, were found to be quite broad-spectrum.

Table 1. Mean zone diameter of the antibacterial effects of the extracts (in mm)

Çizelge 1. Ekstraktların antibakteriyel etkilerinin ortalama zon çapı (mm)

|          | EC     | KP      | BC      | SA      | PA     | SE     | SEN     | BS     | PM      | LM      |
|----------|--------|---------|---------|---------|--------|--------|---------|--------|---------|---------|
| DMSO     | ND     | ND      | ND      | ND      | ND     | ND     | ND      | ND     | ND      | ND      |
| KAN      | 15±0   | 16±0.04 | 15±0.04 | 16±0.04 | 15±0   | 16±0   | 17±0.04 | 19±0.2 | 10±0.09 | 18±0.1  |
| Pa-EtOH  | 9±0    | 8±0.05  | 8±0.05  | 9±0     | 9±0    | 7±0    | ND      | 7±0    | 9±0     | 10±0.05 |
| Pa-MeOH  | 10±0   | ND      | 8±0.05  | 8±0.05  | 8±0.05 | 7±0    | ND      | 8±0.05 | 9±0.05  | 11±0.05 |
| Pa-Chl   | 8±0    | 8±0.05  | 9±0.05  | 9±0.05  | 9±0.05 | 8±0.05 | ND      | 9±0.05 | 9±0     | 13±0.05 |
| Pa-Ace   | 8±0    | 8±0.05  | ND      | 9±0.05  | 8±0    | ND     | ND      | ND     | 7±0     | 14±0.05 |
| Pa-AcOEt | 9±0    | 9±0.05  | 9±0.05  | 10±0.05 | 9±0    | 8±0.05 | ND      | 7±0    | 8±0.05  | 11±0.05 |
| Mb-EtOH  | 7±0    | 7±0     | 8±0.05  | ND      | 9±0.05 | ND     | 9±0     | ND     | ND      | ND      |
| Mb-MeOH  | 7±0    | 7±0     | 7±0.05  | 7±0     | 9±0    | ND     | 9±0.05  | ND     | ND      | ND      |
| Mb-Chl   | 8±0    | 7±0     | 7±0.04  | ND      | 7±0    | 8±0.05 | 8±0.01  | 10±0   | ND      | ND      |
| Mb-Ace   | 8±0.01 | ND      | 7±0.09  | ND      | 9±0.02 | ND     | 8±0.01  | 8±0.01 | ND      | ND      |
| Mb-AcOEt | 8±0.04 | ND      | 7±0.05  | 9±0.02  | 8±0.05 | 7±0.01 | ND      | 9±0    | ND      | ND      |
| Ga-EtOH  | 8±0    | 7±0     | 7±0     | 10±0    | ND     | ND     | 7±0     | 8±0    | 8±0     | 8±0     |
| Ga-MeOH  | 8±0    | 8±0     | 8±0     | 10±0    | 7±0    | 10±0   | 7±0     | ND     | 9±0     | 10±0    |
| Ga-Chl   | 9±0    | 7±0     | 7±0     | 12±0.05 | 9±0    | 10±0   | ND      | 9±0    | 9±0     | 9±0     |
| Ga-Ace   | 7±0    | 10±0.02 | ND      | 9±0     | ND     | ND     | 8±0     | ND     | 10±0    | 9±0     |
| Ga-AcOEt | 9±0    | 9±0     | 9±0     | 10±0.05 | 9±0    | 9±0    | 7±0     | ND     | 10±0    | 8±0     |

The diameter of the discs used in the study is 6 millimetres. (ND: Not Detected, EC: *E. coli*, BC: *B. cereus*, KP: *K. pneumoniae*, SA: *S. aureus*, PA: *P. aeruginosa*, SE: *S. epidermidis*, SEN: *S. enterica*, BS: *B. subtilis*, PM: *P. mirabilis*, LM: *L. monocytogenes*, DMSO: Dimethyl sulfoxide, KAN: Kanamycin, EtOH: Ethanol, MeOH: Methanol, Chl: Chloroform, Ace: Acetone, AcOEt: Ethyl acetate, Pa: *P. alpinum* Mb: *M. bourgaei*, Ga: *G. alakirensis*)

## CONCLUSION

In this study, the antioxidant activity, total phenolic content, total flavonoid content, and antibacterial activity of extractions with different solvents of *P. alpinum*, *G. alakirensis*, and *M. bourgaei* were investigated, and it was found that the use of different solvents for extraction may have various effects on the biological activity of the samples. The best antioxidant activity and total phenolic and flavonoid content generally varied between ethanol, methanol, and acetone extracts. However, in *M. bourgaei*, ethyl acetate showed the highest total flavonoid content compared to the other extracts. Ethanol, methanol, chloroform, and ethyl acetate extracts showed the highest antibacterial activity. It is extremely important to identify these plants growing naturally in Türkiye and to investigate their potential use in medical and industrial fields. There are no or insufficient extraction studies with these species used in this study. From this point of view, this study can fill an important gap in the literature and provide a starting point for research on the important pharmacological properties of species of these genera, but the results of this *in vitro* study cannot be transferred to the clinical field without an *in vivo* study.

## Author's Contributions

Eda DELİK, Berfin EROĞLU and Asst. Prof. Burcu

Emine TEFON ÖZTÜRK has designed the study and collected the data. The collection of plant materials was done by Mertcan GÜLBEN and Eda DELİK and the identifications were made by Prof. Candan AYKURT and Mertcan GÜLBEN. Eda Delik, Berfin EROĞLU and Asst. Prof. Burcu Emine TEFON ÖZTÜRK executed the experiment. Eda DELİK, Berfin EROĞLU, and Mertcan GÜLBEN wrote the article, and critically reviewed it by Asst. Prof. Burcu Emine TEFON ÖZTÜRK and Prof. Candan AYKURT.

## Statement of Conflict of Interest

Authors have declared no conflict of interest.

## Ethical Approval

Not required

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## Biological Activities of *Elaeagnus umbellata* Methanol Extract

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### ABSTRACT

*Elaeagnus umbellata*, known as Autumn olive and growing widely in Asia and Southern Europe, is a shrub tree used in the traditional treatment of many diseases, including cancer. In this study, it was aimed to investigate the several biological effects of methanol extract of *E. umbellata*. The activity of the extract on 11 microorganisms was determined by the agar well diffusion technique. The anti-quorum sensing activity of the extract was tested using *Chromobacterium violaceum* ATCC 12472. The anti-biofilm and anti-swarming activities were tested using *Pseudomonas aeruginosa* PAO1. Cytotoxic effect of the extract against pancreatic tumoral cell line (AR42J), breast cancer cell line (MDA-MB-231), lung adenocarcinoma cell line (A549), and normal epithelial cell line (Vero), and antiviral effect of the extract against herpes simplex virus type 1 was analyzed using the MTT method. It was determined that the extract was moderately effective against 6/11 microorganisms and showed anti-quorum sensing activity. While the extract did not have a cytotoxic effect on cancer cell lines, it was found to have a cytotoxic effect on Vero cells at concentrations of 100 µg/mL and above. However, no anti-biofilm, anti-swarming, and antiviral activity of the extract was observed. The study shows that *E. umbellata* fruit has limited biological activity.

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## *Elaeagnus umbellata* Metanol Ekstraktının Biyolojik Aktivitesi

### ÖZET

Asya ve Güney Avrupa'da yaygın olarak yetişen ve Sonbahar zeytini olarak bilinen *Elaeagnus umbellata*, kanser dahil olmak üzere birçok hastalığın geleneksel tedavisinde kullanılan bir çalı ağacıdır. Bu çalışmanın amacı *E. umbellata* ekstraktının çeşitli biyolojik etkilerinin belirlenmesidir. Ekstraktın 11 mikroorganizma üzerindeki aktivitesi agar kuyucuk difüzyon tekniği ile belirlendi. Ekstraktın anti-quorum sensing aktivitesi *Chromobacterium violaceum* ATCC 12472, anti-biyofilm ve anti-swarming aktiviteleri ise *Pseudomonas aeruginosa* PAO1 kullanılarak test edildi. Ekstraktın pankreatik tümöral hücre hattı (AR42J), meme kanseri hücre hattı (MDA-MB-231), akciğer adenokarsinoma hücre hattı (A549) ve normal epitel hücre hattı (Vero)'nasitotoksik etkisi ve herpes simpleks virüs tip-1'e karşı antiviral etkisi MTT yöntemi ile araştırıldı. Ekstraktın 6/11 mikroorganizmaya karşı orta düzeyde etkili olduğu ve anti-quorum sensing aktivite gösterdiği belirlendi. Ekstraktın kanser hücre hatlarında sitotoksik etkisi görülmezken, Vero hücrelerine 100 µg/mL ve üzeri konsantrasyonlarda sitotoksik etkisi olduğu tespit edildi. Bununla birlikte ekstraktın anti-biyofilm, anti-swarming ve antiviral aktivitesi görülmedi. Yapılan çalışma *E. umbellata* meyvesinin sınırlı biyolojik aktivitesinin olduğunu göstermektedir.

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## INTRODUCTION

Plants (especially medicinal ones), have been used in many different civilizations from past years to the present. It has been serving the field of medicine since ancient times (Temel et al., 2018; Saraç and Özpinar, 2024). They have been included in the human diet not only for their nutritional value but also for use as prophylactic and therapeutic agents in the treatment of different diseases. (Ak et al., 2022) *E. umbellata* is found at an altitude of 1200–2100 m above sea level and grows at temperatures ranging from 43 to 55 °C and a pH range of 5.5–9.5 (Bhat et al., 2023).

*Elaeagnus umbellata* Thunb., Fl. Jap. (Thunberg) 66, t. 14 (1784) is belonging to the Elaeagnaceae family. *E. umbellata* fruit is rich in vitamins A, C, and E, minerals, flavonoids, and fatty acids (Wu et al., 2011; Patel et al., 2015). *Elaeagnus* berries have a lot of bioactive compounds such as lutein,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and  $\alpha$ -cryptoxanthin. (Patel et al., 2015).

*Elaeagnus* species have traditionally been used as antioxidant, anticancer, antinociceptive, anti-inflammatory, antimutagenic, antiulcerogenic, antimicrobial, antidiabetic, and neuroprotective agents (Nazir et al., 2020).

It is known to be effective on many types of cancer as well as many different diseases. It has also been reported to be used as an antipyretic (Ahmad et al., 2005). It is reported that *E. umbellata* fruits are potentially effective in bacterial infections and complications related to type 2 diabetes (Nazir et al., 2018; Nazir et al., 2021). The flowers and seeds of *E. umbellata* are very useful as they are used as a tonic to cure cough. It is known that the oil of *E. umbellata* seeds is preferred in the treatment of infection. At the same time, its essential oil has antioxidant anticholinesterase and antidiabetic activity (Nazir et al., 2021).

In the study, anti-quorum sensing, anti-microbial, antiviral, and anti-cancer activities of *E. umbellata* methanol extract were investigated.

## MATERIAL and METHOD

### Collection of autumn olive fruits and preparation of their extracts

*E. umbellata* fruits were obtained from the high regions of Rize and dried in a Pasteur oven at 55°C overnight. Methanol extracts were obtained using the Macerate. Briefly, 10 to 20 g of fruit were weighed, ground into powder in a mortar, placed in a conical flask, and 100 mL of methanol was added. Incubated overnight on a magnetic stirrer at room temperature. Then, it was filtered through filter paper and evaporated in the evaporator at 40 °C, followed by the extracts dissolved in DMSO (Dimethyl sulfoxide) to 50

to 100 mg/mL. These extracts were stored at -20 °C until use (Eksi et al., 2020).

### Antimicrobial activity

*Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Mycobacterium smegmatis* (ATCC 607), *Chromobacterium violaceum* (ATCC 12472), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella thymurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter haemolyticus* (ATCC 19002), *Candida albicans* (ATCC 10231) and *Candida parapsilosis* (ATCC 22019) strains were used.

The antimicrobial activities were investigated by agar diffusion method. Minimal Inhibitory Concentration (MIC) was calculated using Mueller Hinton broth-II (Woods et al., 2003). The well located before the well where growth started was determined as the MIC well and the value in this well was written as the MIC value. To determine the Minimum Bactericidal Concentration (MBC) value, 50  $\mu$ L was taken from the MIC well and the three wells before it and planted on agar medium. Incubated overnight at 37 °C. Plantings from the MIC well and other wells were evaluated and the lowest value at which no growth was observed was determined as MBC (Gür, 2016).

### Anti-quorum sensing activity

To determine the violacein suppression activities of the extract, firstly the Sub-MIC value was determined in *C. violaceum* ATCC 12472 strain. *C. violaceum* 12472 strain was cultured in 5 mL of Luria Bertani (LB) broth for 8 hours in a 175 RPM shaking incubator. Then, 50  $\mu$ L of *C. violaceum* 12472, which was left for shaking incubation, was taken and added to 5 mL of soft LB agar and poured into LB agar petri dishes. Wells were opened on the dried petri dish. 50  $\mu$ L of the Sub-Mic concentration was added to the well and incubated overnight. The formation of a transparent zone with growth in the petri dish but no violacein pigment was determined as positive (Eksi et al., 2020).

### Anti-swarming activity

First, the MIC of the extract in *P. aeruginosa* PAO1 strain was determined. Values below the MIC were used in the study. Concentrations below the MIC value were added to tubes containing 5 mL LB soft agar, poured onto petri dishes containing LB agar, and allowed to solidify. A colony from the fresh culture of *P. aeruginosa* PAO1 was picked with a sterile toothpick placed in the middle of the prepared LB plates and incubated overnight at 37 °C (Rashid and Kornberg 2000). The activity was determined by measuring the colony spread of bacteria growing from the point where *P. aeruginosa* was inoculated. *P. aeruginosa* PAO1 without added extract was used as a

control and the measured zones were evaluated by comparing them with this zone.

### Anti-biofilm activity

To test the biofilm inhibition of the extract, *P. aeruginosa* PAO1 strain was adjusted to 0.5 McFarland. Extract and *P. aeruginosa* PAO1 were added to microplates containing LB medium. After 24 hours of incubation, microplates were washed with distilled water and 0.3% crystal violet was added to each well. Finally, the microplates were washed with distilled water and treated with ethanol for 15 minutes. Absorbances were measured at 570 nm in spectrophotometer (Truchado et al., 2009; Üreyen Esertaş et al., 2022).

### Cell culture, virus, and standard drug

Breast cancer cell line (MDA-MB-231), pancreatic tumoral cell line (AR42J), lung adenocarcinoma cell line (A549), and normal epithelial cell line (Vero) from Karadeniz Technical University, Medical Microbiology Department culture collection, originally obtained from American Type Culture Collection (ATCC, USA) were used in the study. AR42J, A549, and Vero cell lines were maintained in Dulbecco's Modified Eagles Medium (DMEM), MDA-MB-231 cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin solution. Cultures were incubated at 37 °C with 5% CO<sub>2</sub>.

The HSV-1 Wal strain was originally obtained from the University of Sheffield (England).

### Cytotoxicity of the extract

The cytotoxic effect of the extract on pancreatic tumoral cell line (AR42J), breast cancer cell line (MDA-MB-231), lung adenocarcinoma cell line (A549) and normal epithelial cell line (Vero) was examined by 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as previously described (Cora et al., 2023). Concentrations of 3.12-400 µg/mL of the extract were placed on the cells in a 96-well plate containing 1x10<sup>4</sup> cells in each well, with three wells of each concentration. After the incubation for 72 h at 37 °C with 5% CO<sub>2</sub>, the MTT assay was performed. Wells contained untreated cells were used as negative control. The results were evaluated in Microsoft Excel with reference to the control wells.

### Antiviral activity of the extract

Concentrations of 50 µg/mL and below, which are not cytotoxic to Vero cells, were added to the cells infected with the virus at a concentration of 1TCID<sub>50</sub>. After three days incubation at 37 °C with 5% CO<sub>2</sub> the MTT assay was performed. Acyclovir was used as a positive control, wells containing Vero cells infected with the

virus were used as a negative control, and wells containing Vero cells were used as reproductive control. The data were evaluated using the Microsoft Excel program and the viability of the cells in the wells was calculated as a percentage compared to the growth control (Cora et al., 2023).

### Statistical analysis

Statistical analysis was conducted with the SPSS 15.0 software. Mean ± standard error was employed to depict continuous variables conforming to a normal distribution. In cases where a normal distribution was not evident, the median value was utilized.

## RESULTS and DISCUSSION

The use of plants and herbal products in treatment by humans has been of great importance since ancient times. *E. umbellata*, is one of the medicinal plants widely used among the public for various ailments (Bhat et al., 2023). Today, with the developing technology, the chemical content of *E. umbellata* has been investigated and it has been understood that the plant contains different secondary metabolites and accordingly exhibits different pharmacological properties such as antiviral, anticancer and antioxidant activity (Bhat et al., 2023).

### Preparation of the extract

*E. umbellata* fruits were kept in a Pasteur oven overnight to remove water. Then, the extraction process of the dried fruits was carried out (Figure 1). Methanol extraction is a method used to isolate compounds from plant materials, such as essential oils, pigments, or other chemical substances (Parekh et al., 2005). It's one of several solvent extraction techniques, each with its own applications, benefits, and drawbacks (Methanol extraction is highly effective for certain laboratory applications and non-consumable products, but for consumable goods, safer solvents like ethanol or mechanical/physical methods like CO<sub>2</sub> extraction are preferred due to their lower toxicity and environmental impact (Nauman and Arshad, 2011).

### Antimicrobial activity results

Antimicrobial activity results of *E.umbellata* extract show that it has activity against six microorganisms (Table 1). When the zone diameters and MIC values of these microorganisms are examined, it is seen that the antimicrobial activity of the extract is at a medium level.

Although different methods and solvents were used, anti-bacterial properties of *E. umbellata* against *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *E. coli*, *Propionibacterium acnes*, and *Proteus mirabilis* were demonstrated (Kang et al., 2020; Zulfikar et al., 2022). Uddin & Rauf (2012) investigated the antimicrobial

activity of extracts prepared from *E. umbellata* using different solvents and found that MeOH extract was only effective against Gram-positive bacteria (Uddin &

Rauf 2012). In this study, the extract was found to be effective against Gram-positive bacteria as well as Gram-negative bacteria such as *K. pneumoniae*, *S. thymurium*, *C. violaceum*, *P. aeruginosa*.

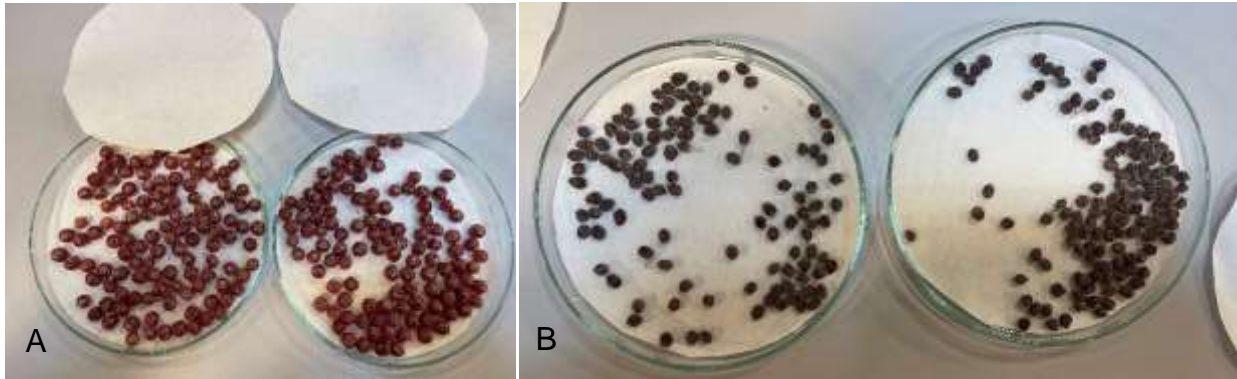


Figure 1. Images of *E.umbellata* before and after incubation A; Before, B; After  
 Şekil 1. *E.umbellata*'nın inkübasyon öncesi ve sonrası görüntüleri A; Önce B; Sonra.

Table 1. Antimicrobial activity results of *E. umbellate* MeOH extract

Çizelge 1. *E. umbellate* MeOH ekstraktının antimikrobiyal aktivite sonuçları

| Microorganisms  | <i>K. pneumoniae</i> | <i>S. thymurium</i> | <i>C. violaceum</i> | <i>B. subtilis</i> | <i>P. aeruginosa</i> | <i>B. cereus</i> |
|-----------------|----------------------|---------------------|---------------------|--------------------|----------------------|------------------|
| Zone (mm)       | 10.33±2.08           | 10.0±1.0            | 14.66±0.57          | 12.66±0.57         | 13.33±2.08           | 14±0.0           |
| MIC/MBC (ug/mL) | 500/1000             | 500/1000            | 250/500             | 500/1000           | 500/1000             | 500/1000         |

#### Anti-quorum sensing activity results

It has been determined that *E. umbellata* extract suppresses violacein pigment, one of the quorums sensing steps. (Table 2, Figure 2).



Figure 2. Anti-violacein activity assay result of the extract

Şekil 2. Ekstraktın anti-violasin aktivite deneyi sonucu

#### Anti-biofilm activity

Considering the biofilm suppression results it was determined that the *E. umbellata* extract had a low level of anti-biofilm activity (Figure 3).

Table 2. Quorum sensing activity assay result of the extract

Çizelge 2. Ekstraktın Quorum sensing aktivite deneyi sonucu

|                            | <i>C. violaceum</i> ATCC 12472 |
|----------------------------|--------------------------------|
| <i>E. umbellata</i>        | +                              |
| Positive control (Vanilin) | +                              |

While studies to examine its antimicrobial activity began in 2007 (Sabir et al., 2007), studies involving quorum sensing activity are still lacking today. Antibiofilm activity literature data show that *Elaeagnus angustifolia* plant extract has been studied and that the plant has anti-biofilm activity. Antibiofilm literature with *E. umbellata* is not yet available. The study is among the first scans in this regard. Likewise, while it was determined that *E. angustifolia* extracts were studied in the literature in terms of quorum sensing scans and the activity was low compared to other plants, *E. umbellata* data will be added to this study (Erdozmezve et al., 2016). The results show that there is activity, albeit at a low level. This is promising for further studies.

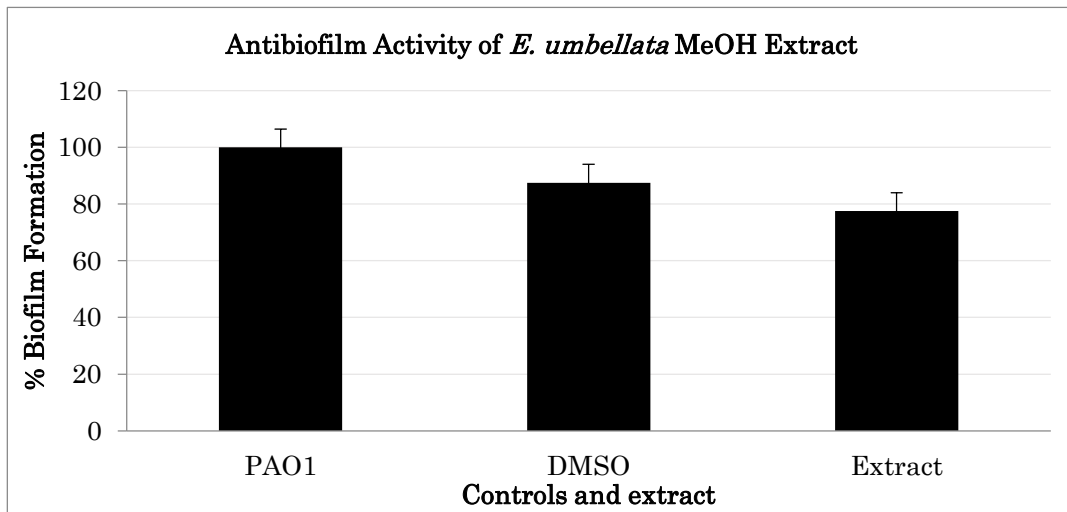


Figure 3. Anti-biofilm activity of *E. umbellata* MeOH extract  
Şekil 3. *E. umbellata* MeOH özütünün anti-biofilm aktivitesi

### Results of cytotoxicity assay

It was determined that *E. umbellata* MeOH had mild cytotoxic activity against Vero cells at concentrations of 100 µg/mL and above. On the other hand, it was

determined that the extract had no cytotoxic effect on AR42J cells, MDA-MB-231 cells, and A549 cells. The results of the experiment were summarized in Figure 4.

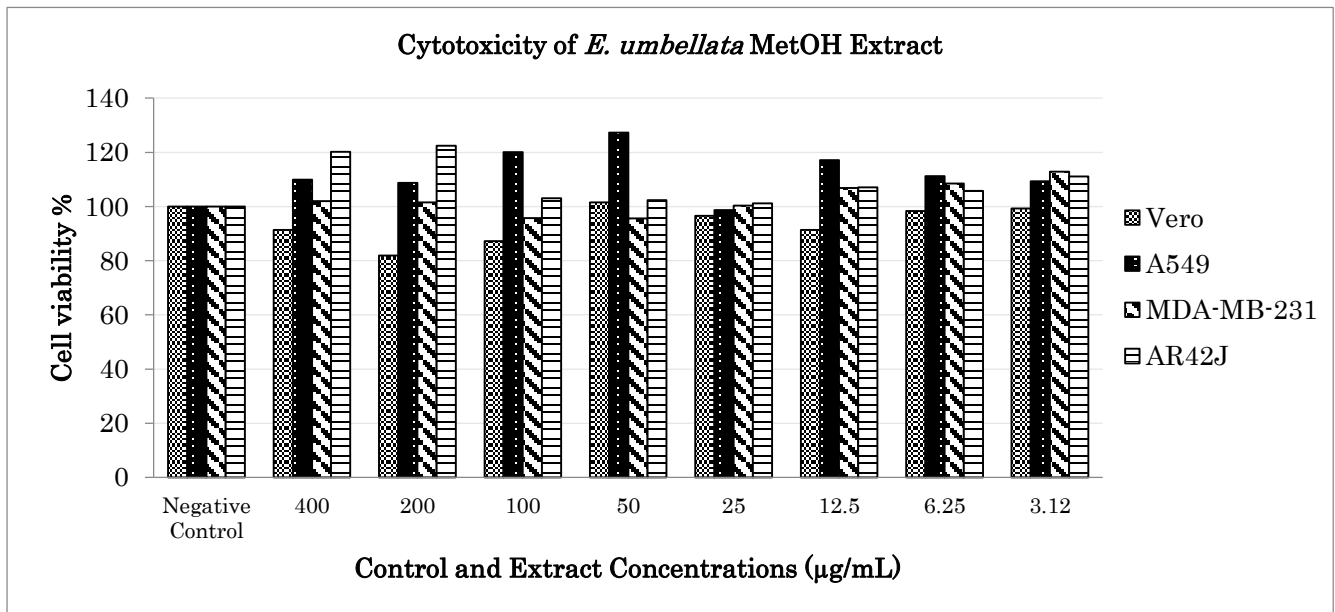


Figure 4. The cytotoxic effect of the extract on different cell lines  
Şekil 4. Ekstraktın farklı hücre dizileri üzerindeki sitotoksik etkisi

Wang et al. (2007) investigated the antiproliferative properties of six different genotypes of *E. umbellata* and found that all six genotypes inhibited proliferation of human leukemia cancer cells and human lung epithelial cancer. Aziz et al. (2015) investigated the activity of fruit and leaf parts of *E. umbellata* on human cervical cancer cell line (HeLa) and human colorectal adenocarcinoma cell line (HT29) cells and determined that leaves were more effective than fruits. In current

study, the effect of the extract prepared only from the fruit of *E. umbellata* plant on AR42J, MDA-MB-231, A549, and Vero cell lines was investigated, and it was observed that the extract had no cytotoxic effect on the cancer cell lines studied. However, it was found that the extract had a dose-dependent effect on Vero cells.

### Antiviral activity assay results

It was observed that the extract included in the study



did not show antiviral activity against HSV-1. The results of the experiment were shown in Figure 5. There are very few studies on other members of the

genus in terms of antiviral studies. A study involving the *Elaeagnus rhamnoides* species reported that the antiviral potential of the genus may be high (Olas and Skalski et al., 2022).

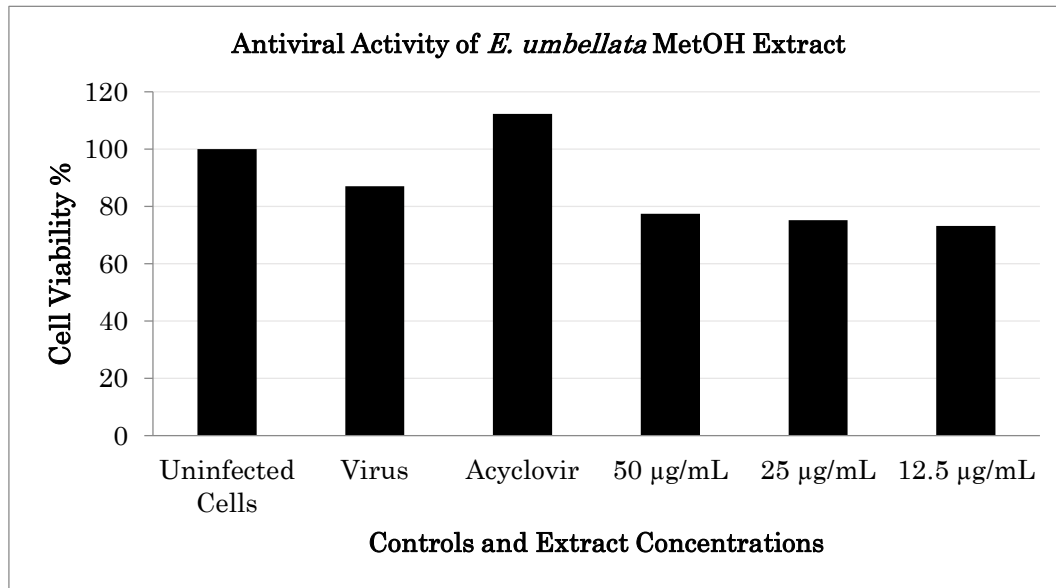


Figure 5. The antiviral activity of the extract on HSV-1. Uninfected cells; uninfected Vero cells, virus; Vero cells infected with virus, acyclovir; positive control

Şekil 5. Ekstraktın HSV-1 ile enfekte olmamış hücreler üzerindeki antiviral aktivitesi; enfekte olmamış Vero hücreleri, virüs; Virüs, asiklovir ile enfekte olmuş Vero hücreleri; pozitif kontrol.

## CONCLUSION

Although *E. umbellata* methanol extract had no antiproliferative activity on the cell lines studied and no antiviral activity against HSV-1, it is valuable that it showed antimicrobial activity. In addition, it is noteworthy that small activity was observed in the biofilm study. In line with the data obtained, it suggests that the plant can be evaluated in terms of active substance and is worthy of research.

## 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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## Tarım Atıklarından Aktif Karbon Üretimi ve Atıksudan Boya Giderimi: Karakterizasyon, Kinetik ve Denge Çalışmaları

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### ÖZET

Tarım atıkları, sürdürülebilir ve çevre dostu atık yönetimi açısından büyük bir potansiyele sahiptir. Bu çalışmada, tarım atıklarından pamuk sapları kullanılarak aktif karbon adsorbenti üretilmiştir. Bu adsorbent ile atık sudan tehlikeli bir boyar madde olan malahit yeşilinin adsorpsiyon prosesi ile giderilmesi incelenmiştir. Adsorbentin BET, SEM ve FT-IR analizleri ile karakterizasyonu yapılmıştır. Adsorpsiyon etkinliğini belirlemek için boya konsantrasyonu ve pH gibi değişkenlerin optimizasyonu gerçekleştirilmiştir. Malahit yeşili adsorpsiyonunun doğası hakkında daha iyi bir anlayış elde etmek için kinetik ve denge çalışmaları yapılmıştır. Yapılan çalışmalar sonucunda, yalancı ikinci dereceden kinetik modelin adsorpsiyon sürecini en iyi şekilde temsil ettiği, Langmuir izoterminin ise denge özellikleri için en uygun model olduğu belirlenmiştir. Langmuir izoterm verilerine dayanarak, maksimum adsorpsiyon kapasitesi ( $q_{max}$ ) 69.06 mg g<sup>-1</sup> olarak belirlenmiştir. Bu çalışma, atık pamuk saplarından üretilen adsorbanın çevre dostu, ekonomik ve etkili bir su arıtım malzemesi olarak potansiyelini ortaya koymaktadır.

### Çevre Bilimi

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi : 03.05.2024

Kabul Tarihi : 06.06.2024

### Anahtar Kelimeler

Tarımsal Atıklar

Aktif Karbon

Su arıtma

Adsorpsiyon

## Activated Carbon Production from Agricultural Wastes and Dye Removal from Wastewater: Characterization, Kinetic and Equilibrium Studies

### ABSTRACT

Agricultural wastes have a great potential for sustainable and environmentally friendly waste management. In this study, activated carbon adsorbent was produced using cotton stalks from agricultural wastes. With this adsorbent, the removal of malachite green, a hazardous dyestuff, from wastewater by the adsorption process was investigated. The adsorbent was characterized by BET, SEM, and FT-IR analysis. Optimization of variables such as dye concentration and pH were carried out to determine the adsorption efficiency. Kinetic and equilibrium studies were carried out to obtain a better understanding of the nature of malachite green adsorption. As a result of the studies, it was determined that the pseudo-second-order kinetic model best represents the adsorption process, while the Langmuir isotherm is the most suitable model for equilibrium properties. Based on the Langmuir isotherm data, the maximum adsorption capacity ( $q_{max}$ ) was determined as 69.06 mg g<sup>-1</sup>. This study reveals the potential of adsorbent produced from waste cotton stalks as an environmentally friendly, economical, and effective water treatment material.

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## GİRİŞ

Tarımsal atıklar tarım faaliyetlerinin yan ürünleri

olup, hasat artıkları, işlenmemiş organik materyaller, üretim süreçlerinden kaynaklanan atıklar, pazar atıkları ve ambalaj atıkları gibi çeşitli kaynaklardan

gelirler. Bu atıklar doğru şekilde yönetilmediğinde çevresel sorunlara neden olabilir; ancak doğru şekilde işlendiklerinde gübre olarak geri dönüştürülebilir, biyogaz üretimi için kullanılabilir veya diğer endüstrilerde hammadde olarak değerlendirilebilirler. Bu nedenle, tarımsal atıkların etkili bir şekilde yönetilmesi ve değerlendirilmesi önemlidir (Teo ve ark., 2022). Günümüzde, artan endüstrileşme ve tarımsal faaliyetlerin yaygınlaşmasıyla birlikte, tarım atıklarının sürdürülebilir atık yönetimi ve su arıtımı alanlarında alternatif kaynaklar olarak değerlendirilmesi giderek artan bir öneme sahiptir. Bu atıklar, sadece çevresel kirliliği azaltmakla kalmayıp aynı zamanda ekonomik ve çevresel açıdan sürdürülebilir çözümler sağlamak potansiyeline sahiptir (Crist ve ark., 2017).

Temiz ve güvenli içme suyunun giderek azaldığı endişe verici bir çağda yaşamaktayız. Bu kıtlık, nehirler ve göller gibi doğal su kaynaklarının üzerindeki büyük baskının, kirlilik ve aşırı kullanım sonucu doğrudan ortaya çıkmasından kaynaklanmaktadır. Dünya genelinde bazı bölgelerde ise temiz içme suyuna erişim, hayatta kalmak için günlük bir mücadele haline gelmiştir. Maalesef, iklim değişikliği kuraklık riskini artırarak mevcut su kıtlığını daha da derinleştirmekte ve bu durumun daha da kötüleşmesi beklenmektedir. Dahası, birçok topluluk temiz suyu arıtmak ve dağıtmak için gerekli kaynak ve altyapıya sahip değildir, bu da onları kirlenme riskine karşı savunmasız hale getirmektedir. Dolayısıyla, değerli su kaynaklarımızın korunması ve dünya çapında temiz içme suyuna erişim sağlanması için acil eylemler alınması gereklidir (Adeleye ve ark., 2023; United Nations, 2019). Dünya genelinde su kaynaklarının kirlenmesi artmakta olup, bunun başlıca nedeni pestisitler (Rodríguez-Bolaña ve ark., 2023), boyalar (Yildiz ve ark., 2023), farmasötikler (Khan & Jabin, 2023), ağır metaller (Aftab ve ark., 2023), deterjanlar (Pack ve ark., 2023), kişisel bakım ürünleri (Doherty ve ark., 2023) ve fenolik maddeler (Piryaei & Abolghasemi, 2022). Son yıllarda, çevre kirliliğine önemli bir katkı sağladığı için boya kirliliğinin giderilmesi konusunda kapsamlı araştırmalar yapılmaktadır (Ji ve ark., 2020; Stjepanović ve ark., 2021; Yıldiz, 2024). Boyaların su sistemlerine sızması, çevre üzerinde olumsuz etkilere neden olabilir. Boyaların suya karışması, suyun insan tüketimi için uygun olmayan hale gelmesine ve arıtma sürecinde zorluklara yol açabilir. Boyalar, gıda ve içecek, tekstil ve kozmetik gibi çeşitli endüstrilerde yaygın olarak kullanılmaktadır. Ancak, bu yaygın kullanıma rağmen, boyaların çevreye yayılma ve bozunma süreci oldukça zordur. Bu durumun temel nedeni, boyaların karmaşık kimyasal bileşimleri ve sentetik boyaların endüstrilerde geniş çapta kullanılmasıdır, bu da onları son derece dayanıklı ve doğal yıkıma karşı dirençli hale getirir (Deniz & Yıldiz, 2019; Tang ve ark., 2017a;

Yildiz ve ark., 2023).

Günlük hayatta giysi ve kozmetik ürünlerinde sıkça kullanılan boyalar, canlı renklerin oluşturulmasında önemli bir rol oynamaktadır. Ancak, bu boyaların üretimi ve kullanımı yüksek düzeyde renkli atık suyun oluşmasına neden olabilir. Bu durum özellikle tekstil, plastik ve baskı gibi sektörlerde yaygın olarak karşılaşılan bir durumdur. Boya üretimi ve kullanımının çevresel etkilerini en aza indirmek için uygun yönetim ve atık su arıtma sistemlerinin uygulanması gereklidir. Bu şekilde, çevresel etkiler azaltılabilir ve su kaynakları korunabilir (Tang ve ark., 2017a). Su içindeki boyaları arıtmak için biyolojik arıtma, çökeltme, flotasyon, ileri oksidasyon, elektrodializ, iyon değişimi, adsorpsiyon, biyosorpsiyon ve membran filtrasyonu gibi çeşitli teknikler kullanılmaktadır (Yildiz ve ark., 2024). Ancak, her bir yöntemin tasarımı, verimliliği ve maliyeti gibi sınırlamaları bulunmaktadır, özellikle düşük giderim verimliliği ve yüksek işletme maliyetleri gibi zorluklarla karşılaşılabılır (Deniz & Yıldiz, 2019a; Valli Nachiyar ve ark., 2023b; Yıldiz ve ark., 2024).

Bununla birlikte, diğer yöntemlerle (örneğin, flokülasyon/koagülasyon, biyolojik arıtma, ileri oksidasyon işlemleri, ozonlama ve membran filtrasyonu gibi) karşılaştırıldığında, adsorpsiyon en uygun olanıdır. Çünkü adsorpsiyon yöntemi çok yönlüdür, özelleştirilebilir ve maliyet etkinidir. Adsorpsiyon, kirleticilerin giderilmesi ve değerli metallerin geri kazanılması gibi çeşitli görevler için kullanılabilir. Ayrıca, karmaşık ekipman veya prosedürler gerektirmez, basit bir işlemdir (Sharifi Pajaie ve ark., 2018; Tang ve ark., 2017b; Uddin & Nasar, 2020; H. Yıldiz ve ark., 2023). Kuşkusuz, adsorpsiyon verimliliğinde en önemli rolü, kullanılan adsorban malzemenin giderim verimliliği, yüksek gözenek boyutu, dayanıklılığı ve ekonomik ömrü gibi faktörler oynamaktadır (Kutluay ve ark., 2019a; Philip, 2023). Bu bağlamda, adsorpsiyon işleminde aktif karbon (Alorabi, 2021), zeolit (Imessaoudene ve ark., 2023), silika (Ni'mah ve ark., 2024), grafit (Saleh & Ali, 2018) gibi çeşitli malzemeler kullanılmaktadır. Aktif karbon özellikle çok yönlü bir adsorbant olarak öne çıkar. Büyük yüzey alanı ve yüksek reaktiviteye sahiptir ve farklı kaynaklardan molekülleri çekme ve bağlama yeteneği ile bilinir. Ancak, yenilenemeyen hammaddelerden sentezlenen ticari aktif karbon son derece maliyetlidir (Malik ve ark., 2020).

Aktif karbon, birçok endüstriyel prosesin çok yönlü ve değerli bir bileşenidir. Gazların saflaştırılmasından suyun arıtılmasına (Kausar ve ark., 2023), enerji depolamaya (Sevilla ve ark., 2010), katalizörlere (Fuente ve ark., 2001) ve farmasötik ürünlere (Song ve ark., 2017) kadar geniş bir yelpazede kullanılmaktadır. Son on yılda, araştırmacılar daha ekonomik aktif karbon üretim yöntemleri geliştirmek



için çaba sarf etmektedirler. Bu amaçla, tarımsal atıklar (Yıldız ve ark., 2022), bitkiler (Pathania ve ark., 2017) ve hatta meyve kabukları (Şahin ve ark., 2016) gibi çeşitli biyolojik malzemelerin kullanımı denetlenmektedir. Sonuç olarak, bu karbon kaynakları artık hem çevre dostu hem de ekonomik olan aktif karbonlar üretmek için kullanılmaktadır.

Bu çalışmada, tarımsal atıklardan elde edilen pamuk sapı, malahit yeşili boyasını su ortamından gidermek için aktif karbon adsorbenti olarak kullanılmıştır. Türkiye, uzun süredir dünya pamuk üretiminde önemli bir rol oynamaktadır. Nispeten küçük boyutuna rağmen, Türkiye, Hindistan, Çin, Amerika Birleşik Devletleri, Brezilya ve Pakistan'ın ardından altıncı sırada yer alarak dünyanın en büyük pamuk üreticileri arasında yer almaktadır (Erdoğan & Sağlan, 2023). Türkiye'nin en önemli pamuk üretim bölgesi, ülkenin güneydoğusunda bulunan Güneydoğu Anadolu Projesi (GAP) bölgesidir. Bu bölgede, Türkiye'nin toplam pamuk üretiminin yarısından fazlası gerçekleştirilmekte olup, yaklaşık 2,9 milyon dekar ekili arazi bulunmaktadır (Ugur & Bayhan, 2023).

Malahit yeşili, deri, tekstil, kauçuk ve kâğıt gibi farklı endüstriyel işlemlerde renklendirici olarak kullanılan ve birçok sağlık ve çevre sorununa neden olan zararlı bir boyadır. Ayrıca, balık yetiştiriciliğinde ve bakteriyolojide güçlü bir ajan olarak kullanılmaktadır (Arora ve ark., 2020). Bu nedenle, MG gibi zararlı boyaların giderilmesi, yaşamsal faaliyetlerin ve ekolojik istikrarın sağlanması için önemlidir (Adeyi ve ark., 2019).

Başlangıç pH'ı ve başlangıç çözelti konsantrasyonunun deneysel parametreleri, adsorpsiyon üzerindeki etkilerini belirlemek için araştırılacaktır. Malahit yeşili açısından adsorbentin kinetiği ve denge modellemesini analiz etmek için ek araştırmalar yapılacaktır.

Çalışma, boya içeren atık sularla ve bunların çevresel etkileriyle mücadele etmek için çevre dostu adsorbanların önemini vurgulamaktadır. Uygun atık yönetimi, kirliliğin çevre ve insan sağlığı üzerindeki olumsuz etkilerini azaltmada kritik bir rol oynamaktadır. Tarım atıklarından elde edilen adsorbanların kullanımı, su kirliliğinin azaltılmasına ve sürdürülebilir kalkınmanın teşvik edilmesine yardımcı olacaktır.

## MATERYAL ve METOD

### Adsorbanın Hazırlanması

Adsorpsiyon işleminde kullanılan tüm malzemeler analitik saflıktaydı ve adsorbanın üretiminde kullanılması amaçlanan tarımsal atık pamuk sapları Şanhurfa, Türkiye'deki yerel üreticilerden temin edildi. İlk olarak atık pamuk sapları öğütülerek 0.5 mm boyuta elenmiştir. Elenen materyal (1g) daha

sonraki modifikasyonu, adsorpsiyon kapasitesini ve yüzey çıkıntılarını artırmak için 24 saat 100 mL 0,3 M Sodyum hidroksit (NaOH) ile muameleyi içermiştir. Modifiye edilen ve aktivasyon aşaması için hazırlanan malzeme bir kül fırınında 600°C'de 45 dakika boyunca aktive edilmiştir. Nihai aktif karbon, malahit yeşili adsorpsiyonu için adsorban olarak kullanılmaya hazır bir şekilde cam bir şişede dikkatlice saklandı.

### Karakterizasyon Çalışmaları

Tarım atıklarından üretilen aktif karbonun yüzey alanı ve gözenek hacmi, yüzey morfolojisi ve fonksiyonel grup profilini karakterize etmek için Brunauer-Emmett-Teller analizi (BET) (BET, Quantachrome Nova 1200), Fourier Transform Infrared Spektrometresi (FT-IR, Perkin Elmer Spectrum 400) ve Taramalı Elektron Mikroskobu (SEM, ZEISS-EVO 50 cihazı) kullanılmıştır.

Malahit yeşili boyası (CAS No: 2437-29-8) Sigma-Aldrich şirketi tarafından temin edilmiştir. Adsorpsiyon işleminde kullanılmak üzere malahit yeşili boya stok çözeltisi (1000 mg L<sup>-1</sup>) hazırlanmıştır. Deneysel çalışmalar için çözeltiler (50-250 mg L<sup>-1</sup>) stoktan distile su ile seyreltilerek elde edildi. Deney test çözeltisinde pH'yı hassas bir şekilde ayarlamak için hem sodyum hidroksit hem de hidroklorik asitin 0,1 mol L<sup>-1</sup> çözeltileri kullanılmıştır. (Sodyum hidroksit (NaOH) ve hidroklorik asit (HCl) sırasıyla Scharlau şirketi, Sinopharm kimyasal reaktif şirketi ve Sigma-Aldrich şirketi tarafından tedarik edilmiştir).

### Adsorpsiyon Deneyleri

Adsorpsiyon deneyleri için başlangıç pH'ı (pH 2-10) ve boya konsantrasyonu (C<sub>0</sub> 50-250 ppm) belirlenmiştir. Çözelti konsantrasyonları 616 nm dalga sayısında UV-VIS spektrofotometre (Hitachi U-0080D) ile ölçülmüştür. Denge durumunda aktif karbon tarafından malahit yeşili adsorpsiyon miktarı (q<sub>e</sub>) Eşitlik 1 ile belirlenmiştir.

$$q_e = \frac{(C_0 - C_e)V}{w} \quad (1)$$

Burada, C<sub>0</sub> (ppm) ve C<sub>e</sub> (ppm) sırasıyla çözeltideki başlangıç boya konsantrasyonu ve dengedeki boya konsantrasyonu, V (mL) çözelti hacmi, w (g) adsorban miktarıdır.

### Adsorpsiyon Kinetiği

Adsorpsiyon sürecinin dinamik etki mekanizmasını belirlemek ve karakterize etmek için sözde birinci dereceden (SBD) (Lagergren, 1898), sözde ikinci dereceden (SİD) (Weber & Morris 1963a) partikül içi difüzyon (PİD) (HO, 2006), ve Elovich (Weber & Morris 1963b).kinetik modelleri kullanılmıştır. Kullanılan kinetik modeller Eşitlik (2), (3), (4) ve (5) ile verilmiştir:

$$q_t = q_e(1 - e^{-k_1t}) \quad (2)$$

$$q_t = \frac{(k_2q_e^2t)}{(1+k_2q_et)} \quad (3)$$

$$q_t = k_{id}t^{\frac{1}{2}} + C \quad (4)$$

$$q_t = \frac{1}{\beta} \ln(\alpha\beta t + 1) \quad (5)$$



Şekil 1. Atık pamuk saplarından aktif karbon üretimi ve adsorpsiyon süreci

Figure 1. Activated carbon production and adsorption process from waste cotton stalks

Burada  $q_e$  ( $\text{mg g}^{-1}$ ) ve  $q_t$  ( $\text{mg g}^{-1}$ ) sırasıyla denge ve  $t$  (dk) zamanındaki adsorpsiyon kapasitesidir. SBD, SİD ve PİD modellerinin adsorpsiyon hız sabitleri sırasıyla  $k_1$  ( $\text{dk}^{-1}$ ),  $k_2$  ( $\text{g mg}^{-1} \text{dk}^{-1}$ ) ve  $k_{id}$  ( $\text{mg g}^{-1} \text{dk}^{-1/2}$ ) ile temsil edilirken,  $C$  ( $\text{mg g}^{-1}$ ) sınır tabaka kalınlığı ile ilgili bir sabittir.  $\alpha$  ( $\text{mg g}^{-1} \text{dk}^{-1}$ ) parametresi adsorpsiyon sürecinin başlangıcındaki hız olarak tanımlanır.  $\beta$  ( $\text{g mg}^{-1}$ ) parametresi bu süreçle ilişkili desorpsiyon sabitidir.

### Adsorpsiyon İzotermi

Adsorpsiyon izoterm çalışması, bir adsorbent materyalin adsorpsiyon kapasitesini ölçerek süreci daha iyi anlamayı amaçlamıştır. Adsorpsiyon izotermi için elde edilen veriler, iyi bilinen dört izoterm modeli kullanılarak değerlendirilmiştir; Freundlich (Freundlich, 1906), Langmuir (Langmuir 1918), Temkin (Temkin & Pyzhev 1940) ve Dubinin ve Radushkevich (DR) (Dubinin & Radushkevich, 1947) dört denklemlerle Eşitlik (6,7,8,9).

$$q_e = \frac{(q_m K_L C_e)}{(1 + K_L C_e)} \quad (6)$$

$$q_e = K_F C_e^{1/n_F} \quad (7)$$

$$q_e = B_T \ln A_T + B_T \ln C_e, \quad B_T = \frac{RT}{b} \quad (8)$$

$$q_e = q_m e^{-B\epsilon^2} \quad (9)$$

Burada,  $q_e$  ( $\text{mg g}^{-1}$ ) ve  $q_m$  ( $\text{mg g}^{-1}$ ) sırasıyla adsorbent denge ve maksimum boya adsorpsiyon kapasitelerini ve  $C_e$  ( $\text{mg L}^{-1}$ ) denge çözelti konsantrasyonunu temsil eder.  $K_L$  ( $\text{L mg}^{-1}$ ) adsorpsiyon enerjisi ile ilgili Langmuir denge sabitini,  $K_F$  ( $\text{mg g}^{-1} (\text{L mg}^{-1})^{1/n_F}$ ) ve  $n_F$  sırasıyla adsorpsiyon kapasitesi ve yoğunluğunun Freundlich model sabitlerini temsil eder.  $B_T$  ( $\text{L g}^{-1}$ ), bir malzemenin adsorpsiyon kapasitesinin bir ölçüsü olan Temkin izoterm sabitidir.  $A_T$  ( $\text{mg L}^{-1}$ ) denge bağlanma sabitidir ve adsorbat moleküllerinin adsorbent yüzeyine ne kadar güçlü bağlandığını gösterir.  $b$  ( $\text{J mol}^{-1}$ ) Temkin sabitidir ve sıcaklığın adsorpsiyon sürecini nasıl etkilediği hakkında bilgi verir.  $B$  ( $\text{mol}^2 \text{kJ}^{-2}$ ) ve  $\epsilon$  ( $RT \ln(1+1/C_e)$ ) adsorpsiyon süreçlerinin verimliliğini değerlendirmek için kullanılan iki önemli parametredir.  $B$ , bir molekülün bir yüzeye bağlı kalması için gereken enerjinin bir ölçüsü olan adsorpsiyon enerjisi anlamına gelir.  $\epsilon$  ise, iki molekül arasındaki bağlanmanın termodinamiğini hesaplamak için kullanılan Polanyi potansiyelinin kısaltmasıdır. Bu, adsorbe edilen malzeme miktarının değişen sıcaklıklarla nasıl değişeceğini tahmin etmek için kullanılabilir olduğundan özellikle önemlidir.  $R$  ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ) gaz sabiti olup, bir gazın basıncının sıcaklığı ve içerdiği molekül sayısı ile doğru orantılı olduğunu belirten ideal gaz yasasında önemli bir faktördür.  $T$  (K) Mutlak Sıcaklıktır ve bir sistemde mevcut olan termal enerji miktarını ölçer.

### Adsorpsiyon istatistiği

Deneysel ve modelleme sonucu elde edilen değerler arasındaki sapmayı değerlendirmek için iki hata ölçütü kullanılmıştır. Sıfır olmayan veri noktaları için ortalama bağıl mutlak hata (OBMH) modeli, Eşitlik

$$\text{OBMH model} = \frac{1}{N} \sum_{i=1}^N \left( \frac{|\text{deneysel de\u0131er} - \text{modellenmi\u015f de\u0131er}|}{\text{deneysel de\u0131er}} \times 100 \right) \quad (10)$$

$$\text{NKOKH model} = \frac{\sqrt{\frac{1}{N} \sum_{i=1}^N (\text{deneysel de\u0131er} - \text{modellenmi\u015f de\u0131er})^2}}{\text{deneysel de\u0131er}} \times 100 \quad (11)$$

### BULGULAR ve TARTI\u015MA

#### Adsorbent Karakterizasyonu (BET, SEM ve FT-IR Analizleri)

BET cihazı, adsorbanın yüzey alanını ve yüzey hacmini kesin olarak belirlemek için kullanıldı. Aktif

(10) ile belirtilmiştir. Ayrıca, normalize edilmiş kök ortalama kare hatası (NKOKH) modeli, Eşitlik (11) ile tanımlanmış olup, deneysel değerlerin tahmin edilmesindeki genel hatayı değerlendirmek için kullanılmıştır (Açın Ok & Kutluay, 2023).

karbon için hesaplanan BET yüzey alanları Çizelge 1'de verilmiştir: Yüzey alanı  $547.169 \text{ m}^2/\text{g}^{-1}$  ve toplam gözenek hacmi  $0.299 \text{ cm}^3/\text{g}^{-1}$ 'dir. Adsorbanın geniş yüzey alanı ve gözenek hacmi, kirleticileri etkin bir şekilde uzaklaştırma yeteneğini gösterir (Wang ve ark., 2023).

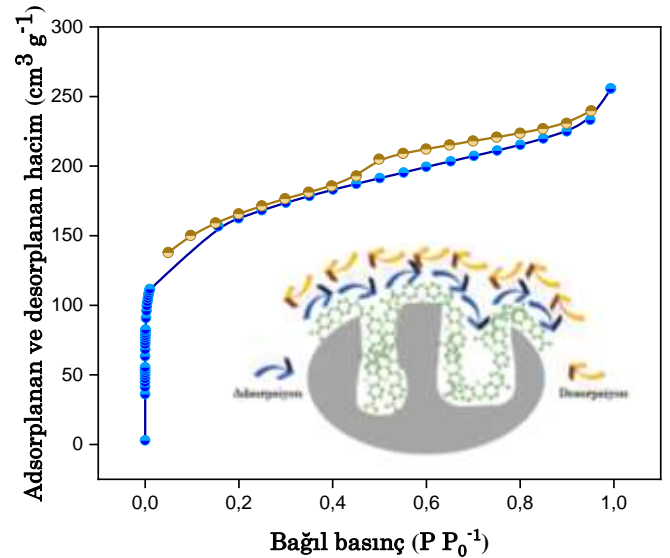
Çizelge 1. Aktif karbonun yüzey alanı, gözenek hacmi ve mikro gözenekliliğin yüzdesi

Table 1 Surface area of activated carbon, volume of pores, and percentage of microporosity

| Yüzey Alanı ( $\text{m}^2 \text{ g}^{-1}$ ) | Gözenek Hacmi ( $\text{cm}^3 \text{ g}^{-1}$ ) |                     | Mikro porozite (%) |
|---|--|---------------------|--------------------|
|   | $V_{\text{mikro}}$                             | $V_{\text{toplam}}$ |                    |
| 547.196                                     | 0.162  | 0.299               | 54.18              |

Şekil 2, aktif karbonun adsorpsiyon ve desorpsiyon izotermelerini göstermektedir ve genellikle mikro ve mezopor materyallerde gözlenen I-IV tipi izotermi içermektedir. Tip I izoterm karakteristik özelliği, düşük basınçlarda azot adsorpsiyon kapasitesinde belirgin bir artışa işaret eden güçlü mikroporlar ile azot molekülleri arasındaki etkileşimlerdir (Kutluay ve ark., 2019b). Bu özellik, hava arıtımı ve su filtrasyonu gibi çeşitli uygulamalarda etkili bir seçenek sunar. Orta ve yüksek bağıl basınçlarda, izoterm tip IV'e dönüşür ve bu dönüşüm, mezoporların oluşumunu ve  $\text{N}_2$  moleküllerinin kapiler kondensasyonunu gösterir (Teğin ve ark., 2020). Kapiler kondensasyon sırasında, artan basınç nedeniyle adsorbe gaz molekülleri mikro gözeneklerde sıkıştırılır, böylece yoğun bir faz oluşturur. Bu yoğun faz, basınç değiştikçe çökebilir ve yeniden oluşturabilir, bu da izotermelerde gözlemlenen histerezis davranışına yol açar (Zhang ve ark., 2021). Bu fenomenin anlaşılması, birçok endüstriyel uygulama için kritiktir. Ayrıca, bağıl basınç arttıkça  $\text{N}_2$  adsorpsiyon kapasitesinde kademeli bir artış gözlenir, bu da adsorban malzemesinde önemli miktarda mikropor varlığına işaret eder. Bu durum, adsorpsiyon kapasitesinin artmasına ve daha etkili Ayrıca, bu maddenin mikro-morfolojik yapısı ve aktif bölge bileşimi, taramalı elektron mikroskobu (SEM) ve Fourier dönüşümü kızılötesi (FT-IR) cihazları kullanılarak incelenmiştir. Bu veriler, adsorban ve Malahit yeşili iyonları arasındaki potansiyel

adsorpsiyon uygulamalarına olanak tanır (Kaouah ve ark., 2013).

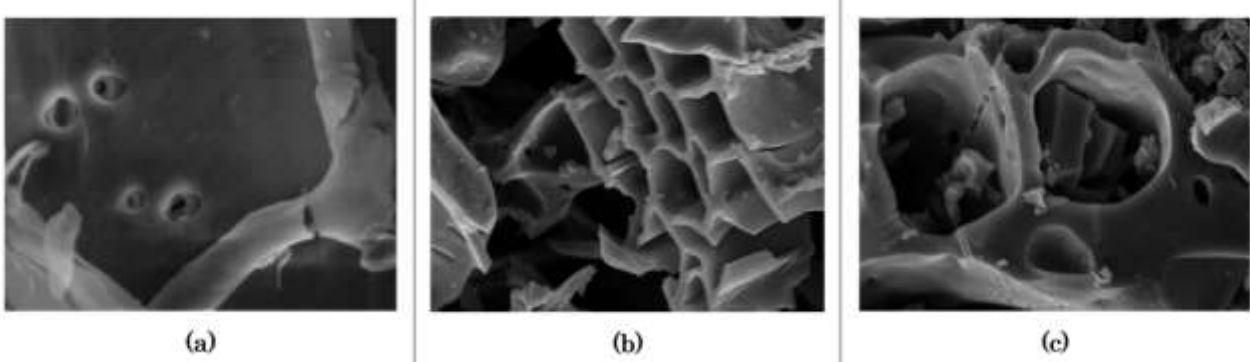


Şekil 2. Adsorpsiyon-desorpsiyon izotermi  
Figure 2. Adsorption-desorption isotherm

etkileşimleri belirlemek için kullanılmış ve adsorpsiyon sürecine ışık tutmuştur. Şekil 3a'da görüldüğü gibi, aktif karbon üretiminde kullanılan hammaddenin SEM görüntüsü boşluksuz bir yüzey ortaya koymaktadır. Üretilen aktif karbonun

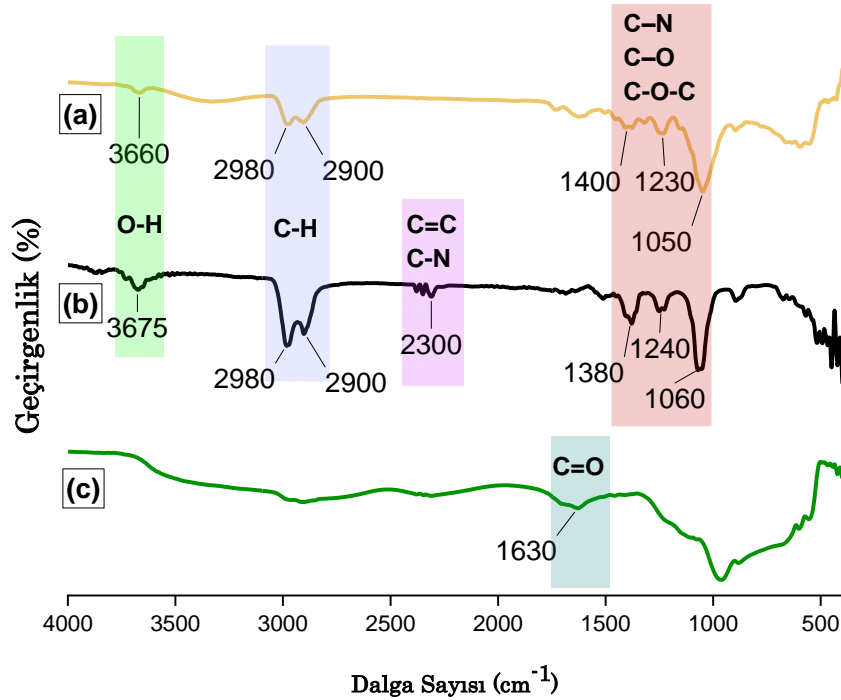
yüzeyinin daha ayrıntılı incelenmesi (Şekil 3b), heterojen olduğunu ve gözenekli bir yapı oluşturan büyük kıvrımlara sahip olduğunu göstermektedir. Bu tür bir yapı, yüksek yüzey alanı nedeniyle adsorpsiyon

performansını artırmaktadır (Şekil 3c). Gözenekler kanal görevi görerek bir sıvı veya gaz akışından moleküllerin adsorpsiyonuna izin verir ve aktif karbonun bunları yakalayıp depolamasını sağlar.



Şekil 3. (a) Ham malzemenin, (b) aktif karbonun ve (c) adsorpsiyon sonrası aktif karbonun taramalı elektron mikroskobu (SEM) görüntüleri (büyütme 500x).

Figure 3. Scanning electron microscopy (SEM) images of (a) raw material, (b) activated carbon and (c) activated carbon after adsorption (magnification 500x).



Şekil 4. (a) Ham malzemenin, (b) aktif karbonun ve (c) boya adsorpsiyonu sonrası aktif karbonun FT-IR spektrumları

Figure 4. FTIR spectra of (a) raw material, (b) activated carbon and (c) activated carbon after dye adsorption

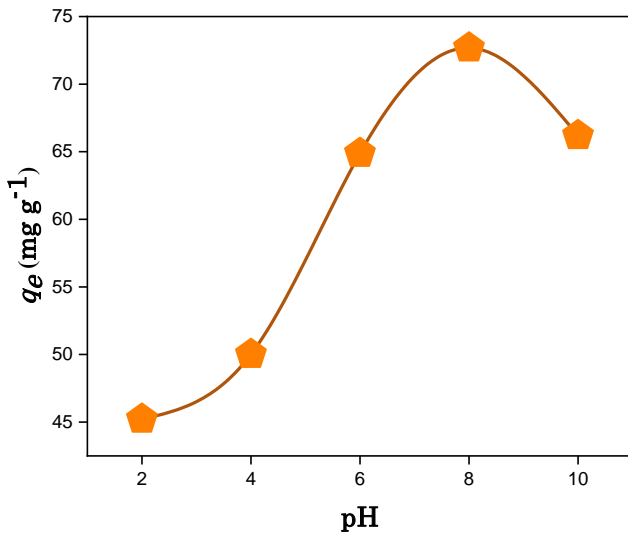
Şekil 4'te işlenmemiş ham madde, adsorpsiyondan önce aktif karbon ve Malahit yeşili adsorpsiyonundan sonra aktif karbon için Fourier dönüşümlü kızılötesi (FTIR) spektrumlarının karşılaştırılması gösterilmektedir. Spektrum analizi, 3660-33675  $\text{cm}^{-1}$  arasındaki piklerin O-H hidroksil gruplarını (Valencia ve ark., 2022), 2980-2900  $\text{cm}^{-1}$  arasındaki piklerin C-H fonksiyonel grubunu temsil ettiğini ortaya koymaktadır (Roy & Rhim, 2019). 2300  $\text{cm}^{-1}$  aralığında meydana gelen pikler CC ve C-N gerilmesini temsil etmektedir (Kumari ve ark., 2023).

Ayrıca, 1630  $\text{cm}^{-1}$  civarındaki piklerin C=O fonksiyonel grubu ile ilişkili olduğu belirlenmiştir (Cheng ve ark., 2016). Bununla birlikte, 1400, 1380, 1240-30 ve 1060  $\text{cm}^{-1}$  civarındaki piklerin C-N, C-O ve C-O-C gruplarını temsil ettiği tespit edilmiştir (S. Liu ve ark., 2018; Nasab ve ark., 2019). 1000  $\text{cm}^{-1}$ 'den daha düşük piklerin ise aromatik halkalarla ilişkili fonksiyonel grupların varlığını gösterdiği görülmüştür (Yildiz & Yuksel, 2023).



## Boya Adsorpsiyonu

Adsorbanın adsorpsiyon verimliliği, sulu ortamdan boyanın uzaklaştırılmasındaki etkinliğini değerlendirmek amacıyla detaylı bir şekilde incelenmiştir. Bu çalışmanın hedefleri doğrultusunda, boya konsantrasyonu ve pH seviyesi gibi ana deneysel parametreler titizlikle ele alınmıştır. Çözeltinin asidik veya alkali olma durumuna bağlı olarak, adsorbanın yüzeyinde çeşitli fonksiyonel gruplar ve yüzey kimyaları tespit edilebilir. Bu sebeple, adsorbanın etkinliğini değerlendirirken çözeltinin başlangıç pH seviyesini göz önünde bulundurmak gereklidir (Liu ve ark., 2021). İlk olarak, farklı başlangıç pH (2-10) değerlerinin malahit yeşili adsorpsiyonu üzerindeki etkisi incelenmiştir (Şekil 5).



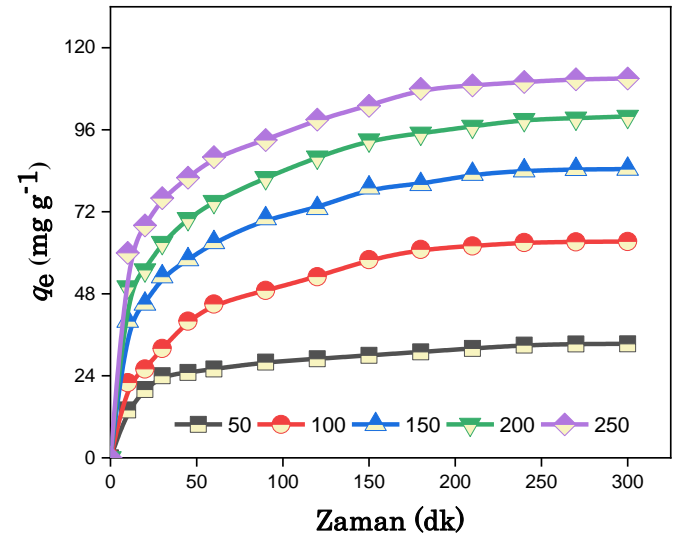
Şekil 5. pH'nın Etkisi (T: 25 °C, V: 100 mL, m: 0.1 g, Co: 100 mg g<sup>-1</sup>)

Figure 5. Effect of pH (T: 25 °C, V: 100 mL, m: 0.1 g, Co: 100 mg g<sup>-1</sup>)

Araştırmanın sonuçları, boya adsorpsiyon kapasitesinin başlangıç pH değerinin artmasıyla belirgin şekilde arttığını göstermektedir. Başlangıç pH değerinin 2 olması, en düşük adsorpsiyon kapasitesiyle sonuçlanırken, başlangıç pH değerinin 8 olması en yüksek adsorpsiyon kapasitesini sağlamıştır. Düşük pH değerlerinde, çözeltideki H<sup>+</sup> iyonlarının seviyesi belirgin şekilde yüksektir ve bu da asidik bir ortama neden olur. Bu durum, adsorban yüzeyinde protonasyonun meydana gelmesine yol açar, yani H<sup>+</sup> iyonları adsorban malzemenin yüzeyine bağlanır. H<sup>+</sup> iyonları yüzeyde yer kapladıkları için ve adsorbe edilebilecek diğer moleküllerin miktarını sınırladıkları için, bu durum malzemenin adsorpsiyon kapasitesinde bir azalmaya neden olabilir (Zhou ve ark., 2019). Öte yandan, hidroksit (OH<sup>-</sup>) konsantrasyonu pH 10'da artarak adsorban yüzeyinin deprotonasyonuna neden olmuştur. Bu durum, adsorbanın adsorpsiyon kapasitesinde bir düşüşe yol açmıştır. Adsorpsiyon için optimum pH değeri olarak 8

belirlenmiştir.

Çözeltinin başlangıç pH seviyesi belirlendikten sonra, Malahit yeşili adsorpsiyonu üzerindeki etkilerini anlamak amacıyla çeşitli başlangıç boya konsantrasyonları (50-250 mg L<sup>-1</sup>) incelenmiştir. Adsorpsiyon hızı ile boya konsantrasyonu arasındaki ilişkiyi anlamak için boya konsantrasyonları çeşitli değerlerde değiştirilmiştir. Şekil 6, boya başlangıç konsantrasyonları (mg L<sup>-1</sup>) ile aktif karbon üzerine adsorpsiyon kapasiteleri (qe) arasındaki ilişkiyi açıkça göstermektedir. Başlangıç konsantrasyonu arttıkça, adsorpsiyon kapasitesi de artmaktadır. Bu gözlem, yüksek Malahit yeşili konsantrasyonlarının sudan uzaklaştırılması için aktif karbondan daha büyük bir adsorpsiyon kapasitesi gerektirdiğini ortaya koymaktadır.



Şekil 6. Boya konsantrasyonunun ve adsorpsiyon süresinin adsorpsiyon süreci üzerindeki etkileri (T: 25°C, V: 100 mL, m: 0,1 g, pH: 8)

Figure 6. Effects of dye concentration and adsorption time on the adsorption process (T: 25°C, V: 100 mL, m: 0,1 g, pH: 8)

Bir adsorban malzemenin adsorpsiyon kapasitesi, büyük ölçüde gözeneklerinin boyutuna ve şekline bağlıdır. Gözenekler daha fazla molekülü adsorbe edebildiğinde, adsorpsiyon kapasitesi hızla artar ve daha fazla gözenek doldukça, adsorpsiyon kapasitesindeki artış oranı azalır. Bu, gözeneklerin doldukça, kalan boş gözeneklere erişimin zorlaşması ve adsorpsiyon oranında bir azalma meydana gelmesiyle açıklanabilir. Bu durum, adsorpsiyon kapasitesinde bir denge noktasına ulaşana kadar devam eder (Liu ve ark., 2010; Yao ve ark., 2023).

## Adsorpsiyon kinetik modelleri

Tarım atıklarından elde edilen aktif karbonun, Malahit yeşili boyası adsorpsiyonu için yapılan kinetik modelleme çalışmasının sonuçları Çizelge 2'de sunulmuştur. Adsorpsiyon kinetiği ve reaksiyon

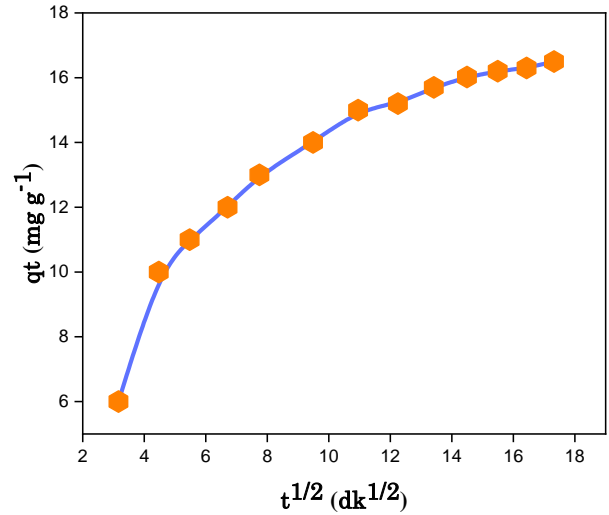
dinamikleri, kimyasal reaksiyonların davranışını anlamada iki temel unsurdur. Bu süreçleri değerlendirmek için kullanılan hız sabitleri, reaktanların ürünlere dönüştürülme hızını ve adsorpsiyonun ne ölçüde gerçekleştiğini ölçer. Adsorpsiyon kinetik modelleri, belirli bir kirleticinin zaman içindeki konsantrasyonunu analiz ederek, kirleticinin adsorban malzeme ile nasıl etkileşime girdiği hakkında bilgi sağlayabilir. Bu, özellikle farklı malzemelerin adsorpsiyon süreçleri için kullanıldığında nasıl performans gösterebileceğini anlamada yardımcı olur (Matos ve ark., 2022).

Çizelge 2. Malahit yeşili adsorpsiyonu için kinetik değerlendirme sonuçları

Table 2. Kinetic evaluation results for malachite green adsorption

| Model   | Parametre  | Değer  |
|---------|--|--------|
| SBD     | $q_e$ (mg g <sup>-1</sup> )                        | 8.947  |
|         | $k_1$ (min <sup>-1</sup> )                         | 0.0000 |
|         | $R^2$  | 0.9860 |
|         | Adj $R^2$  | 0.9847 |
|         | RMSE   | 0.2791 |
|         | OBMH (%)   | 101.11 |
|         | NKOKH (%)  | 91.98  |
| SİD     | $q_e$ (mg g <sup>-1</sup> )                        | 17.18  |
|         | $k_2$ (g mg <sup>-1</sup> min <sup>-1</sup> )      | 0.0037 |
|         | $R^2$  | 0.9975 |
|         | Adj $R^2$  | 0.9973 |
|         | RMSE   | 1.154  |
|         | OBMH (%)   | 58.24  |
|         | NKOKH (%)  | 44.75  |
| PİD     | $C$ (mg g <sup>-1</sup> )                          | 7.180  |
|         | $k_{id}$ (mg g <sup>-1</sup> min <sup>-1/2</sup> ) | 0.6081 |
|         | $R^2$  | 0.8560 |
|         | Adj $R^2$  | 0.8429 |
|         | RMSE   | 16.95  |
| Elovich | $a$ (mg g <sup>-1</sup> min <sup>-1</sup> )        | 17.09  |
|         | $\beta$ (g mg <sup>-1</sup> )                      | 0.351  |
|         | $R^2$  | 0.9663 |
|         | Adj $R^2$  | 0.9632 |
|         | RMSE   | 0.1599 |

Kinetik modellerden elde edilen istatistiklerin, deneylerden elde edilen verilerle uyumu regresyon katsayısı ( $R^2$ ), düzeltilmiş regresyon katsayısı (Adj $R^2$ ) ve ortalama karesel hata (RMSE) testleri ile analiz edilmiştir. Bu istatistiksel analiz, boya adsorpsiyonunun deneysel kinetik verilerinin, yüksek  $R^2$  ve düşük OBMH ve NKOKH değerlerine dayanarak sözde ikinci dereceden (SİD) kinetik modeline uygun olduğunu göstermiştir ( $R^2$ : 0.99, Düzeltmeyle Ayarlanmış  $R^2$ : 0.99 ve RMSE: 1.154). Bu model (SİD), aktif karbon yüzeyindeki Malahit yeşili adsorpsiyonunun kimyasal adsorpsiyon olduğunu göstermektedir (de Souza ve ark., 2022).



Şekil 7. PİD kinetik modelinin grafiği  
Figure 7. Plot of IPD kinetic model

Boya moleküllerinin adsorpsiyon hızı üzerindeki etkisi, PİD kinetik modeli kullanılarak değerlendirildi. Şekil 7'de, kinetik modelin grafiği gösterilmektedir. Bu grafik, Orijin üzerinden geçmemiştir ve tek bir çizgide değildir. Yapılan araştırma, boyanın adsorpsiyon mekanizmasının çeşitli hız kontrol adımlarından etkilendiğini göstermiştir (Li ve ark., 2018). Elovich modeli, moleküllerin değişen adsorpsiyon kapasitelerini dikkate alırken, zamana bağlı olarak değişen adsorpsiyon oranını açıklayan bir denklem üzerine kurulmuştur (Shahat ve ark., 2023).

#### Adsorpsiyon izoterm modelleri

Adsorpsiyona ait deneysel izoterm verileri Freundlich, Langmuir, Temkin ve D-R izoterm modelleri kullanılarak değerlendirilmiştir (Çizelge 3). Çizelge 3'te sunulan yüksek regresyon ( $R^2$ ) katsayılarına ve düşük OBMH ve NKOKH değerlerine dayanarak, adsorpsiyon denge verilerinin Langmuir izotermiyle etkili bir şekilde uyumlu olduğu gözlemlenebilir. Bu model, adsorpsiyonun homojen bölgelerde gerçekleştiğini önerdiğinden, tek katmanlı bir adsorpsiyon sürecini desteklemektedir (Zaini ve ark., 2023). En iyi modeli seçerken sadece  $R^2$  değerlerine bakmak yeterli değildir. En doğru kararı vermek için, denge verilerini hesaplanan maksimum adsorpsiyon kapasitesi ( $q_{max}$ ) ile karşılaştırmalıyız (Yağmur & Kaya 2021). Maksimum adsorpsiyon kapasitesi ( $q_{max}$ ) 69.06 mg g<sup>-1</sup> olarak bulunmuştur.

#### SONUÇ ve ÖNERİLER

Bu çalışmada, tarımsal atık olan pamuk sapları kullanılarak üretilen bir adsorbentten Malahit yeşili boyasının su ortamından giderimi detaylı bir şekilde incelenmiştir. Karakterizasyon bulguları, üretilen adsorbentin birçok çıkıntı ve boşluk içeren heterojen

bir yüzey morfolojisine ve zengin bir fonksiyonel grup özelliğine sahip olduğunu göstermektedir. Adsorpsiyon süreci, pH (2-10) ve çözelti başlangıç konsantrasyonu (50-250 mg L<sup>-1</sup>) parametrelerinin incelenmesi ile başlar. pH 8'in etkili adsorpsiyon için uygun olduğu bulunmuştur. Adsorpsiyon prosesinin kinetik ve denge modelleme çalışmaları, SİD ve Langmuir modellerinin adsorbentten boya giderim davranışını açıklamada en etkili modeller olduğunu göstermiştir.

Çizelge 3. Malahit yeşili adsorpsiyonu için izoterm değerlendirme sonuçları

Table 3. Kinetic evaluation results for malachite green adsorption

| Model                       | Parametre  | Değer                        |
|-----------------------------|--|------------------------------|
| Freundlich                  | $K_f$ (mg g <sup>-1</sup> (L mg <sup>-1</sup> ) <sup>1/n<sub>F</sub></sup> ) | 0.0358                       |
|                             | $n_F$  | 0.58                         |
|                             | $R^2$  | 0.9666                       |
|                             | Adj $R^2$  | 0.9555                       |
|                             | RMSE   | 0.0178                       |
|                             | OBMH (%)   | 114.87                       |
|                             | NKOKH (%)  | 85.11                        |
| Langmuir                    | $q_{max}$ (mg g <sup>-1</sup> )  | 69.06                        |
|                             | $R^2$  | 0.9966                       |
|                             | Adj $R^2$  | 0.9955                       |
|                             | RMSE   | 0.084                        |
|                             | OBMH (%)   | 62.13                        |
|                             | NKOKH (%)  | 46.22                        |
|                             | Temkin   | $B_T$ (J mol <sup>-1</sup> ) |
| $K_T$ (L mg <sup>-1</sup> ) |  | 0.030                        |
| $R^2$                       |  | 0.7942                       |
| Adj $R^2$                   |  | 0.7256                       |
| RMSE                        |  | 26.65                        |
| OBMH (%)                    |  | 101.55                       |
| NKOKH (%)                   |  | 98.23                        |
| D-R                         | $q_m$ (mg g <sup>-1</sup> )  | 107.88                       |
|                             | $E_{DR}$ (kJ mol <sup>-1</sup> )   | 36.73                        |
|                             | $R^2$  | 0.8126                       |
|                             | Adj $R^2$  | 0.7502                       |
|                             | RMSE   | 0.7505                       |
|                             | OBMH (%)   | 121.44                       |
|                             | NKOKH (%)  | 87.24                        |

Çizelge 4. Farklı aktif karbonların maksimum adsorpsiyon kapasiteleri

Table 4. Maximum adsorption capacity of different activated carbons

| Hammadde                                   | $q_{max}$ (mg g <sup>-1</sup> ) | Kaynak                    |
|--|---------------------------------|---------------------------|
| Pamuk Sapı                                 | 69.06                           | Bu çalışma                |
| Gat sapı ( <i>Catha edulis</i> )           | 5.62                            | (Abate ve ark., 2020)     |
| Hindistan cevizi lifi                      | 27.44                           | (Uma ve ark., 2013)       |
| Kavak ağacı talaşı                         | 15.0                            | (Yıldız ve ark., 2023)    |
| Irak hurması çekirdeği                     | 7.04                            | (Jawad & Khadim, 2022)    |
| Guava ( <i>Psidium guajava</i> ) tohumları | 31.82                           | (Elwardany ve ark., 2023) |
| Tohum kabuğu ( <i>Jatropha curcas</i> L.)  | 8.40                            | (Mohammad ve ark., 2018)  |

#### Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış

#### Karşılaştırma Çalışması

Çizelge 4, Malahit yeşili için Langmuir izoterm modeline dayalı olarak farklı hammaddelerden üretilen aktif karbonların maksimum adsorpsiyon kapasitelerini ( $q_{max}$ ) göstermektedir. Üretilen adsorban, yakın zamanda literatürde bildirilen diğer birçok adsorbandan daha yüksek malahit yeşili adsorpsiyon kapasitesine sahiptir. Bu çalışma, kirliliğin giderilmesi ve çevrenin korunmasının yanı sıra, atık yönetimi ve çevre dostu, uygun maliyetli ve verimli adsorbanların üretilebileceğini göstermiştir.

Ortalama adsorpsiyon serbest enerjisi ( $E_{DR}$ ) 36.73 kJ mol<sup>-1</sup> olarak hesaplanmıştır; bu, adsorpsiyon sürecinin kimyasal mekanizmalar tarafından yönlendirilebileceğini göstermektedir ( $E_{DR} = 1/\sqrt{2B}$ ). Bu değer,  $E_{DR}$  değeri, adsorpsiyon mekanizması hakkında bilgi sağlar;  $E_{DR}$  değerleri > 16 kJ mol<sup>-1</sup> kemisorpsiyonu gösterirken,  $E_{DR} < 8$  kJ mol<sup>-1</sup> fizisorpsiyon olarak kabul edilir (Fawzy ve ark., 2022).

Temkin modeli, tek katmanlı adsorbantın heterojen bölgeler üzerinde dağılımını, bağlanma enerjisinin düzgün dağılımını ve Langmuir modeli gibi adsorban ve çözünen madde arasında tutarlı bir etkileşimi karakterize eder. Temkin izotermine göre, 90.69 J mol<sup>-1</sup>lik ısı adsorpsiyonu ( $B_T$ ) değerinin pozitif olduğu gözlemlenmiştir, bu da adsorpsiyon sisteminin endotermik bir süreçten geçtiğini göstermektedir (Vargas ve ark., 2011; Wu ve ark., 2009).

Langmuir izotermine göre  $q_{max}$  69.06 mg g<sup>-1</sup> olarak belirlenmiştir. Atık pamuk sapından üretilen yeni adsorbent malzemenin atık sulardan sentetik boyaların gideriminde oldukça etkili olduğu görülmüştür. Bu adsorbent, farklı kirleticilerin giderilmesinde oldukça etkili olduğu kanıtlandığı için atık su kirliliği sorununa potansiyel bir çözüm olarak kullanılabilir. Sadece sentetik boyaların uzaklaştırılmasıyla sınırlı olmayıp, ağır metaller, farmasötikler ve organik bileşikler gibi diğer kirleticilerin atık sudan uzaklaştırılması için de kullanılabilir. Bu sayede, tarımsal atıkların yeniden değerlendirilmesiyle çevremizde bulunan kirletici miktarı önemli ölçüde azaltılabilir ve daha temiz ve sağlıklı bir geleceğe katkıda bulunabiliriz.

olduklarını beyan eder.

## Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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## Evaluation of the *in vitro* enzyme inhibition and antioxidant activity of *Clinopodium betulifolium* (Boiss. & Balansa) Kuntze

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### ABSTRACT

*Clinopodium betulifolium* (Boiss. & Balansa) Kuntze is a perennial herb belonging to the Lamiaceae family. There are few studies on *C. betulifolium*, except for its essential oil. In this study, Alzheimer's and cosmetic-related enzyme inhibitory activity and antioxidant activity of *C. betulifolium* species were evaluated. This study extracted *C. betulifolium* aerial parts by maceration using 70% methanol and water. Antioxidant [DPPH scavenging assay, ABTS cation decolorization, and iron chelating activity] and enzyme inhibition (acetyl-, and butyrylcholine esterase, and tyrosinase) activities of *C. betulifolium* extracts were evaluated using Elisa microplate reader at 2 mg mL<sup>-1</sup> stock concentration. *C. betulifolium* aqueous extract gave high antioxidant activity (IC<sub>50</sub>: 34.24 ± 5.01 µg mL<sup>-1</sup>) in the ABTS method, while its 70% methanol extract (IC<sub>50</sub>: 100.75 ± 2.62 µg mL<sup>-1</sup>) was higher than the aqueous extract (IC<sub>50</sub>: 131.83 ± 4.70 µg mL<sup>-1</sup>) in the DPPH method. *C. betulifolium* aqueous and 70% methanol extract have moderate anti-tyrosinase activity. Both 70% methanol and aqueous extracts showed similar and high activity against acetylcholinesterase with the IC<sub>50</sub> values of 73.94 ± 2.78 µg mL<sup>-1</sup> and 81.71 ± 9.38 µg mL<sup>-1</sup>, respectively. *C. betulifolium* 70% methanol extract (IC<sub>50</sub>: 64.08 ± 1.04 µg mL<sup>-1</sup>) showed higher inhibitory activity than the aqueous extract (IC<sub>50</sub>: 146.6 ± 8.27 µg mL<sup>-1</sup>) against butyrylcholinesterase. These results provide basic information for studies that will yield positive results in the development of pharmaceutical formulations or food supplements to be used to treat Alzheimer's and oxidative stress-related diseases.

### Botany

### Research Article

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## *Clinopodium betulifolium* (Boiss. & Balansa) Kuntze'nin *in vitro* enzim inhibisyonu ve antioksidan aktivitesinin değerlendirilmesi

### ÖZET

*Clinopodium betulifolium* (Boiss. & Balansa) Kuntze, Lamiaceae familyasına ait çok yıllık bir bitkidir. *C. betulifolium* üzerine uçucu yağ dışında pek fazla çalışma bulunmamaktadır. Bu çalışmada *C. betulifolium* türünün Alzheimer ve kozmetik ile ilişkili enzim inhibitör aktivitesi ve antioksidan aktivitesi değerlendirildi. Bu çalışmada *C. betulifolium*'un toprak üstü kısımlarından %70 metanol ve su kullanılarak maserasyon tekniği ile ekstraksiyon işlemi yapıldı. *C. betulifolium* ekstrelerinin antioksidan [DPPH radikal süpürücü aktivite, ABTS katyonik renk giderici ve demir şelatlama aktivitesi] ve enzim inhibisyonu (asetil/bütirilcholinesteraz ve tirozinaz) aktiviteleri, 2 mg mL<sup>-1</sup> stok konsantrasyonunda Elisa mikroparka okuyucusu kullanılarak değerlendirildi. *C. betulifolium* su ekstresi ABTS yönteminde yüksek antioksidan aktivite verirken (IC<sub>50</sub>: 34.24 ± 5.01 µg mL<sup>-1</sup>), DPPH yönteminde %70 metanol ekstresi (IC<sub>50</sub>: 100.75 ± 2.62 µg mL<sup>-1</sup>) sulu ekstresinden (IC<sub>50</sub>: 131,83 ± 4,70 µg mL<sup>-1</sup>) daha yüksek aktivitede bulunmuştur. *C. betulifolium* su ve %70 metanol ekstreleri orta derecede

### Botanik

### Araştırma Makalesi

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### Anahtar Kelimeler

*Clinopodium betulifolium*  
Antikolinesteraz  
Anti-tirozinaz  
Antioksidan aktivite



anti-tirozinaz aktivitesine sahiptir. Hem %70 metanol hem de su ekstraktları sırasıyla  $73,94 \pm 2,78 \mu\text{g mL}^{-1}$  ve  $81,71 \pm 9,38 \mu\text{g mL}^{-1}$  IC<sub>50</sub> değerleriyle asetilkolinesteraza karşı benzer ve yüksek aktivite gösterdi. *C. betulifolium* %70 metanol ekstresi (IC<sub>50</sub>:  $64,08 \pm 1,04 \mu\text{g mL}^{-1}$ ), bütirikolinesteraza karşı su ekstresinden (IC<sub>50</sub>:  $146,6 \pm 8,27 \mu\text{g mL}^{-1}$ ) daha yüksek inhibitör aktivite gösterdi. Bu sonuçlar Alzheimer ve oksidatif strese bağlı hastalıkların tedavisinde kullanılacak farmasötik formülasyonların veya gıda takviyelerinin geliştirilmesinde olumlu sonuçlar verecek çalışmalar için temel bilgiler sağlamaktadır.

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## INTRODUCTION

For the Lamiaceae family, Turkey is recognized as a significant center of biodiversity. The family is represented by 45 genera, 546 species, and 731 taxa in Turkey (Satil et al., 2011). The Lamiaceae family also includes the genus *Clinopodium* L, a few of which are found in Africa, tropical Asia, and Indo-Malaysia. Still, they are mostly distributed in the New World and temperate Eurasia (Kadereit, 2004).

The initial revision of the genus *Clinopodium* was carried out by Davis and Leblebici (Davis & Leblebici, 1982) for the "Flora of Turkey" and contained just two species. The number of recognized taxa in Turkey has increased to 38 as a result of the taxonomic investigations. *C. troodi* (Post) Govaerts subsp. *grandiflorum* (Hartvig and Å.Strid) Govaerts and *C. troodi* (Post) Govaerts subsp. *vardaranum* (Leblebici) Govaerts are endemic plants in Turkey and East Mediterranean elements (Ayla, 2017).

Several *Clinopodium* species have been studied for their biological and medical applications. This genus has been used for different infections in Ecuadorian folk medicine (De la Torre et al., 2008). Also, in Bulgaria, *C. vulgare* L. has been utilized for the healing of wounds and has shown anti-bacterial properties (Dzhambazov et al., 2002). In traditional medicine, aerial parts of *C. tomentosum* (Kunth) Govaerts have been used to treat respiratory diseases, inflammation, and gastrointestinal problems (Tubon et al., 2020). In Mersin, Turkey, *C. dolichodontum* (P.H.Davis) Bräuchler & Heubl is used for gallstones, analgesic, gastrointestinal pain, relaxant, cold, and flu (Sargin, 2015). *C. vulgare* L. subsp. *vulgare* is used for abdominal pain by wrapping the area in Izmit (Kizilarslan & Özhatay, 2012). *C. acinos* (L.) Kuntze, *C. congestum* (Boiss. & Hausskn. ex Boiss.) Kuntze, *C. graveolens* (M.Bieb.) Kuntze subsp. *graveolens* is used internally for flu and cold in various regions of Turkey. *C. dolichodontum* (P.H.Davis) Bräuchler & Heubl shortness of breath and eye diseases, *C. nepeta* (L.) Kuntze subsp. *glandulosum* (Req.) Govaert snake

bites, *C. serpyllifolium* (M.Bieb.) Kuntze subsp. *barbatum* (P.H.Davis) Bräuchler internally colic, externally antiseptic wound healing, *C. serpyllifolium* (M.Bieb.) Kuntze subsp. *brachycalyx* (P.H.Davis) Bräuchler diuretic, antiseptic, stomach ache, *C. serpyllifolium* (M.Bieb.) subsp. *fruticosum* (L.) Bräuchler is used for cough and stomach ache in Turkey (Selvi et al., 2022).

Previous phytochemical research on the *Clinopodium* species has revealed the presence of several components, including phenylpropanoids, flavonoids, triterpenoid saponins, and diterpenes, as well as fatty acids and essential oils, that are showing various biological effects (Sarıkurkcu et al., 2015; Zeng et al., 2016; Tubon et al., 2020). There are not many studies on *C. betulifolium* (Boiss. & Balansa) Kuntze, known as "kızıl fesleğen" in Turkey, except for taxonomical, and morphological studies, and essential oil research (Kürkçüoğlu et al., 2007; Sevim & Atila, 2009).

Antioxidants can protect the body from the damaging effects of free radicals and reactive oxygen species. They prevent the progression of many serious diseases. Researching alternative, and secure sources of antioxidants as well as looking for natural antioxidants, particularly those with a plant origin, have received a lot of attention in recent years (Gulcin, 2020). There are antioxidant activity studies on *C. sericeum*, *C. vulgare*, *C. nubigenum*, *C. brownei* essential oils (Tepe et al., 2007; Noriega et al., 2018; Benites et al., 2021; P. Noriega et al., 2023). It has been determined that the antioxidant activity of *C. nepeta* and *C. vulgare* extracts is strong (Beddiar et al., 2021; Bektašević et al., 2022).

Acetyl/butyrylcholinesterase are enzymes that break down acetylcholine, which is associated with Alzheimer's disease and plays an important role in the pathophysiology of the disease (Koçyiğit et al., 2022). The disease, which is especially common in the elderly population, has become a major health problem. Therefore, the discovery of new cholinesterase inhibitors is important in the treatment of the disease

(Erdogan Orhan et al., 2015; Güçlü et al., 2022). Tyrosinase (TYR) is a copper-containing enzyme that plays a role in the biosynthesis of melanin dark pigment, and TYR inhibitors are being investigated as skin-whitening agents in the cosmetic industry (Qian et al., 2020). Among *Clinopodium* species, the enzyme inhibition effects of *C. vulgare* against acetylcholinesterase, *C. nepeta* against cholinesterase and TYR enzyme, and *C. gilliesii* (Benth.) Kuntze against cholinesterase was evaluated (Beddiar et al., 2021; Bektašević et al., 2022; Fernández-Galleguillos et al., 2023).

*Clinopodium* has potential therapeutic activities including hemostasis, anti-bacteria, anti-inflammation, immunoregulation, lowering blood glucose, antioxidation, and anti-tumor (Yao et al., 2020). Although the traditional uses of the *Clinopodium* genus have been reported, research on the phytochemical investigation and biological activity of this genus is still needed. A comprehensive study regarding the biological effects of *C. betulifolium* has not been published until now. With the aim of searching for the importance of *C. betulifolium* in pharmacognosy, the bioactivity examinations on the aerial parts of *C. betulifolium* were carried out for the first time. The extracts with different polarity solvents of *C. betulifolium* were evaluated through antioxidant [1,1-Diphenyl-2-picrylhydrazyl (DPPH) quenching assay and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation decolorization, and iron chelating activity] and enzyme inhibition (acetyl-, and butyrylcholine esterase, and tyrosinase) activities.

## MATERIALS and METHODS

### Plant Material

*C. betulifolium* was collected from Mersin (C4 Mersin: Taurus Mountains, Güzeldere valley, 50m above the rock; 20.06.2018; Herbarium No: S Dogu & Y Bagci 3076) and identified by Prof. Dr. Yavuz Bagci.

### Extraction Methods

The shade-dried and powdered aerial parts of *C. betulifolium* were separately macerated with 70% methanol and water 3 times. Macerates were filtered through pleated filter paper and evaporated to dryness with a Rotary evaporator at 40°C. The extracts were stored in the refrigerator for biological activity experiments.

### Total Phenol and Flavonoid Determination

The Folin Ciocalteu (F-C) method was employed to measure total phenol content (TPC) in the extracts (Clarke et al. 2013). The calibration curve of absorbance versus concentration was determined to be mg gallic acid equivalent (GAE) g<sup>-1</sup> extract for all analyses, which were carried out in triplicate. Aluminium chloride colorimetric method was used to

measure the total flavonoid content (TFC) in the extracts. The findings were expressed as mg quercetin equivalent (QE) g<sup>-1</sup> extract (H. Yang et al., 2011).

### Antioxidants Assay

For the ABTS radical scavenging activity of the extracts, 15 mL of 7 mM ABTS and 264 µL of 140 mM potassium persulfate solution are combined to create ABTS•+ radical stock solution. The working solution for ABTS•+ is prepared to provide an absorbance of 0.70 ± 0.02 at 734 nm. 50 µL of sample is combined with 100 µL of ABTS•+ working solution. After mixing for 10 minutes, absorbance is measured at 734 nm (Re et al., 1999). After adding 20 µL of test solution on a 96-well plate, 180 µL of DPPH solution is added. Using an Elisa reader (Multiscan Sky, USA), the plate is measured at 540 nm after 15 min incubation at 25°C in the dark (Clarke et al. 2013). Ascorbic acid was used as a positive control in DPPH and ABTS assay. The iron chelating activity of the extracts was assessed by the interaction of ferrosine-Fe<sup>2+</sup> complex. Ethylenediaminetetraacetic acid (EDTA) was used as positive control (Chai et al., 2014). All the antioxidant activity analyses were performed in triplicate.

### Enzyme Inhibition Assay

The inhibition activity of acetyl- and butyrylcholinesterase enzymes was conducted out according to the Ellman et al.'s approach with some modifications. 10 µL of 3 mM 5,5'-dithio-bisnitrobenzoic acid (DTNB); 140 µL of 0.1 mM phosphate buffer (pH 6.8); 20 µL of the enzyme (0.22 U mL<sup>-1</sup> for acetylcholinesterase/0.1 U mL<sup>-1</sup> for butyrylcholinesterase) produced in phosphate buffer; and 20 µL of test sample/reference standard of varied quantities. 10 µL of the substrate (0.71 mM acetylthiocholine iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer) was added to start the reaction. At 412 nm, absorbance was measured (Epoch, USA). Galantamine served as the positive reference (Ellman et al., 1961; Šinko et al., 2007). According to Yang et al.'s approach, the extracts' TYR inhibitor activities were assessed. To summarize, 20 µL of the sample solution, 20 µL of the enzyme solution (20 µL of mushroom tyrosinase in phosphate buffer), and 100 µL of phosphate buffer (0.1 M, pH 6.8) were added to a 96-well plate. The mixture was then incubated for 30 minutes at 25°C after 20 µL of a 3 mM L-tyrosine solution prepared in phosphate buffer was added as the substrate. At 492 nm, the absorbance was measured using a microplate reader. Kojic acid served as the positive reference (Yang et al., 2012). All the enzyme inhibition analyses were performed in triplicate.

### Statistical analysis

The data are represented as the mean ± standard

deviation, with each analysis performed in triplicate. Statistical significance was set at  $p < 0.05$ .

## RESULTS and DISCUSSION

In this study, *C. betulifolium* total phenol quantification was found to be  $40.48 \pm 1.51$ ; and  $56.37 \pm 3.70$  mg GAE  $g^{-1}$  extract in 70% methanol and aqueous extracts, respectively. TFC content of *C. betulifolium* 70% methanol extract ( $3.19 \pm 4.98$  mg QE  $g^{-1}$  extract) was found quercetin equivalent. In addition, free radical scavenging and iron chelating activities of *C. betulifolium* extracts were investigated.  $IC_{50}$ , or half-maximal inhibitory concentration, is the concentration of an extract required to scavenge free radical by 50%. The lower the  $IC_{50}$  value calculated in the antioxidant activity assays, the higher the antioxidant activity. As a result, *C. betulifolium* aqueous extract showed higher antioxidant activity ( $IC_{50}$ :  $34.24 \pm 5.01$   $\mu g mL^{-1}$ ) than its 70% methanol extract ( $IC_{50}$ :  $62.65 \pm 0.68$   $\mu g mL^{-1}$ ). In the ABTS method, while its 70% methanol extract ( $IC_{50}$ :  $100.75 \pm 2.62$   $\mu g mL^{-1}$ ) exhibited higher DPPH radical scavenging activity than its aqueous extract ( $IC_{50}$ :  $131.83 \pm 4.70$   $\mu g mL^{-1}$ ). Iron chelation activities of *C. betulifolium* extracts were found close to each other and low (Table 1). Additionally, the findings on the extracts appear to have a strong positive correlation

between TPC and DPPH, and ABTS radical scavenging activities (Figure 1).

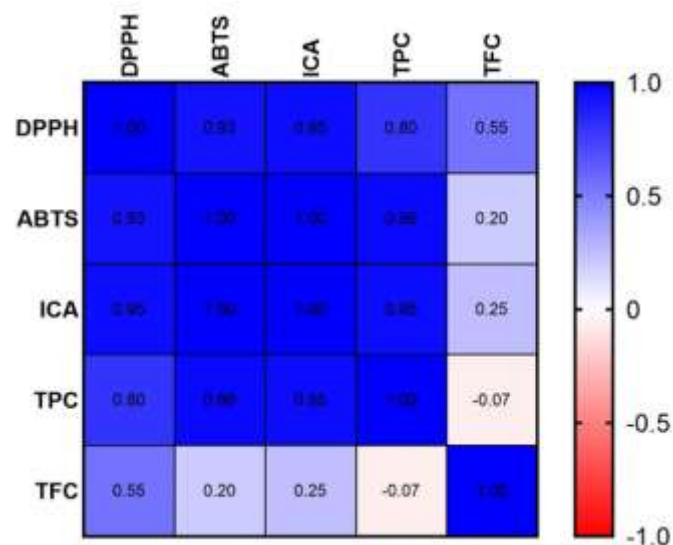


Figure 1. Heatmap of correlations between the analyzed antioxidant parameters\*

Şekil 1. Analizlerin antioksidan parametreleri arasındaki korelasyonların ısı haritası

\*DPPH ( $r = 0.80$ ,  $r = 0.55$ ,  $p < 0.0001$ ) and ABTS ( $r = 0.96$ ,  $r = 0.20$   $p < 0.0001$ ) radical scavenging, and iron chelating ( $r = 0.95$ ,  $r = 0.25$ ,  $p < 0.0001$ ) activities.

Table 1. Extract yield, total phenol and flavonoid contents, and antioxidant activity of *C. betulifolium* extracts.  
Çizelge 1. *C. betulifolium* ekstrelerinin ekstre verimi, toplam fenol ve flavanoit içerikleri ve antioksidan aktivitesi

| Plant extract                               | Extract yield %g $g^{-1}$ | TPC mg GAE $g^{-1}$ extract | TFC mg QE $g^{-1}$ extracts | ABTS (inhibition percentage $\pm$ S.D.) 2 mg $mL^{-1}$                     | DPPH (inhibition percentage $\pm$ S.D.) 2 mg $mL^{-1}$                      | Iron-chelating activities (inhibition percentage $\pm$ S.D.) 2 mg $mL^{-1}$ |
|---|---------------------------|-----------------------------|-----------------------------|--|---|---|
| <i>C. betulifolium</i> 70% methanol extract | 5.24                      | $40.48 \pm 1.51$            | $3.19 \pm 4.98$             | $86.15 \pm 0.28^{***}$<br>( $IC_{50}$ : $62.65 \pm 0.68$ $\mu g mL^{-1}$ ) | $83.84 \pm 0.53^{***}$<br>( $IC_{50}$ : $100.75 \pm 2.62$ $\mu g mL^{-1}$ ) | $9.93 \pm 2.66^{**}$  |
| <i>C. betulifolium</i> aqueous extract      | 14.03                     | $56.37 \pm 3.70$            | -                           | $87.17 \pm 0.28^{***}$<br>( $IC_{50}$ : $34.24 \pm 5.01$ $\mu g mL^{-1}$ ) | $53.67 \pm 3.69^{***}$<br>( $IC_{50}$ : $131.83 \pm 4.70$ $\mu g mL^{-1}$ ) | $9.55 \pm 3.17^{**}$  |
| Ascorbic acid (2 mg $mL^{-1}$ )             | -                         | -                           | -                           | $87.51 \pm 0.17^{***}$   | $93.91 \pm 0.14^{***}$  | -   |
| EDTA (2 mg $mL^{-1}$ )                      | -                         | -                           | -                           | -  | -   | $87.06 \pm 0.34^{***}$  |

The data are represented as the mean  $\pm$  standard deviation of three replicates. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

In the results on enzyme inhibition activity, *C. betulifolium* aqueous (41.18%) and 70% methanol (29.73%) extracts have moderate anti-TYR activities. Both extracts similarly showed high inhibition activities against acetylcholinesterase ( $IC_{50}$  values of 70% methanol extract and aqueous extracts:  $73.94 \pm 2.78$ , and  $81.71 \pm 9.38$   $\mu g mL^{-1}$ , respectively). *C. betulifolium* 70% methanol extract ( $IC_{50}$ :  $64.08 \pm 1.04$   $\mu g mL^{-1}$ ) showed higher inhibitory activity than the aqueous extract ( $IC_{50}$ :  $146.6 \pm 8.27$   $\mu g mL^{-1}$ ) against butyrylcholinesterase (Table 2).

Free radicals and reactive oxygen species cause diseases such as neurodegenerative, atherosclerosis, cancer, and inflammatory disorders by growing damage to important macromolecules such as DNA, protein, and lipid (Na et al., 2011). Studies have proven that plant extracts and natural ingredients reverse the negative effects of these radicals. Antioxidant activity studies are particularly common on phenolic compounds and there are very few studies on other classes of natural products as antioxidant agents (El-Sayed et al., 2008)



In a study on *Clinopodium* genus, the antioxidant effect of *C. nepeta* extracts was evaluated by six methods (DPPH, ABTS<sup>+</sup>, GOR, CUPRAC, Phenanthroline, FRAP), where the BuOH extract was the most active. Phenolic compounds such as apigenin (21.75 ± 0.41 µg g<sup>-1</sup>), myricetin (72.58 ± 0.57 µg g<sup>-1</sup>), and rosmarinic acid (88.51 ± 0.55 µg g<sup>-1</sup>) were detected in *C. nepeta* (Beddiar et al., 2021). Contraversely, the

antioxidant activities of *C. nepeta* BuOH extract (DPPH• IC<sub>50</sub>: 8.12 ± 0.11 µg mL<sup>-1</sup>; ABTS•<sup>+</sup> IC<sub>50</sub>: 12.82 ± 2.62 µg mL<sup>-1</sup>) was found to be high than antioxidant activities of *C. betulifolium* MeOH and water extracts (DPPH• IC<sub>50</sub>: 100.75 ± 2.62 µg mL<sup>-1</sup>, 131.83 ± 4.70 µg mL<sup>-1</sup>; ABTS•<sup>+</sup> IC<sub>50</sub>: 62.65 ± 0.68 µg mL<sup>-1</sup>, 34.24 ± 5.01 µg mL<sup>-1</sup>), respectively.

Table 2. Enzyme inhibition activity of *C. betulifolium* extracts.

Çizelge 2. *C. betulifolium* ekstralarının enzim inhibisyonu.

| Plant extract                                  | TYR<br>(percentage ± S.D. <sup>a</sup> )<br>2 mg mL <sup>-1 b</sup> | AChE<br>(percentage ± S.D. <sup>a</sup> )<br>2 mg mL <sup>-1 b</sup>      | BChE<br>(percentage ± S.D. <sup>a</sup> )<br>2 mg mL <sup>-1 b</sup>      |
|--|---|---|---|
| <i>C. betulifolium</i><br>70% methanol extract | 29.73 ± 0.33***   | 72.60 ± 5.87***<br>(IC <sub>50</sub> : 73.94 ± 2.78 µg mL <sup>-1</sup> ) | 89.24 ± 3.44***<br>(IC <sub>50</sub> : 64.08 ± 1.04 µg mL <sup>-1</sup> ) |
| <i>C. betulifolium</i><br>aqueous extract      | 41.18 ± 2.17***   | 81.62 ± 5.20***<br>(IC <sub>50</sub> : 81.71 ± 9.38 µg mL <sup>-1</sup> ) | 61.82 ± 2.80***<br>(IC <sub>50</sub> : 146.6 ± 8.27 µg mL <sup>-1</sup> ) |
| Kojic acid                                     | 80.96 ± 0.51***   | -   | -   |
| Galantamine<br>hydrobromide                    | -   | 99.10 ± 1.18***   | 84.34 ± 4.85***   |

<sup>a</sup> Standard deviation, <sup>b</sup> Stock concentration. The data are represented as the mean ± standard deviation of three replicates. \*p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

In another study on *C. vulgare*, the total phenolic content, DPPH, and FRAP values of the extract were found 27.9 ± 0.4 mg gallic acid equivalents per g sample, 0.114 ± 0.0004 mg mL<sup>-1</sup>, and 1556 ± 3 µM trolox equivalents per g sample, respectively. Bektašević et al. examined the chemical composition of *C. vulgare* hot water and methanol extract using spectroscopic and HPLC/DAD techniques. Among sixteen identified and quantified phenolic compounds the dominant compounds were rosmarinic (26.63 and 34.21 mg g<sup>-1</sup>) and ellagic acid (23.11 and 29.31 mg g<sup>-1</sup>) of hot water and methanol extract, respectively (Bektašević et al., 2022). The TPC content of *C. vulgare* hot water (145.5 ± 4.2 mg GAE g<sup>-1</sup> extract) and methanol (170.0 ± 3.9 mg GAE g<sup>-1</sup> extract) extracts was higher than the TPC content of our extracts. Controversially, *C. vulgare* methanol extract was shown higher TPC content than water extract, while *C. betulifolium* water extract was found higher methanol content.

*C. nepeta* extracts showed moderate inhibition on acetylcholinesterase, butyrylcholinesterase, tyrosinase, and α-amylase. *C. nepeta* dichloromethane extract gave the highest activity in both acetyl (IC<sub>50</sub>: 170.1 ± 1.58 µg mL<sup>-1</sup>), and butyrylcholinesterase (IC<sub>50</sub>: 73.06 ± 0.83 µg mL<sup>-1</sup>) (Beddiar et al., 2021). *C. betulifolium* methanol extract showed higher activity against cholinesterase than *C. nepeta* dichloromethane extract. IC<sub>50</sub> values of all *C. nepeta* extracts against TYR were higher than 200 µg mL<sup>-1</sup>. Since no inhibition above 50% occurred in this *C. betulifolium* extract, IC<sub>50</sub> could not be calculated. Hot

water and methanol extract of *C. vulgare* were not inhibited against butyrylcholinesterase. Acetylcholinesterase inhibition of *C. vulgare* extracts was weak (Sarikurkcü et al., 2015). *C. vulgare* subsp. *vulgare* methanol and water extracts had no TYR enzyme inhibitory activity. In this study, *C. betulifolium*, methanol (29.73 ± 0.33%), and water extracts (41.18 ± 2.17%) had moderate inhibitory activity.

## CONCLUSIONS

In this study, enzyme inhibition and antioxidant activities of *C. betulifolium* were carried out for the first time. *C. betulifolium* extracts showed remarkable radical scavenging activity in both ABTS and DPPH methods. On the contrary, iron chelating capacity of *C. betulifolium* extracts was found to be low. Moreover, *C. betulifolium* extracts showed moderate anti-TYR inhibitory activity. Otherwise, *C. betulifolium* extracts showed significant inhibitory activity against Alzheimer-related enzymes. As a conclusion, our findings revealed that 70% methanol extract of *C. betulifolium* may be promising natural source to conduct advanced studies. The following study will be on the determination the phytochemical contents of *C. betulifolium* and identification of the phytoconstituents, responsible for the mentioned biological activities.

## 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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## Myxobiota of the İskenderun Gulf (Mediterranean Sea/Türkiye) and its Environment

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### ABSTRACT

This study aims to determine the myxobiota of the İskenderun Gulf and its environment. This study was carried out on samples collected from 9 different stations in the İskenderun Gulf district in 2019-2022. Myxomycetes samples were collected from leaves, tree bark, and decayed and non-rotting plant materials. This aim is to detect both the myxobiota of this region and the halophytic myxomycetes species. The collected samples have developed myxomycete sporophores by using the moist chamber technique. In addition, myxomycetes which were developed in their natural environment were collected. As a result of the field and laboratory studies, 161 samples from different localities were collected and processed and 111 myxomycete samples were obtained from these samples. 41 species belonging to 6 ordo, 12 families, and 20 genera have been defined. 3 species were obtained only from the natural environment, 30 species were obtained only from moist chamber culture, and 8 species were obtained from both moist chamber culture and natural area. *Arcyria cinerea*, *Didymium difforme*, *D. squamulosum*, and *Physarum pusillum* were determined as abundant. While the number of species is rare in the seaside regions, it was observed that the further away from the sea, the number of species increased, especially in forest areas. *A. cinerea*, *D. difforme*, and *D. dubium* are the most common species on the beach and near the seaside. While cosmopolitan species were observed in abundance, Physarida members were found to be common in this results area. This study has contributed to the myxobiota of Türkiye.

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## İskenderun Körfezi (Akdeniz/Türkiye) ve Çevresinin Myxobiota'sı

### ÖZET

Bu çalışma İskenderun Körfezi ve çevresinin mikrobiyotasının belirlenmesini amaçlamaktadır. Bu çalışma 2019-2022 yıllarında İskenderun Körfezi ve çevresinde 9 farklı istasyondan toplanan numuneler üzerinde yapılmıştır. Yaprak, ağaç kabuğu, çürümüş ve çürümeyen bitki materyalleri üzerinden miksomiset örnekleri toplandı. Amacımız hem bu bölgenin Myxobiota'sını hem de halofitik miksomiset türlerini tespit etmektir. Toplanan örneklerde nemli oda tekniği kullanılarak miksomiset sporoforları geliştirilmiştir. Ayrıca doğal ortamlarında gelişen miksomisetler de toplanmıştır. Arazi ve laboratuvar çalışmaları sonucunda farklı lokalitelerden 161 adet örnek toplanarak işlenmiş ve bu örneklerden 111 adet miksomiset örneği elde edilmiştir. 6 ordo, 12 familya ve 20 cinse ait 41 tür tanımlanmıştır. 3 tür sadece doğal ortamdan, 30 tür sadece nemli oda kültüründen, 8 tür hem nemli oda kültürü hem de doğal ortamdan elde edilmiştir. *Arcyria cinerea*, *Didymium difforme*, *D. squamulosum* ve *Physarum pusillum* bol olarak belirlendi. Denize kıyaslı olan bölgelerde tür sayısı az bulunurken, denizden uzaklaştıkça özellikle ormanlık alanlarda tür sayısının arttığı gözlemlendi. *A. cinerea*, *D. difforme* ve *D. dubium*, sahilde ve deniz kıyısına yakın yerlerde en yaygın türlerdir. Kozmopolit türler bol miktarda gözlenirken çalışma alanımızda Physarida üyelerinin de yaygın olduğu görülmüştür. Bu çalışmada Türkiye mikrobiyotasına katkı sağlanmıştır.

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## INTRODUCTION

Myxomycetes have been known worldwide for 300 years and 6 orders, 14 families, 68 genera, and approximately 1100 species have been identified until today (Lado, 2005-2023). Studies on myxomycetes first began with the description of the *Lycogala epidendrum* by Pankow in 1654. In 1887, De Bary distinguished between true slime molds and cellular slime molds according to plasmodium formation. This researcher showed both groups within Mycetozoa, reporting that true slime molds were closer to Protozoa than fungi (Alexopoulos et al., 1996). Plasmodial slime molds, also referred to as acellular or true slime molds, are considered a monophyletic taxon. In the current classification of living organisms, they are placed in the phylum Amoebozoa of the kingdom Protozoa as the class Myxomycetes or Myxogastrea (Adl et al., 2019). They are a group of amoeboid eukaryotes that produce fungus-like fruiting bodies (Leontyev et al., 2019). De Bary (1859) was the first to discover some protozoan properties of such organisms, calling them Mycetozoa (Rao & Chen, 2023). Myxomycetes are a group of eukaryotic microorganisms in terrestrial ecosystems, that are common in bark, woody debris, litter, lianas, bryophytes, dung, and soil (Liu et al., 2015). They are currently recorded in tropical, subtropical, temperate, and boreal forests, tundra, grasslands, deserts, and alpine snowbanks (Schnittler et al., 2017). However, in terms of the distribution of the world's climatic zones, the distribution in tropical and temperate climates is far more extensive than that of subtropical humid climates, and the species of myxomycetes in subtropical forests have not been sufficiently studied. Subtropical regions have a humid climate and diverse vegetation, and they are located in the transition region between tropics and temperate zones. Therefore, they should have abundant resources of myxomycete species (Li et al., 2021). The main influencing factors of species diversity of myxomycetes are divided into abiotic factors, such as temperature, humidity, altitude, water retention, and pH, and biological factors, such as vegetation type, bacterial composition, fungal composition (Liu et al., 2015; Xavier de Lima & de Holanda Cavalcanti, 2015).

Türkiye shows different climatic, topographic, and geological differences, it is located in an area where three different geographical regions such as the Mediterranean, Iran-Turan, and Euro-Siberia intersect, is in the transition zone between the European and Asian continents, is a gene center for many living things, it is rich in biodiversity since it has different habitats such as lakes, streams, swamps,

mountains, and plains (FAO, 2019). Now the number of species has reached 309 today. These species belong to 6 orders, 13 families, and 45 genera (Baba & Sevindik, 2023). The Mediterranean Region is one of the seven geographical regions of Türkiye. It stretches along the Mediterranean coast in the south of Anatolia. İskenderun Gulf is the easternmost point of the Mediterranean, nestled between the provinces of Hatay and Adana (Figure 1). There are 9 districts in the Gulf, namely Karataş, Yumurtalık, and Ceyhan belonging to the province of Adana, and Erzin, Dört Yol, Payas, İskenderun, Belen, and Arsuz belonging to the province of Hatay.



Figure 1. Study area  
Şekil 1. Çalışma alanı

The town of Arsuz (36°24'43.88" N - 35°53'25.11" E) is located between the Amanos Mountains and the Mediterranean coast. District lands are generally covered with flat areas. High hills, valleys, and plateaus are covered with forests in the parts close to the Amanos Mountains. 50.5% of Arsuz district lands are mountains, 45.7% plains, and 5.3% plateaus. The highest point within the borders of the Arsuz district is Daz Hill with 1.755 meters and Çobandede Hill with a height of 1.722 meters. The most important streams of the district are Zilli and Arsuz Streams (Anonymous, 2021a).

Belen (36°27'36.90" N - 36°09'14.20" E) is a district of Hatay province in the Adana Section of the Mediterranean Region. Belen Pass, whose coordinates and altitude are one of the most important passes in Türkiye, is within the borders of the district and has rich forest areas. The altitude of the district center, which is 50 km away from Antakya, is approximately 700 meters. Since Belen is located within the borders of the Mediterranean Region, the air masses and



movements that are effective in the Mediterranean Region also affect the climatic characteristics of the district. The annual average temperature in Belen district is 16.8°C. The annual average rainfall is 697.5 mm in Belen. The month with the highest average precipitation in Belen is December (Anonymous, 2021a).

İskenderun district (36°35'04.47" N - 36°10'36.10" E) was established on the skirts of the Nur Mountains, on an area of 5 km<sup>2</sup>, on the edge of the Mediterranean. Coordinates and altitude Mediterranean climate are observed in İskenderun. The warmest month average is 32-34 °C, the coldest month average is 10-12 °C. The annual average temperature is 18 °C. The most precipitation falls in the winter, the least in the summer. Annual rainfall varies according to altitude. The average is between 600-1000 mm (Anonymous, 2021a).

Payas (36°44'39.42" N - 36°17'04.98" E) district center was established on a flat area on the Mediterranean coast. *Citrus* spp. production and agriculture are widespread. Its coordinates and altitude The most important streams of the district are Kozludere and Payas Streams. The Mediterranean climate is seen in the Payas district. Summers are hot and dry, and winters are cold and rainy (Anonymous, 2021a).

The district of Dörtüol (36°49'07.96" N - 36°17'10.17" E) is located in the North-South direction between the Nur Mountains (Amanos), which is the extension of the Eastern Taurus Mountains, and the İskenderun Gulf of the Mediterranean Sea, and consists of the Payas Alluvial plains. In its coordinates and altitude the climate is hot and dry in summer, mild and rainy in winter, and a typical Mediterranean climate prevails. Precipitation in the district is in the form of rain and it is one of the centers that receive the most precipitation after Rize in Türkiye. In the district, snow falls in high mountain areas (Anonymous, 2021a).

Erzin (36°57'13.90" N - 36°12'18.82" E) district, located on the western skirt of the Amanos mountains in the north of Hatay province, facing the Mediterranean Sea, in the triangle of Erzin, Adana Osmaniye Hatay, with its back abutting the Amanos mountains and its feet extending towards the Mediterranean. Coordinates and altitude in the district, which has a warm and rainy climate in winter, the temperature and humidity increase in summer. *Citrus* spp. production is widespread in Erzin, whose economy is largely based on agriculture (Anonymous, 2021a).

The town of Ceyhan (37°01'43.58" N - 35°48'44.88" E) is 43 km from Adana and 30 km from the Mediterranean. Coordinates away and altitude Climate is a typical Mediterranean climate. The amount of precipitation in the Ceyhan Plain is 600–750 mm. While it is between 750–1000 mm in mountainous areas, 50% of precipitation falls in winter, 27% in

spring, 18% in autumn, and 5% in summer. The most important stream, the Ceyhan River, passes by the district and its length is 509 km. The vegetation of Ceyhan consists of maquis. Since Ceyhan is a large plain, forest areas have been destroyed and turned into agricultural areas. Located in the Ceyhan district of Adana, Botaş was established on the beach by the sea, and its beach is 50 meters wide and 400 meters long (Anonymous, 2021b).

Yumurtalık (36°46'07.05" N - 35°47'17.56" E) district, 81 km from Adana. It is located on the western shores of İskenderun Gulf in the southwest. It is a coastal settlement surrounded by the Mediterranean to the east and south of the district. Coordinates and altitude located in the center of the district, there is an extremely clear and clean natural beach, which is especially popular with local tourists in summer. The district also has a wide bay that gets shallower as you go in and consists of swamps in places. Yumurtalık Lagoon National Park, established on 16 December 2008, is a former National Park and today a nature conservation area in the Yumurtalık district of Adana province in the Mediterranean Region of Türkiye. The lagoon has a total area of 16,430 hectares. It creates a protected natural environment for the plant and animal species in the lake lagoons on the Seyhan-Ceyhan delta, especially for the rare plant species known as Aleppo pine (*Pinus halepensis* Mill.) (Anonymous, 2021b).

The district of Karataş (36°39'52.37" N - 35°15'33.21" E) was established within the natural borders of the Seyhan and Ceyhan rivers in the Eastern Mediterranean region of Adana. The lands of the district are located in the southern part of Adana Province, which protrudes towards the Mediterranean. The lands of the district, whose coordinates and altitude are located in Çukurova, have a completely flat plain land structure. The district has natural beaches on the Mediterranean coast. There are lagoons between the coastal dune banks and the sea (Anonymous, 2021b).

In this context, it has been determined that no studies have been carried out on myxomycetes, especially on the coastline. In this study, the myxomycete diversity of the research area was revealed, and the species that spread in saline environments were determined in line with the results obtained.

## MATERIAL and METHOD

### Research Area

9 districts located in the Gulf of İskenderun, especially in the coastal area, are homogeneously determined (Konacık, Arsuz, Büyükdere, Belen, Karaağaç, İskenderun, Sariseki, Denizciler, Azganlık, Karayılan, Payas, Yakacık, Dörtüol, Yeşilkent, Yeni yurt, Kurtkulağı village, Ceyhan, Botaş coast, Erzin free

zone, Yumurtalık, Yumurtalık lagoon forest and Karataş) plant substrates and natural samples collected especially from the seaside and its immediate surroundings were used as materials. Trips were organized to the land between February 2019 and January 2022, covering all four seasons.

### Samplings and Identifications

In the context of field studies, various samples of myxomycetes were gathered from different regions within the İskenderun Gulf district. These samples were carefully extracted from their original environments using a cutting tool and subsequently transported to the laboratory in small carton boxes. Samples were collected from various sources, such as tree bark, cut tree stumps, rotting leaves, twigs, cones, fruits, and vegetable residues, with the intention of excluding myxomycetes and sporophore materials. These samples were carefully placed in individual lock storage bags and subsequently transported to the laboratory for further analysis. Subsequently, within the laboratory setting, the researchers successfully achieved fructification formation using the Moist Chamber Technique, as established by Gilbert & Martin in 1933. In the application of the Moist Chamber Technique, a dual layer of sterile filter paper was positioned a top of the petri dishes or transparent storage containers. Subsequently, the samples were placed on the filter paper, followed by the addition of distilled water. The anticipated outcome was the gradual expansion of the samples throughout 24 to 48 hours. Efforts have been made to acquire sporophores through the utilization of a stereomicroscope, with periodic observations conducted under diffuse lighting conditions. The samples were prepared by placing one or two layers of blotting paper in the petri dishes containing storage containers, followed by air-drying at ambient temperature. Following the completion of the drying process, the material transformed fungarium material (Baba et al., 2020). The study involved a comprehensive investigation of various aspects of the spore, including its general structure, shape, color, macroscopic measurements, capillitium, presence of pseudocapillitium and columella (if present), as well as detailed analysis of its shape, measurements, color, size, and spore ornamentations. The identification of specimens was conducted through the utilization of reference books (Martin & Alexopoulos, 1969; Farr, 1981; Thind, 1977; Martin et al., 1983; Neubert et al., 1993; Neubert et al., 1995; Neubert et al., 2000; Stephenson & Stempen, 1994; Lado & Pando, 1997; Ing, 1999). The fungarium materials of the identified samples are stored in the laboratory of the Department of Biology of HMKU Faculty of Science and Arts.

## RESULTS and DISCUSSION

In the study conducted in the district of İskenderun Gulf (Adana, Hatay) in 2019-2021, 161 samples from different localities were collected and processed in the laboratory. A total of 111 myxomycete samples were obtained from these samples. As a result of the identification of myxomycete samples obtained from the natural environment and moist chamber culture, 41 species belonging to 6 ordos, 12 families and 20 genera have been defined. 3 species were obtained only from the natural environment, 30 species were obtained only from moist chamber culture, and 8 species were obtained from both moist chamber culture and natural area.

The taxa determined by the field studies are listed. Habitat, geographical location, sample numbers, way of obtaining methods, and substrates of the samples are indicated.

### Annotated List of Species

Domain: Eukaryota  
Kingdom: Protista  
Phylum: Amoebozoa  
Infraphylum: Mycetozoa

1. Classis: Protostelia  
Order: Protosteliida  
Family Ceratiomyxaceae  
Genus: *Ceratiomyxa*
    1. *Ceratiomyxa fruticulosa* (O.F. Müll.) T. Macbr.  
Distribution of species: Arsuz, on wood, Baba 10.
  2. Classis: Myxogastria or Myxomycetes  
Order: Echinosteliida  
Family Echinosteliaceae  
Genus: *Echinostelium*
    2. *Echinostelium minutum* de Bary  
Distribution of species: Karatas beach, on wood, Baba 137; Yumurtalık lagoon forest, on debris, Baba 102.
- Order Liceida  
Family Cribrariaceae  
Genus: *Cribraria*
  3. *Cribraria argillaceae* (Pers. ex J.F. Gmel.) Pers.  
Distribution of species: Karataş beach, on wood, Baba 147.
  4. *C. violaceae* Rex  
Distribution of species: Yumurtalık lagoon forest, on wood, Baba 100.
- Family Dictydiaethaliaceae  
Genus: *Dictydiaethalium*
  5. *Dictydiaethalium plumbeum* (Schumach.) Rostaf.  
Distribution of species: Arsuz, on bark, Baba 6; Karatas beach, on wood, 129; Kurtkulağı village

Ceyhan, on wood, Baba 85.

Family Liceaceae

Genus: *Licea*

6. *Licea kleistobolus* G.W. Martin

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 99.

Family Reticulariaceae

Genus: *Lycogala*

7. *Lycogala epidendrum* (L.) Fr.

Distribution of species: Yumurtalık lagoon forest, on wood, natural, Baba 96.

Order Trichiida

Family Arcyriaceae

Genus: *Arcyria*

8. *Arcyria cinerea* (Bull.) Pers.

Distribution of species: Karaağaç, on wood, natural, Baba 15; Konacık, on wood, natural, Baba 106; Yumurtalık lagoon forest, on wood, Baba 92, 95, 98; on debris, Baba 92, 98, 99, 104.

9. *A. incarnata* (Pers. ex J.F. Gmel.) Pers.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 105.

10. *A. insignis* Kalchbr. & Cooke,

Distribution of species: Denizciler, on debris, Baba 28; Karataş beach, on wood, Baba 129; Konacık, on wood, Baba 106; Yumurtalık lagoon forest, on wood, Baba 105.

11. *A. obvelata* (Oeder) Onsberg

Distribution of species: Yumurtalık lagoon forest, on wood, natural, Baba 101.

12. *A. pomiformis* (Leers) Rostaf.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 94, 99.

Genus: *Perichaena*

13. *Perichaena chryosperma* (Curr.) Lister

Distribution of species: Payas, on dung, Baba 44. Yumurtalık lagoon forest, on bark, Baba 105.

14. *P. depressa* Lib.

Distribution of species: Kurtkulağı village Ceyhan, on wood, Baba 85; Yumurtalık lagoon forest, on wood, natural, Baba 101.

Family Dianemataceae

Genus: *Calomyxa*

15. *Calomyxa metallica* (Berk.) Nieuwl.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 94, Kurtkulağı village Ceyhan, on wood, Baba 85.

Family Trichiaceae

Genus: *Metatrichia*

16. *Metatrichia vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop.

Distribution of species: Yumurtalık lagoon forest, on

wood, Baba 91.

Genus: *Trichia*

17. *Trichia favoginea* (Batsch) Pers.

Distribution of species: Dörtüol beach, on wood, Baba 57.

Order Physarida

Family Didymiaceae

Genus: *Diderma*

18. *Diderma hemisphaericum* (Bull.) Hornem.

Distribution of species: Denizciler, on twig, Baba 24; Karataş beach, on debris, Baba 128.

Genus: *Didymium*

19. *Didymium annulisporum* H.W. Keller & Schokn.

Distribution of species: Kurtkulağı village Ceyhan, on bark, Baba 85.

20. *D. bahiense* Gottsb.

Distribution of species: Karaağaç, on twig, Baba 16, 17, 18; Karataş, on bark, Baba 140, 145; Konacık, on wood, Baba 108.

21. *D. difforme* (Pers.) Gray

Distribution of species: Botaş beach, on debris, Baba 78; Denizciler, on bark, Baba 24, 29; Erzin free zone, on filter paper, Baba 70; Karaağaç, on petri dishes, Baba 16; Karatas beach, on bark, Baba 132, Konacık, on dung, Baba 109; Kurtkulağı village Ceyhan, on dung, Baba 81, Yumurtalık lagoon forest, on wood, natural, Baba 105.

22. *D. dubium* Rostaf.

Distribution of species: Arsuz, on bark, Baba 5, Ceyhan, on debris, Baba 86, Ceyhan Erzin road, on debris, natural, Baba 89, Karaağaç, on twig, Baba 12; Karatas beach, on wood, Baba 127, Payas, on bark, Baba 40, Kurtkulağı village, on bark, natural, Baba 84.

23. *D. melanospermum* (Pers.) T. Macbr.

Distribution of species: Karaağaç, on twig, Baba 14, Payas, on filter paper, Baba 43.

24. *D. minus* (Lister) Morgan

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 102.

25. *D. squamulosum* (Alb. & Schwein.) Fr. & Palmquist

Distribution of species: Arsuz, on filter paper, Baba 7, on wood, natural, Baba 10, Denizciler, on wood, natural, Baba 24, Konacık, on petri dishes, Baba 109, Kurtkulağı village Ceyhan, on petri dishes, Baba 86, Yumurtalık lagoon forest, on debris, Baba 90, 98, 101, 105.

Genus: *Mucilago*

26. *Mucilago crustacea* P. Micheli ex F.H. Wigg.

Distribution of species: Karataş, on wood, natural, Baba 144.

Family Physaraceae

Genus: *Badhamia*

27. *Badhamia dubia* Nann.-Bremek.

Distribution of species: Yumurtalık lagoon forest, on bark, Baba 94.

28. *B. foliicola* Lister

Distribution of species: Kurtkulağı village Ceyhan, on debris, Baba 88.

29. *B. macrocarpa* (Ces.) Rostaf.

Distribution of species: Belen, on debris, Baba 35.

Genus: *Physarum*

30. *Physarum album* (Bull.) Chevall.

Distribution of species: Erzincan region 3, on wood, natural, Baba 61; Yumurtalık lagoon forest, on dung, Baba 104, 105.

31. *P. bethelii* T. Macbr. ex G. Lister

Distribution of species: Dörtöyl beach, on wood, Baba 58.

32. *P. cinereum* (Batsch) Pers.

Distribution of species: Erzincan free zone, on petri dishes, Baba 67; Payas beach, on wood, Baba 44, 48; Yumurtalık lagoon forest, on dung, Baba 104, 105.

33. *P. compressum* Alb. & Schwein.

Distribution of species: Arsuz, on wood, Baba 5, 10; Konacık, on filter paper, Baba 106.

34. *P. melleum* (Berk. & Broome) Masee

Distribution of species: Arsuz, on wood, Baba 4.

35. *P. pusillum* (Berk. & M.A. Curtis) G. Lister

Distribution of species: Arsuz, on wood and debris, Baba 5, 7, 9, 10; Botaş beach, on wood, Baba 74; Denizciler, on debris, Baba 25; Karaağaç, on wood, natural, Baba 14; Karatas, on wood, Baba 125; Kurtkulağı village Ceyhan, on debris, Baba 85, Payas, on wood, Baba 44, Yumurtalık lagoon forest, on wood, natural, Baba 104.

Stemonitida

Stemonitidaceae

Genus: *Comatricha*

36. *Comatricha ellae* Härk.

Distribution of species: Arsuz, on wood, Baba 4; Botaş, on debris, Baba 74.

37. *C. nigra* (Pers. ex J.F. Gmel.) J. Schröt.

Distribution of species: Konacık, on wood, natural, Baba 102, Erzincan region 3, on wood, Baba 70; Yumurtalık lagoon forest, on wood, natural, Baba 94.

Genus: *Macbrideola*

38. *Macbrideola cornea* (G. Lister & Cran) Alexop.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 91, 95.

Genus: *Stemonitis*

39. *Stemonitis axifera* (Bull.) T. Macbr.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 94.

Genus: *Stemonitopsis*

40. *Stemonitopsis amoena* (Nann.-Bremek.)  
Nann.-Bremek.

Distribution of species: Erzincan Region 5, on wood, Baba 67.

41. *S. hyperopta* (Meyl.) Nann.-Bremek.

Distribution of species: Yumurtalık lagoon forest, on wood, Baba 91, 104.

Analysis of the prevalence of 41 species and 20 genera demonstrated that the S/G value was determined as 2.05. A low rate of these results indicates that taxonomic diversity is high (Stephenson et al., 1993). It was reported that S/G values in temperate or tropical regions are between 2.2 and 4.6 (Stephenson et al., 2000). The present study findings were consistent with the findings of other studies conducted in the Mediterranean region. The ratio of the number of species to the number of genera (S/G) is used as an indicator of taxonomic diversity. In previous studies in Türkiye, this rate was found to be 2.7 in Northern Adana (Baba et al., 2016), 1.75 in Osmaniye (Baba, 2017), 2.6 in Gaziantep (Baba et al., 2021b) and 2.75 in Batman (Baba et al., 2021c) in the vicinity of İskenderun Gulf. In the 9 districts where we collected samples in the İskenderun Gulf, we see that the microbiota diversity in this results area is rich, since the sea level, warm, and humidity ratios are suitable for myxomycete development, as well as forested and scrub areas are common.

The distribution of the species determined in this results area is Physarida 18 species, Trichiida 10 species, Stemonitida 6 species, Liceida 5 species the other two orders have one species. In the study conducted in Afyon/Türkiye, Physarida (24%), Trichiida (27%), and Stemonitida (30%) were found (Ocak, 2015). Trichiida (16.7%), Stemonitida (16.7%), and Physarida (57%) were found in Gaziantep/Türkiye (Baba et al., 2021b). Liceida (40%), Trichiida (26%), Physarida (6.7%), and Stemonitida (22%) were found in Northern Adana/Türkiye (Baba et al., 2016).

In this study, Physaraceae 9, Didymiaceae 9, Arcyriaceae 7, Stemonitidaceae 6, Trichiaceae 2, Cribrariaceae 2, Liceaceae, Echinosteliaceae, Dictydiaethaliaceae, Reticulariaceae, Dianemataceae and Ceratiomyxaceae 1 species were determined. In previous studies in Türkiye, the most common families are Stemonitidaceae, Physaraceae, Arcyriaceae, Trichiaceae, and Didymiaceae. 72.4% of the species in Manisa (Baba & Tamer, 2007), 78% in Hatay (Baba et al., 2013), 70% in Adana (Baba et al., 2016) and 74.1% in Konya (Yağız & Afyon, 2007) are these families. In these results, the rate of these 5 families was found to be 80%. Stemonitidaceae, Physaraceae, Didymiaceae, and Arcyriaceae family's rate were 88% in Gaziantep (Baba et al., 2021b). The results show parallelism with previous studies in Türkiye.

In this study the most common genera are *Didymium* (7 species), *Physarum* (6 species), and *Arcyria* (5



species). The most common genera were reported as *Cribraria*, *Arcyria*, *Stemonitis*, *Physarum*, *Comatricha*, *Licea*, and *Trichia* in Adana (Baba et al., 2016). The most common genera were reported *Arcyria*, *Comatricha*, *Didymium*, *Echinostelium*, and *Physarum* in Osmaniye (Baba, 2017). The most common genera were reported as *Didymium*, *Badhamia*, and *Physarum* in Gaziantep (Baba et al., 2021b). The most common genera were reported as *Didymium*, *Physarum*, *Badhamia*, *Comatricha*, and

*Licea* in Batman (Baba et al., 2021c). It is important that these genera contain the most common species, as well as having cosmopolitan species, as well as having a large number of species. The lime (calcium carbonate) contained in the genus *Physarida* may also cause them to tolerate sea salt on the beach.

Da Silva & Cavalcanti (2010) reported 4 species of Abundant (Over %7), 2 species of Common (5-7%), 5 species of Occasional (2-4%), and 30 species of Rare (0.5-1 %) (Table 1).

Table 1. Abundance class of species

Çizelge 1 Türlerin bolluk sınıfı .....

| Abundance class    | Species   |
|--------------------|---|
| Abundant (Over %7) | <i>Arcyria cinerea</i> , <i>Didymium difforme</i> , <i>D. squamulosum</i> , <i>Physarum pusillum</i>  |
| Common (5-7%)      | <i>Didymium dubium</i> , <i>D. bahiense</i>   |
| Occasional (2-4%)  | <i>Arcyria insignis</i> , <i>Comatricha nigra</i> , <i>Dictydiaethalium plumbeum</i> , <i>Physarum album</i> , <i>P. cinereum</i>   |
| Rare (0.5-1 %)     | <i>Arcyria incarnata</i> , <i>A. obvelata</i> , <i>A. Pomiformis</i> , <i>Badhamia dubia</i> , <i>B. Foliicola</i> , <i>B. macrocarpa</i> , <i>Calomyxa metallica</i> , <i>Ceratiomyxa fruticulosa</i> , <i>Comatricha ellae</i> , <i>Cribraria argillaceae</i> , <i>C. Violaceae</i> , <i>Diderma hemisphaericum</i> , <i>Didymium annulisporum</i> , <i>D. melanospermum</i> , <i>D. minus</i> , <i>Echinostelium minutum</i> , <i>Licea kleistobolus</i> , <i>Lycogala epidendrum</i> , <i>Macbrideola cornea</i> , <i>Metatrichia vesparia</i> , <i>Mucilago crustacea</i> , <i>Perichaena chrysosperma</i> , <i>P. depressa</i> , <i>Physarum bethelii</i> , <i>P. compressum</i> , <i>P. melleum</i> , <i>Stemonitis axifera</i> , <i>Stemonitopsis amoena</i> , <i>S. hyperopta</i> , <i>Trichia favoginea</i> |

In this study, *Arcyria cinerea*, *Didymium difforme*, *D. squamulosum*, and *Physarum pusillum* were abundant species. It has been reported that these species are the most common species in studies conducted in the immediate environment. In a study conducted in Northern Adana/Türkiye, *Arcyria pomiformis*, *Ceratiomyxa fruticulosa*, *Cribraria argillaceae*, *C. cancellata*, *C. vulgaris*, *Enerthenema papillatum*, *Lycogala epidendrum* and *Trichia favoginea* were reported to be most common species (Baba et al., 2016). In the study conducted in Batman/Türkiye, *Didymium annulisporum*, *D. difform*, *D. megalosporum*, *D. squamulosum*, and *Lamproderma arcyrioides* were reported to be the most common species (Baba et al., 2021c). Also, previous studies have reported that *Arcyria cinerea*, *Didymium difforme*, and *D. squamulosum* can grow on all kinds of substrates and in almost all parts of the world. Geographically, *E. minutum*, *A. cinerea*, *A. denudata*, and *D. difforme* are prevalent globally on all types of substrates (Martin & Alexopoulos 1969; Stephenson & Stempen, 1994; Alves et al., 2010; Baba, 2012; Baba, 2015; Ergül et al., 2016).

## CONCLUSION

In this study, 41 species of 12 families and 20 genera were determined. While the number of species is rare in the seaside regions, It was observed that the further away from the sea, the number of species increased, especially in forest areas. While cosmopolitan species were observed in abundance, *Physarida* members were

found to be common in this results area. The above species can be said to be Halophilic species. However, there is a need for more research, morphological, systematic, and physiological studies on this subject.

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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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## A New Record for The Flora of Türkiye: *Cousinia mazu-shirinensis* Rech.f.

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### ABSTRACT

*Cousinia mazu-shirinensis* taxon was recorded for the first time in the Flora of Türkiye from Şırnak province, located in the southeast of Türkiye. The morphological characteristics of the *C. mazu-shirinensis*, the map of the distribution area, and the photographs of its natural habitat are also presented in the study.

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## Türkiye Florası için Yeni Bir Kayıt: *Cousinia mazu-shirinensis* Rech.f.

### ÖZET

*Cousinia mazu-shirinensis* taksonu Türkiye Florasına ilk kez Türkiye'nin güneydoğusunda yer alan Şırnak ilinden kaydedilmiştir. *C. mazu-shirinensis* taksonunun morfolojik özellikleri, yayılış alanını gösteren harita, ve doğal habitatında çekilmiş fotoğrafları da çalışmada sunulmuştur.

### Botanik

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## INTRODUCTION

The genus *Cousinia* Cass. was described by the French botanist and naturalist Alexandre Henri Gabriel de Cassini (Cassini, 1827). According to APG IV, the genus *Cousinia* is in the subfamily of *Carduoideae* (Stevens, 2001; APG, 2016). According to the Compositae Global Database data, the genus is represented in the world by approximately 700 taxa (CWG, 2023). The *Cynaroideae* Bunge is the largest section of the genus *Cousinia* and represents 113 species (Restegar et al., 2018), and is distributed in Lebanon, Syria, Türkiye, the Caucasus, Iraq, Iran, Turkmenistan, Afghanistan, and Pakistan. This is characterized by the number of chromosomes as  $2n = 24$ , ± spiny-winged leaves and appendaged phyllaries

(Mehregan & Kadereit, 2008).

According to the latest revision study of *Cousinia*, the genus is represented by 39 species, one of which is a dubious record, in 6 sections in the Turkish flora, and 26 of these species are endemic (Tugay, 2012). Then, three new taxa were found in three separate studies; the first is hybrid, the second is a new species and the last one is a new record taxon published by Türkiye (İlçim et al., 2013; Fidan et al., 2019; Tugay et al., 2019). Nine of these 41 taxa are included in the *Cynaroideae* section (Huber-Morath, 1975; Tugay, 2012; Fidan et al.; 2019).

In 2015, during the field studies carried out around the Balveren town, Şırnak province, a population of the



genus *Cousinia*, which is interesting in terms of flower color, was encountered. In the field studies carried out in the same area in the following years, the same population could not be found anymore due to grass cutting. In 2018 and 2019, in the biodiversity study conducted in the province of Şırnak, another population consisting of the same specimens was found approximately 10 km east of the first region. Individuals from the population were photographed and collected in their natural habitats. Since the specimens could not be identified from the flora of Türkiye (Huber-Morath, 1975), they were identified from the Flora Iranica because they were close to the region. As a result of the diagnosis, it was concluded that the specimens belonged to the *Cousinia mazu-shirinensis* Rech.f. species, which has not been recorded before in the flora of Türkiye but is found in Iraq.

## MATERIAL and METHODS

The material of the study consists of specimens of the genus *Cousinia* collected from the towns of Şenoba and Balveren, Şırnak province, Türkiye, in 2015, 2018 and 2019. The plant specimens were photographed in their natural habitat (Figure 1) collected from the field and dried. First of all, Flora of Turkey was consulted for the identification of the collected specimens (Huber-Morath, 1975). When the specimens could not be identified using the Flora of Turkey, the relevant volumes of the Flora Iranica were consulted and identified from there (Rechinger, 1972; 1979). Life span, stem and leaf characteristics, involucre (especially phyllary and appendage characters), and floral characteristics were used when identifying the taxon. Herbarium specimens found in international digital herbaria (E, K, and W) were compared with the collected specimens (Figure 2). Specimens deposited in Van Yüzüncü Yıl University Herbarium (VANF) and Siirt University Flora and Fauna Center Herbarium (SUFAF) (herbaria abbreviation follows Thiers, 2024). The detailed structures of the phyllaries and flowers of the specimens were examined under a Leica EZ4 brand/model stereo microscope (Figure 3).

Morphological examinations and measurements of the collected specimens were made. By combining these measurements with the description in the Flora Iranica, the description of the taxon was expanded.

The map showing the known distribution area of the taxon was prepared using Google Earth (Google Inc., 2024) (Figure 4).

## RESULT and DISCUSSION

*Cousinia mazu-shirinensis* Rech.f., Fl. Iranica [Rechinger] 90: 226 (1972).

**Typus:** WHEELER-HAINES 2046, E (photo!)

**Description:** Biannual or perennial monocarpic herbs,

25–46 cm tall, usually branched from the base. Stems slender, cylindrical, densely arachnoid-tomentose in base, sparsely arachnoid-tomentose above. Leaves coriaceous-herbaceous, reticulate, dentate, decreasing from bottom to top, densely arachnoid-tomentose below, sparsely arachnoid pubescent above, with spines ca. 1–3 mm long; basal leaves elliptical, broadly oblong to oblanceolate, 15–30 × 4–7 cm, simple or 2–3 pinnatifid, segments elliptically ovate, triangular or lanceolate, upper segment bigger than others, broadly ovate, rachis ca. 1–2 mm broad; stem leaves decurrent, pinnatisect at the lower part of the stem, simple at the upper part of the stem, decurrent into wings up to 4.5–10 mm (including spines); lower leaves elliptical, 5–10 × 2–3 cm, pinnatisect; middle stem leaves narrowly ovate, 2–5 × 1.5–3 cm, upper leaves ovate to linear-lanceolate, 0.8–3 × 0.5–1.5 cm, including spines. Capitula 6–12, single on the top of branches, 40–60 flowered, involucre ovoid-globose to subglobose, 20–35 × 20–55 mm including spines; phyllaries 40–90(-120), glabrous, densely imbricate, inner phyllaries linear, subulate, 19–30 × 1–3.5 mm, middle phyllaries narrowly oblong, 3–13.5 × 3–5.5 mm, outer phyllaries narrowly oblong, 1.5–3 × 2–5 mm, patent or recurved, broadly rhomboid to rhomboid-flabellate appendage; appendages 8–16.5 × 4–15 mm, carinate, sharply attenuate into a 1–3.5 mm long spine at apex, with 0.5–1.5 mm, up to 1–3 rows tiny spines on both sides, median vein of appendage prominent, outer surface glabrous, papillate, inner sparsely arachnoid-tomentose, enlarged middle part of appendage long beard like hairy; bristles of receptacle smooth, 9–16 mm long; flowers wine purple, 20–26 mm long; anther tubes wine purple, glabrous; bristles of pappus barbellate, 3.5–5.5 mm long; achenes black-brown, obovate, 4.5–5.5 × 2–3.5 mm.

**Turkish name:** The genus *Cousinia* is known as “Kızan” in Turkish (Tugay, 2012). The authors suggest the name of the species as “Erbilkızanı”, as it was first described in Erbil province, Iraq according to Menemen et al. (2016).

**Distribution:** The taxon is distributed in the northern region of Iraq and southeast of Türkiye (Figure 4).

**Habitat:** The taxon is distributed in oak areas and steppes open to oaks.

**Phenology:** The flowering season of the taxon is mid-June and the fruiting season of the taxon is July.

**Examined specimens:** —TÜRKİYE. C9 Şırnak: Balveren Town, Cevizli Hamlet, Piştâ Guva Bapire Miste district, oakyards, 37° 29' 31" N, 42° 39' 07" E, 14 July 2015, *M. Fidan* 1848 (VANF!, SUFAF!); Uludere, Şenoba Town, Şenoba to Uludere 1 km, oakyards, steppe, 950 m, 37° 27' 30" N, 42° 43' 55" E, 28 June 2019, *M. Fidan*, *M. Pınar*, *H. Eroglu*, MMH 1623 (VANF!, SUFAF!). —IRAK, Kurdistan, Arbil, Kani Mazu Shirin, 5000 ft., 20.6.1961, *Agnew, A.D.Q.*

& Hadač & Wheeler Haines, R., 2046 [E (E00383837) photo!; isotypes: K (K000778377) photo!, W

(W19720001074) photo!].

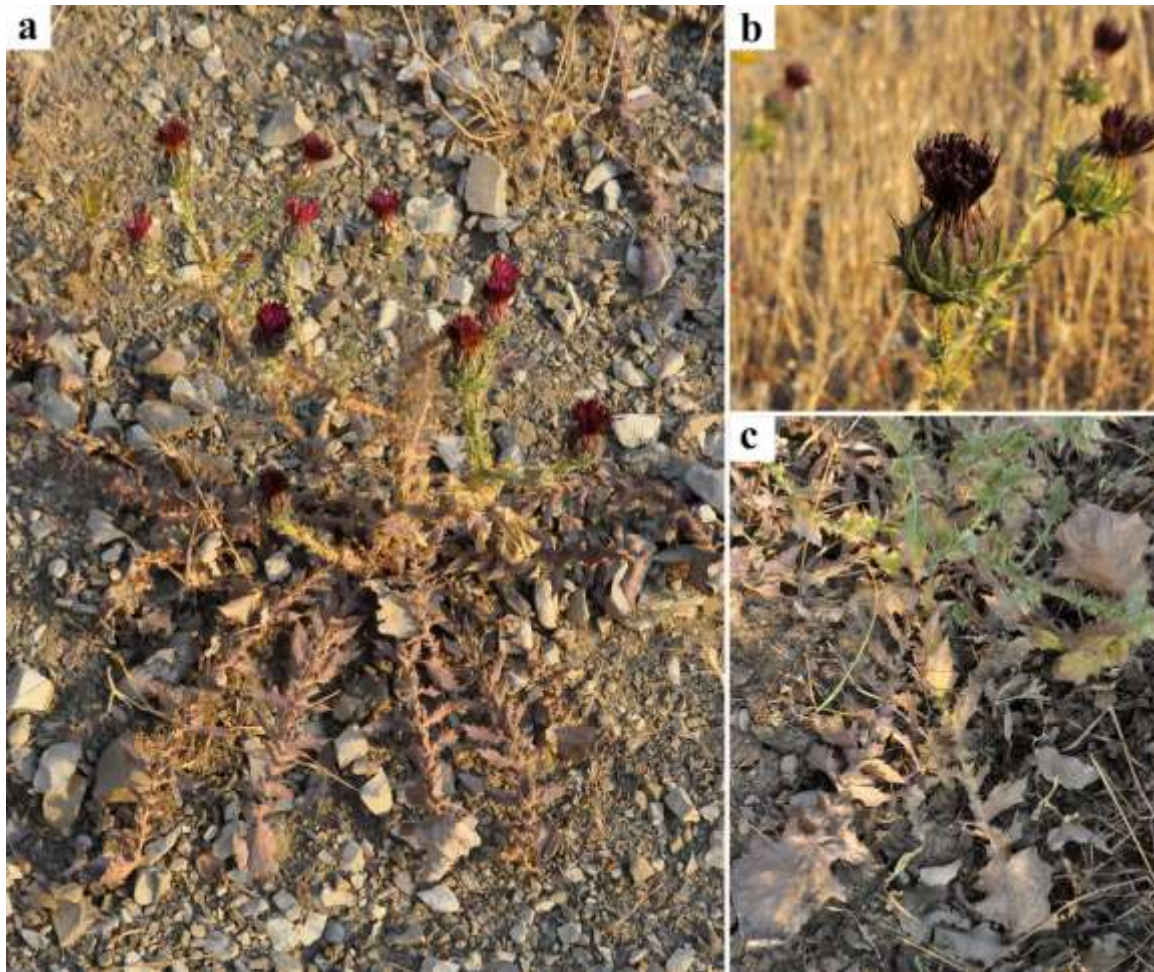


Figure 1. *Cousinia mazu-shirinensis* a. habit, b. capitula, c. basal, and stem leaves

Şekil 1. *Cousinia mazu-shirinensis* a. genel görünüş, b. kapitula, c. gövde ve taban yaprakları

*Cousinia mazu-shirinensis* species was considered a synonym of the *Cousinia odontolepis* DC. subsp *odontolepis* taxon with the studies carried out after the writing of the Flora Iranica. With this study, the number of taxa in the *Cynaroidae* section was sharply (110 to 31) reduced (Mehregan & Kadereit, 2008). This study is mostly based on examining the morphological characteristics of materials in herbaria. According to this study, not only *C. mazu-shirinensis* but also many taxa previously recorded in Turkey have become synonymous with other taxa. However, in subsequent studies, this concept was not widely accepted because involucre characters were ignored and sufficient population observations were not made (Attar & Djavadi, 2010; Tugay, 2012; Restegar et al., 2018).

When the distribution of *Cousinia mazu-shirinensis* taxon is screened in preliminary studies, it is seen that it is distributed in the southeast of the Bitlis-Zagros suture zone (Rechinger, 1979; Hüsing et al., 2009; Yeşilova & Helvacı 2012) (Figure 4). *Cousinina mazu-shirinensis* was described from a region very close to

the Turkish border. In other words, it is inevitable that this taxon exists in Türkiye. The authors predict that the taxon will probably be found in the south of Hakkari province, Türkiye, in future studies.

*Cousinia mazu-shirinensis* can be easily distinguished from other *Cousinia* taxa distributed in the region, especially by its wine-purple flower color (Figure 1). This was the most distinctive feature that attracted the authors' attention in the field.

With this study, the number of taxa of the genus *Cousinia* in Türkiye increased to 42 one of which is a dubious record, 28 of those are endemic.

#### ACKNOWLEDGEMENTS

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field studies within the scope of the terrestrial and inland water ecosystems biodiversity inventory and monitoring project of Şırnak Province, and the Şırnak

Provincial Directorate of Nature Conservation and National Parks and its employees, who assisted in the field studies.



Figure 2. Isotype specimen of *Cousinina mazu-shirinensis* in the Wienn Herbarium (W19720001074).  
Şekil 2. *Cousinina mazu-shirinensis*'in Wienn Herbaryumunda yer alan isotip örneği (W19720001074).

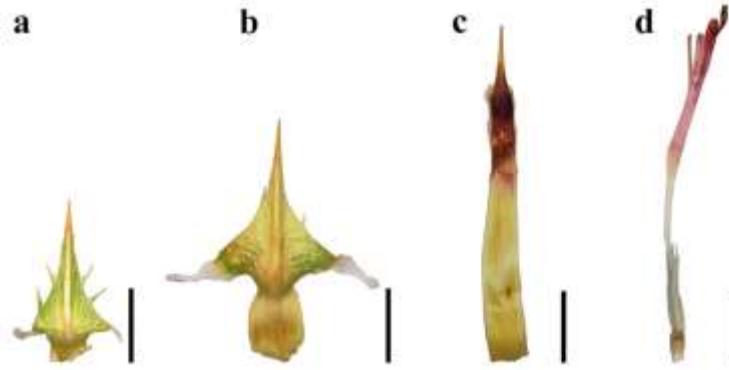


Figure 3. Phyllaries and flower images of *Cousinia mazu-shirinensis* a. outer phyllary b. middle phyllary, c. inner phyllary d. flower (bars 5 mm).

Şekil 3. *Cousinia mazu-shirinensis*'in fillari ve çiçek görüntüleri a. dış fillariler b. orta fillariler, c. iç fillariler d. çiçek (barlar 5 mm).

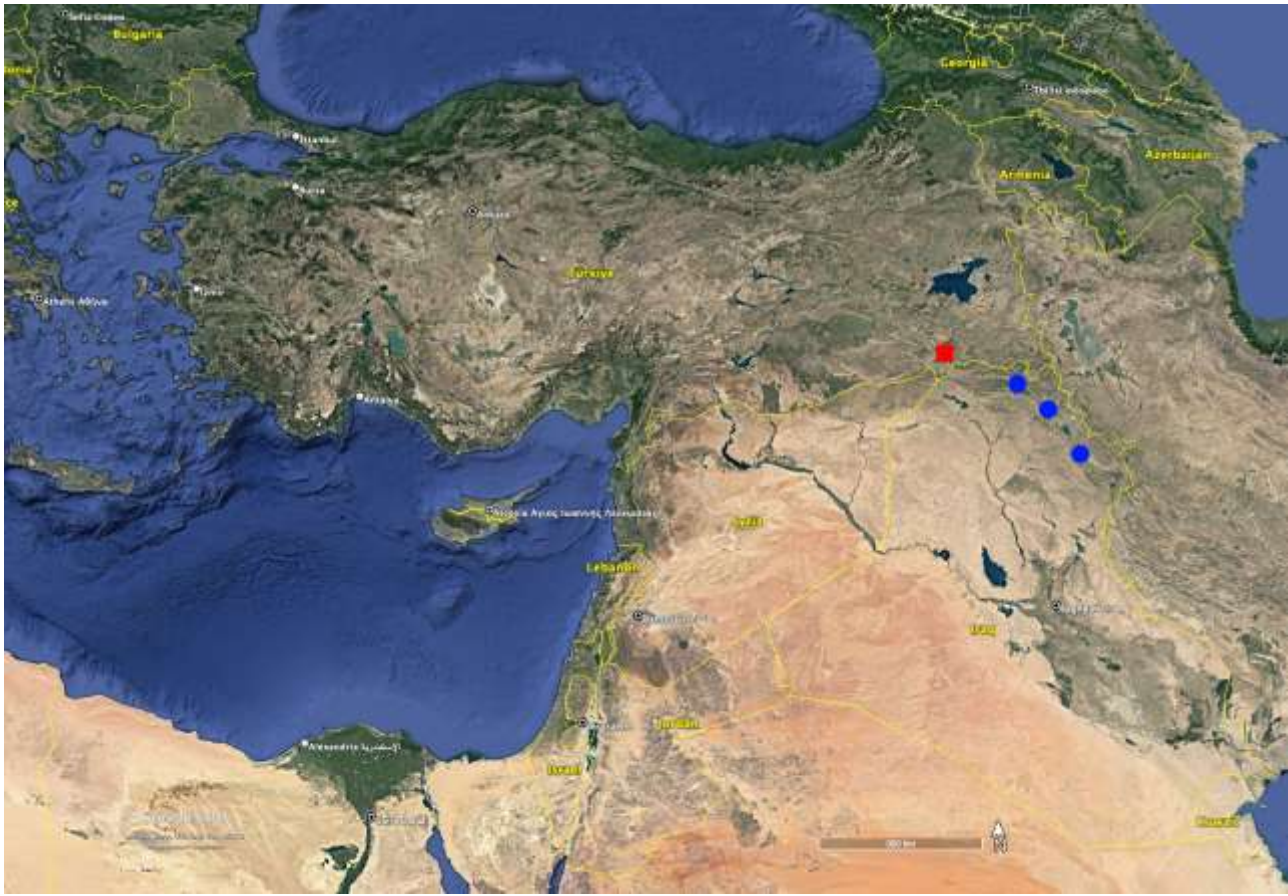


Figure 4. Map showing the distribution area of *Cousinia mazu-shirinensis*. Red square: the region where the species was first recorded in Türkiye; Blue circles: literature records showing the species' previous distribution area (Rechinger, 1972) (Map taken from Google Earth software).

Figure 4. *Cousinia mazu-shirinensis*'in dağılım alanını gösteren harita. Kırmızı kare: türün Türkiye'de ilk kaydedildiği bölge; Mavi daireler: türün önceki dağılım alanını gösteren literatür kayıtları (Rechinger, 1972) (Harita Google Earth yazılımından alınmıştır).

#### Researchers' Contribution Rate Declaration Summary

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

#### Conflict of Interest Declaration

The authors of the article declare that they do not have

any conflict of interest.

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## Initial Report of *Inocybe costinitii* in Türkiye with Morphological and Molecular Data

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### ABSTRACT

*Inocybe* specimens were collected from Ankara University Beşevler 10. Yıl Campus, (Ankara, Türkiye) on October 19, 2022. As a result, the samples were identified as *I. costinitii*, a new record for Turkish *Inocybe*. This study presents a detailed description of this newly recorded species, covering aspects such as its location, observations of its habitat, geographical coordinates, the date of collection, and photographs that highlight its macroscopic and microscopic characteristics. Moreover, the study features the species' drawings and some of its microscopic traits. The study is enhanced by images obtained from a scanning electron microscope (SEM), briefly examining the spore features and discussed briefly.

### Mycology

### Research Article

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### Keywords

*Inocybe costinitii*

*Inocybaceae*

New record

Türkiye

## Morfolojik ve Moleküler Verilerle *Inocybe costinitii*'nin Türkiye'deki İlk Raporu

### ÖZET

*Inocybe* örnekleri Ankara Üniversitesi Beşevler 10. Yıl Kampüsü'nden (Ankara, Türkiye) 19 Ekim 2022 tarihinde toplanmıştır. Sonuç olarak, bu örnekler Türkiye *Inocybe* cinsi için yeni bir kayıt olan *I. costinitii* olarak tanımlanmıştır. Bu çalışma, yeni kaydedilen bu türün konumu, yaşam alanı gözlemleri, coğrafi koordinatları, toplanma tarihi ve makroskobik ve mikroskobik özelliklerini vurgulayan fotoğraflar gibi hususları kapsayan ayrıntılı bir tanımını sunmaktadır. Araştırmada ayrıca türün bazı mikroskobik özelliklerinin çizimleri de yer almaktadır. Çalışma, spor özelliklerinin derinlemesine incelenmesini sağlayan taramalı elektron mikroskobundan (SEM) elde edilen görüntülerle zenginleştirilmiş ve kısaca tartışılmıştır.

### Mikoloji

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi : 12.04.2024

Kabul Tarihi : 11.06.2024

### Anahtar Kelimeler

*Inocybe costinitii*

*Inocybaceae*

Yeni kayıt

Türkiye

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## INTRODUCTION

Belonging to the family *Inocybaceae*, the genus *Inocybe* is recognized as one of the most diversely populated taxa within the *Agaricales*, comprising around 1050 documented species (Dovana et al., 2023). Recent studies indicate that this genus embarked on a swift and widespread evolutionary journey approximately 52 to 79 million years ago (Fachada et al., 2024). The genus exhibits a broad ecological amplitude, inhabiting a spectrum of climatic zones from tropical to Arctic regions (Altuntaş et al., 2019). Ecologically, *Inocybe* species predominantly colonize many forest habitats, spanning deciduous and

coniferous forests (Akata et al., 2023).

Morphologically, *Inocybe* species are identified by their relatively diminutive and subtly hued basidiomata (Kuyper, 1985). The pileus is characterized by its dry texture and may exhibit surface features such as scales, fibrils, or cracks. The lamellae generally present a muted brown hue, complemented by the presence of a stipe. A distinctive feature of the genus members is its unique odor, which plays a crucial role in their identification. The spores bear a muted brown coloration, are encased by somewhat thickened and smooth walls, and manifest in a variety of morphologies including angular, nodulose, or spinose

forms, conspicuously lacking a germ pore and the presence of cystidia, are often adorned with apices embedded with calcium oxalate crystals (Kuyper, 1986; Matheny & Kudzma, 2019; Bandini et al., 2020; 2021).

Fachada et al. (2024) estimate that the genus exhibits substantial diversity, with a global species count ranging from 3,000 to 5,000. Akata et al. (2023) state that, thus far, 92 *Inocybe* species have been reported from Turkey. Among them, 11 species have been reclassified into different genera: 7 have been transferred to *Inosperma* (Kühner) Matheny & Esteve-Rav., and 4 to *Mallochybe* (Kuyper) Matheny, Vizzini & Esteve-Rav. Due to recent updates, the count of *Inocybe* species recorded in Turkey has been revised to 81 (Akata et al., 2023; Matheny et al., 2020).

The objective of the present study is to contribute to the mycobiota of Turkey.

## MATERIALS and METHODS

This study utilized a comprehensive approach, combining traditional and cutting-edge molecular methods to analyze and categorize samples collected from Beşevler 10. Yıl Campus of Ankara University in Ankara, Türkiye. It involved thoroughly examining both the samples' macroscopic and microscopic attributes, enhanced by studying ribosomal DNA (rDNA) sequences using Internal Transcribed Spacer (ITS) sequencing techniques.

### Morphological Characterization

*Inocybe* samples were collected from the study area, and a comprehensive evaluation of their macroscopic and environmental attributes was conducted in their natural habitat. Subsequently, these specimens were subjected to microscopic examination in a controlled laboratory environment. A Euromex Oxion Trinocular light microscope facilitated the observation of the specimens' fine details at a magnification of 100X. To ensure the precision of the observations, each microscopic characteristic was quantified approximately 30 times. Some chemical agents, including 5% potassium hydroxide (KOH), and Congo red, were employed for the analysis process. The collected data from these measurements underwent statistical analysis. For scan electron microscope (SEM) analysis, small fragments of the specimens were affixed to stubs using double-sided adhesive tape and sputter-coated with gold. These prepared samples were then analyzed using an EVO 40XVP SEM, produced by LEO Ltd. in Cambridge, UK, with the device operating at an accelerating voltage of 20 kV.

The methodologies for morphologically identifying the samples were based on the protocols described in research studies by Bizio et al. (2016) and Bandini et al. (2021). Following their identification, the samples

were preserved at the Fungarium of Ankara University, located within the Faculty of Science, Biology Department.

### Molecular Characterization

#### *Determination of the ITS rDNA sequences*

The genomic DNA of the samples, identified as ANK AKATA 8687, was extracted employing the CTAB method, following the protocol described by Rogers & Bendich (1994). The quality and quantity of the extracted DNA were assessed using a Nanodrop Lite Thermo Scientific spectrophotometer. This DNA then served as the template for the PCR amplification of the Internal Transcribed Spacer (ITS) rDNA regions, using the ITS1 forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3') universal primers, according to the approach outlined by Martin & Rygielwicz (2005). The PCR amplification products were subjected to agarose gel electrophoresis, followed by purification with the Expin Gel PCR and CleanUp SV Kit (GeneAII). For Sanger dideoxy sequencing, the purified samples were processed using the BigDye™ Direct Cycle Sequencing Kit (Thermo Fisher Scientific) with the ITS1 and ITS4 primers. The sequenced PCR products were then analyzed on an ABI Prism 3130 Genetic Analyzer. The methodologies for agarose gel electrophoresis and Sanger sequencing were implemented by the protocols provided by Chen et al. (2014).

### Molecular Phylogeny Study

The sequencing data derived from the ITS1 and ITS4 primers were compiled using the Clustal Omega online sequence alignment tool (<https://www.ebi.ac.uk/jdispatcher/msa/clustalo>). This compiled data was subjected to a BLASTn search to identify its similarity index. Based on the BLAST search results, sequences corresponding to the target group (in-group) and a reference group (out-group) were sourced from the NCBI GenBank database. These sequences were aligned with the compiled ones using the ClustalW algorithm within the MEGAX software, following the method outlined by Kumar et al. (2018). This alignment served as the foundation for subsequent phylogenetic analysis. The evolutionary relationships of the sample series ANK AKATA 8687 were inferred using the Maximum Likelihood method, employing the T92+G model for nucleotide substitution as introduced by Tamura (1992). The accuracy of this phylogenetic assessment was improved by using a bootstrap technique with 1000 repetitions (Felsenstein, 1985). The resulting phylogenetic tree provides insights into the evolutionary relationships among the samples, placing them within the broader framework of fungal classification.



## RESULTS

The recent report of a newly recorded species is meticulously documented, highlighting critical data, such as the collection date, precise location, environmental habitat, and geographical coordinates, alongside the unique collection numbers. The description covers the species' macroscopic and microscopic traits, providing a thorough understanding of its morphology. Moreover, the utilization of scanning electron microscopy (SEM) to capture images of the spores presents an in-depth look

at the intricate features that define this species.

### Taxonomic overview

*Fungi*

*Basidiomycota* R.T. Moore

*Agaricales* Underw.

*Inocybe costinitii* Bizio, Ferisin & Dovana (2016), (Figure1-6).

Bizio et al. (2016) provided an in-depth examination of type collections.



Figure 1. Fruit bodies of *Inocybe costinitii*.

Şekil 1. *Inocybe costinitii*'nin fruktifikasyonları.

### Macroscopic and microscopic features

**Pileus** 20–30 mm diam., campanulate, occasionally featuring a wide, blunt umbo. Initially, the margin is inflexed, becoming erect and frequently displaying fissures. **Surface** smooth, fibrous texture with minute, radially arranged scales, displaying a color gradient from grayish brown to pale brown, with the center being lighter, uniformly enveloped by a delicate, white veil persisting on the surface for an extended duration. **Lamellae** sparsely distributed, and adnate, transitioning in color from beige grey to pale brownish, ultimately turning brown, edges eroded and exhibit a whitish hue. **Stipe** 30–40 × 5–6 mm, solid, tapering to cylindrical towards the base, culminating in a subtly margined, diminutive bulb, apex pruinose, with its coloration being predominantly white, albeit with a

slight brownish tint near the base, and longitudinally striated. **Flesh** firm and whitish. **Odor** spermatic. **Taste** non-distinctive. **Spores** (9.6–) 10–11.3 (–12) × (5.7–) 6–7 (–7.3) µm, Q = (1.50–) 1.55–1.74 (–1.80), Qav = 1.64, smooth, yellow-brown to brownish, amygdaliform to sub-amygdaliform, featuring a conical to slightly papillate apex. **Basidia** 34–41 × 12–16 µm, hyaline, clavate, typically with four, but occasionally two sterigmata. **Pleurocystidia** 55–74 × 18–24 µm, characterized by a thick-walled composition, changing color to a yellowish or pale yellow-green when exposed to 5% potassium hydroxide (KOH), predominantly fusiform or subfusiform, occasionally clavate or somewhat lageniform or subcylindrical, often with an extended peduncle, either small or large calcium oxalate crystals at the apex.



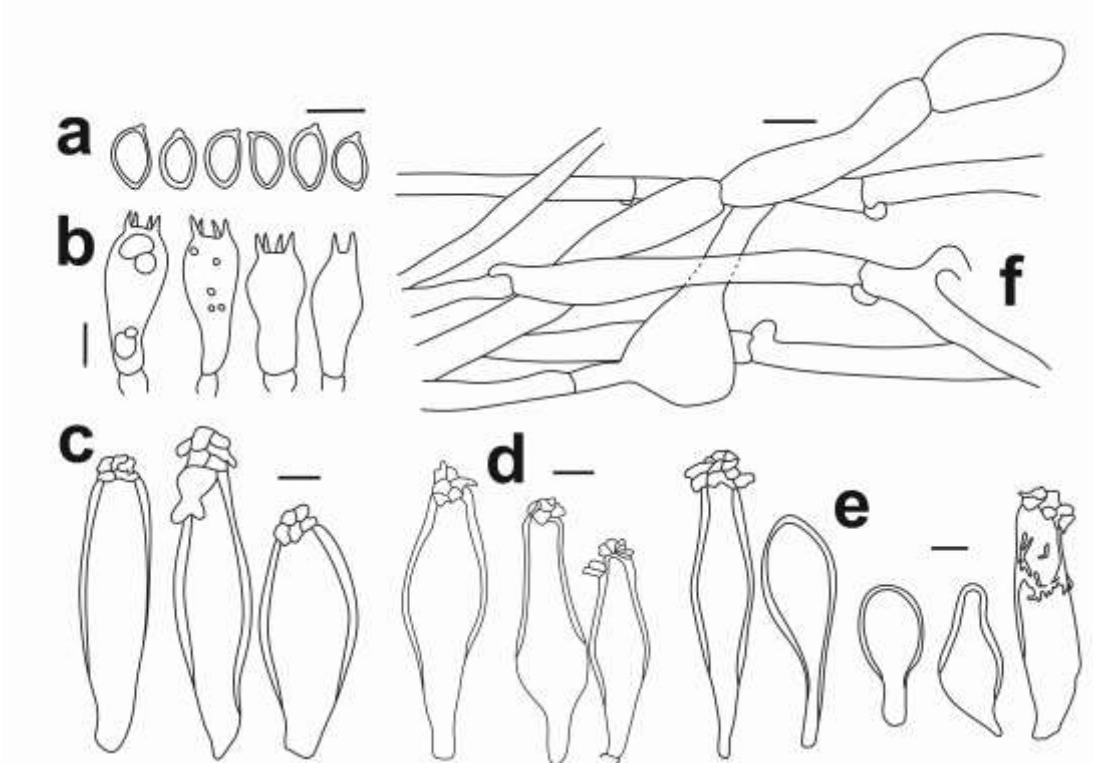


Figure 2. *Inocybe costinii*: a. spores, b. basidia, c. pleurocystidia, d. cheilocystidia, e. caulocystidia, f. pileipellis (scale bars: 10 µm).  
Şekil 2. *Inocybe costinii*: a. sporlar, b. basidyumlar, c. pleurosistidyumlar, d. keylolosistidyumlar, e. kaulosistidyumlar, f. pileipellis (ölçek:10 µm).

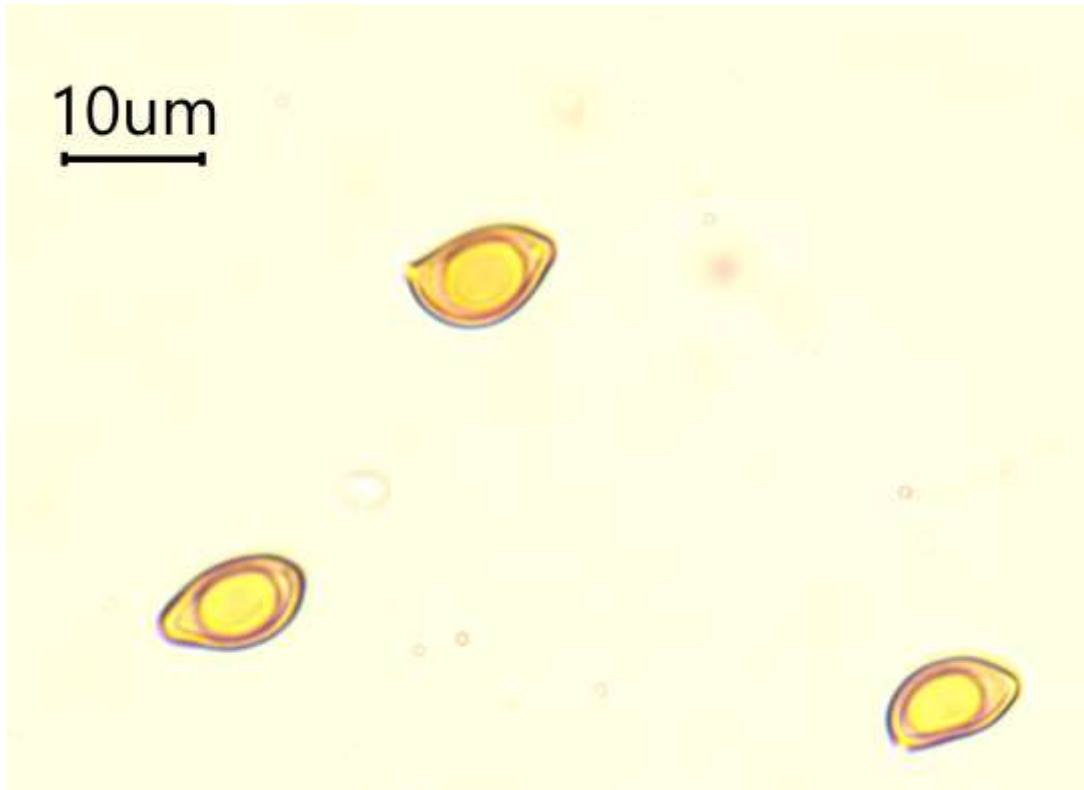


Figure 3. Spores of *Inocybe costinii* (LM, in KOH).  
Şekil 3. *Inocybe costinii*'nin sporları (LM, KOH'ta).

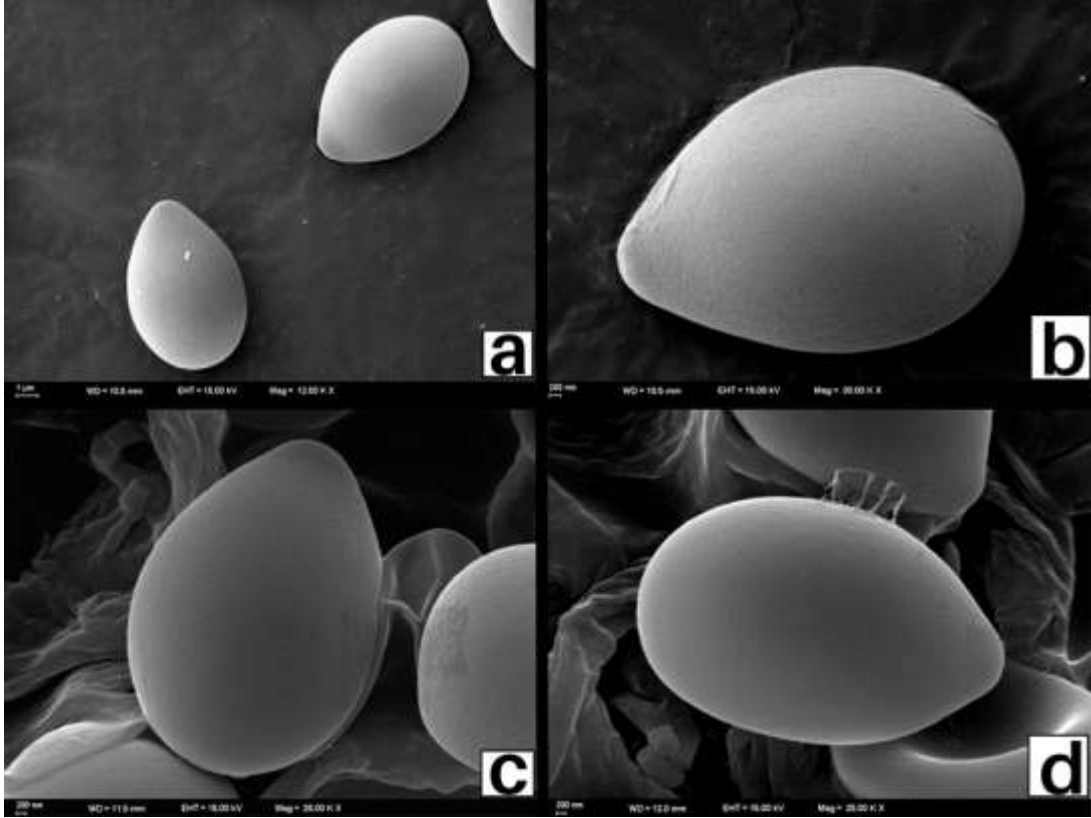


Figure 4. *Inocybe costinitii*: a-d. spores (SEM).  
Şekil 4. *Inocybe costinitii*: a-d. spollar (SEM).

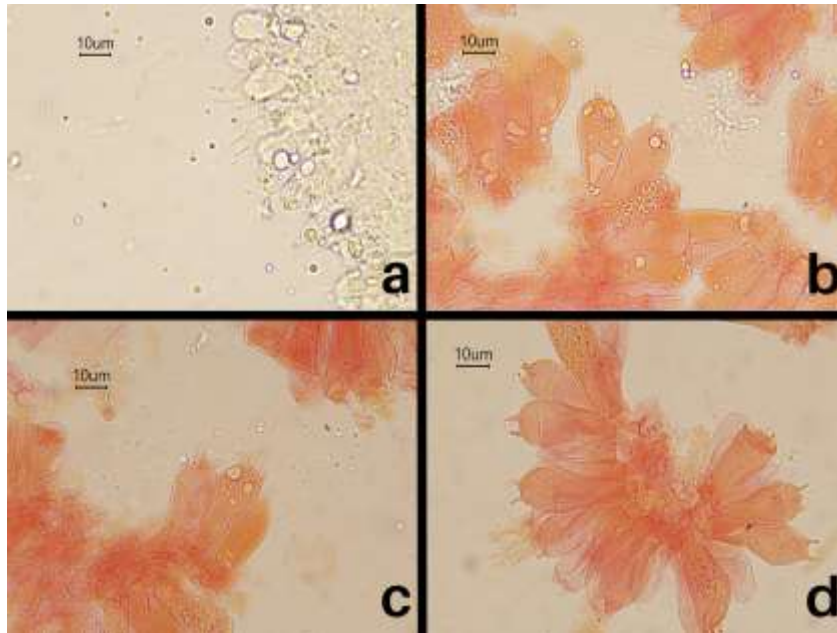


Figure 5. Basidia of *Inocybe costinitii* (LM): a. in KOH, b-d. in Congo red.  
Şekil 5. *Inocybe costinitii*'nin basidyumları (LM): a. KOH'ta, b-d. Kongo kırmızısı'nda.

**Cheilocystidia** 48–62 × 12–20 µm, similar to pleurocystidia but marginally shorter and thicker. **Caulocystidia** 35–75 × 14–22 µm, clavate to fusiform, and occasionally ventricose, some smooth, while others with crystalline formations at the apex, rarely urticoid-like structures observed, found mainly at the apex of the stipe. **Pileipellis** 5–14 µm broad, thick-walled,

consisting of periclinal hyphae, clamp connections present in all tissues.

**Material examined:** TÜRKİYE— Ankara, Ankara University Beşevler 10. Yıl Campus, under pine, 39° 56' N, 32° 50' E, 860 m, 19.10.2022, ANK AKATA 8687. (nrITS rDNA sequence GenBank accession number: PP494209.1).

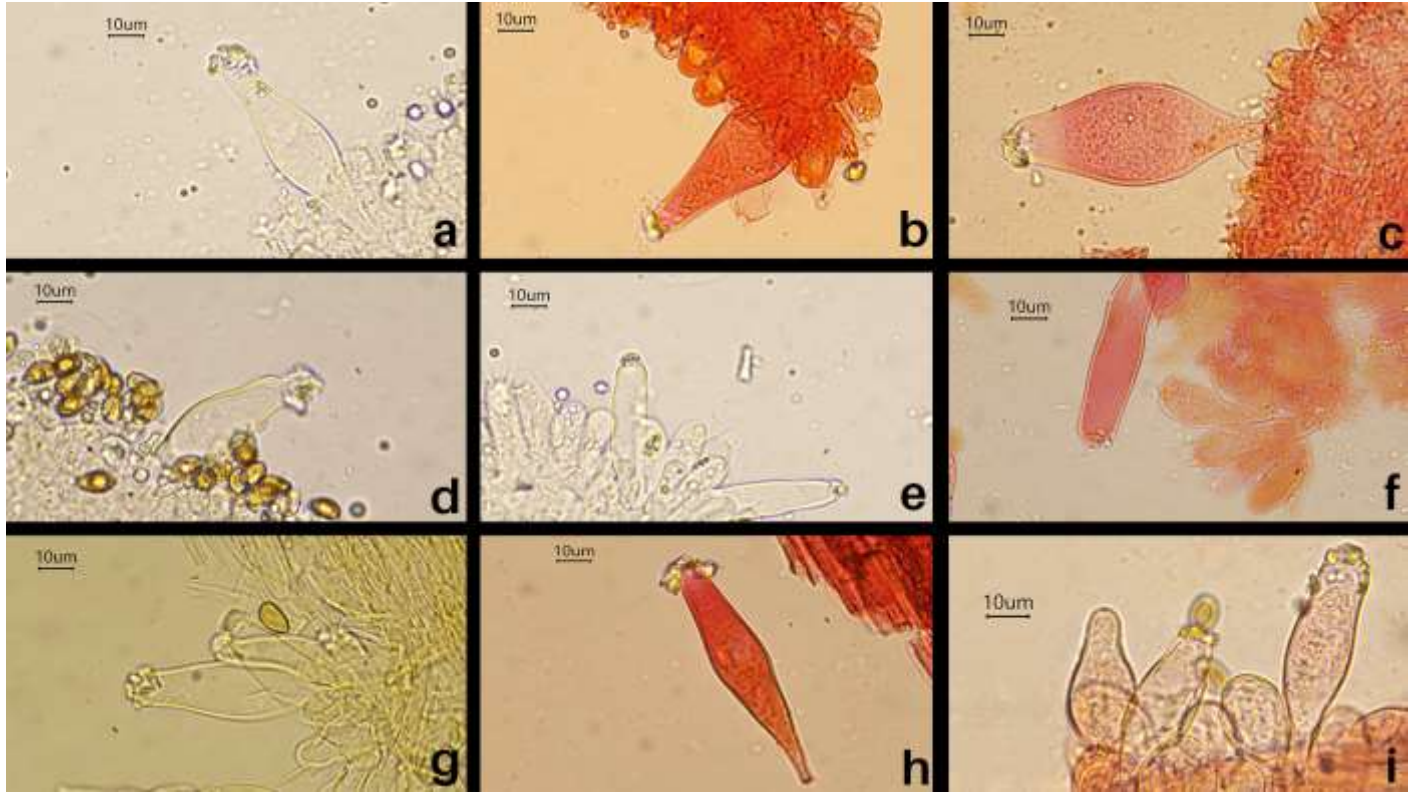


Figure 6. Cystidia of *Inocybe costinitii* (LM): a. pleurocystidium (in KOH), b,c. pleurocystidium (in Congo red), d. cheilocystidium (in KOH), e. cheilocystidia (in KOH), f. cheilocystidium (in Congo red), g. caulocystidium (in KOH), h. caulocystidium (in Congo red), i. caulocystidia (in Congo red).

Şekil 6. *Inocybe costinitii* (LM) sistidyumları: a. pleurosistidyum (KOH'ta), b,c. pleurosistidyumlar (Kongo kırmızısı'nda), d. keylosistidyum (KOH'ta), e. keylosistidyum (KOH'ta), f. keylosistidyum (Kongo kırmızısı'nda), g. kaulosistidyum (KOH'ta), h. kaulosistidyum (Kongo kırmızısı'nda), i. kaulosistidyumlar (Kongo kırmızısı'nda).

### Evolutionary History of ANK AKATA 8687

The evolutionary lineage of the specimen ANK Akata 8687 was explored based on its nrITS rDNA sequence, obtained using standard molecular techniques and archived in the NCBI GenBank under the accession number PP494209.1. To investigate its evolutionary connections, nrITS rDNA sequences from various *Inocybe* genus members were chosen for comparison, with the nrITS rDNA sequence of *Peziza montirivicola* serving as an outgroup. Molecular phylogenetic analysis revealed nine distinct clades, including Clade 7, consisting of the isolates of *Inocybe costinitii* (KX686581.1, PP794435.1) and ANK Akata 8687. Other clades (Clades 1-6 and Clades 8-9) comprised different *Inocybe* species. *Peziza montirivicola* formed a separate branch, indicating its outgroup status. BLAST analyses revealed a similarity of over 99% between the nuclear ITS rDNA sequences of ANK Akata 8687 and a single isolate of *I. costinitii* (Bizio et al., 2016). Phylogenetic analyses affirmed the close relationship between ANK Akata 8687 and *I. costinitii*, with a bootstrap value of 100%, indicating the reliability of their grouping.

### DISCUSSION and CONCLUSION

The genetic diversity of fungal species far exceeds their morphological diversity, prompting the integration of genetic information with traditional morphological methods for more accurate species identification. Various genetic markers, including rRNA gene regions such as nrITS, nrSSU, and nrLSU, along with sequences of protein-coding genes, have been employed in molecular systematic studies for decades (Raja et al., 2017). ITS is widely used in fungal molecular taxonomy among these markers, offering valuable insights (White et al., 1990; Akata & Erdoğan, 2020; Akata et al., 2024; Altuntaş et al., 2021). Furthermore, advancements in high throughput sequencing technologies and bioinformatics tools allow for whole genome comparisons and phylogenomic analyses among fungal taxa, potentially replacing molecular phylogenetic analyses based on a few marker genes shortly (Marian et al., 2024). In our research, nuclear ITS rDNA sequences were utilized for the molecular identification of ANK Akata 8687. This approach revealed a similarity of over 99% between the specimen (GenBank ID: PP494209.1) and *I. costinitii*. (Figure 7).



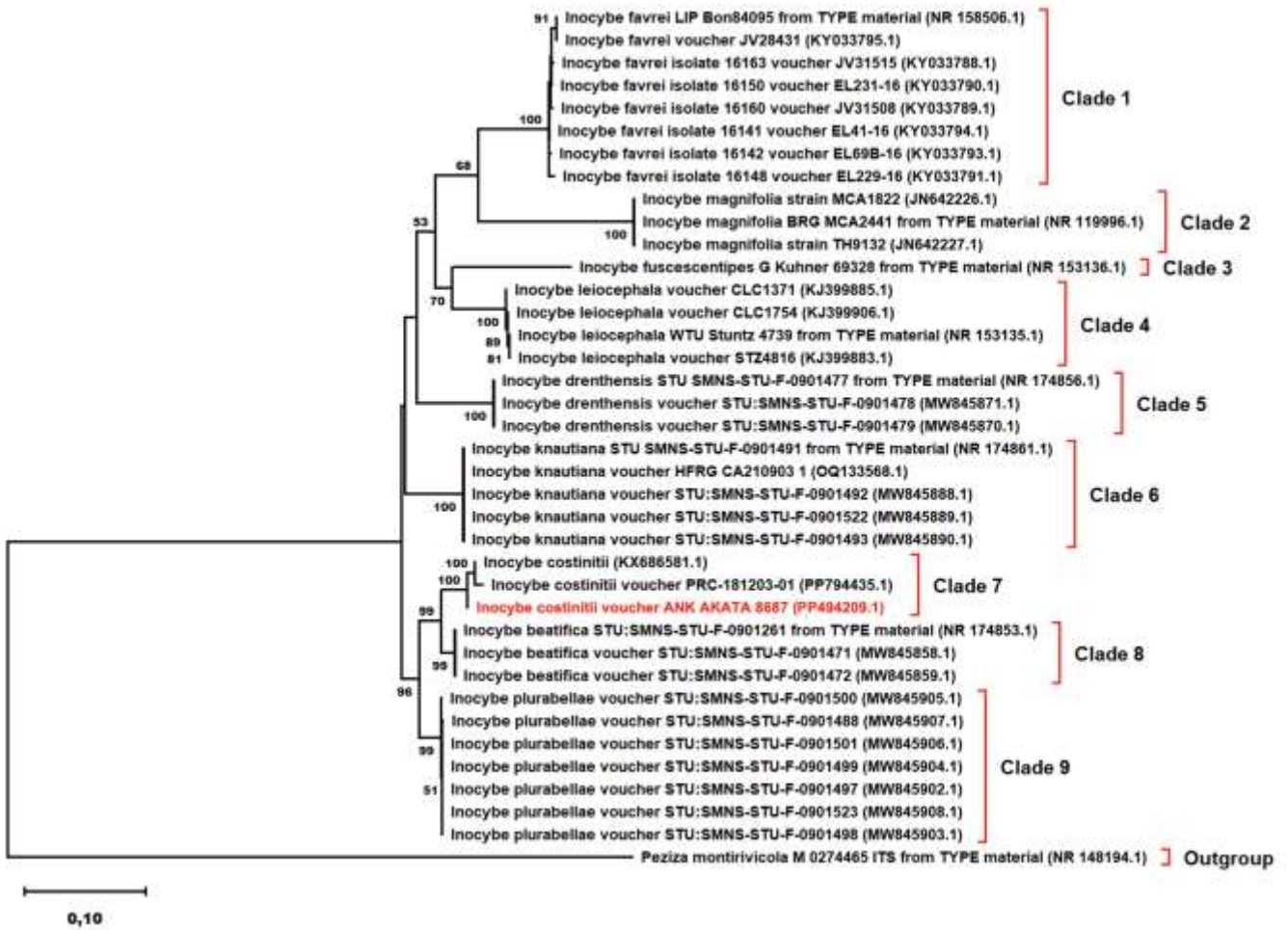


Figure 7. The evolutionary relationships among 38 fungal specimens were depicted through a phylogenetic tree constructed using the nrITS rDNA region and the maximum likelihood (ML) method. Bootstrap rates ( $\geq 50$ ) are assigned to each branch to indicate confidence levels. The sequences utilized in tree construction were sourced from the NCBI GenBank, except for ANK Akata 8687. Moreover, *Peziza montirivicola* was incorporated into the phylogenetic tree as the outgroup representative. GenBank accession numbers accompany each sequence, and a scale bar in the lower left corner represents a genetic distance of 0.10.

Şekil 7. 38 mantar örneği arasındaki evrimsel ilişkiler, nrITS rDNA bölgesi ve maksimum olabilirlik (ML) yöntemi kullanılarak oluşturulan bir filogenetik ağaç üzerinde tasvir edilmiştir. Güven seviyelerini göstermek için her bir dalın yanında en az %50'lik önyükleme oranları belirtildi. ANK Akata 8687 dışında kalan ve ağaç oluşturulurken kullanılan diziler NCBI GenBank'ten alındı. Ayrıca, *Peziza montirivicola*, filogenetik ağaçta dış grup temsilcisi olarak dahil edildi. Her dizinin yanında GenBank erişim numaraları bulunmaktadır. Sol alt köşede bulunan genetik mesafe ölçeği, 0.10 genetik uzaklığı temsil eder.

*Inocybe costinitii* is distinguishable on a larger scale by its moderately sized basidiomata, which feature pileus of a beige-ocher hue, enveloped in a dense, white veil. The stipe is smooth, whitish, and exhibits a swollen base. This species emits a spermatic odor and thrives in sandy, grassy areas adjacent to *Pinus halepensis* Mill. during the winter months. On a microscopic level, it is identified by its subamygdaliform spores that feature a conical to subpapillate tip, along with its fusiform cystidia (Bizio et al., 2016).

Bizio et al. (2016) described the caulocystidia as

clavate to sub-fusiform or sub-ovoid, characterized by thin walls and scarcity or complete absence of apical crystals, predominantly found at the stipe's apex. In contrast, the caulocystidia observed in Turkish specimens were noted to vary from clavate to fusiform, with occasional ventricose shapes and thick walls. While some were smooth, others exhibited crystalline formations at the apex. urticoid-like structures were seldom observed.

*Inocybe costinitii* shares similar morphological and ecological characteristics with *I. griseotarda* Poirier



and *I. griseovelata* Kühner (Bandini & Huijser, 2017; Sesli, 2019; Akata et al., 2023). Distinguished by its considerable size and sturdy form, *I. griseotarda* features a greyish-white veil, with its stem initially presenting a waxy white appearance before adopting a coloration akin to the cap, predominantly covered in fine white frost-like particles. This species typically forms extensive groups during winter, thriving in symbiosis with pine and strawberry (Bizio et al., 2017). Despite these similarities, *I. griseotarda* sets itself apart with a more pronounced robustness, a fibrously cracked pileus around the center, a quickly disappearing veil, and a stem entirely dusted with fine particles and displays pinkish tones at the top. It also features spores that are longer and slimmer, pleurocystidia with thin walls, and the presence of caulocystidia not just at the apex of the stipe but along its entire length (Bandini & Huijser, 2017; Bizio et al., 2016; 2017). *I. griseovelata* is characterized by its distinctive velipellis, which is whitish to grayish and densely covers the pileus. The surface of the pileus is generally smooth, though it may exhibit some innate fibrils. The stipe features light powdery deposits limited to the uppermost part. This species is recognized for its relatively large spores and typically has elongated hymenial cystidia with broad necks. Moreover, it exhibits long and slender caulocystidia. It is frequently found in areas with calcareous soil and can be associated with broad-leaved species and conifers (Akata et al., 2023; Bandini et al., 2022). This species diverges from *I. costinitii* due to its darker pileus, a veil of beige-grey coloration, a uniform thick stipe, and a pruinose apex. The lower part of the stipe is adorned with greyish fibrils. Moreover, it is characterized by its distinct subcylindrical cystidia (Bizio et al., 2016).

In the current study, *Inocybe costinitii* has been identified and documented from Türkiye for the first time, adding to the diversity of Turkish *Inocybe*. This report increases the number of *Inocybe* species known in the country's border to 82.

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### 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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## Effects of Melatonin Application on Post-Harvest Quality and Shelf Life of Kabarcık Grape Variety

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### ABSTRACT

In viticulture, post-harvest is a very important period to ensure and protect grape quality and health. The storage of the Kabarcık variety, which is of great importance for Dulkadiroğlu grape growers, after harvest and its short road endurance period, constitute a real problem expressed by the growers. In this research, it was aimed to maximize grape cluster health and post-harvest quality protection with melatonin application. Different doses of melatonin were applied to grape clusters of Kabarcık variety obtained from Dulkadiroğlu producer vineyards in the 2023-24 production season. Fruit weight loss, brix (sugar content), pH, and titratable acid (tartaric acid) amounts were determined in the clusters subjected to melatonin doses in a total of four different periods (days 0, 5, 10, and 15). As a result, the highest weight loss was seen in the control and 6-hours 25 µmol L<sup>-1</sup> melatonin applications, while the least weight loss was seen in the 3-hours 250 µmol L<sup>-1</sup> melatonin application. Thus, while the 25 µmol L<sup>-1</sup> dose of melatonin was seen as too low and ineffective, the 250 µmol L<sup>-1</sup> dose of melatonin was determined to be an effective solution in preventing weight loss in grape berries. This study highlights the important effects of melatonin in improving fruit quality and extending shelf life.

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Grape cluster development

## Melatonin Uygulamasının Kabarcık Üzüm Çeşidinde Hasat Sonrası Kalite ve Raf Ömrü Üzerine Etkileri

### ÖZET

Bağcılıkta hasat sonrası, üzüm kalitesi ile sağlığının sağlanması ve korunması için çok önemli bir dönemdir. Dulkadiroğlu üzüm yetiştiricileri için büyük öneme sahip olan Kabarcık çeşidinin, hasat edildikten sonra muhafazası ve yola dayanım süresinin kısa oluşu yetiştiriciler tarafından dile getirilen gerçek bir problem oluşturmaktadır. Bu çalışmada, hasat sonrası melatonin uygulaması ile üzüm salkım sağlığı ile üzüm kalitesinin korunması ve en üst düzeye çıkartılması amaçlanmıştır. 2023-2024 üretim sezonunda Dulkadiroğlu üretici bağlarından elde edilen Kabarcık çeşidi üzüm salkımlarına farklı melatonin dozları uygulanmıştır. Melatonin dozlarına tabi tutulan salkımlar toplam 4 farklı zaman dilimlerinde (0, 5, 10, ve 15. günler) olmak üzere meyve ağırlık kaybı, briks (şeker içeriği), pH ve titre edilebilir asit (tartarik asit) miktarları tespit edilmiştir. Sonuç olarak, en fazla ağırlık kaybı kontrol ve 6 saat 25 µmol L<sup>-1</sup> melatonin uygulamalarında görülürken, en az ağırlık kaybı 3 saat 250 µmol L<sup>-1</sup> melatonin uygulamasında görülmüştür. Böylece, 25 µmol L<sup>-1</sup> melatonin çok düşük ve etkisiz olarak görülürken, 250 µmol L<sup>-1</sup> melatonin üzüm tanesinde ağırlık kaybını önlemede etkili bir çözüm olarak tespit edilmiştir. Bu çalışma, melatoninin meyve kalitesinin iyileştirilmesi ve raf ömrünün uzatılması açısından önemli etkilerini vurgulamaktadır.

### Bahçe Bitkileri

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## INTRODUCTION

In Türkiye, approximately 4165000 tons of grapes were produced from a total area of 384537 ha in 2022 (FAOSTAT, 2024). In Kahramanmaraş, a total of 64681 tons of grapes were produced in 2022, especially in Dulkadiroğlu (25265 tons), Onikişubat (17304 tons), Pazarcık (12860 tons) districts (TUIK, 2024). Kabarcık, which is the most produced by farmers in Kahramanmaraş, is a variety with a very short shelf life after harvest. Numerous plant species contain melatonin, also known as N-acetyl-5-methoxytryptamine, a multifunctional signaling chemical that is widely dispersed (Ze et al., 2021). Plants use melatonin for a variety of physiological processes, such as fruit development (Wang et al., 2016), ripening (Sun et al., 2015), and senescence (Li et al., 2019). The senescence and degeneration of post-harvest fruit, including pear (Liu et al., 2019), sweet cherry (Wang et al., 2019), and peach (Gao et al., 2016), were greatly delayed during storage when exogenous melatonin administration was applied. Melatonin, a harmless and safe substance, thus shows promise for use in the fruit supply chain after harvest. Applying a melatonin solution by soaking or spraying is currently the main postharvest management method. Rapid soaking or spraying appears to have more potential for large-scale use in the postharvest fruit during storage and marketing, based on the viability of commercial technology (Ze et al., 2021). Improving shelf life and preserving nutritional levels mostly depends on reducing the senescence processes after harvest. Berry deterioration following harvest is primarily caused by components oxidizing, respiration consuming compounds, and softening of cell walls (Shehata et al., 2019). It has been found that exogenous melatonin is helpful in some plant tolerance to a range of environmental stresses such as toxic chemicals, salt, and drought (El-Bauome et al., 2022). Melatonin has been reported to greatly affect crop ripening in broccoli (Cano et al., 2022) and some other horticulture crops (Ze et al., 2021). Additionally, melatonin application externally postponed the fruit senescence, softened some fruits, losing on the total fruit weight by decreasing losing water status, postponing the decay and respiration proportions (Wang et al., 2019; Liu et al., 2018; Onik et al., 2021; Aghdam & Fard, 2017; Yang et al., 2019). It has been discovered that melatonin applied postharvest can effectively delay ripening and senescence. The study regarding grape berries examined the effects of two postharvest melatonin doses (50 and 100  $\mu\text{mol}$ ) on the quality, bioactive components, and enzyme activities of grape berries cv "Crimson," which were kept for 35 days at 0

$\pm 1$  °C and 90% relative humidity (RH). According to their findings, adding melatonin to berries prolongs their shelf life by preventing weight loss and preserving titratable acidity (TA), total soluble solids (TSS), berry adherence strength, and firmness (Nasser et al., 2022). In order to promote improvements in sustainable practices and a more complex knowledge of agricultural systems, this research study aims to explain the complex interactions between melatonin and grapevine physiology. In the face of changing environmental challenges and market demands, this research attempts to fill fundamental knowledge gaps and open up access to new methods that can boost grapevine productivity, durability, and post-harvest quality maintenance. Therefore, this study is aimed at improving the self-life of Kabarcık cultivar berries by exogenous melatonin applications at room temperature (22 °C) were examined to maintain berry quality in this study.

## MATERIALS AND METHOD

### Plant material

The research was carried out at the Kahramanmaraş Sutcu Imam University, Horticulture Department laboratory during the period from 2023-2024 growing season. The selected grape cultivar for this study was the Kabarcık grafted onto Rupestris du Lot rootstock, and clusters were collected from a trained system with a spacing of 3 m x 2 m vineyard. Although the Kabarcık is suitable for wine and must, it is also widely consumed as a table grape in the region. Another reason for choosing this variety is that the berry shell is thin and the interaction between the application of melatonin and the extension of the shelf life of the variety will be better understood.

### Experiment Design and Treatments

The study employed a randomized complete block design for its execution, comprising three replicates, each consisting of 3 clusters per replicate. Clusters were harvested at a ripe stage (17-18 Brix) in September free from mechanical damage, insect damage, and any outer decay. Clusters were randomly divided into five groups of similar color, size, and form, then clusters were immersed in control (distilled water), 25  $\mu\text{mol L}^{-1}$  melatonin for 3-hours, 250  $\mu\text{mol L}^{-1}$  melatonin for 3-hours, 25  $\mu\text{mol L}^{-1}$  melatonin for 6-hours, 250  $\mu\text{mol L}^{-1}$  melatonin for 6-hours. Each cluster was left until its surfaces dried at room temperature at  $23 \pm 5$  °C.



### Determination of Cluster Weights, Brix (sugar content), pH, and Titratable Acidity

The cluster weights, Brix (sugar content), pH, and titratable acidity values of the control group without melatonin application and melatonin applied samples, stored at 23°C room temperature, were determined on the 0, 5<sup>th</sup>, 10<sup>th</sup>, and 15<sup>th</sup> days. The weights of the cluster were measured every 5 days using a Radwag brand AS 310 R.2 model precision scale and the weight values were recorded. Water loss of berries was determined by the formula:

$$\text{Weight loss(\%)} = \frac{(\text{Harvest Weight} - \text{Last Weight})}{\text{Harvest Weight}} * 100$$

Brix, pH, and titratable acidity values of the samples were calculated using the juices obtained from grape berries. Brix values of the samples were measured with an Atago brand PAL<sup>-1</sup> model digital handheld refractometer, and the pH value of the fruit was measured with a Hanna brand HI-2550 model pH meter. 5 ml of the sample and some pure water were taken into an erlenmayer and 2 drops of 1% phenolphthalein indicator were added to the solution and titrated with 0.1 N NaOH solution. % titratable acidity was calculated using the formula below.

$$\% \text{ Titratable Acidity} = \frac{S \times N \times E \times F}{C} \times 100$$

S is the amount of NaOH used in titration (ml),

N= Normality of NaOH used in titration,

E= Equivalent value of the relevant acid

F= Factor of NaOH used

C= Amount of sample used (ml)

### Statistical Analysis

All descriptive analyses were carried out in R Studio using the agricolae package. The significance of treatments and days and their interactions with berry characteristics (weight loss, brix, pH and titratable acidity) were analyzed through Analysis of Variance (ANOVA) in R Studio. All data were checked for normality using the chi-square test before analysis. Linear models were employed to evaluate the main effects (treatments and days) on berry characteristics (weight loss, brix, pH and tartaric acid). Post-hoc analysis utilizing Tukey HSD was conducted with the agricolae package in R Studio. Principal Component Analyses (PCAs) for berry characteristics (weight loss, brix, pH and tartaric acid) datasets were carried out using ggbiplot2 within R Studio. The heatmap was generated using the pheatmap package in R Studio, providing a visual representation of relationships and variations in the analyzed datasets (R Core, 2013).

## RESULTS AND DISCUSSION

Weight loss, brix, pH and titratable acidity were affected by treatments and days. About weight loss, it

was statistically different in treatments ( $p=0.0101$  \*) and days ( $p<2e-16$  \*\*\*). Control and 6-hours 25  $\mu\text{mol L}^{-1}$  treatments, (31.91% and 32.41%, respectively) were higher predominantly weight loss than treatment of 3-hours 250  $\mu\text{mol L}^{-1}$  (24.08%). 3-hours 25  $\mu\text{mol L}^{-1}$  (29.41%) and 6-hours 250  $\mu\text{mol L}^{-1}$  treatments (25.75%) were not statistically different. Day 15, (54.06 %) was consistently higher weight loss than day 0 (control, 0.00%). And day 10 (36.93 %) and day 5 (22.86 %) with  $p<2e-16$  \*\*\* were between these treatments. Regarding Brix, it was the predominant greater (with  $p<2e-16$  \*\*\*) in 6-hours 25  $\mu\text{mol L}^{-1}$  treatment (17.95) while 3-hours 250  $\mu\text{mol L}^{-1}$  treatment (16.60) and control (16.62) were the least. In addition, 6-hours 250  $\mu\text{mol L}^{-1}$  (16.90) and 3-hours 25  $\mu\text{mol L}^{-1}$  (16.97) were between these treatments. Brix (with  $p<2e-16$  \*\*\*) ranged from day 0 (15.30) to day 15 (20.40). Day 10 (18.50) and day 5 (15.46) were between these days. For pH, it was the predominant greater (with  $2.54e-08$  \*\*\*) in 3-hours 250  $\mu\text{mol L}^{-1}$  treatment (4.30), while control and 6-hours 25  $\mu\text{mol L}^{-1}$  treatment (4.15 for both treatments) were least. And 3-hours 25  $\mu\text{mol L}^{-1}$  (4.18) and 6-hours 250  $\mu\text{mol L}^{-1}$  (4.23) were between treatments. pH (with  $p<2.60e-11$  \*\*\*) dramatically increased from day 0 (4.15) to day 15 (4.33). Lastly related to titratable acidity, the biggest value was obtained from the treatment of 3-hours 25  $\mu\text{mol L}^{-1}$  and 3-hours 250  $\mu\text{mol L}^{-1}$  (0.43 for both treatments) while the lowest from 6-hours 25  $\mu\text{mol L}^{-1}$  treatment with 0.39. In titratable acidity (with  $p<2e-16$  \*\*\*) greater value was obtained from day 10 with 0.51 and the lowest value was obtained from day 0 (0.35) (Table 1).

All variables are shown, different color circles representing the method are distributed along the vertical axis of PC1. The purple circle represents control treatment and has a great contribution to Weight loss and Brix while 3-hours 0.25  $\mu\text{mol L}^{-1}$  treatment has the lowest to total variance. The other treatments were between control and 3-hours 0.25  $\mu\text{mol L}^{-1}$  cultivars and have contributions differently (Fig. 2A). This shows that treatments and days provide the greatest contribution to Weight loss (%), Brix, pH and Titratable acidity (as Tartaric acid). On the other hand, day 10 and day 15 were more widely distributed than days 0 and 5 along the horizontal axis, indicating that in these days had a greater effect on Weight loss and Brix than the other days. This shows that day 0 and day 5 have the lowest contribution to the Weight loss and Brix, while three bud spurs represents a significant portion of the total variance (Fig. 2B). For the variables, Dim1 and Dim2 states for 60.7% and 22.3% of the variance, respectively. Weight loss, Brix and pH were located on the positive side Dim1, having strong correlation, while tartaric acid was on the negative side of Dim1 (Fig.2C). Furthermore, a robust positive association was discovered among pH, brix, and weight reduction, as indicated by the dark blue

circles. Conversely, tartaric acid was found to have a pronounced negative correlation with weight loss, brix and pH, as indicated by the light blue circles (Fig. 2D).

Table 1. Weight loss (%), Brix, pH and Titratable acidity (as Tartaric acid) of grape berry of Kabarcık in EL-38 phenological stage.

Çizelge 1. EL-38 fenolojik döneminde Kabarcık üzümü salkım ağırlık kaybı (%), SÇKM (Suda Çözünebilir Kuru Madde), pH ve Titre edilebilir asitliği (Tartarik asit olarak).

|                                  | Weight Loss (%)   | Brix                                       | pH           | Titratable acidity ( Tartaric acid)      |
|----------------------------------|-------------------|--|--------------|--|
|                                  | Ağırlık Kaybı (%) | SÇKM (Suda Çözünebilir Kuru Madde Miktarı) | pH           | Titre edilebilir asitlik (Tartarik asit) |
| Treatments <sup>W</sup> (Tr)     |                   |  |              |  |
| Control                          | 31.91±1.85a       | 16.62±0.03c                                | 4.15±0.02c   | 0.42±0.00ab                              |
| 3-hours 25 µmol L <sup>-1</sup>  | 29.41±1.92ab      | 16.97±0.02b                                | 4.18±0.01bc  | 0.43±0.00a                               |
| 3-hours 250 µmol L <sup>-1</sup> | 24.08±1.89b       | 16.60±0.01c                                | 4.30±0.02a   | 0.43±0.00a                               |
| 6-hours 25 µmol L <sup>-1</sup>  | 32.41±1.95a       | 17.95±0.03a                                | 4.15±0.01c   | 0.39±0.00b                               |
| 6-hours 250 µmol L <sup>-1</sup> | 25.75±1.55ab      | 16.90±0.02b                                | 4.23±0.02b   | 0.41±0.00ab                              |
| Days <sup>T</sup> (D)            |                   |  |              |  |
| Day 0                            | 0.00±0.00d        | 15.30±0.01d                                | 4.15±0.01b   | 0.35±0.00c                               |
| Day 5                            | 22.86±1.75c       | 15.46±0.02c                                | 4.17±0.01b   | 0.42±0.00b                               |
| Day 10                           | 36.93±1.90b       | 18.50±0.03b                                | 4.29±0.02a   | 0.51±0.00a                               |
| Day 15                           | 54.06±1.95a       | 20.40±0.05a                                | 4.33±0.02a   | 0.37±0.00c                               |
| Significance                     |                   |  |              |  |
| Tr                               | 0.0101 *          | <2e-16 ***                                 | 2.54e-08 *** | 0.0222 *                                 |
| D                                | <2e-16 ***        | <2e-16 ***                                 | 2.60e-11 *** | < 2e-16 ***                              |
| Tr x D                           | 0.8559            | <2e-16 ***                                 | 0.368        | 7.1e-11 ***                              |

W, Mean separation in Treatments; D, Mean separations in Days. Tr, Treatments; D, Days; Tr x D, interactions; For a given factor (different letters within a column represent significant differences (Tukey test, \*, Significant at *p*-value <0.05; \*\*\*, Significant at *p*-value < 0.001). Data are stated as averages of the data and their standard deviations.

A hierarchical clustering heatmap illustrating the relative concentrations of berry characteristics in grape samples across treatments and days. Berry characteristics are clustered at the bottom of the heatmap, revealing similarities and dissimilarities between them. Weight loss and brix emerge as closely related, indicating similar concentration patterns across samples. Conversely, compounds like tartaric acid exhibits lower concentrations in these same samples, as shown by the deep blue color. The pH was between these groups. Grape samples, labeled with days and treatments are vertically clustered on the right. It shows that the treatments were strongly correlated among berry characteristics, while day 0 (as control), day 5, day 10 and day 15 were separated in the heatmap depending on the amount of the berry features such as weight loss, brix, pH and tartaric acid (Fig. 2E).

The association of melatonin with grapevine physiology is part of a comprehensive investigation into the consequences for berry quality and postharvest protection. To make clear the intricate processes by which melatonin influences the growth and maturity of grapevines, and therefore the characteristics of fruit quality. It has been discovered

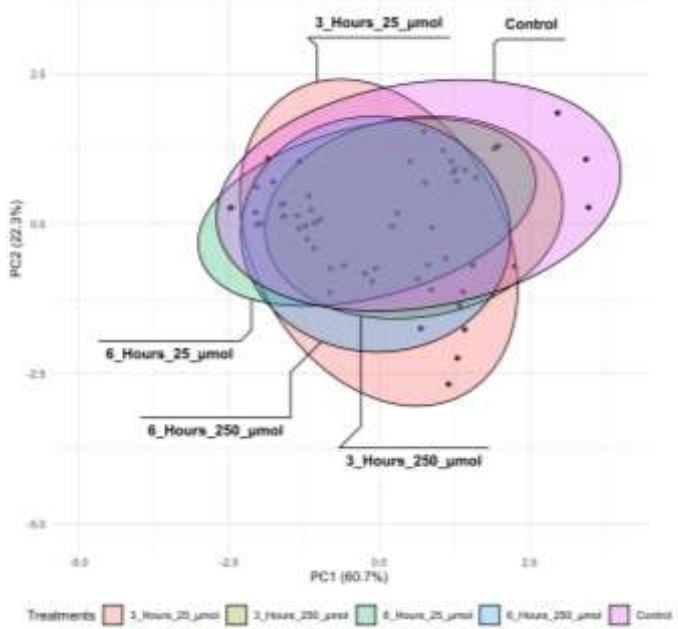
that melatonin applied postharvest can effectively delay ripening and senescence. The study regarding grape berries examined the effects of two postharvest melatonin doses (50 and 100 µmol) on the quality, bioactive components, and enzyme activities of grape berries cv "Crimson," which were kept for 35 days at 0 ± 1 °C and 90% relative humidity (RH). According to their findings, adding melatonin to berries prolongs their shelf life by preventing weight loss and preserving titratable acidity (TA), total soluble solids (TSS), berry adherence strength, and firmness (Nasser et al., 2022). The findings from this study are in parallel with the studies. Results from this research indicated that from this research the control and 6-hours 25 µmol L<sup>-1</sup> treatments, (31.91% and 32.41%, respectively) were higher predominantly weight loss than treatment of 3-hours 250 µmol L<sup>-1</sup> (24.08%). This showed that waiting too long (6 hours) in solution with low melatonin concentration (250 µmol L<sup>-1</sup>) is not affected to weight loss as it was not statistically different than control. However, waiting enough time (3 hours) with an adequate melatonin dose (250 µmol L<sup>-1</sup>) had a great contribution to weight loss (Table 1).

Moreover, some other researches are supported the current study. For example, after harvest, the main issue is weight loss since it deteriorates the quality and

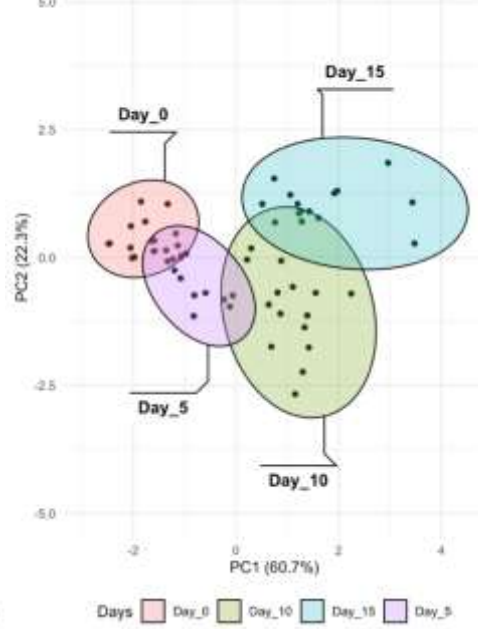
visual appeal of horticulture crops. According to reports, the biggest causes of water loss in fresh vegetables and fruits are transpiration and respiration (El-Mogy et al., 2020). Many studies reported that the application of melatonin has reduced the water loss in crops (Nasser et al., 2022; Liu et al., 2018; Wang et al., 2020). In addition, a study was conducted to protect post-harvest grape quality in cold storage from Türkiye, which is recently reported melatonin dips, in any dosage, slowed down the loss of berry weight, and visual quality, thereby prolonging the minimally processed (stem-excised) grapes' physical and biochemical quality (Sabir et al., 2024). In addition, the melatonin treatment has increased the Brix levels compared to the control in this study (Table 1). The findings from the previous research, additionally, have

reported an increase of Brix level compared to control in grapevine which could be due to a decreasing respiration and a reduction in the loss of Brix (Nasser et al., 2022; Xu et al., 2018). Furthermore, exogenous melatonin application has also reported an increase in Brix other fruits such as strawberries (El-Mogy et al., 2020) and sweet cherries (Wang et al., 2019). Another finding from this study is that the application of melatonin has reduced the loss of TA (except, for 6-hours of waiting with 25 µmol treatment which could be the same scenario in the weight loss of 6-hours waiting with 25 µmol treatment) levels in grape berries from the current results (Table 1). The reduction in the loss of TA has also been reported in grapes and guava fruits (Nasser et al., 2022; Fan et al., 2020).

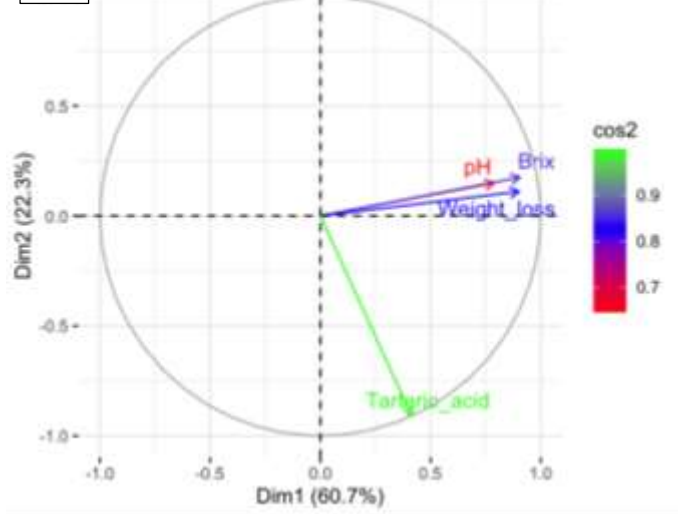
A



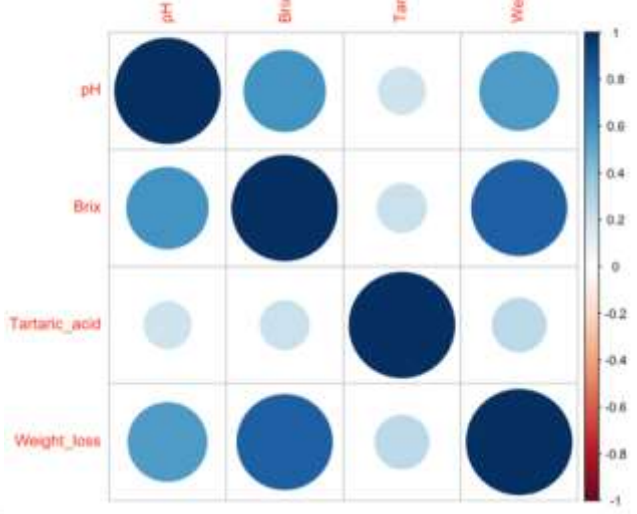
B



C



D



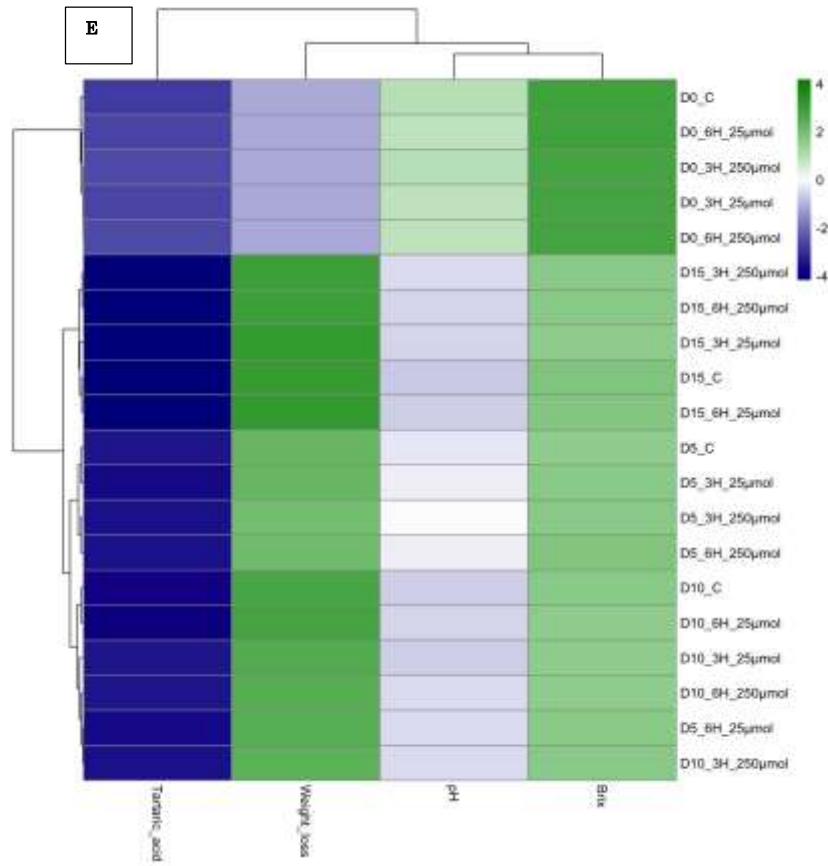


Figure 2. PCA biplot of colored by days and treatments. All application treatments (A), days (B), all variables (C), correlation of all variables (D) and heatmap of variables (E) are demonstrated.

Şekil 2. Günlere ve uygulamalara göre renklendirilmiş PCA biplot'u. Tüm uygulamaları (A), günleri (B), tüm değişkenler (C), tüm değişkenlerin korelasyonu (D) ve değişkenlerin ısı haritası (E) gösterilmektedir.

Furthermore, exogenous melatonin administration has been shown in prior research to reduce TA loss in sweet cherry and grape berries during cold storage (Wang et al., 2019). Melatonin's role in senescence control may account for some of its reduction in TA loss upon administration (Yang et al., 2019).

## CONCLUSION

Post-harvest processes have an important role in grape production to preserve berry quality and health. The short shelf life of the Kabarcık variety after harvest has become a big problem for Dulkadiroğlu grape growers. In this study conducted to solve this problem, as a result, the highest weight loss was found in the control and 6-hours 25  $\mu\text{mol L}^{-1}$  melatonin applications, and the least weight loss was found in the 3-hours 250  $\mu\text{mol L}^{-1}$  melatonin application. As a result of this study, it was determined that keeping grape clusters in solution for a long period of 6-hours was an ineffective method and the applied dose of 25  $\mu\text{mol L}^{-1}$  melatonin was found to be ineffective. However, a 250  $\mu\text{mol L}^{-1}$  dose of melatonin was determined to be an effective solution in preventing weight loss in grape berries. In future studies, grape berries are required to be supported by biochemical and molecular analyses.

## Conflicts of Interest Statement

The author has stated that there is no conflict of interest.

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## Influence of Leaf Water Potential and Defoliation Techniques on Leaf Area Characteristics in 'Merlot'/41B Grapevines

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### ABSTRACT

This study aimed to assess how variations in leaf water potential and different defoliation treatments influence leaf area characteristics. The research was carried out during two consecutive years (2019-2020) on 'Merlot'/41B combination grapevines cultivated in the Tekirdağ, Şarköy vineyards of Chateau Kalpak. Four distinct water stress levels (S0, S1, S2, and S3) were implemented based on measurements of leaf water potential. Additionally, defoliation treatments were applied, including Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). Upon analyzing leaf characteristics, a clear trend emerged, wherein higher stress levels correlated with an increased area of primary, lateral, and total leaves per vine. Concerning leaf removal interventions, the application of FW led to a reduction in all criteria except for the total area of main leaves per vine. While FW causes a decrease in certain leaf parameters under controlled conditions, the stress-induced increase in total leaf area points to the mechanism of plasticity in grapevines and warrants further investigation under different environmental and production dynamics.

### Horticulture

### Research Article

### Article History

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Vitis  
Stress  
Drought  
Canopy  
cv. Merlot

## 'Merlot'/41B Asmalarında Yaprak Su Potansiyeli ve Yaprak Alma Uygulamalarının Yaprak Alanı Özelliklerine Etkisi

### ÖZET

Bu çalışma, yaprak su potansiyelindeki değişikliklerin ve farklı yaprak alma işlemlerinin yaprak alanı özelliklerini nasıl etkilediğini değerlendirmeyi amaçlamıştır. Araştırma, Kalpak Şatosu'nun Tekirdağ, Şarköy bağlarında yetiştirilen 'Merlot'/41B kombinasyonlu asmalarda iki yıl süresince (2019-2020) yürütülmüştür. Yaprak su potansiyeli ölçümlerine dayalı olarak dört farklı su stresi seviyesi (S0, S1, S2 ve S3) uygulanmıştır. Ek olarak, Kontrol (C), Tam Pencere (FW), Sağ Pencere (RW) ve Sol Pencere (LW) olmak üzere dört farklı yaprak alma işlemi uygulanmıştır. Daha yüksek stres seviyelerinin, asma başına artan ana, koltuk ve toplam yaprak artış eğilimine neden olduğu belirlenmiştir. FW uygulaması, asma başına toplam ana yaprak alanı dışında tüm kriterlerde bir azalmaya yol açmıştır. FW, kontrollü koşullarda belirli yaprak parametrelerinde düşüşe neden olurken, toplam yaprak alanında stresin neden olduğu artış, asmalardaki plastidite mekanizmasına işaret etmekte ve farklı çevresel ve üretim dinamikleri altında daha fazla araştırma yapılmasını gerektirdiğini göstermektedir.

### Bahçe bitkileri

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

Vitis  
Stres  
Kuraklık  
Kanopi  
cv. Merlot

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### INTRODUCTION

The grapevine canopy is a complex system of leaves,

stems, and branches that play an important role in photosynthesis, fruit ripening, and water relations.

The regulation of grapevine canopy characteristics, and thus the growth and productivity of grapevines, are influenced by a range of factors, including biotic and abiotic factors such as water availability, temperature, soil nutrients, and pest and disease pressure. However, among these factors, water availability is crucial in determining grapevine growth and productivity as it directly affects the plant's physiological and biochemical processes. Human interaction with grapevine plants also plays a significant role in wine production, the direct manipulation of the grapevines' growth and health through cultivation practices, and the subsequent post-harvest handling and processing techniques meticulously employed by winemakers. Proper care and management of grapevines, including pruning, canopy management, irrigation, and fertilization, can improve vine growth and fruit quality, leading to higher-quality wine production (Brillante et al., 2018; Candar et al., 2020; Mirás-Avalos & Araujo, 2021).

In sustainable viticulture, the regulation of leaf water potential (LWP,  $\Psi_{\text{leaf}}$ ) is essential, and it is a commonly used indicator of plant water status, defined as the difference in water potential between a leaf and its surroundings. Decreased water resources due to global climate change are effective in the grapevine life cycle, and monitoring and management of LWP in grapevine plants affect cluster characteristics, berry, and wine composition by promoting slower leaf growth and higher water use efficiency via leaf characteristics (Rienth & Scholasch, 2019; Deloire et al., 2020).

Defoliation is a common vineyard practice that can affect grapevine water status and productivity. However, defoliation practices can have significant physiological effects on the production-consumption balance of vines (Bowen, 2009). These effects include a decrease in the number of photosynthesis products delivered to the cluster (Poni et al., 2008; Palliotti et al., 2013; Vaillant-Gaveau et al., 2014), limited root growth (Hunter et al., 1995), and decreased water efficiency (Medrano et al., 2007). Removing leaves during berry ripening can eliminate a potential source of carbon (C) and nitrogen (N), which may lead to a reduction in sugar and nitrogen accumulation (Rossouw et al., 2018), ultimately affecting berry quality (Bubola et al., 2022). Moreover, defoliation can reduce the total leaf area of the vine, weakening grapevine growth in the following years, and leading to decreased yields (Bahar et al., 2018). Therefore, grapevine physiological activity, leaf individual size, and the total leaf area on the grapevine are in an interactive relationship with each other (Candar, 2021; Candar, 2022; Candar et al. 2022). Additionally, defoliation can alter the microclimate around grape clusters, affecting fruit quality and ripening (Bubola et al., 2019; Candar, 2019; Stefanovic et al., 2021).

Understanding the effects of LWP and defoliation on

grapevine leaf characteristics can provide valuable insights into the mechanisms underlying grapevine responses to water stress and defoliation. Such insights can help optimize vineyard management practices to improve grapevine productivity and fruit quality.

Therefore, this study aimed to investigate the effects of LWP and defoliation treatments on grapevine canopy characteristics, including individual and total leaf areas of main and lateral leaves, total leaf areas of grapevines, leaf area exposed to direct sun and the sun-exposed leaf area per kilogram of grapes.

## MATERIAL and METHOD

### Location and plant material

The study was carried out in the vineyard of a private winery located in Tekirdağ, Şarköy, during two consecutive vegetation periods in 2019 and 2020. The grapevines utilized in the study were 'Merlot'/41B combination, planted with a 2.1 m and 1.0 m in-row spacing. The grapevines were trained to 70 cm stem height and the double arm cordon training method in the Espalier system.

### Methods

To ensure the accuracy of the study, the vines in each row were carefully selected to have the same age, development stage, and approximate load. After disregarding edge effects, the selected grapevines were considered homogeneous. In the year 2020, when the shoots had grown approximately 25-35 cm, the number of shoots and clusters was found to be the same as the previous year. Routine cultural operations, including tillage, fertilization, weeding, and spraying, were performed in the vineyard throughout the two-year vegetation period from 2019 to 2020.

The experiment was designed using the divided plots trial design with three replications, and each plot was subjected to a specific level of stress measured by leaf water potential (LWP). The study included a total of 144 vines, with 48 vines in each replication, and four different stress levels (S0, S1, S2, and S3) and leaf removal treatments including Control (C, no defoliation), Full Window (FW), Right Window (RW), and Left Window (LW).

Irrigation was performed as required based on the predawn leaf water potential (LWP,  $\Psi_{\text{pd}}$ ) measurements taken at five to seven-day intervals. The predetermined stress levels were used to adjust the irrigation, and the  $\Psi_{\text{pd}}$  was verified the next day to ensure it remained within the desired range. The control treatment, S0, received no irrigation and relied on random precipitation. S1 had a stress level between -0.4 to -0.6 MPa, and irrigation was used to maintain the  $\Psi_{\text{pd}}$  within this range. Similarly, S2 had a stress level between -0.5 to -0.7 MPa, and the  $\Psi_{\text{pd}}$  was

maintained within this range through irrigation. Lastly, S3 was subjected to a stress level of  $\leq -0.7$  MPa and the  $\Psi_{pd}$  was kept below this value through irrigation. The defoliation treatments (DT) were carried out approximately two weeks after the start of veraison. The treatments involved removing shoots and leaves from the eighth node and creating a window by eliminating all the leaves between the seventh and thirteenth nodes. The study consisted of four different defoliation treatments: Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). For the FW treatment, shoots and leaves were removed from the eighth node. For the RW treatment, all the leaves between the seventh and thirteenth nodes on the west side of the row were removed, while for the LW treatment, all the leaves between the seventh and thirteenth nodes on the east side of the row were removed. The C treatment was used as the control, with no defoliation being performed. The defoliation process was conducted with special care to ensure that the grapes were at a 15-17 °Brix level according to Alço (2019).

#### Leaf area analysis and measurements

The main phenological development dates of the bud burst (EL- 05), pre-bloom (EL- 19), full bloom (EL- 23), berry set (EL- 27), veraison (EL- 35) and the harvest (EL- 38) stages were recorded using the method described by Lorenz et al. (1995). Climate data were obtained from the Turkish State Meteorological Service (MGM).

To determine the average leaf area of the main leaves developing from the main shoot and the lateral leaf areas growing from the lateral shoots, the fully grown and healthy leaves were scanned with a scanner after the harvest. The images obtained from the scanner were analyzed using the Fläeche program (Kraft, 1995), and the leaf area was calculated and recorded in  $\text{cm}^2$ .

To calculate the total main leaf ( $\text{cm}^2 \text{vine}^{-1}$ ) and total lateral leaf area per vine ( $\text{cm}^2 \text{vine}^{-1}$ ), the average number of leaves in the shoot and the total number of shoots after harvest were multiplied. The total leaf area per vine was determined by adding the main leaf area per stem and the lateral leaf area per vine, following the method described by Irimia and Tardea (2006) and Sanchez-de-Miguel et al. (2010).

The formula used to calculate the leaf area exposed to direct sun ( $\text{m}^2 \text{da}^{-1}$ ) was:

$$\frac{1000}{RS} * [(H * 2) + CW] * (1 - CD)$$

where RS represents row spacing in meters, H represents height in meters, CW represents canopy width in meters, and CD represents canopy discontinuity (10%).

To obtain the sun-exposed leaf area per kilogram of

grapes ( $\text{m}^2 \text{kg}^{-1}$ ), the leaf area exposed to direct sun ( $\text{m}^2 \text{da}^{-1}$ ) was divided by the yield per decare ( $\text{kg da}^{-1}$ ), using the equation by Carbonneau (1980) and Carbonneau (1983).

#### Trail design and statistical analysis

The experiment was designed using a divided plots trial design, where the main plot represented water stress levels, and each subplot represented defoliation practices. A total of 144 vines were included in the study, with three plants per sub-plot and three replications per combination of four water stress levels and four defoliation treatments.

Statistical data analysis was performed using JMP 13.2.0. Analysis of variance (ANOVA) was used to determine the significance of differences between treatments, and significant differences were further grouped using the LSD test at a 5% significance level. correlations and principal component analysis of selected variables was carried out using R statistical environment (R Core Team, 2016).

## RESULTS and DISCUSSION

#### Climate, phenology, yield, and total soluble solids.

Table 1 shows the viticultural climate indicators of Tekirdağ for the years 1939-2019, as well as for the years 2019 and 2020.

The mean annual precipitation decreased from 589.50 mm between 1939 and 2019 to 378.40 mm in 2019 and further decreased to 290.00 mm in 2020. The precipitation for vegetation, also decreased from 196.70 mm to 129.80 mm to 83.60 mm, respectively. The average temperature for 2019 was 15.60°C, while the average for 2020 was 15.30°C, while the long-term average temperature was 14.00°C. The mean temperature of the hottest month increased from 23.80 °C for 1939-2019 to 25.30 °C in 2019 and remained stable at 25.00 °C in 2020. These trends suggest that the region is becoming drier, which could have implications for grape production.

The Huglin index (HI) increased from 2128.20 °C in 1939-2019 to 2324.07 °C in 2019 but decreased slightly to 2229.21 °C in 2020. The Winkler index (WI-GDD) increased from 1872.00 degree-days to 2157.00 degree-days in 2019 and slightly decreased to 2124.00 degree-days in 2020. The Hydrothermal Index (HyI), which combines temperature and precipitation, decreased from 3595.20 °C mm for 1939-2019 to 2181.54 °C mm in 2019 and further decreased to 1328.10 °C mm in 2020. These changes indicate that the region is becoming warmer, with increasing heat accumulation during the growing season. The Night Cold Index (CI), increased from 16.00 °C to 17.60 °C to 19.20 °C, respectively, and the Growing Season Temperatures (GST). Finally, the GST, which is the average temperature during the growing season, increased



from 18.91 °C to 20.27 °C to 20.11 °C, respectively (Table 1).

Table 1 Tekirdağ viticultural climate indicators in experimental years.

*Çizelge 1. Deneme yıllarında Tekirdağ bağcılık iklim göstergeleri.*

| Climatic indices                  | Unit       | 1939-2019 | 2019    | 2020    | References            |
|-----------------------------------|------------|-----------|---------|---------|-----------------------|
| Precipitation (Mean Annual)       | mm         | 589.50    | 378.40  | 290.00  | -                     |
| Precipitation (Vegetation)        | mm         | 196.70    | 129.80  | 83.60   | -                     |
| Mean temperature of hottest month | °C         | 23.80     | 25.30   | 25.00   | -                     |
| Huglin index (HI)                 | °C         | 2128.20   | 2324.07 | 2229.21 | (Huglin, 1978)        |
| Winkler index (WI-GDD)            | degree-day | 1872.00   | 2157.00 | 2124.00 | (Winkler et al, 1974) |
| Hydrothermic Index (HyI)          | °C mm      | 3595.20   | 2181.54 | 1328.10 | (Branas, 1946)        |
| Night Cold Index (CI)             | °C         | 16.00     | 17.60   | 19.20   | (Tonietto, 1999)      |
| Growing Season Temperatures (GST) | °C         | 18.91     | 20.27   | 20.11   | (Jones, 2007)         |

The bud burst (EL 05), which corresponds to the appearance of green shoot tips, occurred on April 11, 2019, and on April 15, 2020, indicating that the 2020 bud burst was delayed by four days compared to 2019. The pre-bloom (EL 19) stage, occurred on May 26, 2019, and May 30, 2020, respectively. The full bloom (EL 23), in 2019, occurred on June 2, while in 2020, it occurred on June 8, indicating a six-day delay in the latter year. Berry set (EL 27), was observed on June 9, 2019, and June 14, 2020, respectively. The veraison (EL 35) occurred on July 20, 2019, and July 24, 2020, respectively. The harvest (EL 38), in 2019, occurred on September 15, while in 2020, it occurred on September 16.

Overall, data showed that there were slight variations in the timing of the phenological stages between the two years, with some stages being delayed in 2020 compared to 2019. These differences could be attributed to variations in weather patterns and environmental conditions between the two years.

Since homogeneous grapevines were already selected in both years according to the trial design, the number of shoots and clusters was also homogenized, no statistical difference could be detected in kg yield per grapevine according to defoliation and stress treatments. Yield per grapevine varied between 2.20-2.22 kg per grapevine in defoliation treatments and 2.20-2.26 kg per grapevine in stress treatments.

Although defoliation treatments (FW and LW) had no overall impact on total soluble solids (TSS) accumulation over multiple years, leaf water potential (LWP) treatments did significantly affect TSS. Within the defoliation group, LW resulted in the highest average TSS (24.78 °Brix), while FW had the lowest (24.35 °Brix). Within the LWP group, the S0 treatment with the highest potential (25.00 °Brix) achieved the highest TSS, while S3 had the lowest. Notably, TSS was higher in 2020 (24.76 °Brix) compared to the previous year (24.39 °Brix).

The previous studies determined that the size of the main leaves of the 'Merlot' grape cultivar ranged from 152.29 cm<sup>2</sup> to 237.60 cm<sup>2</sup> (Candar, 2018). However, the available data for experimental years ranged from 91.79 cm<sup>2</sup> to 142.94 cm<sup>2</sup>, with an average of 125.93±22.69 cm<sup>2</sup>. It is known that leaf size is influenced by environmental, developmental, and genetic factors during the formation process. Thus, there can be variations in leaf size from the average appearance to its actual size (Chitwood et al., 2016a). Therefore, the morphological and physiological characteristics of the leaves may be influenced by factors other than the variety itself, such as their position in the shoot or environmental effects.

In the year of 2019, there was a general decrease in the main leaf area as the level of water stress increased. This trend is supported by the declining mean values observed from S0 to S3. The highest main leaf area was recorded under S3 in 2019, which differed significantly from the other stress treatments. However, in 2020, there were no significant differences among the stress treatments. Regarding the defoliation treatments, the mean main leaf area values in the year 2019 did not show any significant differences. However, in 2020, significant differences were observed among the defoliation treatments. The main leaf area was significantly higher in the control group (C) compared to the defoliation treatments in both experimental years. This indicates that defoliation negatively affected the main leaf area (Table 2).

When considering the significance levels for the mean main leaf area concerning the main effect of defoliation treatment (DME), it was observed that the C treatment had the largest leaves, with an average size of 139.66 cm<sup>2</sup>, while the FW treatment had the smallest leaves, averaging 114.97 cm<sup>2</sup>. In terms of the main effect of leaf water potential (LWPME), the S3 treatment exhibited the highest main leaf size, with an average value of 134.01 cm<sup>2</sup>, while the S0 treatment had the lowest main leaf size, averaging 121.01 cm<sup>2</sup> across the experimental years.

### Main leaf area



Table 2 Effects of stress levels and defoliation treatments on leaf area variables.

*Çizelge 2. Stres seviyeleri ve yaprak alma uygulamalarının yaprak alanı değişkenleri üzerindeki etkileri.* Values marked with different letters in the same column and row were statistically significant at  $p < 0.05$  level according to ANOVA and the LSD test. Results expressed as mean of three replications with  $\pm$  SE.

S0; control of stress treatments, S1;  $\Psi_{pd}$  between  $-0.3/-0.5$  MPa, S2;  $\Psi_{pd}$  between  $-0.5/-0.7$  MPa, and S3;  $\Psi_{pd} < -0.7$  MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window. LWPME= stress treatments main effect, DTME=defoliation treatments main effect, YME=year main effect.

It is reasonable to expect that increased water stress would lead to a decrease in the average main leaf area. However, in the case of grapevine plants, which are perennial woody plants, their leaf shape and size differentiation may exhibit adaptation and flexibility to various environmental factors, which may differ from evolutionary and developmental effects (Chitwood et al., 2016b). Therefore, the finding regarding the main effect of leaf water potential (LWPME) aligns with the results reported by Candar (2018). Regarding the main effect of defoliation treatment (DME), it was discovered that the FW treatment resulted in the smallest main leaf areas. This finding differs from the observations of Candar (2018), where it was noted that the main leaf size increased in grapevines with the lowest total leaf area, where only the main leaves remained on the plant. However, the available data indicate that lateral leaves have the potential to contribute to the development of main leaves, and the size of the main leaves may not play a critical role in maintaining total photosynthesis rates. Moreover, the physiological ages of main leaves and axillary leaves were found to be distinct, which is linked to their photosynthetic capacity. Schultz (1996) explained that young leaves exhibit a high photosynthetic capacity until harvest, but their position in the canopy and the light microclimate can influence the overall photosynthesis of the plant. In this regard, the available data are consistent with the findings of Schultz (1996). Furthermore, based on the data from this study, the relationship between the initiation time of stress-induced irrigation practices and leaf maturity could elucidate the observation that leaf size did not exhibit a linear and positive alteration with escalating stress levels.

However, the impact of decreasing soil water reserves becomes evident when comparing the experimental years. The main effect of the year (YME) shows statistical significance. In 2019, the average main leaf area was  $138.56 \text{ cm}^2$ , indicating its higher importance. However, in 2020, the average main leaf area significantly decreased to  $113.29 \text{ cm}^2$ , placing it in the lower-importance group. The reduced precipitation received during the vegetation period in 2020 contributed to the main leaves remaining consistently smaller compared to the previous year. It is believed that this decrease in the average main leaf area in

2020, compared to 2019, has a direct impact on photosynthesis, resulting in reduced yield per vine and, consequently, yield per decare.

### Lateral leaf area

The average size of the lateral leaves of cv. 'Merlot' was determined to be  $31.62 \pm 6.17 \text{ cm}^2$ . Previous studies, on the other hand, report that the lateral leaf area for 'Merlot' grape cultivar varies between  $55.16 \text{ cm}^2$  and  $92.74 \text{ cm}^2$  (Candar, 2018). In the year of 2019, the lateral leaf area generally increased as the level of water stress increased. The highest lateral leaf area was observed under S3 in 2019, which differed significantly from the S0 and S1 treatments. However, in 2020, although S3 had the lowest value, it was significantly different only from S2. The significant differences observed in the main effect of water stress treatments (LWPME) in both experimental years indicate that the lateral leaf area was significantly influenced by the amount of water applied to grapevines (Table 2).

In both 2019 and 2020 years, the lateral leaf area exhibited some variation among the different defoliation treatments. In 2019, the lateral leaf area showed significant differences among the defoliation treatments in the control group (C). However, in 2020, significant differences were not observed between the defoliation treatments. The control group in 2020 and S3 in 2019 displayed higher lateral leaf area compared to the defoliation treatments.

When examining the mean lateral leaf area across the experimental years, the main effect of defoliation treatment (DME) was found to be statistically significant. The highest values were observed in LW with a mean of  $32.76 \text{ cm}^2$  and RW with a mean of  $32.28 \text{ cm}^2$ , while the lowest lateral leaf area size was recorded in the FW treatment with a mean of  $29.77 \text{ cm}^2$ .

Analyzing the average lateral leaf area based on the main effect of leaf water potential (LWPME) across the years, ANOVA showed statistical significance at the 5% level. The S2 treatment had the highest mean value of  $34.68 \text{ cm}^2$ , placing it in the first importance group, while the lower values were observed in the S1, S3, and S0 treatments, respectively, in the last importance group.

Furthermore, when examining the average lateral leaf

area concerning the main effect of the year (YME), statistical significance was observed. The year 2019 exhibited the highest mean value of 34.02 cm<sup>2</sup>, placing it in the first importance group, while the year 2020 was found to be in the last importance group with a mean value of 29.21 cm<sup>2</sup>.

Zinni et al. (2023) observed that complete removal of whole leaves was effective in increasing the average lateral leaf area in the application without tipping. The study indicated that both RW and LW applications were successful in enhancing the average lateral leaf area. The available data corroborate these findings, as similar results were achieved for the year 2019.

### Total main leaf area per grapevine

In both experimental years, there was a general increase in the total main leaf area as the level of water stress increased. The highest total main leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. However, there were no significant differences in the total main leaf area among the control group and the different defoliation treatments in both 2019 and 2020. The mean values for the control group (C) and the defoliation treatments were relatively similar in both years (Table 2).

When evaluating the two-year data of the total main leaf area per grapevine together, it was found that the main effect of defoliation treatment (DME) did not show significant differences between applications with close values. On the other hand, the main effect of leaf water potential (LWPME) was statistically significant, with S3 reaching the highest value when importance levels were examined.

Regarding the main effect of year (YME), while the total main leaf area per grapevine was statistically significant throughout the experimental years, it was determined that 2019 had the highest value of 1.80 m<sup>2</sup> main leaf area per vine, placing it in the first importance group, and 2020 had the lowest value of 1.67 m<sup>2</sup> vine<sup>-1</sup>, placing it in the last importance group.

### Total lateral leaf area per grapevine

In both experimental years, there was a general increase in the total main leaf area as the level of water stress increased. The highest total main leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress

treatments. However, there were no significant differences in the total main leaf area among the control group, and in both 2019 and 2020, there was a general increase in the total lateral leaf area as the level of water stress increased. This is evident from the increasing mean values observed from S0 to S3. The highest total lateral leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. The main effect of stress treatments (LWPME) did not significantly affect the total lateral leaf area, indicating that the interaction between stress treatments did not have a significant impact.

Similarly, the total lateral leaf area did not show significant differences among the different defoliation treatments in both 2019 and 2020. The mean values for the control group (C) and the defoliation treatments were relatively similar in both years (Table 2).

When examining the combination of years for the total lateral leaf area per grapevine, the main effects of leaf water potential (LWPME) and year (YME) were found to be statistically significant. However, the main effect of defoliation treatment (DME) was not found to be statistically significant. Among the defoliation treatments, RW had the highest value, while FW had the lowest value based on DME. According to LWPME, the S3 application was in the first importance group, followed by the other treatments. Considering the combined effect of LWP and defoliation treatments, the year 2020 reached the highest value of 3.38 m<sup>2</sup> vine<sup>-1</sup>, placing it in the first importance group, while the year 2019 was found to be in the last importance group with a value of 2.11 m<sup>2</sup> vine<sup>-1</sup>.

### Total leaf area per grapevine

In both 2019 and 2020, there was a general increase in the total leaf area as the level of water stress increased. This trend is evident from the increasing mean values observed from S0 to S3. The highest total leaf area was consistently observed under S3 in both 2019 and 2020, and it differed significantly from the other stress treatments. The total leaf area also exhibited some variation among the different defoliation treatments in both 2019 and 2020. In 2019, there were no significant differences observed among the defoliation treatments. However, in 2020, significant differences were observed between the defoliation treatments (Table 3).

Table 3 Effects of stress levels and defoliation treatments on total main leaf area, sun-exposed leaf area, and yield-related variables.

*Çizelge 3. Stres seviyeleri ve yaprak alma uygulamalarının toplam ana yaprak alanı, güneşe maruz kalan yaprak alanı ve verime bağlı değişkenler üzerindeki etkileri.*

| Total leaf area per grapevine (m <sup>2</sup> vine <sup>-1</sup> ) | Leaf area exposed to direct sun (m <sup>2</sup> da <sup>-1</sup> ) | Sun exposed leaf area per kilogram of grapes (m <sup>2</sup> kg <sup>-1</sup> ) |
|--|--|---|
|--|--|---|



|                        | 2019        | 2020        | 2019          | 2020          | 2019        | 2020        |
|------------------------|-------------|-------------|---------------|---------------|-------------|-------------|
|                        |             |             | <i>LWPs'</i>  |               |             |             |
| S0                     | 3.60±0.18b  | 4.86±0.21b  | 1051.50±45.49 | 1062.15±45.52 | 0.94±0.04b  | 1.04±0.06   |
| S1                     | 3.44±0.59b  | 4.92±0.22b  | 1056.83±45.47 | 1058.10±44.41 | 0.97±0.03b  | 1.01±0.07   |
| S2                     | 3.75±0.53b  | 4.94±0.14b  | 1055.91±46.34 | 1057.85±44.31 | 1.03±0.03a  | 1.02±0.05   |
| S3                     | 4.72±0.14a  | 5.90±0.14a  | 1058.33±47.50 | 1057.14±45.68 | 1.02±0.04a  | 1.01±0.07   |
| <b>LWPME (LSD0.05)</b> | <b>0.46</b> | <b>0.50</b> | <b>ns</b>     | <b>ns</b>     | <b>0.03</b> | <b>ns</b>   |
|                        |             |             | <i>DTs'</i>   |               |             |             |
| C                      | 3.85±0.19   | 5.55±0.20a  | 1272.58±3.62a | 1271.19±2.31a | 1.17±0.20a  | 1.28±0.06a  |
| FW                     | 3.95±0.15   | 4.86±0.28b  | 841.33±3.72c  | 850.00±2.29c  | 0.80±0.12c  | 0.80±0.03c  |
| RW                     | 3.99±0.20   | 5.20±0.15ab | 1055.24±5.52b | 1054.52±2.59b | 0.99±0.01b  | 0.99±0.01b  |
| LW                     | 3.72±0.29   | 5.01±0.18b  | 1053.33±5.09b | 1059.52±3.73b | 0.99±0.01b  | 1.02±0.03b  |
| <b>DME (LSD0.05)</b>   | <b>ns</b>   | <b>0.50</b> | <b>14.31</b>  | <b>8.54</b>   | <b>0.03</b> | <b>0.13</b> |
|                        |             |             | <i>YME</i>    |               |             |             |
|                        | 3.88±0.10B  | 5.16±0.10A  | 1055.59±22.35 | 1058.81±21.76 | 0.99±0.02   | 1.02±0.03   |
| <b>YME (LSD0.05)</b>   |             | <b>0.23</b> |               | <b>ns</b>     |             | <b>ns</b>   |

Values marked with different letters in the same column and row were statistically significant at  $p < 0.05$  level according to ANOVA and the LSD test. Results expressed as mean of three replications with  $\pm$  SE.

S0; control of stress treatments, S1;  $\Psi_{pd}$  between  $-0.3/-0.5$  MPa, S2;  $\Psi_{pd}$  between  $-0.5/-0.7$  MPa, and S3;  $\Psi_{pd} < -0.7$  MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window. LWPME= stress treatments main effect, DTME=defoliation treatments main effect, YME=year main effect.

Although the year combination did not show statistical significance in terms of the main effect of defoliation treatment (DME), it was found that the highest value for the total leaf area per grapevine was  $4.70 \text{ m}^2 \text{ vine}^{-1}$  in the control (C) treatment, while the lowest value was observed in the FW treatment. When considering the main effect of leaf water potential (LWPME), it was found that the annual incorporation of LWPME is significant for the total leaf area per grapevine at the 5% level. The S3 treatment was placed in the first importance group with a value of  $5.31 \text{ m}^2 \text{ vine}^{-1}$ , followed by the S2, S0, and S1 treatments in order.

Furthermore, the total leaf area per grapevine showed statistical significance at the 5% level in terms of the main effect of year (YME). In the first importance group according to YME, it reached  $5.16 \text{ m}^2 \text{ vine}^{-1}$  in 2020 and  $3.88 \text{ m}^2 \text{ vine}^{-1}$  in 2019 (Table 3).

Delice (2001) and Calo et al. (1999) found a significant positive correlation between grapevine yield and total leaf area, stating that brix was associated with the ratios of total leaf area/leaf area exposed to direct sun and vegetative growth/yield balance. In the present study, it was observed that the total leaf area per vine increased in 2020, and the brix values in 2020 were higher compared to the previous year.

#### Leaf area exposed to direct sun $\text{m}^2 \text{ da}^{-1}$

There were no significant differences in the leaf area exposed to direct sun among the different stress treatments in both 2019 and 2020. The mean values for the leaf area exposed to direct sun were relatively similar across all stress treatments in both years.

However, there were significant differences in the leaf area exposed to direct sun among the different defoliation treatments in both 2019 and 2020. The control group (C) had the highest leaf area exposed to

direct sun, which was significantly different from the other defoliation treatments. Significant differences were also observed among the defoliation treatments themselves, with the FW treatment having the lowest leaf area exposed to direct sun (Table 3).

When examining the combination of years for the main effect of defoliation treatment (DME), it was found to be statistically significant. The C treatment had the highest value of  $1271.88 \text{ m}^2 \text{ ha}^{-1}$ , while the FW treatment had the lowest leaf area exposed to direct sun with a value of  $845.66 \text{ m}^2 \text{ ha}^{-1}$ . In terms of the main effect of leaf water potential (LWPME), it was observed that the S0 treatment had a relatively lower leaf area exposed to direct sun with a value of  $1056.82 \text{ m}^2 \text{ ha}^{-1}$ , while the S3 treatments had a higher leaf area exposed to direct sun with a value of  $1057.73 \text{ m}^2 \text{ ha}^{-1}$ . Regarding the main effect of the year (YME), the year 2019 had a value of  $1055.64 \text{ m}^2 \text{ ha}^{-1}$ , while the year 2020 had a slightly higher value of  $1058.81 \text{ m}^2 \text{ ha}^{-1}$ .

#### Sun-exposed leaf area per kilogram of grapes $\text{m}^2 \text{ kg}^{-1}$

There were significant differences in the sun-exposed leaf area per kilogram of grapes among the different stress treatments in 2019. However, in 2020, no significant differences were observed among the stress treatments.

Similarly, the sun-exposed leaf area per kilogram of grapes showed significant differences among the different defoliation treatments in both 2019 and 2020. The control group generally had the highest sun-exposed leaf area per kilogram of grapes, which differed significantly from the other defoliation treatments. Significant differences were also observed among the defoliation treatments themselves, with the FW treatment having the lowest sun-exposed leaf area per kilogram of grapes (Table 3).



When considering the combination of 2019 and 2020 in terms of the main effect of defoliation treatment (DME), it was determined that the values ranged between  $2.19 \text{ m}^2 \text{ kg}^{-1}$  in the control (C) treatments and  $2.00 \text{ m}^2 \text{ kg}^{-1}$  in the FW treatments. Examining the main effect of leaf water potential (LWPME), it was found to be statistically significant, with the S3 treatments reaching the highest value of  $2.43 \text{ m}^2 \text{ kg}^{-1}$  in the first importance group, followed by the S2, S1, and S0 treatments in order. In terms of the main effect of the year (YME), although statistically significant, it reached a value of  $2.38 \text{ m}^2 \text{ kg}^{-1}$  in 2020 and  $1.77 \text{ m}^2 \text{ kg}^{-1}$  in 2019, with a higher value in 2020.

### Correlations of leaf characteristics, maturation indices and yield

The variable lateral leaf area has a moderate positive correlation with the main leaf area with a correlation coefficient of 0.398. The total main leaf area per grapevine has a strong positive correlation with the main leaf area, a weak positive correlation with the lateral leaf area, and no significant correlation with the remaining variables. The variable total lateral leaf area per grapevine has a weak negative correlation with the main leaf area and no significant correlation with the other variables. The total leaf area per grapevine has no significant correlation with the main leaf area and lateral leaf area, but it has a moderate positive correlation with the total main leaf area per grapevine, a strong positive correlation with the total lateral leaf area per grapevine with a correlation coefficient of 0.944, and no significant correlation with the remaining variables. The leaf area exposed to the direct sun has a moderate positive correlation with the main leaf area and no significant correlation with the other variables. The correlation coefficient between sun-exposed leaf area per kilogram of grapes and leaf area exposed to direct sun is 0.796 and represents a strong positive relationship. The sun-exposed leaf area per kilogram of grapes also has weak positive correlations with total main leaf area per grapevine, total lateral leaf area per grapevine, and total leaf area per grapevine. The maturation indices of  $\text{pH}^2 \times \text{°Brix}$  have a weak positive correlation with the main leaf area, weak negative correlations with total leaf area per grapevine and total lateral leaf area per grapevine, and no significant correlation with the other variables. The yield has no significant correlation with any of the variables except a strong negative correlation with

sun-exposed leaf area per kilogram of grapes (Figure 1).

### Principal component analysis (PCA) of leaf characteristics, maturation indices, and yield

To assess the interaction among stress levels, defoliation treatment, and the leaf characteristics under study, a PCA was conducted. The dataset consisted of a total of eight treatments and nine leaf variables, and the analysis was performed using the covariance matrix. Two distinct biplots were generated to examine the impact of stress levels and defoliation treatments on the leaf variables individually.

According to the cumulative proportion of variance in the LWP biplot, PC1 accounts for 56.80% of the total variance. When PC1 and PC2 are combined, they explain 95.10% of the total variance. Furthermore, PC1, PC2, and PC3 together explain 100% of the total variance. Hence, PC1 and PC2 are the primary components in capturing the variability in the LWP data. Similarly, for the defoliation treatments, PC1 explains 66.80% of the total variance, PC2 explains 23.50% of the total variance, and when PC1, PC2, and PC3 are combined, they account for 100% of the total variance, as shown by the cumulative proportion of variance. Both PCA correlation plots demonstrate a noticeable distinction among the samples based on the treatments and variables, indicating a reasonable separation (Figure 2).

Upon analyzing the LWPs biplot, it is evident that variable S0 has a loading value of -2.08 for Dim.1, indicating a negative correlation between S0 and the first principal component. Similarly, S1 has a loading value of -1.14 for Dim.1, also indicating a negative correlation with the first principal component. In contrast, S2 has a loading value of 0.11 for Dim.1, suggesting a weaker correlation. On the other hand, S3 displays a loading value of 3.11 for Dim.1, indicating a strong positive correlation with the first principal component. Regarding Dim.2, S0 has a loading value of 0.79, suggesting a positive correlation with the second principal component. Similarly, S1 has a loading value of 0.98 for Dim.2, indicating a positive correlation. In contrast, S2 demonstrates a significant negative correlation with the second principal component, as shown by its loading value of 2.78 for Dim.2. Finally, S3 has a loading value of 0.99 for Dim.2, suggesting a positive correlation.

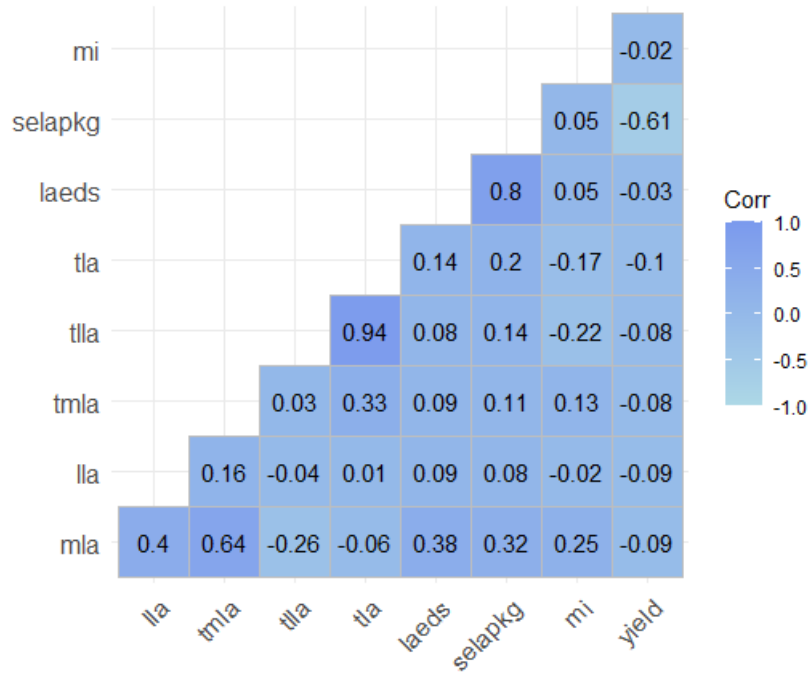


Figure 1. Correlations of selected variables. Coefficient statistical significance indicated by color of squares. mla; main leaf area (cm<sup>2</sup>), lla; lateral leaf area (cm<sup>2</sup>), tmla; total main leaf area per grapevine (m<sup>2</sup>), tlla; total lateral leaf area per grapevine (m<sup>2</sup>), tla; total leaf area per grapevine (m<sup>2</sup>), laeds; leaf area exposed to direct sun (m<sup>2</sup> da<sup>-1</sup>), selapkg; Sun exposed leaf area per kilogram of grapes (m<sup>2</sup> kg<sup>-1</sup>), mi; maturation indice of pH<sup>2</sup> x °Brix, yield; yield per grapevine (kg)

Şekil 1. Seçilmiş değişkenlerin korelasyonları.

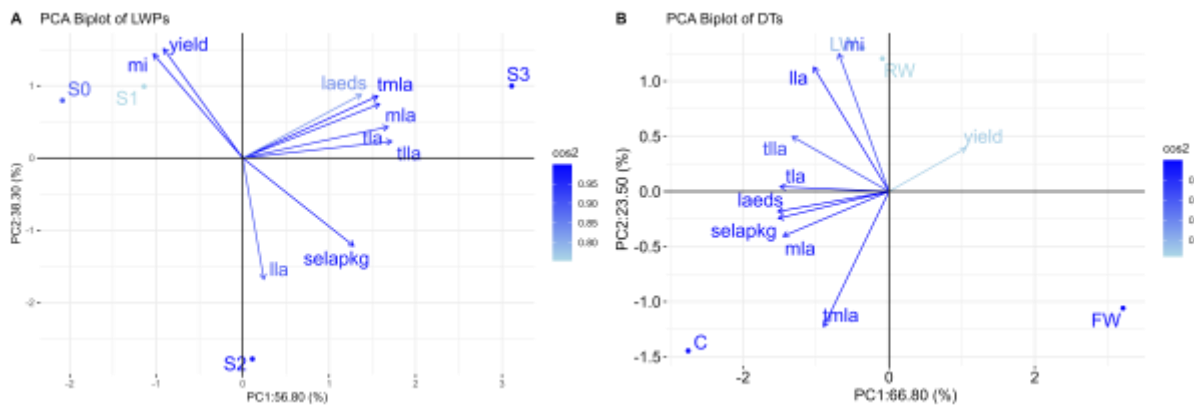


Figure 2. Principal component analysis (PCA) with the mean values of variables. A; PCA biplot of LWP's, B; PCA biplot of DTs'. mla; main leaf area (cm<sup>2</sup>), lla; lateral leaf area (cm<sup>2</sup>), tmla; total main leaf area per grapevine (m<sup>2</sup>), tlla; total lateral leaf area per grapevine (m<sup>2</sup>), tla; total leaf area per grapevine (m<sup>2</sup>), laeds; leaf area exposed to direct sun (m<sup>2</sup> da<sup>-1</sup>), selapkg; sun-exposed leaf area per kilogram of grapes (m<sup>2</sup> kg<sup>-1</sup>), mi; maturation indices of pH<sup>2</sup> x °Brix, yield; yield per grapevine (kg)

Şekil 2. Seçilmiş değişkenlerin birincil bileşen analizi (PCA)

When examining the loading values for the leaf variables in relation to LWP levels, it is evident that the total lateral leaf area per grapevine and the MI<sub>pH<sup>2</sup> x °Brix</sub> variable exhibit strong positive correlations with both Dim.1 and Dim.2. This implies that these variables have a significant influence on multiple aspects captured by the principal components. Their impact extends across different underlying factors represented by Dim.1 and Dim.2. In contrast, variables

such as lateral leaf area and total lateral leaf area per grapevine display contrasting correlations between the two dimensions. Lateral leaf area shows a weak correlation with Dim.1 but a strong positive correlation with Dim.2. On the other hand, total lateral leaf area per grapevine demonstrates a strong positive correlation with Dim.1 but a weak correlation with Dim.2. These distinct patterns suggest that these variables contribute differently to the underlying

factors represented by Dim.1 and Dim.2.

In practical terms, this information can be used to identify key variables that have a consistent and strong impact across multiple dimensions such as total lateral leaf area per grapevine and  $MI_{pH^2 \times \text{Brix}}$ . These variables can be considered influential factors that contribute significantly to the overall structure of the data. Conversely, variables with contrasting correlations like lateral leaf area and total lateral leaf area per grapevine may require further investigation to understand their unique contributions and how they affect different dimensions of the data.

In the DTs biplot, by examining the loading values, it is observed that the variable FW has the highest loading value on Dim.1 with a value of 3.20, indicating a strong positive correlation with the first principal component. This suggests that FW is highly associated with the variability explained by Dim.1. Similarly, LW and RW exhibit positive loadings on Dim.2 by values of 1.29 and 1.20, respectively, indicating a positive correlation with the second principal component. Conversely, variable C demonstrates negative loadings on both Dim.1 and Dim.2, indicating a negative correlation with both principal components. This implies that C is inversely related to the variability explained by both dimensions. These loading values help identify the variables that contribute the most to the respective principal components. In this case, FW appears to have the strongest influence on Dim.1, while LW and RW have significant contributions to Dim.2. Variables such as total leaf area per grapevine, leaf area exposed to direct sun, and sun-exposed leaf area per kilogram of grapes exhibit relatively high loading values in both Dim.1 and Dim.2, indicating strong positive correlations with both principal components. On the other hand, variables like total main leaf area per grapevine, total lateral leaf area per grapevine, and  $MI_{pH^2 \times \text{Brix}}$  display moderate positive loadings in both dimensions. It's important to note that while the loading values for all variables are positive, their magnitudes differ, indicating variations in the strength of their contributions to the principal components. For instance, the leaf area exposed to direct sun and sun-exposed leaf area per kilogram of grapes have higher loading values compared to the main leaf area or yield, suggesting that they contribute more significantly to the explained variability.

## CONCLUSION

Upon analyzing leaf characteristics, an observable trend emerged wherein an elevated stress level corresponded to an increased count of primary, lateral, and overall leaves per vine. This atypical occurrence, rarely documented in existing literature, is believed to activate a stress-mitigating mechanism. This mechanism involves the mobilization of stored materials through internal metabolic processes and

the plastidic effects within the grapevine. Furthermore, the experiment was conducted in a uniform and well-balanced vineyard with consistent cultivation practices and longstanding production objectives. Consequently, certain criteria did not distinctly manifest the effects of the interventions; however, the outcomes did capture the underlying trends.

Regarding leaf removal interventions, the application of FW led to a reduction in all criteria except for the total number of primary leaves per vine.

It is anticipated that disparities between the outcomes will become more conspicuous in vineyards with dissimilar crop loads and/or during years when climatic variables exert more pronounced influences.

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## Author contribution

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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## Bazı Kapyta Biber (*Capsicum annuum* L.) Genotiplerinin Morfolojik Karakterizasyonu

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### ÖZET

*Solanaceae* familyasının bir üyesi olan *Capsicum annuum* (Capia), meyve ve bitki özellikleri bakımından zengin bir genetik çeşitliliğe sahiptir. Bu çalışma, yeni hibrit kapyta biber çeşitlerinin geliştirilmesi için arzu edilen morfolojik özelliklere sahip kapyta biber genotiplerini seçmek amacıyla morfolojik karakterler kullanarak yerel popülasyonlar, standart ve hibrit çeşitlerden oluşturulan genetik havuzu karakterize etmeyi amaçlamıştır. Yüz on iki genotip 15 morfolojik (11 kalitatif ve 4 kantitatif) karakter kullanılarak karakterize edilmiş ve kalitatif karakterler gözlemlenirken kantitatif karakterler ölçülmüştür. Tek bitki seleksiyonu sonucunda belirlenen hatlar arasındaki ilişkiyi belirlemek için Küme ve Temel Bileşen Analizi (PCA) uygulanmıştır. Yapılan küme analizi sonucunda hatlar arasındaki morfolojik benzerliği değerlendirmek için dendrogram oluşturulmuş ve 15 değişkene dayalı olarak on dokuz grup belirlenmiştir. Temel bileşenler analizinde ise altı TB eksenini toplam varyasyonun %66,2' sini açıklamıştır. Çalışma sonucunda, biber hatları arasında yüksek morfolojik varyasyon tespit edilmiştir. Bitki özelliklerinde ki varyabilitenin değerlendirmesi, genetikçilere ve ıslahçılara biber ıslah programlarına dahil etmek üzere arzu edilen özelliklere sahip popülasyonların belirlemelerinde yardımcı olabilir.

### Bahçe Bitkileri

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### Anahtar Kelimeler

Kapyta biber  
Morfolojik karakterizasyon  
Gen koleksiyonu

## Morphological Characterization of Some Capia Pepper (*Capsicum annuum* L.) Genotypes

### ABSTRACT

*Capsicum annuum* (Capia), a member of the *Solanaceae* family, has a rich genetic diversity in fruit and plant traits. To select Capia pepper genotypes with desirable morphological traits for the development of new hybrid capia pepper varieties, this study aimed to characterize genetic accessions generated from local populations, and standard and hybrid cultivars using morphological characters. One hundred and twelve genotypes were identified using 15 morphological traits (11 qualitative and 4 quantitative) and quantitative traits were measured while qualitative traits were observed. Cluster and Principal Component Analysis (PCA) was applied to determine the relationship between the lines identified as a result of single plant selection. As a result of the cluster analysis, a dendrogram was prepared to evaluate the morphological similarity between the lines, and nineteen groups were identified based on 15 variables. In Principal Component Analysis, six PC axes explained 66.2% of the total variation. As a result of the study, high morphological variability was observed among pepper lines. This evaluation of plant trait variability can assist geneticists and breeders in identifying populations with desirable characteristics for inclusion in pepper breeding programs.

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## INTRODUCTION

Pepper (*Capsicum annuum* L.) is a crop with different colors ranging from green at the intermediate stage to yellow or red at maturity. Pepper is an important health protective factor that helps protect against common human diseases, especially due to the phenolic compounds, flavonoids, and carotenoids in its fruits, which can increase the vitamins perspective (Mady et al., 2005). According to Tük (2023), capia pepper is one of the most widely cultivated crops in Turkey and has increased significantly in both open-field and greenhouse cultivation in recent years. In 2022, Faostat reported the world production of pepper at 36.972.494 metric tons and the estimated total production of pepper in Turkey at 3.018.775 metric tons. Considering this production data, Turkey has a rich genetic diversity of peppers with high cultivation value and important importance in terms of research and conservation. Correct identification and classification of botanical species is a crucial step in efficiently managing germplasm collections. Because this stage forms the basis of developing any plant species. In addition, many researchers have stressed the importance of morphological characterization as a fundamental step toward resolving taxonomic conflicts in many plant species (Moura et al., 2013; Ranjit et al., 2013; Gerrano et al., 2017; Olatunji & Afolayan, 2018).

It has been reported that due to the increased cultivation of improved varieties, a large portion of cultivated peppers are in constant danger of extinction along with the loss of genetic diversity (Sunday et al., 2021). In this context, morphological characterization studies are considered an important starting point for genotype selection from cultivars for breeding purposes (Oyelakin et al., 2019). These studies are also a useful genetic guide for selecting parental plants for hybridization (Singh, 2006). Due to the increasing cultivation of improved varieties, a large proportion of cultivated peppers have been reported to be in constant danger of extinction with a loss of genetic diversity (Sunday et al., 2021). Taia (2005) and Karaca (2013) reported that the use of morphological characters is the most valuable tool in selecting genotypes from varieties for crop improvement and breeding purposes. This process involves studying and evaluating the physical and structural characteristics of the plant, such as shape, size, color, and other visible traits. Evaluation of genetic diversity determined after morphological characterization is very important for determining unique traits, genetic variations, and potential sources of resistance to diseases, pests, and environmental stresses. In addition the genotypes identification and differentiation of different genotypes or cultivars based on their physical characteristics, such as plant height, leaf shape, fruit shape, color, and size. This information is crucial for maintaining genetic purity and integrity in breeding programs.

Morphological characterization also provides valuable data for plant breeders to select and develop new pepper cultivars with desirable traits and enables them to make informed decisions on which genotypes to cross in line with breeding aims. Weerakoon and Somaratne (2010) also used morphological traits such as plant height, stem pubescence, fruit weight, flower color, and fruit shape to select pepper different genotypes for hybridization and breeding purposes. Sunday et al. (2021) used morphological traits such as fruit shape and color, fruit length (cm), fruit width (cm), and fruit wall thickness. All these morphological traits are very important in varietal selection and breeding programs. This is because fruit yield is determined by the vegetative characters. Numerous researchers in our country and around the world performed various characterizations on *C. annuum* L. species according to the fruit and plant characteristics, and the existing morphological variations were identified in detail (Başak, 2019; Belay et al., 2019; Lima et al., 2019; Santos et al., 2019; Hernández-Pérez et al., 2020; Khan et al., 2020; Ferdousi et al., 2021; Sunday et al., 2021; Tripodi et al., 2021; Bedjaoui et al., 2022; Gomes et al., 2022; Şahin et al., 2022; Çetin, 2023). To date, many characterization studies have been carried out on pepper, but morphological characterization studies on capia pepper are limited. It is known that breeders of capia pepper develop varieties through the selection of superior parents for various purposes. Therefore, understanding the level of genetic variation among pepper varieties through the use of morphological traits is important for the selection of suitable genotypes for crop improvement and breeding purposes. Accordingly, this study aimed to characterize the genetic pool established to select capia pepper genotypes with desirable morphological traits for future breeding studies.

## MATERIAL and METOD

### Plant material

The study material consisted of 112 Capia pepper genotypes, including 61 local populations from Kırşehir, 1 standard variety (Yalova Yağlık 28), and 50 hybrid varieties. Morphological observations and measurements were reported for 122 lines identified through morphological characterization studies on 775 plants at S1 stage.

### Phenotypic Characters

Data for morphological characteristics were collected according to the UPOV (TG/76/8) protocol (UPOV, 2006). One hundred and twelve Capia pepper genotypes were cultivated in the fully automated R&D greenhouse at Kırşehir Ahi Evran University. On average, 8 plants of each genotype were planted based on seed emergence with spacing of 0.7m x 0.4m x 0.4m.

A total of fifteen phenotypic characters were evaluated. All characters were measured in the greenhouse at normal harvest time. One hundred and twenty genotypes were characterized using 15 morphological characters (11 qualitative and 4 quantitative), with qualitative characters observed and quantitative characters measured.

The qualitative characters included: Anthocyanin coloration in the internode (none/medium/high/very high), Plant grown habit (upright, semi-upright, prostrate), Leaf color (light green, green, dark green), Leaf shape (lanceolate, ovate, broad elliptic), Fruit attitude (erect, horizontal, drooping), Immature fruit color (light green, green, dark green), Mature fruit color (light red, red, dark red), Fruit cross-section shape (elliptic, angular, circular), Fruit tip shape (very acute, moderately acute, rounded, moderately depressed, very depressed), Number of fruit loculus (predominantly two, equally two and three, predominantly three, equally three and four) and fruit stalk tip (absent, shallow, medium, deep, very deep). The quantitative characters were: Fruit length (cm), Fruit width (cm), Fruit flesh thickness (mm), and Seed cavity length (cm). Fruit character analyses were conducted on 3 fruits from each plant.

### Statistical Analysis

The multivariate procedure in Minitab (MINITAB 19, 2019) was used for principal component analysis (PCA) and cluster analysis based on 15 morphological characters. In this analysis, the data obtained from the selected materials were scored according to the UPOV scale values, in order to identify patterns of variation within the *Capsicum* pepper accession groups.

## RESULTS and DISCUSSION

### Phenotypic Traits

Phenotypic characterization parameters showed a high variation in terms of some plant and fruit traits. When plant traits were evaluated, it was determined that 3 lines were prostrate (2.46%), 11 lines were semi-upright (9.02%) and 108 lines were upright (88.52%) in terms of plant attitude. In the study, the peppers that were determined to be prostrate and semi-upright in terms of plant attitude were non-types of village pepper, bell pepper, and ornamental pepper. In the gene collection, which showed high variation in terms of leaf color and shape, 58 lines were dark green (47.54%), 55 lines were green (46.08%) and 9 lines were light green (7.38%). In terms of leaf shape; 6 lines were broad elliptic (4.92%), 2 lines were lanceolate (1.64%) and 114 lines were ovate (93.44%). Finally, according to the intensity of anthocyanin in the internode; 19 lines were low (15.58%), 46 lines were medium (37.70%), 46 lines were high (37.70%) and 11 lines (9.02%) were absent anthocyanin in the internode. In a study conducted on interspecific hybrid populations

of pepper, in terms of plant characteristics, 87 genotypes showed upright growth habits, while 11 genotypes showed semi-upright development. In terms of leaf shape, it was found that 7 genotypes had long leaves, 51 genotypes had medium leaves and 12 genotypes had short leaves, while in terms of leaf color, 53 genotypes had dark green leaves and the remaining genotypes had green leaves. As for anthocyanin in the internode level, while no anthocyanin formation was observed in 45 genotypes, it was reported that out of the remaining 43 genotypes, 24 showed moderate, 14 had low and 5 had very low (Pınar & Dilfiruz, 2022). According to the findings obtained, the gene collection exhibits a wide variation in terms of plant characteristics. This variation is very important in the design of breeding programs.

Upon a general assessment in terms of pomological characteristics, significant differences among the lines have been observed. The research findings indicate that, based on observations of fruit attitude, 2.46% of the fruits are upright, 8.20% are semi-upright, and 89.34% have drooping of the fruit, especially for plants with lower leaf density, which is a desired trait as it makes them more resistant to physiological disorders such as sunburn (Karaağaç, 2006). When examining the pre-maturity fruit color visually, it was observed that the colors range from green to dark green among the lines. 31.15% of the lines have green fruits, while 68.85% consist of dark green fruits. As fruits mature, a color transformation occurs. In *Capsicum* pepper, mature fruit color, which is very important both as a quality and a harvest parameter, was determined as 4.92% light red, 27.87% medium red, and 67.21% dark red. According to the study by Mutlu et al. (2009), the pre-maturity fruit color in most populations was green (92.97%), with 7.03% observed as yellow. The mature fruit color in the populations was reported to be red (54.59%), dark red (38.92%), and light red (5.41%), and in 2 populations (1.08%) a brown mature fruit color was observed.

In our study, when the cross-sectional shape of the fruits of the lines was evaluated, it was observed that the majority of 103 lines (84.43%) were triangular, while 19 lines (15.57%) were ovate. When the fruit tip shape was analyzed, it was determined that 2.46% were very depressed, 39.34% were depressed, 56.56% were peaked and 1.64% were very peaked. In terms of the number of fruit lobes, 35.25% of the lines had three lobes, 62.30% had two lobes and 2.45% had no lobes. Finally, when the stalk tip was analyzed, it was observed that 3 lines (2.46%) were deep, 7 lines (58.20%) were slightly deep and 48 lines (39.34%) had no stalk tip. Karaağaç (2006) reported that 60.7% of the genotypes were ovate-shaped when analyzed in terms of fruit cross-section. It was stated that the types with pointed fruit tips are more preferred in the market and three-lobed fruits are a desirable feature



for the processing industry. However, it has been reported that two-lobed capia pepper fruits are preferred by consumers for roasting. The pomological traits characterized in our study show variation in parameters such as fruit color, fruit posture, fruit tip shape, number of fruit lobes and stem pit at different rates according to the lines. This variation is thought to be because the lines were collected from different locations and from a wide area and foreign pollination, which is common in pepper. This situation shows that the gene pool analyzed has a very heterogeneous structure.

In the study, the lowest seed cavity length was found to be 1.30 cm in line 122, while the highest was 9.20 cm in line 47 (Table 1). When fruit length and diameter values were evaluated, the shortest fruit length was 4 cm in line 8, while the longest fruit length was 20.40 cm in line 80. For fruit diameter, the smallest diameter was 2.10 cm in line 122, while the largest diameter was 6.50 cm in line 79. For flesh thickness, the lowest value was 2.54 mm in line 46, while the highest value was

6.95 mm in line 79 (Table 1). Consistent with our results, Mutlu et al. (2009) reported that in pepper, fruit length ranged from 1.4 to 18.5 cm, fruit diameter ranged from 0.7 to 7.3 cm, and fruit flesh thickness varied from 1 to 7 mm among populations. Similarly, Karaağaç (2006) found that in red pepper, fruit length ranged from 9.1 to 18.5 cm, fruit diameter varied from 4.5 to 6.8 cm, and fruit flesh thickness ranged from 3.3 to 5.8 mm. Panayotov et al. (2000) observed that in capia-type red peppers, fruit length ranged from 10.2 to 15.9 cm, fruit diameter ranged from 2.1 to 5.7 cm, and fruit flesh thickness ranged from 2.1 to 5.5 mm. Genetic relatedness among 122 red capia pepper lines was determined using phenotypic markers at the end of the study. Some markers emphasized the differentiation among pepper genotypes phenotypically, as they showed more significant variations that caused significant distinctions. In conclusion, the importance of phenotypic variation in future pepper breeding programs was emphasized, and phenotypic traits were predicted to be useful in the differentiation of pepper genotypes.

Çizelge 1. S1 kademesindeki seçilen biber hatlarına ait kantitatif özellikler

Table 1. Quantitative characters of selected pepper lines at S1 level

| Characters              | Maximum | Genotype | Minimum | Genotype | Mean  |
|-------------------------|---------|----------|---------|----------|-------|
| Seed cavity length (cm) | 9.20    | 47       | 1.30    | 122      | 3,38  |
| Fruit width (cm)        | 6.50    | 79       | 2.10    | 122      | 4.37  |
| Fruit length (cm)       | 20.40   | 80       | 4.0     | 8        | 12.70 |
| Flesh thickness (mm)    | 6.95    | 79       | 2.54    | 46       | 4.10  |

### Principal Component Analysis

In the study, PCA was also applied to group the pepper lines based on morphological and pomological observations and measured parameters, and to study the relationship between the parameters obtained.

It is reported that the evaluation of PC axes with eigenvalues greater than 1 in PCA analysis is a very reliable indicator (Özdamar, 2004; Kanal & Balkaya, 2021). Total variance ratios and cumulative variance values of the axes obtained as a result of the analysis were determined and interpretations were made accordingly. In the study, as a result of principal component analysis, 15 independent principal component axes were obtained in pepper lines and it was determined that there were 6 PC axes with eigenvalues greater than 1. It was determined that the cumulative variation of PC axes with eigenvalue greater than 1 represented 66.2% of the selected pepper lines (Table 2). When the values of the principal component axis were analyzed, it was found that the first principal component axis explained 20.2% of the total variation, the second principal component axis explained 12.2% of the variation, and the third principal component axis explained 10.4% of the total variation. In the study, it is accepted that the weight coefficient values in the components in terms of the

criteria examined in the principal component analysis have significant weight if they are 0.3 and above (Brown, 1991; Taş, 2020). In this direction, the traits with high coefficients of 0.3 and above on the PC-1 axis were plant attitude (0.326), fruit tip shape (0.334), leaf shape (0.355), fruit attitude (-0.368), fruit length (-0.442) and seed cavity length (-0.355), while stalk tip (0.392), sectional cross-sectional shape (0.473), fruit width (0.438) and fruit flesh thickness (0.486) were found to be significant on PC-2 axis (Table 2). The score plot and loading plot analyses are shown in Figure 1.

The studies conducted by Agyare et al. (2016) and Bozkalfa et al. (2017) emphasize the importance of characterizing genetic diversity in Capsicum species and local pepper genotypes through morphological and agronomic traits. The findings of Agyare et al. (2016) demonstrate that morphological traits effectively reflect genetic diversity in *Capsicum* species, with a genetic variance of 59.61%. The study by Bozkalfa et al. (2017) reveals that genetic distances among local pepper genotypes vary between 62% and 94%. These studies provide significant information for pepper breeding and the conservation of genetic diversity. The results of these research efforts show that the pepper gene pool possesses a high degree of heterogeneity, indicating the potential for its use in breeding

programs aimed at improving resistance to diseases and pests, yield, and certain quality criteria. Supporting these breeding efforts with molecular characterization studies will ensure more effective use of the results obtained. Additionally, cluster analysis based on agronomic traits for distinguishing and

identifying plant participation and group participation based on their similarities increases the possibility of determining heterotic effects from parents in the breeding populations and obtaining new superior genotypes.

Çizelge 2. S1 kademesindeki seçilen biber hatlarında temel bileşen analizine göre faktör grupları ve bunlara karşılık gelen TB eksenleri

Table 2. Factor groups and corresponding PC axes after principal component analysis for selected pepper lines at S1 level

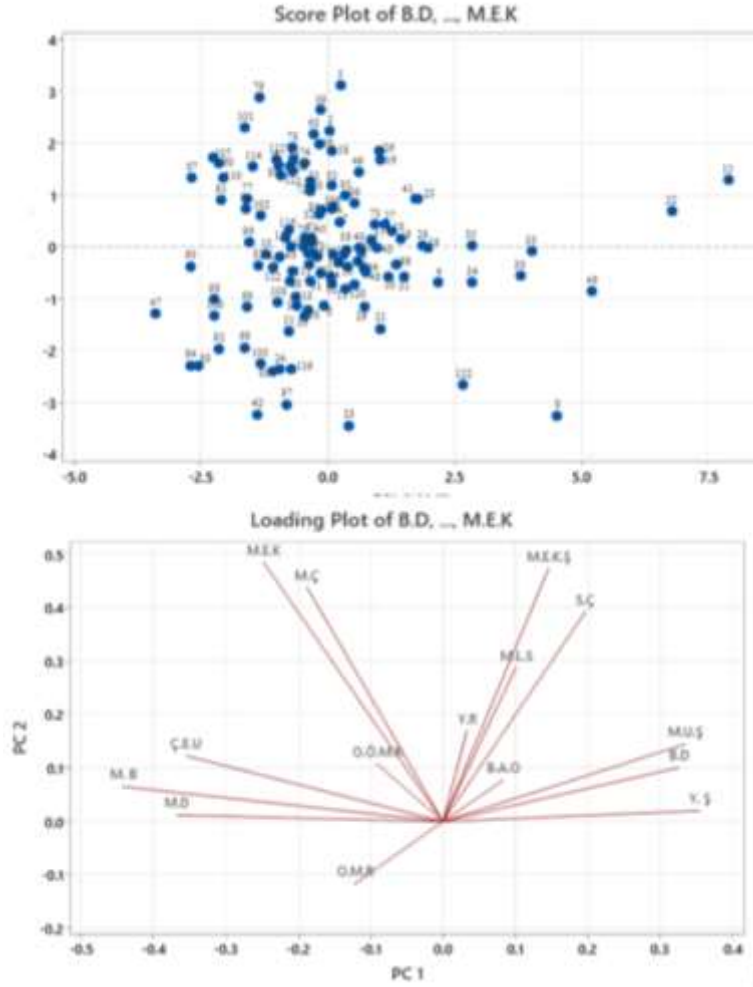
| Eigenanalysis of the Correlation Matrix |        |        |        |        |        |        |
|---|--------|--------|--------|--------|--------|--------|
| Eigenvalue                              | 3.029  | 1.832  | 1.567  | 1.264  | 1.212  | 1.028  |
| Proportion                              | 0.202  | 0.122  | 0.104  | 0.084  | 0.081  | 0.068  |
| Cumulative                              | 0.202  | 0.324  | 0.429  | 0.513  | 0.594  | 0.662  |
| Variable                                |        |        |        |        |        |        |
| Eigenvalue                              | PC1    | PC2    | PC3    | PC4    | PC5    | PC6    |
| P.H                                     | 0.326  | 0.101  | 0.009  | -0.012 | -0.412 | -0.141 |
| L.C                                     | 0.033  | 0.169  | -0.261 | -0.678 | 0.083  | 0.134  |
| L.S                                     | 0.355  | 0.020  | 0.002  | -0.189 | -0.283 | 0.197  |
| A.C.I                                   | 0.084  | 0.078  | -0.518 | 0.472  | -0.094 | 0.062  |
| F.A                                     | -0.368 | 0.012  | -0.166 | 0.247  | 0.197  | -0.211 |
| I.F.C                                   | -0.091 | 0.108  | -0.342 | -0.359 | 0.216  | -0.426 |
| M.F.C                                   | -0.122 | -0.117 | 0.404  | 0.003  | 0.152  | -0.395 |
| F.C.S.S                                 | 0.146  | 0.473  | 0.139  | 0.018  | 0.210  | -0.228 |
| F.T.S                                   | 0.334  | 0.144  | -0.278 | 0.198  | -0.090 | -0.395 |
| N.F.L                                   | 0.101  | 0.288  | 0.024  | 0.093  | 0.536  | 0.446  |
| F.S.T                                   | 0.197  | 0.392  | 0.302  | 0.191  | 0.068  | 0.140  |
| S.C.L                                   | -0.355 | 0.123  | -0.268 | 0.014  | -0.238 | 0.301  |
| F.L                                     | -0.442 | 0.065  | 0.149  | 0.015  | -0.264 | 0.075  |
| F.W                                     | -0.188 | 0.438  | 0.238  | -0.075 | -0.394 | -0.098 |
| F.F.H                                   | -0.248 | 0.486  | -0.129 | 0.022  | -0.031 | -0.066 |

**Explanation:** Plant grown habit (P.H), Leaf color (L.C), Leaf shape (L.S), Anthocyanin coloration in internode(A.C.I), Fruit attitude (F.A), Immature fruit color (I.F.C), Mature fruit color (M.F.C), Fruit cross-section shape (F.C.S.S), Fruit tip shape (F.T.S), Number of fruit loculus(N.F.L), fruit stalk tip (F.S.T), Seed cavity length (S.C.L), Fruit length (F.L), Fruit width (F.W), Fruit flesh thickness (F.F.T), Fruit flesh hardness (F.F.H).

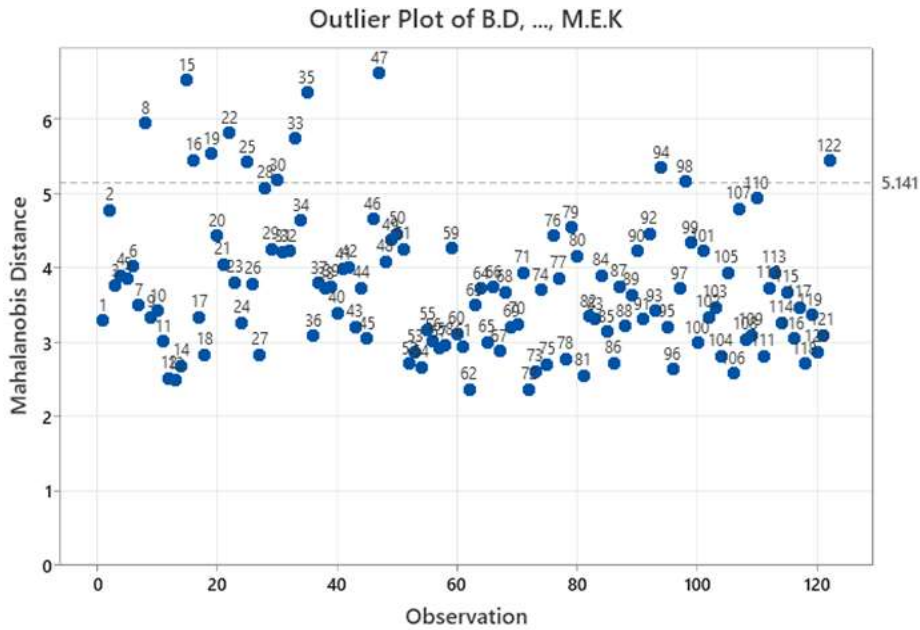
The outlier plot analysis conducted in our research data is also extremely important for detecting outliers containing extreme measurement values. Here, the calculated Mahalanobis distance is one of the statistical approaches used for outlier detection. Mahalanobis distance, which can be interpreted as a measure of distance, takes into account the covariance matrix calculated from the data to compute the distance between two points. Thus, it also considers the behavior of other points. The unit of the obtained distance is in terms of standard deviation along the line passing between two points, which is a value dependent on the data (Anonymous, 2023). Therefore, these lines possessing outliers in the gene pool can be characterized as atypical lines. Here, lines numbered 8, 15, 16, 19, 22, 25, 30, 33, 35, 47, and 122 exceeding a Mahalanobis distance of 5.141 have been identified as outliers (Figure 2).

### Cluster Analysis

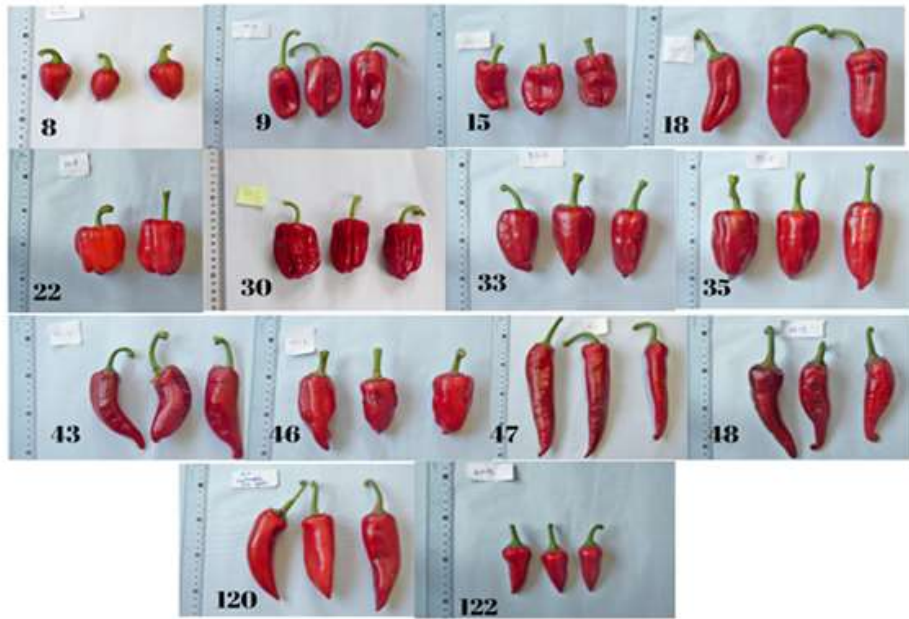
Cluster analysis was applied to the data obtained as a result of characterization. Using the data obtained after characterization studies, similarities-differences and groupings among pepper species, subspecies, or local varieties can be shown by using cluster analysis (Düzyaman & Vural, 2002; Rivera Martinez et al., 2004). It has been reported that cluster analysis is more reliable when 25% or more of the total variation can be explained by the first 2 or 3 axes (Mohammadi & Prassana, 2003). In the dendrogram formed as a result of cluster analysis according to the correlation matrix, the lines were defined as 19 groups. If the dendrogram is examined in general, it can be seen that it consists of 4 main groups. The first main group consists of 7 subgroups, the second main group consists of 3 subgroups, the third main group consists of 3 subgroups and the fourth main group consists of 5 subgroups (Figure 4). The genotypes in the groups formed as a result of the dendrogram are given in Table 3.



Şekil 1: PCA Analizi sonucu oluşturulan score plot ve loading plot analizleri  
Figure 1: Score plot and loading plot analyses prepared based on PCA Analysis



Şekil 2: PCA Analizi sonucu oluşturulan aykırı değer analizi  
Figure 2: Outlier analysis created as a result of PCA Analysis



Şekil 3: Çalışmada tip dışı olarak tespit edilen bazı hatların bir fotoğraf kesiti  
 Figure 3: A photo section of some of the lines identified as non-typical in the study

Çizelge 3: Dendrogram sonucunda oluşan gruplardaki genotipler  
 Table 3: Genotypes in the groups created as a result of the dendrogram

| Group | Lines   | Total Lines (pcs) |
|-------|---|-------------------|
| A     | 1. subgroup (1, 53, 12, 13, 40, 7, 10, 38, 16, 39, 43, 55), 2. subgroup (11, 120, 23, 21, 113, 48, 83, 111), 3. subgroup (76, 77), 4. subgroup (2, 5, 66, 27, 58, 41), 5. subgroup (3, 45, 44, 61, 32, 65, 25, 28, 57, 56, 26, 52, 121, 59), 6. subgroup (4, 18, 73, 85, 19, 118, 34, 99), 7. subgroup (9, 50, 29, 31). | 54                |
| B     | 1. subgroup (8, 122), 2. subgroup (15, 30), 3. subgroup (22, 46, 33, 35).   | 8                 |
| C     | 1. subgroup (6, 94, 97, 98, 103, 17, 51, 42, 108, 96, 109, 24, 36, 105, 65, 119), 2. yan grup (47), 3. subgroup (20, 87, 95, 89, 90, 92), 4. subgroup (80,110).   | 25                |
| D     | 1. subgroup (14, 116, 37, 62, 115, 81, 86, 49, 54, 72, 78, 63, 106, 60, 67, 104, 75, 100, 117, 79, 114), 2. subgroup (71), 3. subgroup (64, 88, 102), 4. subgroup (74, 84, 101), 5. subgroup (68, 70, 82, 93, 112, 91, 107).  | 35                |

The general characteristics of the groups are given in detail below.

**Group A:** It was determined to be the group in which the most genetic material was clustered, with 54 lines among the groups formed as a result of cluster analysis (Table 3). The majority of lines in this group (44 lines) belonged to the local population. In this group, which consisted of seven subgroups, the plant attitude was erect/semi-erect, the leaf shape was oval, the fruit attitude was hanging/semi-erect, the fruit tip shape was pointed/depressed, and the presence of stalk tip was slightly deep/absent. According to the averages among the lines; the fruit length (11.96 cm) of the lines in this group was medium, fruit width (4.25 cm) was medium, seed cavity length (36.92 mm) was medium and fruit flesh thickness (4.51 mm) was medium.

**Group B:** It was identified as the group in which the

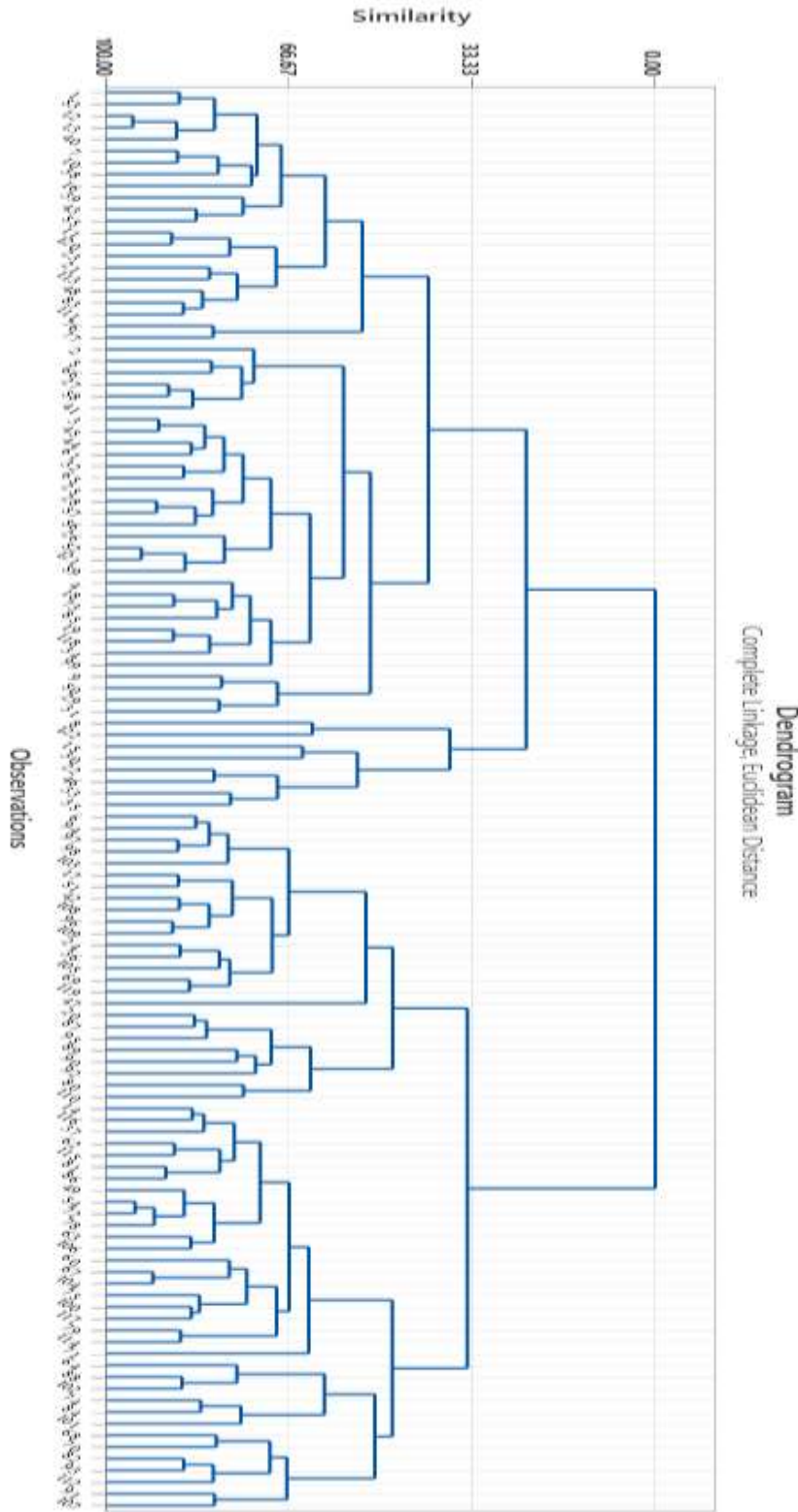
least genetic material was clustered with 8 lines among the groups formed as a result of cluster analysis (Table 3). All lines in this group belonged to the local population and generally consisted of ornamental, dolma, and mini capia pepper types which are considered non-typical. In this group consisting of three side groups, the fruit attitude is upright/semi-upright, the fruit tip shape is depressed/very depressed and the fruit cross-sectional shape is square. According to the averages among the lines; the fruit length (6.91 cm) of the lines in this group was low, the fruit width (4.15 cm) was medium, the seed cavity length (19.13 mm) was low and the fruit flesh thickness (3.02 mm) was thin.

**Group C:** This group consists of a total of 25 lines (Table 3). Most of the lines in this group (17 lines) are hybrid variety extensions in the market. In this group, consisting of four lateral groups, plant attitude was



upright, leaf shape was oval, fruit attitude was drooping, fruit tip shape was pointed/depressed, the number of fruit lobes was two, and the presence of stalk tip was not detected. According to the averages

among the lines; the fruit length (15.64 cm) of the lines in this group was long, the fruit width (4.35 cm) was medium, the seed cavity length (40.06 mm) was high and the fruit flesh thickness (4.22 mm) was medium.



Şekil 4: Cluster analizi sonucunda elde edilen dendrogram

Figure 4: The dendrogram obtained as a result of the cluster analysis.

**Group D:** Among the groups formed in the dendrogram, the group with the highest number of

lines (35 lines) is after group A (Table 3). The majority of lines in this group (30 lines) are commercial hybrids.

In this group, which consists of five subgroups, plant attitude is upright, leaf shape is oval, fruit attitude is drooping, fruit tip shape is pointed, fruit cross-sectional shape is square, and the presence of stem tip is slightly deep. According to the averages among the lines; the fruit length (16.29 cm) of the lines in this group is long, the fruit width (4.70 cm) is large, the seed cavity length (38.97 mm) is medium and the fruit flesh thickness (4.64 mm) is thin.

When the cluster analysis results were analyzed based on genotypes, it was found that in group A, lines 12-13 and 52-121 were very similar to each other, while lines 12-50 and 13-29 were the most distant lines. In group D, lines 54-72 were very similar to each other, while lines 54-64 were the most distant lines. These results will help to eliminate very similar genotypes and save work and time in breeding studies. In addition, the most distant genotypes are important in terms of a high positive heterosis rate as a result of future crosses between them.

## CONCLUSION and RECOMMENDATIONS

*Capsicum annuum* L. is one of the most important vegetable crops grown and stands out both for its fresh consumption and for industrial use, in the world and Turkey. In conclusion, morphological characterization and selection of Capia pepper genotypes are vital steps in crop improvement, genetic diversity conservation, and ensuring the agricultural sustainability of this important crop. These processes enable the development of better-performing varieties, adaptation to changing environmental conditions, and the delivery of high-quality produce to meet market demands and are fundamental for the continued development and advancement of *Capsicum annuum* L. cultivation. This study aimed to characterize the gene pool created from standard, hybrid expansion, and local populations using morphological characters. At the end of the study, it was found that the six PC axes obtained from the PCA analysis represented 66.2% of the cumulative variation. This value indicates that this gene collection can help geneticists and breeders identify populations with desirable traits for inclusion in pepper breeding programs. In addition, four main groups were identified in the cluster analysis. To be used in different breeding studies, if the important criterion is Capia type pepper with long fruit and high fruit flesh thickness, the populations in groups C and D in the dendrogram showing the similarity coefficients between pepper samples can be selected. Again, if the criterion sought is plant and fruit attitude, these groups should be considered. In addition, if the populations that show a high similarity to the varieties we use as standard are included in breeding programs and evaluated, new varieties suitable for the market can be brought to the market. As a result of this study, sufficient morphological

variability was found in the pepper lines. However, while it is difficult to reveal the difference between the types by morphological characterization, this difference can be revealed more easily and precisely by using molecular technical methods. Therefore, in the next stage of the study, characterization of the capia-type red pepper genotypes with these molecular breeding methods in the purpose-oriented breeding studies planned to be carried out in the future will allow more precise decisions to be made in the breeding studies.

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## Author contribution statement

The contributions of all authors are defined as follows: study concept and design: A.N.Şavkan, O.Turkmen; data collection: A.N. Şavkan; analysis and interpretation of results: A.N.Şavkan, O.Turkmen, H. Başak; preparing a draft text: A.N.Şavkan, O.Turkmen. All authors reviewed the results and approved the final version of the article.

## Compliance with ethical standards

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Doğu Akdeniz Bölgesi Modern Seralarında Farklı Örtü Malzemesi ile Farklı Isı Perdeleri Kullanımının Sera Isı Gereksinimine Etkisinin Belirlenmesi

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### ÖZET

Bu çalışmada, Doğu Akdeniz Bölgesindeki Antakya, Mersin, Adana ve Antalya illerinde yer alan ileri teknolojik seralarda ihtiyaç duyulan ısı gereksinim değerleri hesaplanmıştır. Hesaplamalar, farklı örtü malzemesi ve çeşidi ile ısı perdesinin farklı sızdırmazlık özelliklerinin kullanıldığı her bir il için 24 sera tipi için ayrı ayrı yapılmış ve karşılaştırmaları verilmiştir. Isı perdesiz hesaplamalarda optimum ısı enerjisi gereksinimi Adana, Mersin, Antalya ve Antakya için sırasıyla cam seralarda 79.9 kW h m<sup>-2</sup> a<sup>-1</sup>, 80.8 kW h m<sup>-2</sup> a<sup>-1</sup>, 104.8 kW h m<sup>-2</sup> a<sup>-1</sup>, 117.9 kW h m<sup>-2</sup> a<sup>-1</sup>, plastik seralarda ise 78.7 kW h m<sup>-2</sup> a<sup>-1</sup>, 81.9 kW h m<sup>-2</sup> a<sup>-1</sup>, 105.6 kW h m<sup>-2</sup> a<sup>-1</sup>, 116.3 kW h m<sup>-2</sup> a<sup>-1</sup> olarak bulunmuştur. Isı perdeli hesaplamalarda ise optimum ısı enerjisi gereksinimi Mersin, Adana, Antalya ve Antakya için sırasıyla cam seralarda 49.0 kW h m<sup>-2</sup> a<sup>-1</sup>, 53.0 kW h m<sup>-2</sup> a<sup>-1</sup>, 61.4 kW h m<sup>-2</sup> a<sup>-1</sup>, 75.9 kW h m<sup>-2</sup> a<sup>-1</sup>, plastik seralarda ise 53.2 kW h m<sup>-2</sup> a<sup>-1</sup>, 54.6 kW h m<sup>-2</sup> a<sup>-1</sup>, 66.5 kW h m<sup>-2</sup> a<sup>-1</sup>, 79.0 kW h m<sup>-2</sup> a<sup>-1</sup> olarak bulunmuştur. Hesaplamalar sonucunda optimum ısı maliyetini sağlayan sera tipi, cam örtülü seralar için C8, PE örtülü seralar için ise P16 olarak belirlenmiştir. Çelik borulu ısıtma sisteminde boruların seraya yerleştirilme şekli olarak en iyi sonuç sera tabanına yakın yerleştirilmesi ile sağlanırken, sızdırmazlığı iyi ısı perdelerinin kullanılması gerektiği belirlenmiştir. Sonuç olarak, Doğu Akdeniz Bölgesi ileri teknolojik seralarda optimum ısı maliyetinin sağlanması için cam seralarda, çatıda tek kat cam, yan duvarlarda çift kat cam, PE seralarda ise çatı da tek kat PE, yan duvarlarda ise çift kat sert PE (32 mm) kullanılmalıdır. Çelik borulu ısıtma sisteminde borular sera tabanına yakın yerleştirilmeli ve ısı koruma önlemi olarak da ısı perdeleri kullanılmalıdır.

### Biyosistem Mühendisliği

### Araştırma Makalesi

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### Anahtar Kelimeler

Optimum ısı maliyeti

Isı perdesi

Örtü malzemesi

Isı koruma

Enerji verimliliği

## Determination of the Effect of Using Different Covering Materials and Different Thermal Curtains on Greenhouse Heat Requirement in Modern Greenhouses of the Eastern Mediterranean Region

### ABSTRACT

In this study, the heat requirement values needed in advanced technological greenhouses in the provinces of Antakya, Mersin, Adana and Antalya in the Eastern Mediterranean Region were calculated. Calculations were made separately for 24 greenhouse types for each province where different cover materials and types and different sealing properties of the thermal curtain were used, and their comparisons were given. In calculations without thermal curtains, the optimum heat energy requirement was found to be 79.9 kW h m<sup>-2</sup> a<sup>-1</sup>, 80.8 kW h m<sup>-2</sup> a<sup>-1</sup>, 104.8 kW h m<sup>-2</sup> a<sup>-1</sup>, 117.9 kW h m<sup>-2</sup> a<sup>-1</sup>, 78.7 kW h m<sup>-2</sup> a<sup>-1</sup>, 81.9 kW h m<sup>-2</sup> a<sup>-1</sup>, 105.6 kW h m<sup>-2</sup> a<sup>-1</sup>, 116.3 kW h m<sup>-2</sup> a<sup>-1</sup> in glass greenhouses for Adana, Mersin, Antalya and Antakya, respectively. In thermal curtain calculations, the optimum heat energy requirement was found to be 49.0 kW h m<sup>-2</sup> a<sup>-1</sup>, 53.0 kW h m<sup>-2</sup> a<sup>-1</sup>, 61.4 kW h m<sup>-2</sup> a<sup>-1</sup>, 75.9 kW h m<sup>-2</sup> a<sup>-1</sup> for glass greenhouses and 53.2 kW h m<sup>-2</sup> a<sup>-1</sup>, 54.6 kW h m<sup>-2</sup> a<sup>-1</sup>, 66.5 kW h m<sup>-2</sup> a<sup>-1</sup>, 79.0 kW h m<sup>-2</sup> a<sup>-1</sup> for plastic greenhouses for Mersin, Adana, Antalya, and Antakya,

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respectively. As a result of the calculations, the greenhouse type that provides the optimum heat cost was determined as C8 for glass-covered greenhouses and P16 for PE-covered greenhouses. In the steel pipe heating system, the best result is achieved by placing the pipes in the greenhouse close to the greenhouse floor, while it has been determined that heat curtains with good sealing should be used. As a result, in order to ensure optimum heat cost in advanced technological greenhouses in the Eastern Mediterranean Region, in glass greenhouses, single layer glass on the roof, double layer glass on the side walls, and in PE greenhouses, single layer PE on the roof and double layer hard PE (32 mm) on the side walls should be used. In the steel pipe heating system, the pipes should be placed close to the greenhouse floor and heat curtains should be used as a heat protection measure.

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## GİRİŞ

Seralar, bölgenin iklim koşullarına bağlı olarak yılın farklı aylarında ısıtma, havalandırma, gölgeleme, soğutma gibi işlemlere ihtiyaç duyarlar. Bitkiler sıcaklık değişimine, gelişimleri ile hızlı bir şekilde cevap verirler. Serada yetiştirilen bitkiler 10°C – 24°C sıcaklık aralığına pozitif olarak artan bir tepki verdiği bilinmektedir (Nelson, 2002).

Seralarda en fazla üretilen ürünler, çoğunlukla yüksek ısı gereksinimi gösteren sebzelerdir (domates, biber, hıyar, kavun, yeşil fasulye, patlıcan vb.). Seralarda üretilen bu ürünler, ılıman iklim bitkileri olup ortalama 17°C-27°C'ye adapte olmuşlardır. Bu ürünler minimum 12°C'nin altında ve maksimum 32°C'nin üstündeki sıcaklık değerlerinde strese girerler (Nisen ve ark., 1988). Don olayının yaşandığı düşük sıcaklıklarda geri dönüşü olmayan hasarlar ortaya çıkar.

Günlük ortalama sıcaklığın 12°C'nin altına düştüğü koşullarda seralarda ısıtma yapılmalıdır. Türkiye'de seracılığın yaygın olarak yapıldığı Akdeniz ve Ege bölgelerinde, günlük ortalama sıcaklık 12°C'nin altına düştüğünden seralar Aralık, Ocak ve Şubat aylarında ısıtılmalıdır. Verim ve kaliteyi olumlu yönde etkileyen ısıtma, üretim maliyetini arttırmaktadır. Isıtma giderleri sera yapısına, donanımına ve yöre iklimine bağlı olarak işletme giderlerinin % 20-60 arasında değişmektedir (Baytorun ve ark., 2017).

Soğuk iklim bölgelerinde üretim yapılan seralar, yılın büyük bir bölümünde ısıtılmak zorundadır. Isıtma süresi daha kısa olan ılıman iklim bölgelerinde üretim yapılan seralarda ısıtma gereksinimi kış aylarında ortaya çıkmaktadır. Akdeniz gibi ılıman iklim bölgelerinde, ısı gereksinimine sadece kış aylarının gece saatlerinde ihtiyaç duyulmaktadır (Baytorun, 2016).

Bölgenin iklim koşullarına göre inşa edilen seralarda

gerekli ısı tasarruf önlemlerinin alınması, ısıtma giderlerinden kaynaklı üretim maliyetlerinin düşürülmesi açısından önemlidir (Boyacı ve ark., 2016). Bununla birlikte seralar için seçilen ısıtma sisteminin doğru olarak projelenmesi, enerji tasarrufu ve ilk yatırım giderlerinin azaltılması açısından büyük bir öneme sahiptir (Akyüz ve ark., 2017).

Seralarda çatı bölgesinde çift katlı örtü malzemesinin kullanılması enerji tasarrufu sağlarken seraya ulaşan ışık miktarını azaltabilmekte, aynı zamanda CO<sub>2</sub> ve nem sorunu yaratabilmektedir (Tantau, 2012; Baytorun, 2016). Seralarda ısı kayıplarını minimize etmek için yapılan yalıtım sonucunda, sera içindeki nem yükselmesi de göz önüne alınmalıdır. Bu nedenle sera çatı bölgesinde, son yıllarda yaygın olarak tek katlı örtü malzemesi ile gece saatlerinde iyi yalıtılmış hareketli ısı perdelerinin kullanılması önerilmektedir (Baytorun, 2016). İlıman iklim bölgelerinde ısıtma için gerekli olan ısı enerjisinin %100'üne ve soğuk bölgelerde %70-75'ine gece saatlerinde ihtiyaç duyulduğundan, sistem iyi projelenip malzeme iyi seçildiğinde ısı perdeleri ile önemli düzeyde ısı tasarrufu sağlanabilir (Von Zabeltitz, 1982, Baytorun, 2016). Aynı zamanda don olayı riskini azaltmak için de ısıtma yapılmayan seralarda ısı perdeleri kullanılabilir (Teitel ve ark., 1996). Isı perdelerinin seçiminde malzemenin, küçük yüzey alanına sahip olmasına, kolay monte edilebilmesine ve çekme gerilmesinin az olmasına dikkat edilmelidir (Meyer, 1982).

Rath (1992) çalışmalarında alüminyum dokusu az olan ısı perdesi ile %40, alüminyum dokusu fazla olan ısı perdesi ile %50 oranında tasarruf edilebileceğini hesaplamıştır. Tek kat enerji perdesi yardımıyla seralarda %32 enerji tasarrufu sağlanabilirken, bu oranın çift kat perde ile %48'e ve çift kat enerji perdesi ile %52'ye çıkarılabilmektedir. Serada sadece gündüz perdelerinin kullanılması durumunda gece saatlerinde

ihtiyaç duyulan ısı enerjisi gereksinimini %25, gündüz saatlerinde ise %9 azaltılabildiği belirlenmiştir (Domke, 2011).

Seralarda kullanılan perdelerin ısı tasarruf oranları perdelerin sızdırmazlıklarına bağlı olarak değişmektedir (Meyer, 1984; Müller, 1987). Isı perdelerinin yan duvar ve sera cepheleriyle birleştiği yerler sızdırmaz olmalıdır. Aksi takdirde ısınan havanın yükselerek bu aralıklardan geçip çatı örtü malzemesi tarafından dış ortama taşınması ısı tasarrufunu azaltmaktadır (Çaylı ve ark., 2016; Önder & Baytorun, 2016). Aynı zamanda ısı perdesi sızdırmazlığı iyi olan seralarda rüzgar hızına bağlı olarak ortaya çıkan ısı kayıpları en düşük düzeydedir (Schmidt ve ark., 2011).

Seralarda ısı gereksinimi kullanılan ısıtma sistemlerinin tipi ve sera donanımına bağlı olarak değişmektedir (Baytorun ve ark., 2018a; Baytorun ve ark., 2018b). Isı gereksinimi hesaplamaları, serada ortaya çıkan gerçek sıcaklık ve seranın özelliğine bağlı sıcaklık yükselmesi dikkate alınarak saatlik iklim değerlerine göre yapılmalıdır (Baytorun ve ark., 2018c). Isı gereksinimi hesaplamalarında doğru sonuçlar elde edebilmek için iklim verilerinin, araştırmanın yapıldığı yerden toplanması da oldukça önemlidir (Çaylı ve ark., 2018).

Yapılan bu çalışmada bölge iklim koşullarında kurulacak ve düzenli olarak ısıtılacak yüksek teknolojik seralarda, farklı örtü malzemelerine göre ısı enerjisi gereksiniminin hesaplanması, ısı perdesi gibi ısı koruma önlemleri ile ısı tasarruf oranlarının belirlenmesi ve çelik borulu ısıtma sisteminde boruların seraya en uygun yerleşim şeklinin belirlenmesi amaçlanmıştır. Çalışmada, seracılık faaliyetlerinin yoğun olarak yapıldığı Antalya ili ile diğer illerdeki ısı gereksinim değerleri karşılaştırılarak üreticilerin rekabet edebilmeleri için alması gereken önlemler belirlenmiştir. Ayrıca çelik borulu ısıtma sisteminin, serada farklı konumlarda kullanılması durumunun ısı enerjisi gereksinimine etkisi de ortaya konulmuştur.

## MATERYAL ve METOD

### Doğu Akdeniz Bölgesi

Doğu Akdeniz bölgesi, Adana, Antalya, Burdur, Hatay, Isparta, Mersin, Osmaniye ile Konya'nın Beyşehir, Seydişehir, Bozkır, Taşkent, Hadim, Derebucak, Yalhöyük ve Ahırılı ilçelerini kapsamaktadır. Bu çalışmada ISIGER-SERA (Baytorun ve ark., 2016) uzman sisteminde verileri olan ve Akdeniz'e doğrudan kıyısı bulunan Adana, Antalya, Antakya ve Mersin illerine ait hesaplamalar yapılmıştır.

### Sera Özellikleri

Isı gereksiniminin hesaplanmasında son yıllarda modern seralarda kullanılan sera ölçüleri göz önüne

alınmıştır. Çalışmada, uzunluğu 50 m, genişliği 9.6 m, yan duvar yüksekliği 4.5 m, mahya yüksekliği 6.5 m olan 10 adet bloktan oluşan taban alanı 4800 m<sup>2</sup> olan, plastik örtü malzemeli (yay çatılı), cam örtü malzemeli (gotik çatılı) olan seralar kullanılmıştır. Yapılan hesaplamalarda, serada sıcaklık yükselmesi 7 °C, ısı depolama katsayısı cam örtülü sera için 1, plastik örtülü sera için ise 0.5 olarak alınmıştır. Havalandırma sıcaklığı ise 23°C olarak alınmıştır. Hesaplamalar için farklı örtü malzemelerinin kullanıldığı sera tipleri oluşturulmuş ve bu sera tipleri Çizelge 1'de verilmiştir.

### Isı Perdesi

Seralarda kullanılan ısı perdelerinin dokularında bulunan alüminyum malzemenin oranı, serada ısıma yoluyla ortaya çıkan ısı kayıplarının azaltılması açısından büyük bir öneme sahiptir. Yapılan çalışmada enerji tasarruf oranı %50 olan ısı perdesi hesaplamalarda esas alınmıştır. Isı perdelerinin sağladığı ısı tasarrufu perdelerin sızdırmazlıklarına bağlıdır. Yapılan çalışmada %50 tasarruf oranına sahip ısı perdesinin sızdırmazlığına bağlı olarak hesaplamalarda esas alınan düzeltme faktörleri Çizelge 2'de verilmiştir (Rath 1992).

### Isıtma Sistemi

Çalışmada ısıtma sistemi olarak çelik borulu ısıtma sistemi seçilmiştir. Hesaplama kullanılan ısıtma sistemine ait özellikler Çizelge 3'de verilmiştir.

### Isı Gereksiniminin Hesaplanması

Saatlik ölçülmüş iklim verileri kullanılarak, seranın özelliklerine ve serada kullanılan donanıma bağlı olarak ısı enerjisi gereksinimi, Rath (1992) tarafından DIN 4701 standartları esas alınarak geliştirilen 1 nolu eşitlik yardımı ile hesaplanmıştır.

$$Q = \sum_{n=1}^{8760} \left( (t_{in} - t_{i,OH_n} - \Delta t_{sp_n}) \cdot U_{cs} \cdot A_c \cdot (1 - EE_{ES_n}) \right) \cdot t_{si} \quad [1]$$

Eşitlikte;

Q : Seranın yıllık ısı gereksinimi [Wh]

$t_{in}$  : Serada istenen sıcaklık [°C]

$t_{i,OH}$  : Isıtmasız serada ortaya çıkan gerçek sıcaklık [°C]

$\Delta t_{sp}$  : Serada depolanan ısıya bağlı ortaya çıkan sıcaklık yükselmesi [°C]

$U_{cs}$  : Isı gereksinim katsayısı [W m<sup>-2</sup> K<sup>-1</sup>]

$A_c$  : Sera örtü yüzey alanı [m<sup>2</sup>]

$EE_{ES}$  : Serada kullanılan enerji koruma önleminin tasarruf oranı [-]

n: Yılın saatleri [h]

1 nolu eşitlikteki  $\Delta t_{sp}$ , gündüz saatlerinde serada depolanan enerji dikkate alınarak belirlenir.  $\Delta t_{sp}$ 'nin belirlenmesinde 2 ve 3 nolu eşitlikler kullanılmıştır.

$$\Delta t_{sp,pot} = \frac{Z_d}{\max(Z_{2...365})} * \Delta t_{sp,max} \quad [2]$$

2 nolu eşitlikteki  $Z_d$ , 3 nolu eşitlik yardımı ile elde edilmiştir:

$$Z_d = \overline{t_{i,OH,Gündüz_d-1}} - \overline{t_{i,OH,Gece_d}} \quad [3]$$

$Z_d$ : Gündüz gece sıcaklık ortalama farkı [°C]

Elde edilen verilere göre gün boyu depolanan enerjiye bağlı olarak serada ortaya çıkan sıcaklık yükselmesi ( $\Delta t_{sp}$ ) aşağıdaki mantıksal eşitlikler yardımı ile belirlenmiştir (Rath 1992).

Çizelge 1. Örtü malzemelerine göre oluşturulan sera tipleri  
Table 1. Greenhouse types created according to covering materials

| Sera Tipi | Örtü Malzemesi    |                                 | Isı Perdeleri ve Sızdırmazlık Durumu |
|-----------|-------------------|---------------------------------|--------------------------------------|
|           | Çatı              | Yan Duvar                       |                                      |
| C1        | Tek katlı cam     | Tek katlı cam                   | Yok                                  |
| C2        | Tek katlı cam     | Tek katlı cam                   | Var, Kötü                            |
| C3        | Tek katlı cam     | Tek katlı cam                   | Var, Orta                            |
| C4        | Tek katlı cam     | Tek katlı cam                   | Var, İyi                             |
| C5        | Tek katlı cam     | Çift katlı cam                  | Yok                                  |
| C6        | Tek katlı cam     | Çift katlı cam                  | Var, Kötü                            |
| C7        | Tek katlı cam     | Çift katlı cam                  | Var, Orta                            |
| C8        | Tek katlı cam     | Çift katlı cam                  | Var, İyi                             |
| P1        | Tek katlı plastik | Tek katlı plastik               | Yok                                  |
| P2        | Tek katlı plastik | Tek katlı plastik               | Var, Kötü                            |
| P3        | Tek katlı plastik | Tek katlı plastik               | Var, Orta                            |
| P4        | Tek katlı plastik | Tek katlı plastik               | Var, İyi                             |
| P5        | Tek katlı plastik | Çift katlı plastik              | Yok                                  |
| P6        | Tek katlı plastik | Çift katlı plastik              | Var, Kötü                            |
| P7        | Tek katlı plastik | Çift katlı plastik              | Var, Orta                            |
| P8        | Tek katlı plastik | Çift katlı plastik              | Var, İyi                             |
| P9        | Tek katlı plastik | Çift katlı sert plastik (16 mm) | Yok                                  |
| P10       | Tek katlı plastik | Çift katlı sert plastik (16 mm) | Var, Kötü                            |
| P11       | Tek katlı plastik | Çift katlı sert plastik (16 mm) | Var, Orta                            |
| P12       | Tek katlı plastik | Çift katlı sert plastik (16 mm) | Var, İyi                             |
| P13       | Tek katlı plastik | Çift katlı sert plastik (32 mm) | Yok                                  |
| P14       | Tek katlı plastik | Çift katlı sert plastik (32 mm) | Var, Kötü                            |
| P15       | Tek katlı plastik | Çift katlı sert plastik (32 mm) | Var, Orta                            |
| P16       | Tek katlı plastik | Çift katlı sert plastik (32 mm) | Var, İyi                             |

Çizelge 2. Isı perdesinin sızdırmazlıklarına bağlı düzeltme katsayıları  
Table 2. Correction coefficients depending on the sealing of the thermal curtain

| Isı Perdeleri Sızdırmazlığı | Düzeltilme Katsayısı |
|-----------------------------|----------------------|
| İyi                         | 6.80                 |
| Orta                        | 11.05                |
| Kötü                        | 29.43                |
| Isı perdesi yok             | 0.00                 |

Çizelge 3. Isıtma sistemlerine bağlı olarak hesaplamalarda kullanılan düzeltme faktörleri  
Table 3. Correction factors used in calculations depending on heating systems

| Isıtma Sistemi  | Düzeltilme Faktörü |
|---|--------------------|
| Yükseğe yerleştirilmiş borulu ısıtma sistemi                      | 1.17               |
| Masa altına yerleştirilmiş borulu ısıtma sistemi                  | 1.06               |
| Yan duvarlara yerleştirilmiş borulu ısıtma sistemi                | 1.16               |
| Tabana ve bitki masuralarına yerleştirilmiş borulu ısıtma sistemi | 0.96               |
| Delikli plastik tüplü hava ısıtıcısı                              | 1.00               |

$$\left. \begin{array}{l} \Delta t_{sp,pot} \geq 25 \text{ ve } q_G > 0 \text{ ise} \\ t_i - t_{i,OH} \leq \Delta t_{sp,pot} < 25 \text{ ve } q_G > 0 \text{ ise} \\ 0 < \Delta t_{sp,pot} < t_i - t_{i,OH} < 25 \text{ ve } q_G > 0 \text{ ise} \\ \text{Değilse} \end{array} \right\} \Delta t_{sp} = \begin{cases} \Delta t_{sp,pot} \\ \Delta t_{sp,pot} \\ \frac{\Delta t_{sp,pot} \cdot (t_i - t_{i,OH} - 25)}{\Delta t_{sp,pot} - 25} \\ 0 \end{cases}$$



1 nolu eşitlikte ısıtılmayan serada ortaya çıkan sıcaklık değerinin ( $t_{i,OH}$ ) belirlenmesi için, güneş radyasyonuna bağlı serada ulaşılan teorik sıcaklık değerinin ( $t_{i,th}$ ) hesaplanması gereklidir. Serada teorik sıcaklık 4 nolu eşitlik ile hesaplanmıştır.

$$t_{i,th} = \frac{q_G * D_G * \eta * A_G}{U_{CS} * (1 - EE_{ES}) * A_C} + t_a \quad [4]$$

Eşitlikte:

- $t_{i,th}$ : Isıtılmayan ve havalandırılmayan serada ortaya çıkan teorik sıcaklık [°C]  
 $q_G$ : Güneş radyasyonu [W m<sup>-2</sup>]  
 $D_G$ : Örtü malzemesinin geçirgenlik oranı [%]  
 $t_a$ : Dış sıcaklık [°C]  
 $\eta$ : Serada enerji dönüşüm faktörü (duyulur/gizli) (Standart =0,7)

Serada ortaya çıkan teorik sıcaklık ve serada istenen havalandırma sıcaklığına ( $t_L$ ) bağlı olarak, 1 nolu eşitlikteki ısıtılmayan serada iç sıcaklık değeri ( $t_{i,OH}$ ), aşağıdaki mantıksal eşitlikler yardımı ile belirlenmiştir.

$$t_{i,th} \geq t_L \text{ ve } t_L \geq t_a \text{ ise } \left. \begin{array}{l} t_{i,th} < t_L \text{ ve } t_{i,th} > t_a \text{ ise} \\ \text{Değilse} \end{array} \right\} t_{i,OH} = \begin{cases} t_L \\ t_{i,th} \\ t_a \end{cases}$$

Eşitlikte;

- $t_L$ : Havalandırma sıcaklığı [°C]  
 $t_{i,S}$ : Serada arzulanan iç sıcaklık değeri [°C]

Serada gerçek iç sıcaklık değeri ( $t_i$ ) aşağıdaki koşullara göre belirlenmiştir.

$$t_{i,OH} \leq t_{i,S} \text{ ise } \left. \begin{array}{l} \text{Değilse} \end{array} \right\} t_i = \begin{cases} t_{i,S} \\ t_{i,OH} \end{cases}$$

Eğer serada gece gündüz farklı sıcaklık değerleri arzu ediliyorsa bu durumda iç sıcaklık değeri ( $t_i$ ) aşağıdaki koşullara göre belirlenir.

$$q_G > 0 \text{ ve } t_{i,OH} \leq t_{i,S_{Gün}} \text{ ise } \left. \begin{array}{l} q_G = 0 \text{ ve } t_{i,OH} \leq t_{i,S_{Gece}} \text{ ise} \\ \text{Değilse} \end{array} \right\} t_i = \begin{cases} t_{i,S_{Gündüz}} \\ t_{i,S_{Gece}} \\ t_{i,OH} \end{cases}$$

Serada ısı gücü ve ısı enerjisi gereksiniminin belirlenmesinde kullanılan toplam ısı iletim katsayısı ( $U_{CS}$ ), örtü malzemesinin özelliğine, sera tipine, ısıtma, sulama sistemine, rüzgâr hızına ve gökyüzünün bulutlu ve açık olmasına bağlı olarak değişim göstermektedir. Yapılan hesaplamalarda toplam ısı iletim katsayısı 4 m s<sup>-1</sup> rüzgâr hızında tek katlı PE plastik için 7 W m<sup>-2</sup> K<sup>-1</sup>, cam sera için 7.65 W m<sup>-2</sup> K<sup>-1</sup> olarak alınmış (Zabeltitz, 1986; Tantau, 1983; Meyer, 1981, 1982) ve hesaplamaların yapıldığı saatteki rüzgâr hızına bağlı olarak yeniden belirlenmiştir.

## BULGULAR ve TARTIŞMA

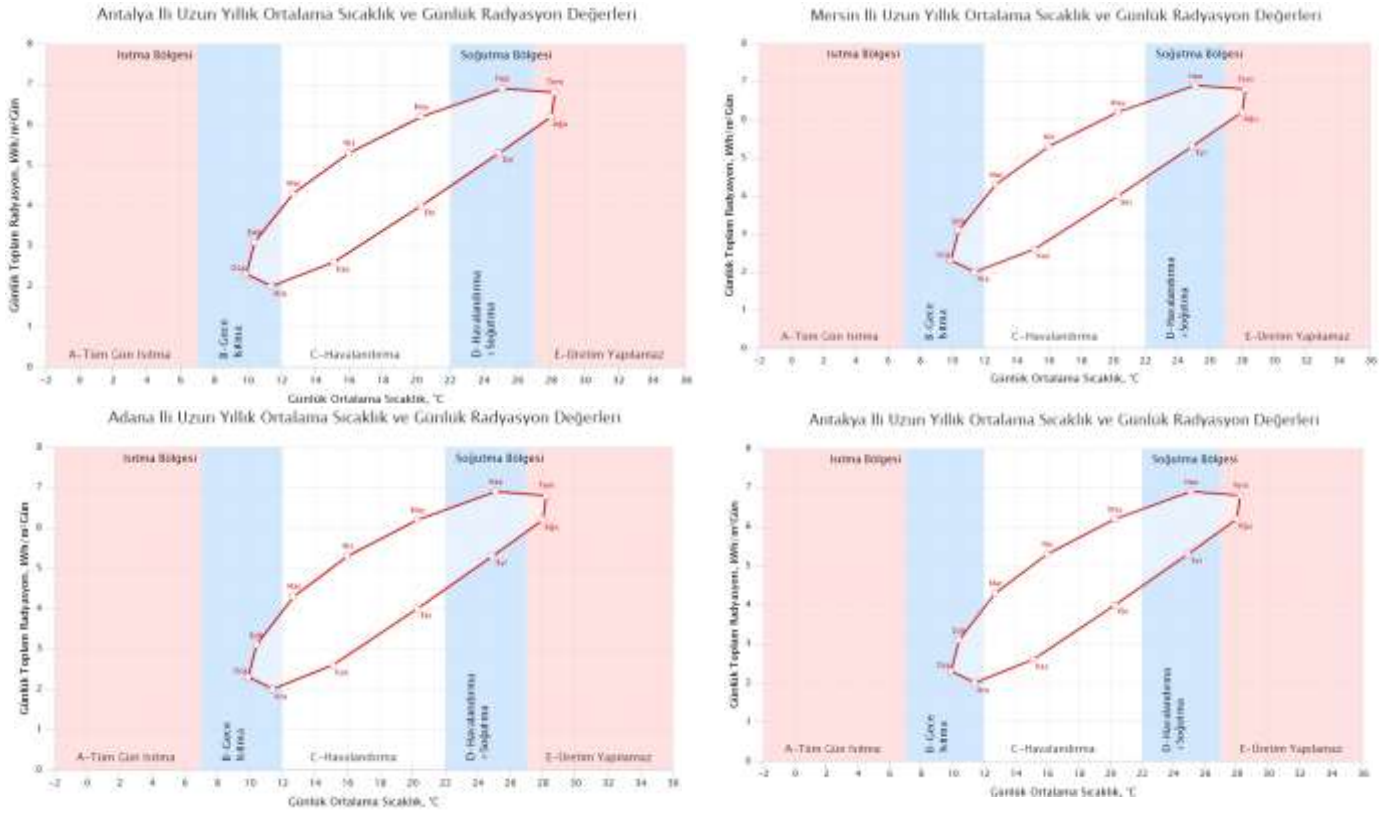
Meteoroloji Genel Müdürlüğünden elde edilen iklim değerleri incelendiğinde Doğu Akdeniz Bölgesi proje illerinde hava sıcaklığının 7°C'nin altına düşmediği görülmektedir. Bu koşullarda çalışmada kullanılan tüm illerde seralarda Aralık-Şubat döneminde sadece

gece saatlerinde ısıtma, Mart-Nisan-Mayıs-Ekim-Kasım aylarında sadece havalandırma, Haziran ve Eylül aylarında havalandırma ve günün belirli saatlerinde soğutma yapılması gerekmektedir. Seralarda Temmuz ve Ağustos aylarında ortaya çıkan yüksek sıcaklıklar nedeniyle üretim yapılamamaktadır (Şekil 1).

Modern seralarda ısı perdesi ve çift kat örtü malzemesi kullanımı gibi ısı tasarruf önlemleri uygulanmaktadır. Ancak günümüzde örtü altı yetiştiriciliğin yaygın olarak yapıldığı Akdeniz bölgesindeki seraların % 3'ünde modern seracılık yapılmaktadır (Baytorun, 2016). Bu durum göz önüne alındığında bölgedeki seraların çoğunluğunda ısıtma yapılmadığı veya modern olmayan seralarda üretim yapıldığı anlaşılmaktadır.

Seralarda yapılacak fizibilite hesaplamalarında maksimum ısı gücünün hesaplanması yanında sera kurulacak yerin iklimine bağlı yıllık ısı enerjisi gereksiniminin bilinmesi büyük önem arz etmektedir. Yapılan çalışmada, Doğu Akdeniz Bölgesi illeri teknolojik seracılık faaliyetlerinin yapıldığı proje illerinde saatlik iklim değerleri kullanılarak gece ve gündüz farklı sıcaklık değerleri için gerekli ısı gücü ve yıllık ısı enerjisi gereksinimi hesaplanarak Çizelge 4'te verilmiştir. Çizelge 4'ten görüleceği gibi farklı sıcaklık değerlerinde farklı sera tipleri için hesaplanan maksimum ısı gücü gereksinimleri değişim gösterirken, ısı perdesinin sızdırmazlık durumuna göre hesaplanan maksimum ısı gücü değerlerinde değişim gözlenmemektedir. Bununla birlikte ısı perdesinin kullanılmadığı durumlarda sadece Antalya ilinde maksimum ısı gücü gereksinimi, ısı perdesi kullanılan durumlara göre cam seralarda %0.6, plastik seralarda %0.3 daha fazla hesaplanmıştır. Hesaplanan yıllık ısı enerjisi gereksinimi, en fazla ısı perdesinin kullanılmadığı durumlarda ortaya çıkmıştır.

Çizelge 4'te çelik borulu ısıtma sisteminde boruların yerleşim durumları incelendiğinde, boruların en uygun yerleşim şeklinin tabana yakın yerleştirme olduğu görülmektedir. Boruların masa altına yerleştirilmesi ikinci sırada, tabana yakın yerleştirilmesi ise üçüncü sırada yer almıştır. Bu durumda çelik borulu ısıtma sisteminde boruların sera tabanına yakın yerleştirilmesi, optimum ısıtma maliyetini sağlamaktadır. Seralarda ısıtma borularının taban yakın yerleştirilmesi durumunda ısı enerjisi gereksinimi en fazla Antakya ilinde ısı perdesinin kullanılmadığı cam örtülü sera tipinde 130.8 kW h m<sup>-2</sup> a<sup>-1</sup> olarak hesaplanırken, Antalya, Adana ve Mersin illerinde ise sırasıyla 115.58 kW h m<sup>-2</sup> a<sup>-1</sup>, 89.68 kW h m<sup>-2</sup> a<sup>-1</sup>, 88.98 kW h m<sup>-2</sup> a<sup>-1</sup> olarak hesaplanmıştır. Plastik seralarda ise Antakya, Antalya, Adana ve Mersin illeri için sırasıyla 135.858 kW h m<sup>-2</sup> a<sup>-1</sup>, 121.758 kW h m<sup>-2</sup> a<sup>-1</sup>, 94.258 kW h m<sup>-2</sup> a<sup>-1</sup>, 92.658 kW h m<sup>-2</sup> a<sup>-1</sup> olarak hesaplanmıştır.



Şekil 1. Çalışmada kullanılan illerin uzun yıllık günlük ortalama sıcaklık ve günlük toplam güneş radyasyonu değerlerine bağlı olarak serada alınması gereken iklimlendirme önlemleri

Figure 1. Climatization measures to be taken in the greenhouse depending on the long-year daily average temperature and daily total solar radiation values of the provinces used in the study

Table 4. The heat power required for regularly heated greenhouses in the Eastern Mediterranean Region and the heat energy requirement values needed throughout the production period.

Çizelge 4. Doğu Akdeniz Bölgesinde düzenli olarak ısıtılan seralar için gerekli ısı gücü ve üretim periyodu boyunca ihtiyaç duyulan ısı enerjisi gereksinimi değerleri.

| Sera Tipi | Antalya                                      |       | Mersin |      | Adana |      | Antakya |       |
|-----------|--|-------|--------|------|-------|------|---------|-------|
|           | 1  | 2     | 1      | 2    | 1     | 2    | 1       | 2     |
|           | Tabana Yakın, Çelik Borulu Isıtma Sistemleri |       |        |      |       |      |         |       |
| C1        | 100.5  | 115.5 |        | 88.9 |       | 89.6 |         | 130.8 |
| C2        |  | 102.8 |        | 79.9 |       | 81.7 |         | 118.5 |
| C3        | 99.9   | 83.2  | 86.9   | 65.4 | 92.8  | 69.5 | 115.8   | 99.5  |
| C4        |  | 63.5  |        | 50.9 |       | 57.4 |         | 80.5  |
| C5        | 91.5   | 104.8 |        | 80.8 |       | 79.9 |         | 117.9 |
| C6        |  | 94.2  |        | 73.2 |       | 73.2 |         | 107.6 |
| C7        | 90.6   | 77.8  | 78.6   | 61.1 | 83.8  | 63.1 | 104.8   | 91.8  |
| C8        |  | 61.4  |        | 49.0 |       | 53.0 |         | 75.9  |
| P1        | 101.8  | 121.7 |        | 94.2 |       | 92.6 |         | 135.8 |
| P2        |  | 109.4 |        | 85.4 |       | 85.0 |         | 124.0 |
| P3        | 101.4  | 90.1  | 88.3   | 71.1 | 93.8  | 73.1 | 117.4   | 105.5 |
| P4        |  | 70.8  |        | 56.8 |       | 61.3 |         | 87.1  |
| P5        | 95.2   | 113.5 |        | 88.1 |       | 85.6 |         | 126.0 |
| P6        |  | 102.7 |        | 80.3 |       | 78.9 |         | 115.5 |
| P7        | 94.1   | 85.7  | 82.4   | 67.7 | 87.4  | 68.5 | 108.9   | 99.3  |
| P8        |  | 68.7  |        | 55.2 |       | 58.0 |         | 83.1  |
| P9        | 93.8   | 111.5 |        | 86.4 |       | 83.9 |         | 123.3 |
| P10       |  | 101.0 |        | 78.9 |       | 77.4 |         | 113.3 |
| P11       | 92.3   | 84.6  | 80.5   | 66.8 | 86.1  | 67.3 | 106.6   | 97.6  |

|   |       |       |       |       |       |       |       |       |
|---|-------|-------|-------|-------|-------|-------|-------|-------|
| P12                                       |       | 68.2  |       | 54.6  |       | 57.3  |       | 82.0  |
| P13                                       | 88.9  | 105.6 |       | 81.9  |       | 78.7  |       | 116.3 |
| P14                                       |       | 96.1  |       | 75.2  |       | 72.8  |       | 107.2 |
| P15                                       | 87.3  | 81.3  | 76.5  | 64.2  | 81.1  | 63.7  | 100.6 | 93.1  |
| P16                                       |       | 66.5  |       | 53.2  |       | 54.6  |       | 79.0  |
| Yan Duvar, Çelik Borulu Isıtma Sistemleri |       |       |       |       |       |       |       |       |
| C1  | 120.9 | 138.9 |       | 106.9 |       | 107.8 |       | 157.3 |
| C2  |       | 123.6 |       | 96.1  |       | 98.2  |       | 142.4 |
| C3  | 120.1 | 100.0 | 104.5 | 78.6  | 111.6 | 83.6  | 139.2 | 119.6 |
| C4  |       | 76.3  |       | 61.2  |       | 69.0  |       | 96.8  |
| C5  | 110.0 | 126.0 |       | 97.1  |       | 96.1  |       | 141.8 |
| C6  |       | 113.2 |       | 88.0  |       | 88.1  |       | 129.3 |
| C7  | 108.9 | 93.5  | 94.5  | 73.5  | 100.8 | 75.9  | 126.0 | 110.3 |
| C8  |       | 73.8  |       | 58.9  |       | 63.7  |       | 91.3  |
| P1  | 123.1 | 147.0 |       | 113.9 |       | 111.9 |       | 164.1 |
| P2  |       | 132.2 |       | 103.2 |       | 102.7 |       | 149.8 |
| P3  | 122.5 | 108.8 | 106.7 | 86.0  | 113.4 | 88.4  | 141.9 | 127.5 |
| P4  |       | 85.5  |       | 68.7  |       | 74.0  |       | 105.2 |
| P5  | 115.1 | 137.2 |       | 106.4 |       | 103.5 |       | 152.2 |
| P6  |       | 124.1 |       | 97.1  |       | 95.3  |       | 139.6 |
| P7  | 113.8 | 103.6 | 99.5  | 81.9  | 105.7 | 82.7  | 131.6 | 120.0 |
| P8  |       | 83.0  |       | 66.6  |       | 70.1  |       | 100.4 |
| P9  | 113.4 | 134.8 |       | 104.4 |       | 101.4 |       | 149.0 |
| P10                                       |       | 122.1 |       | 95.4  |       | 93.5  |       | 136.8 |
| P11                                       | 111.5 | 102.3 | 97.2  | 80.7  | 104.0 | 81.4  | 128.8 | 118.0 |
| P12                                       |       | 82.5  |       | 66.0  |       | 69.2  |       | 99.0  |
| P13                                       | 107.4 | 127.6 |       | 99.0  |       | 95.1  |       | 140.6 |
| P14                                       |       | 116.1 |       | 90.8  |       | 87.9  |       | 129.6 |
| P15                                       | 105.4 | 98.2  | 92.4  | 77.6  | 98.0  | 77.0  | 121.5 | 112.5 |
| P16                                       |       | 80.3  |       | 64.3  |       | 66.0  |       | 95.5  |
| Masa Altı, Çelik Borulu Isıtma Sistemleri |       |       |       |       |       |       |       |       |
| C1  | 110.7 | 127.2 |       | 97.9  |       | 98.7  |       | 144.1 |
| C2  |       | 113.2 |       | 88.0  |       | 89.9  |       | 130.5 |
| C3  | 110.0 | 91.6  | 95.7  | 72.0  | 102.2 | 76.5  | 127.5 | 109.6 |
| C4  |       | 69.9  |       | 56.0  |       | 63.2  |       | 88.7  |
| C5  | 100.7 | 115.4 |       | 88.9  |       | 88.0  |       | 129.9 |
| C6  |       | 103.7 |       | 80.6  |       | 80.7  |       | 118.5 |
| C7  | 99.7  | 85.6  | 86.5  | 67.3  | 92.3  | 69.5  | 115.4 | 101.0 |
| C8  |       | 67.6  |       | 53.9  |       | 58.4  |       | 83.6  |
| P1  | 112.5 | 134.4 |       | 104.1 |       | 102.3 |       | 150.0 |
| P2  |       | 120.8 |       | 94.3  |       | 93.8  |       | 136.9 |
| P3  | 111.9 | 99.5  | 97.5  | 78.5  | 103.6 | 80.7  | 129.7 | 116.5 |
| P4  |       | 78.1  |       | 62.7  |       | 67.6  |       | 96.2  |
| P5  | 105.1 | 125.4 |       | 97.3  |       | 94.5  |       | 139.1 |
| P6  |       | 113.4 |       | 88.7  |       | 87.1  |       | 127.6 |
| P7  | 104.0 | 94.6  | 90.9  | 74.8  | 96.5  | 75.6  | 120.2 | 109.7 |
| P8  |       | 75.9  |       | 60.9  |       | 64.1  |       | 91.8  |
| P9  | 103.6 | 123.1 |       | 95.4  |       | 92.7  |       | 136.2 |
| P10                                       |       | 111.5 |       | 87.2  |       | 85.4  |       | 125.1 |
| P11                                       | 101.9 | 93.5  | 88.8  | 73.7  | 95.0  | 74.3  | 117.7 | 107.8 |
| P12                                       |       | 75.4  |       | 60.3  |       | 63.2  |       | 90.5  |
| P13                                       | 98.2  | 116.6 |       | 90.4  |       | 86.9  |       | 128.4 |
| P14                                       |       | 106.1 |       | 83.0  |       | 80.4  |       | 118.4 |
| P15                                       | 96.3  | 89.8  | 84.4  | 70.9  | 89.5  | 70.3  | 111.0 | 102.8 |
| P16                                       |       | 73.4  |       | 58.8  |       | 60.3  |       | 87.2  |

<sup>1</sup> Maksimum ısı gücü gereksinimi ( $W m^{-2}$ ), <sup>2</sup> Isı enerjisi gereksinimi ( $kWh m^{-2} a^{-1}$ )

Çizelge 4'te görüldüğü gibi en az ısı enerjisi gereksinimi cam örtülü seralar için Mersin ilinde 88.9 kW h m<sup>-2</sup> a<sup>-1</sup>, plastik örtülü seralar için Adana ilinde 92.6 kW h m<sup>-2</sup> a<sup>-1</sup> olarak hesaplanmıştır. Cam ve plastik seralarda hesaplanan değerler incelendiğinde Mersin ve Adana illerinin değerleri çok küçük değişiklikler ile benzerlik göstermektedir. Mersin ilinde C1 ile P1 sera tipi için hesaplanan ısı enerjisi gereksinim değerine, Adana ilinde C1 ile P1, Antalya ilinde C3 ile P3 ve Antakya ilinde ise C4 ile P4 sera tipi kullanılarak sağlanabilmektedir. Benzer durumlar yan duvarlarda kullanılan farklı örtü malzemeleri ile ısı perdesinin kullanıldığı durumlarda da

görülmektedir. Bu durum, ısı perdesi gibi ısı koruma önlemleri kullanılarak ısı enerjisi gereksinimi için ortaya çıkan ısı maliyeti ile farklı bölgeler için rekabet edilebilir ürünler üretilebilir.

Seralarda ısıtma borularının taban yerleştirilmesi durumunda ısı enerjisi gereksinimi en fazla Antakya ilinde ısı perdesinin kullanılmadığı cam sera tipi için 130.8 kW h m<sup>-2</sup> a<sup>-1</sup> (C1), plastik sera tipi için ise 135.858 kW h m<sup>-2</sup> a<sup>-1</sup> (P1) değerleri kullanılarak ısı perdesinin sızdırmazlık durumlarına göre hesaplanan tasarruf oranları Çizelge 5'te verilmiştir.

Table 5. Thermal energy requirement and heat curtain sealing savings rates when heating pipes are placed at the base

Çizelge 5. Isıtma borularının tabana yerleştirilmesi durumunda ortaya çıkan ısı enerjisi gereksinimi ve ısı perdesi sızdırmazlık tasarruf oranları

| Sera Tipi | Antalya |      | Mersin |      | Adana |      | Antakya |      |
|-----------|---------|------|--------|------|-------|------|---------|------|
|           | 1       | 2    | 1      | 2    | 1     | 2    | 1       | 2    |
| C1        | 0.0     | 11.7 | 0.0    | 32.0 | 0.0   | 31.5 | 0.0     | 0.0  |
| C2        | 11.0    | 21.4 | 10.1   | 38.9 | 8.9   | 37.6 | 9.4     | 9.4  |
| C3        | 28.0    | 36.4 | 26.5   | 50.0 | 22.4  | 46.9 | 23.9    | 23.9 |
| C4        | 45.0    | 51.5 | 42.8   | 61.1 | 36.0  | 56.1 | 38.4    | 38.4 |
| C5        | 9.2     | 19.9 | 9.2    | 38.3 | 10.8  | 38.9 | 9.9     | 9.9  |
| C6        | 18.4    | 28.0 | 17.7   | 44.0 | 18.3  | 44.0 | 17.8    | 17.8 |
| C7        | 32.7    | 40.5 | 31.3   | 53.3 | 29.6  | 51.8 | 29.9    | 29.9 |
| C8        | 46.9    | 53.1 | 44.9   | 62.6 | 40.9  | 59.5 | 42.0    | 42.0 |
| P1        | 0.0     | 10.4 | 0.0    | 30.6 | 0.0   | 31.8 | 0.0     | 0.0  |
| P2        | 10.1    | 19.5 | 9.3    | 37.1 | 8.3   | 37.4 | 8.7     | 8.7  |
| P3        | 26.0    | 33.7 | 24.5   | 47.6 | 21.1  | 46.2 | 22.3    | 22.3 |
| P4        | 41.8    | 47.9 | 39.7   | 58.2 | 33.9  | 54.9 | 35.9    | 35.9 |
| P5        | 6.7     | 16.4 | 6.5    | 35.2 | 7.6   | 37.0 | 7.3     | 7.3  |
| P6        | 15.6    | 24.4 | 14.8   | 40.9 | 14.9  | 41.9 | 15.0    | 15.0 |
| P7        | 29.6    | 36.9 | 28.1   | 50.1 | 26.1  | 49.6 | 26.9    | 26.9 |
| P8        | 43.5    | 49.4 | 41.5   | 59.4 | 37.4  | 57.3 | 38.8    | 38.8 |
| P9        | 8.4     | 17.9 | 8.3    | 36.4 | 9.4   | 38.2 | 9.2     | 9.2  |
| P10       | 17.0    | 25.6 | 16.2   | 41.9 | 16.5  | 43.0 | 16.6    | 16.6 |
| P11       | 30.5    | 37.7 | 29.1   | 50.8 | 27.3  | 50.4 | 28.1    | 28.1 |
| P12       | 43.9    | 49.8 | 42.0   | 59.8 | 38.2  | 57.9 | 39.7    | 39.7 |
| P13       | 13.2    | 22.3 | 13.1   | 39.7 | 15.1  | 42.1 | 14.4    | 14.4 |
| P14       | 21.0    | 29.3 | 20.3   | 44.7 | 21.4  | 46.4 | 21.1    | 21.1 |
| P15       | 33.2    | 40.2 | 31.9   | 52.7 | 31.3  | 53.1 | 31.4    | 31.4 |
| P16       | 45.4    | 51.0 | 43.5   | 60.8 | 41.1  | 59.8 | 41.8    | 41.8 |

<sup>1</sup> Yıllık ısı gücü tasarruf oranları (%), <sup>2</sup> Isı perdesi tasarruf oranları (%)

Çizelge 5 incelendiğinde, cam seralar için en uygun sera tipi C8, plastik seralar için en uygun sera tipi ise P16 olarak belirlenmiştir. Bu durumda Doğu Akdeniz Bölgesi cam seralarda çatıda tek kat cam, yan duvarlarda ise çift kat cam plastik seralarda ise çatıda tek kat PE, yan duvarlarda çift katlı sert PE (32 mm) ile ısı perdesinin "iyi" sızdırmazlık durumunun kullanılması optimum ısı maliyetinin ortaya çıkmasını sağlayacaktır.

Çizelge 5'te cam seralarda ısı perdesinin

kullanılmadığı durumda Antakya ili ısı enerjisi gereksinimine göre karşılaştırıldığında, Adana, Mersin ve Antalya illeri için sırasıyla %31.5, %32.0, %11.7, plastik seralarda ise %31.8, %30.6, %10.4 daha az ısı enerjisi gereksinimine ihtiyaç duyulmaktadır.

Çizelge 5'te verilen optimum ısı maliyetini sağlayan ısı perdesinin sızdırmazlık durumlarına göre, cam ve plastik seralar için ortaya çıkan tasarruf oranları Çizelge 6'da verilmiştir. Isı perdeleri en fazla ısı koruma oranını Antalya ilinde sağlarken diğer iller



sırasıyla Mersin, Antakya ve Adana olarak belirlenmiştir.

Çizelge 6 incelendiğinde, ısı perdeleri cam seralarda daha iyi sonuç vermektedir. Isı perdesi sızdırmazlığının “iyi” olması durumunda cam

seralarda ısı enerjisi gereksiniminde %40, plastik seralarda ise %35 oranında tasarruf sağlanabilmektedir. Bu sonuçlar seralarda kullanılacak ısı perdelerinin sızdırmazlık oranlarının göz önüne alınması gerektiğini göstermektedir.

Table 6. Heat curtain sealing rates of greenhouse types calculated for optimum heat cost  
Çizelge 6. Optimum ısı maliyeti için hesaplanan sera tiplerinin ısı perdesi sızdırmazlık oranları

| Örtü<br>Malzemesi | Antalya |      |      | Mersin |      |      | Adana |      |      | Antakya |      |      |
|-------------------|---------|------|------|--------|------|------|-------|------|------|---------|------|------|
|                   | 1       | 2    | 3    | 1      | 2    | 3    | 1     | 2    | 3    | 1       | 2    | 3    |
| Cam               | 10.2    | 25.8 | 41.5 | 9.3    | 24.3 | 39.4 | 8.4   | 21.0 | 33.7 | 8.8     | 22.2 | 35.6 |
| Plastik           | 9.0     | 23.0 | 37.0 | 8.2    | 21.6 | 35.0 | 7.5   | 19.0 | 30.6 | 7.8     | 19.9 | 32.1 |

<sup>1</sup> Isı perdesi sızdırmazlığı kötü, <sup>2</sup> Isı perdesi sızdırmazlığı orta, <sup>3</sup> Isı perdesi sızdırmazlığı iyi

Çaylı (2014), tek kat PE serada, ısı perdesinin sızdırmazlık durumuna göre değişmekle birlikte % 17 oranında tasarruf sağlanabileceğini bildirmiştir. Diğer araştırmacılar ise ısı perdesi kullanarak % 20–70 arasında ısı tasarrufu sağlanabileceğini bildirmişlerdir (Arinze ve ark., 1986; Chandra ve Albright, 1980; Critten ve Bailey, 2002; Le Quillec ve ark., 2005; Meyer, 1984; Nijskens ve ark., 1984). Sızdırmazlık oranı iyi olan ısı perdesi kullanılması durumunda Antalya, Mersin, Antakya ve Adana illerindeki cam serada sırasıyla % 42, % 39, %36 ve % 34, plastik serada ise sırasıyla %37, %35, %32 ve %31 oranında ısı tasarruf edilebileceği hesaplanmıştır. Bu değerler literatürdeki değerler ile benzerlik göstermektedir.

Seralarda ısıtma sistemlerinde kullanılacak olan kazan kapasitelerinin belirlenmesi için maksimum ısı gücü değeri ile maksimum ısı gücü değerlerine yılın kaç saatinde ihtiyaç duyulduğunun bilinmesi, ısıtma sistemlerinin kapasite tayinlerinde büyük öneme sahiptir. Yapılan çalışmada optimum ısı maliyeti sağlayan cam örtülü C8 ile plastik örtülü P16 sera tiplerinde iyi derecede ısı perdesi yalıtımına sahip sera için hesaplanmış ısı gücü değerleri ve yılın kaç saatinde bu değerlere ihtiyaç duyulduğu Çizelge 7’de verilmiştir.

Çizelge 7’den de görüleceği gibi Adana ilinde çatısı tek kat PE, yan duvarları çift kat sert PC ile kaplı serada (P16) sıcaklığın üretim periyodu boyunca gündüz/gece 21/16°C’de tutulmak istenmesi durumunda gereksinim duyulan ısı gücü 90 W m<sup>-2</sup> olmaktadır. Ancak bu ısı gücüne yılın sadece 3 saatinde ihtiyaç duyulmaktadır. Serada 60 W m<sup>-2</sup> ısı gücüne sahip ısıtma sisteminin kurulması durumunda yılın sadece 88 saatinde sıcaklık istenilen değerlerin altında seyredecektir. Belirtilen nedenle serada ısı gereksiniminin %80’ini karşılayacak ana kazan ve bitkileri dona karşı koruyacak ikincil bir kazanın seçilerek ısıtma sisteminin planlanması daha sağlıklı olacaktır.

Sera ısıtma sisteminde fosil enerji kullanılması

durumunda yakıt depolama kapasitesinin belirlenmesi için aylık ısı enerjisi gereksiniminin bilinmesi gereklidir. Yapılan çalışmada optimum ısı maliyetinin sağlandığı çelik borulu ısıtma sisteminde boruların sera tabanına yakın yerleştirilmiş seraların farklı donanımlar için aylık ısı enerjisi gereksinimleri Çizelge 8’de verilmiştir.

Hesaplamalarda ısı enerjisi gereksinimi olmayan Haziran, Temmuz, Ağustos ve Eylül ayları Çizelge 8’de verilmemiştir. Bununla birlikte çizelge incelendiğinde Nisan, Mayıs ve Ekim aylarında ortaya çıkan ısı enerjisi gereksinimi çok küçük değerler olduğu için ihmal edilebilir. Sonuç olarak Doğu Akdeniz Bölgesi ileri teknolojik proje seralarında Ocak, Şubat, Mart, Kasım ve Aralık ayları olmak üzere beş (5) ay ısı enerjisi gereksinimi ortaya çıktığı belirlenmiştir.

## SONUÇ ve ÖNERİLER

Doğu Akdeniz Bölgesinde yer alan ileri teknolojik seralarda Antalya, Mersin, Adana ve Antakya illerinin ölçülmüş saatlik değerlerine göre farklı sera tipleri için ısı enerjisi gereksinimi değerleri farklı gece-gündüz sıcaklıklar için hesaplanmıştır. Hesaplamalar sonucunda optimum ısı maliyeti, cam seralar için çatıda tek kat cam, yan duvarlarda çift kat örtü malzemesi, plastik seralarda ise çatıda tek kat PE, yan duvarlarda ise çift kat sert PE (32 mm) örtü malzemesi ile ısı perdesi kullanılması ile sağlandığı belirlenmiştir. Çalışmada çelik borulu ısıtma sisteminde boruların sera tabanına yakın yerleştirilmesi gerektiği belirlenmiştir.

Sonuç olarak Doğu Akdeniz Bölgesindeki ileri teknolojik seralarda örtü malzemesi olarak cam malzemenin ve sızdırmazlığı iyi olan ısı perdelerinin kullanılması, eğer bölgede plastik örtülü sera tercih edilecek ise çatıda tek kat PE, yan duvarlarda ise çift kat sert PE (32 mm) malzemenin kullanılması önerilmektedir. Ayrıca ısıtma sisteminde çelik borular kullanılacak ise boruların sera tabanına yakın yerleştirilmesi önerilmektedir.

Table 7. Heat power requirements of greenhouse types that provide optimum heat costs by province  
*Çizelge 7. Optimum ısı maliyeti sağlayan sera tiplerinin illere göre ısı gücü gereksinimi tekerrürleri*

| Isı Gücü Gereksinimi<br>W m <sup>-2</sup> | Antalya |         | Mersin |         | Adana |         | Antakya |         |
|---|---------|---------|--------|---------|-------|---------|---------|---------|
|   | Cam     | Plastik | Cam    | Plastik | Cam   | Plastik | Cam     | Plastik |
| 0   | 2609    | 2605    | 2319   | 2333    | 2280  | 2251    | 2670    | 2650    |
| 10  | 2248    | 2284    | 1911   | 1965    | 1919  | 1916    | 2303    | 2332    |
| 20  | 1760    | 1865    | 1466   | 1568    | 1520  | 1539    | 1924    | 1989    |
| 30  | 1190    | 1396    | 836    | 1055    | 1006  | 1120    | 1485    | 1602    |
| 40  | 420     | 716     | 256    | 425     | 440   | 589     | 887     | 1113    |
| 50  | 177     | 191     | 120    | 110     | 158   | 148     | 376     | 535     |
| 60  | 132     | 118     | 81     | 78      | 99    | 88      | 246     | 216     |
| 70  | 98      | 92      | 49     | 38      | 62    | 55      | 179     | 169     |
| 80  | 57      | 47      | 22     | 18      | 33    | 22      | 151     | 131     |
| 90  | 24      | 19      | 6      | 5       | 7     | 3       | 114     | 97      |
| 100                                       | 5       | 4       |        |         | 1     |         | 74      | 51      |
| 110                                       |         |         |        |         |       |         | 33      | 18      |
| 120                                       |         |         |        |         |       |         | 7       | 2       |

Table 8. Monthly heat energy requirements for different equipment of greenhouses with pipes placed close to the greenhouse floor in the steel pipe heating system where optimum heat cost is achieved

*Çizelge 8. Optimum ısı maliyetinin sağlandığı çelik borulu ısıtma sisteminde boruların sera tabanına yakın yerleştirilmiş seraların farklı donanımlar için aylık ısı enerjisi gereksinimleri*

| Sera Tipi          | Ocak | Şubat | Mart | Nisan | Mayıs | Ekim | Kasım | Aralık |
|--------------------|------|-------|------|-------|-------|------|-------|--------|
| <b>Antalya İli</b> |      |       |      |       |       |      |       |        |
| C1                 | 31.2 | 25.2  | 17.4 | 6.4   | 0.5   | 0.5  | 10.6  | 23.8   |
| C2                 | 27.9 | 22.4  | 15.5 | 5.6   | 0.3   | 0.5  | 9.3   | 21.3   |
| C3                 | 22.5 | 18.1  | 12.6 | 4.6   | 0.3   | 0.4  | 7.5   | 17.1   |
| C4                 | 17.2 | 13.9  | 9.7  | 3.6   | 0.2   | 0.3  | 5.6   | 13.0   |
| C5                 | 28.3 | 22.8  | 15.8 | 5.8   | 0.4   | 0.5  | 9.6   | 21.6   |
| C6                 | 25.6 | 20.5  | 14.2 | 5.2   | 0.3   | 0.4  | 8.5   | 19.5   |
| C7                 | 21.1 | 17.0  | 11.8 | 4.3   | 0.2   | 0.4  | 7.0   | 16.0   |
| C8                 | 16.6 | 13.4  | 9.4  | 3.5   | 0.2   | 0.3  | 5.5   | 12.6   |
| P1                 | 32.6 | 26.3  | 18.3 | 6.9   | 0.5   | 0.6  | 11.4  | 25.0   |
| P2                 | 29.4 | 23.7  | 16.5 | 6.2   | 0.4   | 0.5  | 10.1  | 22.5   |
| P3                 | 24.2 | 19.5  | 13.7 | 5.2   | 0.3   | 0.5  | 8.3   | 18.5   |
| P4                 | 19.0 | 15.3  | 10.8 | 4.1   | 0.3   | 0.4  | 6.4   | 14.4   |
| P5                 | 30.4 | 24.6  | 17.1 | 6.5   | 0.5   | 0.5  | 10.6  | 23.3   |
| P6                 | 27.6 | 22.2  | 15.5 | 5.8   | 0.4   | 0.5  | 9.5   | 21.2   |
| P7                 | 23.0 | 18.6  | 13.0 | 4.9   | 0.3   | 0.4  | 7.9   | 17.6   |
| P8                 | 18.4 | 14.9  | 10.5 | 4.0   | 0.2   | 0.4  | 6.3   | 14.0   |
| P9                 | 29.9 | 24.1  | 16.8 | 6.4   | 0.5   | 0.5  | 10.5  | 22.9   |
| P10                | 27.1 | 21.9  | 15.3 | 5.7   | 0.4   | 0.5  | 9.3   | 20.8   |
| P11                | 22.7 | 18.3  | 12.8 | 4.8   | 0.3   | 0.4  | 7.8   | 17.4   |
| P12                | 18.3 | 14.8  | 10.4 | 4.0   | 0.2   | 0.4  | 6.2   | 13.9   |
| P13                | 28.3 | 22.8  | 15.9 | 6.0   | 0.5   | 0.5  | 9.9   | 21.7   |
| P14                | 25.8 | 20.8  | 14.5 | 5.5   | 0.3   | 0.5  | 8.9   | 19.8   |
| P15                | 21.8 | 17.6  | 12.3 | 4.7   | 0.3   | 0.4  | 7.5   | 16.7   |
| P16                | 17.8 | 14.4  | 10.1 | 3.9   | 0.2   | 0.3  | 6.1   | 13.6   |

| Mersin İli |      |      |      |     |     |     |     |      |
|------------|------|------|------|-----|-----|-----|-----|------|
| C1         | 27.3 | 20.4 | 12.2 | 2.2 | 0.0 | 0.1 | 6.6 | 20.0 |
| C2         | 24.7 | 18.4 | 11.0 | 1.9 | 0.0 | 0.1 | 5.9 | 17.9 |
| C3         | 20.5 | 15.1 | 9.1  | 1.6 | 0.0 | 0.1 | 4.7 | 14.3 |
| C4         | 16.2 | 11.8 | 7.1  | 1.3 | 0.0 | 0.1 | 3.5 | 10.8 |
| C5         | 24.8 | 18.5 | 11.1 | 2.0 | 0.0 | 0.1 | 6.0 | 18.2 |
| C6         | 22.6 | 16.8 | 10.1 | 1.8 | 0.0 | 0.1 | 5.4 | 16.4 |
| C7         | 19.1 | 14.1 | 8.5  | 1.5 | 0.0 | 0.1 | 4.4 | 13.4 |
| C8         | 15.6 | 11.3 | 6.8  | 1.2 | 0.0 | 0.1 | 3.5 | 10.5 |
| P1         | 28.5 | 21.5 | 13.0 | 2.5 | 0.0 | 0.1 | 7.3 | 21.2 |
| P2         | 26.0 | 19.5 | 11.8 | 2.2 | 0.0 | 0.1 | 6.6 | 19.1 |
| P3         | 21.9 | 16.3 | 9.9  | 1.8 | 0.0 | 0.1 | 5.4 | 15.6 |
| P4         | 17.8 | 13.0 | 8.0  | 1.5 | 0.0 | 0.1 | 4.2 | 12.2 |
| P5         | 26.7 | 20.1 | 12.2 | 2.3 | 0.0 | 0.1 | 6.9 | 19.8 |
| P6         | 24.5 | 18.3 | 11.1 | 2.1 | 0.0 | 0.1 | 6.2 | 18.0 |
| P7         | 20.9 | 15.5 | 9.4  | 1.8 | 0.0 | 0.1 | 5.1 | 14.9 |
| P8         | 17.3 | 12.6 | 7.7  | 1.4 | 0.0 | 0.1 | 4.1 | 11.9 |
| P9         | 26.2 | 19.7 | 11.9 | 2.3 | 0.0 | 0.1 | 6.7 | 19.4 |
| P10        | 24.0 | 18.0 | 10.9 | 2.0 | 0.0 | 0.1 | 6.1 | 17.7 |
| P11        | 20.6 | 15.2 | 9.3  | 1.7 | 0.0 | 0.1 | 5.1 | 14.7 |
| P12        | 17.1 | 12.5 | 7.7  | 1.4 | 0.0 | 0.1 | 4.0 | 11.8 |
| P13        | 24.8 | 18.6 | 11.3 | 2.2 | 0.0 | 0.1 | 6.4 | 18.4 |
| P14        | 22.9 | 17.1 | 10.4 | 1.9 | 0.0 | 0.1 | 5.8 | 16.8 |
| P15        | 19.8 | 14.6 | 8.9  | 1.7 | 0.0 | 0.1 | 4.9 | 14.2 |
| P16        | 16.6 | 12.2 | 7.5  | 1.4 | 0.0 | 0.1 | 4.0 | 11.5 |
| Adana İli  |      |      |      |     |     |     |     |      |
| C1         | 28.0 | 19.4 | 11.2 | 3.3 | 0.0 | 0.1 | 6.8 | 20.8 |
| C2         | 25.6 | 17.7 | 10.2 | 3.0 | 0.0 | 0.1 | 6.1 | 19.0 |
| C3         | 21.9 | 15.0 | 8.6  | 2.6 | 0.0 | 0.1 | 5.2 | 16.1 |
| C4         | 18.2 | 12.3 | 7.1  | 2.3 | 0.0 | 0.1 | 4.3 | 13.1 |
| C5         | 25.0 | 17.3 | 10.0 | 2.9 | 0.0 | 0.1 | 6.0 | 18.6 |
| C6         | 23.0 | 15.8 | 9.1  | 2.7 | 0.0 | 0.1 | 5.5 | 17.0 |
| C7         | 19.9 | 13.6 | 7.8  | 2.4 | 0.0 | 0.1 | 4.7 | 14.6 |
| C8         | 16.8 | 11.4 | 6.5  | 2.1 | 0.0 | 0.1 | 3.9 | 12.2 |
| P1         | 28.7 | 20.0 | 11.6 | 3.6 | 0.0 | 0.1 | 7.2 | 21.5 |
| P2         | 26.4 | 18.3 | 10.7 | 3.3 | 0.0 | 0.1 | 6.5 | 19.7 |
| P3         | 22.9 | 15.7 | 9.1  | 2.9 | 0.0 | 0.1 | 5.6 | 16.8 |
| P4         | 19.3 | 13.1 | 7.6  | 2.5 | 0.0 | 0.1 | 4.7 | 14.0 |
| P5         | 26.5 | 18.5 | 10.8 | 3.3 | 0.0 | 0.1 | 6.6 | 19.8 |
| P6         | 24.5 | 17.0 | 9.9  | 3.1 | 0.0 | 0.1 | 6.0 | 18.3 |
| P7         | 21.4 | 14.7 | 8.6  | 2.7 | 0.0 | 0.1 | 5.2 | 15.8 |
| P8         | 18.2 | 12.4 | 7.2  | 2.4 | 0.0 | 0.1 | 4.4 | 13.3 |
| P9         | 26.0 | 18.1 | 10.6 | 3.2 | 0.0 | 0.1 | 6.5 | 19.4 |
| P10        | 24.1 | 16.7 | 9.7  | 3.0 | 0.0 | 0.1 | 5.9 | 17.9 |
| P11        | 21.0 | 14.5 | 8.4  | 2.7 | 0.0 | 0.1 | 5.1 | 15.5 |
| P12        | 18.0 | 12.3 | 7.1  | 2.3 | 0.0 | 0.1 | 4.3 | 13.1 |
| P13        | 24.3 | 17.0 | 9.9  | 3.1 | 0.0 | 0.1 | 6.1 | 18.2 |
| P14        | 22.6 | 15.7 | 9.2  | 2.9 | 0.0 | 0.1 | 5.6 | 16.8 |
| P15        | 19.9 | 13.7 | 8.0  | 2.5 | 0.0 | 0.1 | 4.9 | 14.7 |
| P16        | 17.1 | 11.7 | 6.8  | 2.2 | 0.0 | 0.0 | 4.1 | 12.5 |

| Antakya İli |      |      |      |     |     |     |      |      |
|-------------|------|------|------|-----|-----|-----|------|------|
| C1          | 40.3 | 26.8 | 15.2 | 3.5 | 0.2 | 0.7 | 13.1 | 31.1 |
| C2          | 36.8 | 24.3 | 13.7 | 3.1 | 0.1 | 0.6 | 11.8 | 28.0 |
| C3          | 31.7 | 20.5 | 11.5 | 2.6 | 0.1 | 0.5 | 9.6  | 23.1 |
| C4          | 26.6 | 16.6 | 9.2  | 2.1 | 0.1 | 0.4 | 7.4  | 18.1 |
| C5          | 36.0 | 24.2 | 13.8 | 3.2 | 0.1 | 0.7 | 11.9 | 28.0 |
| C6          | 33.1 | 22.2 | 12.6 | 2.9 | 0.1 | 0.5 | 10.8 | 25.5 |
| C7          | 28.8 | 19.0 | 10.7 | 2.4 | 0.1 | 0.4 | 9.0  | 21.3 |
| C8          | 24.5 | 15.7 | 8.8  | 2.0 | 0.1 | 0.4 | 7.2  | 17.2 |
| P1          | 41.0 | 27.8 | 16.0 | 3.9 | 0.2 | 0.8 | 13.9 | 32.2 |
| P2          | 37.8 | 25.4 | 14.6 | 3.5 | 0.1 | 0.6 | 12.7 | 29.2 |
| P3          | 32.9 | 21.7 | 12.4 | 3.0 | 0.1 | 0.6 | 10.5 | 24.5 |
| P4          | 28.0 | 17.9 | 10.2 | 2.4 | 0.1 | 0.5 | 8.4  | 19.7 |
| P5          | 37.8 | 25.8 | 14.9 | 3.6 | 0.2 | 0.7 | 13.0 | 29.8 |
| P6          | 35.0 | 23.7 | 13.7 | 3.3 | 0.1 | 0.6 | 11.9 | 27.3 |
| P7          | 30.7 | 20.4 | 11.7 | 2.8 | 0.1 | 0.5 | 10.0 | 23.1 |
| P8          | 26.3 | 17.1 | 9.8  | 2.3 | 0.1 | 0.5 | 8.2  | 18.9 |
| P9          | 37.0 | 25.3 | 14.6 | 3.6 | 0.2 | 0.7 | 12.8 | 29.2 |
| P10         | 34.2 | 23.3 | 13.4 | 3.2 | 0.1 | 0.6 | 11.7 | 26.8 |
| P11         | 30.1 | 20.1 | 11.5 | 2.7 | 0.1 | 0.5 | 9.9  | 22.7 |
| P12         | 25.9 | 16.9 | 9.6  | 2.3 | 0.1 | 0.4 | 8.1  | 18.6 |
| P13         | 34.7 | 23.9 | 13.8 | 3.4 | 0.1 | 0.7 | 12.1 | 27.6 |
| P14         | 32.2 | 22.1 | 12.7 | 3.0 | 0.1 | 0.6 | 11.2 | 25.4 |
| P15         | 28.5 | 19.2 | 11.0 | 2.6 | 0.1 | 0.5 | 9.5  | 21.7 |
| P16         | 24.7 | 16.3 | 9.3  | 2.2 | 0.1 | 0.4 | 7.9  | 18.0 |

### Teşekkür

Bu çalışmanın hazırlanmasında “Seralarda Isıtma Sistemlerinin Modellemesi ve Karar Verme Aşamasında Bilimsel Verilere Dayalı Uzman Sistemin Geliştirilmesi” adlı 1140533 nolu proje ile TÜBİTAK tarafından desteklenerek geliştirilen ISIGER-SERA yazılımı kullanılmıştır. Bu olanağı sağlayan TÜBİTAK’a teşekkür ederiz.

### Araştırmacıların Katkı Oranı Beyan Özeti

Yazar(lar) makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

### Çıkar Çatışması Beyanı

Makale yazar(lar)ı aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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## Determination of Population Dynamics of Cicadellidae (Hemiptera) Species and Their Relationship with Climatic Parameters in Organic Cotton Fields in Hatay Province, Türkiye

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### ABSTRACT

In this study, population dynamics of leafhopper (Hemiptera: Cicadellidae) species in organic cotton-growing areas of Hatay province were investigated along with their associations with climatic parameters. Weekly sampling using a sweep net (100 sweep net/parcel) identified nine species within the Cicadellidae family. Among these, *Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* (Paoli) displayed high population densities throughout the entire vegetation period of cotton, while *Zyginidia sohrab* (Zatchvakin), *Psammotettix striatus* (Linnaeus), *Orosius orientalis* (Matsumura), *Anaceratagallia laevis* (Ribaut), and *Anaceratagallia sinuata* (Mulsant & Rey) were present in both vegetative and reproductive phases of cotton, with *Z. sohrab* reaching significant population levels. *Cicadulina bipunctella* (Matsumura) and *Balclutha hebe* (Kirkaldy) were found only during the reproductive phase, yet they achieved considerably high populations. Correlation and regression analyses have shown a moderate to high level of positive correlation between the population development of five leafhopper species and various temperature (°C) parameters ( $r=0.578-0.790$ ,  $p<0.05$ ). Additionally, a high level of positive correlation has been observed between the population development of *O. orientalis* and maximum humidity (%) ( $r=0.732$ ,  $p=0.003$ ), and a middle level of negative correlation between *P. striatus* and minimum temperature ( $r=0.650$ ,  $p=0.011$ ). These findings indicate that leafhopper species exhibit variations in population presence and densities throughout the cotton vegetation periods, and certain climatic parameters may influence the population development of leafhoppers. The findings from this study may assist in developing more cost-effective and efficient pest management strategies to timely detect and suppress pest leafhopper species in cotton cultivation areas.

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## Hatay İlindeki Organik Pamuk Tarlalarında Cicadellidae (Hemiptera) Türlerinin Popülasyon Dinamikleri ve İklim Parametreleri ile İlişkilerinin Belirlenmesi

### ÖZET

Bu çalışmada, Hatay ilinde organik pamuk yetiştirilen alanlardaki yaprakpıresi (Hemiptera: Cicadellidae) türlerinin popülasyon dinamikleri ve iklim parametreleriyle olan ilişkileri araştırılmıştır. Atrap ile yapılan haftalık örneklemeler (100 atrap/parsel) sonucunda Cicadellidae familyasına bağlı dokuz tür belirlenmiş ve bunlar arasında *Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* (Paoli)'in popülasyon yoğunluğunun pamuğun tüm vejetasyon dönemi boyunca yüksek seyrettiği, *Zyginidia sohrab* (Zatchvakin), *Psammotettix striatus* (Linnaeus), *Orosius orientalis* (Matsumura), *Anaceratagallia laevis* (Ribaut) ve *Anaceratagallia sinuata* (Mulsant & Rey)'nin pamuğun her iki vejetasyon aşamasında varlık gösterdiği ve *Z. sohrab*'ın popülasyon yoğunluğunun önemli seviyelere ulaştığı görülmüştür. *Cicadulina bipunctella* (Matsumura) ve *Balclutha hebe*

### Entomoloji

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Organik pamuk  
Hatay

(Kirkaldy)'nin pamuğun yalnızca generatif döneminde varlık gösterdiği ancak önemli ölçüde yüksek popülasyonlara ulaştığı belirlenmiştir. Korelasyon ve regresyon analizleri, beş yaprakpiresi türünün popülasyon gelişimi ile farklı sıcaklık (°C) parametreleri arasında orta ya da yüksek düzeyde pozitif korelasyon olduğunu ( $r=0.578-0.790$ ,  $p<0.05$ ), buna ek olarak *O. orientalis*'in popülasyon gelişimi ile maksimum nem (%) arasında yüksek düzeyde pozitif korelasyon olduğunu ( $r=0.732$ ,  $p=0.003$ ) ve *P. striatus* ile minimum sıcaklık arasında orta düzeyde negatif korelasyon olduğunu ( $r=0.650$ ,  $p=0.011$ ) göstermiştir. Bu sonuçlar yaprakpiresi türlerinin, popülasyon varlıklarının ve yoğunluklarının, pamuğun vejetasyon dönemleri içerisinde farklılık gösterdiğini ve bazı iklim parametrelerinin yaprakpirelerinin popülasyon gelişimi üzerinde etkili olabileceğini göstermiştir. Çalışmadan elde edilen bulgular, pamuk yetiştirilen alanlarda bulunan zararlı yaprakpiresi türlerinin varlıklarını zamanında tespit etmek ve bunların popülasyonlarını baskı altına alabilmek için daha ekonomik ve etkili zararlı yönetim stratejileri geliştirilmesine yardımcı olabilir.

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## INTRODUCTION

Cotton (*Gossypium hirsutum* L.), a pivotal member of the Malvaceae family, is a crucial fiber crop cultivated in approximately 100 countries across temperate and tropical regions (Ozyigit & Gozukirmizi, 2009; Datta et al., 2020; USDA, 2020).

Cotton holds significant economic value, offering wide-ranging applications, contributing to value creation, and providing employment opportunities in producer countries (Majumdar et al., 2019; Rehman et al., 2019). As a versatile raw material, cotton supports various sectors: its fibers are essential to the textile industry, while its seeds are valuable for both oil and feed industries. Additionally, its byproducts, such as lint, are used in paper manufacturing (Ozyigit, 2009; Munir et al., 2020). The oil derived from cottonseed is also finding increasing use as a feedstock in biodiesel production, serving as an alternative to petroleum-based fuels (Sharma et al., 2020; Sundar & Udayakumar, 2020).

In the 2021/22 season, Türkiye achieved a significant standing in the global cotton market, ranking third in the world for cotton yield per harvested hectare at 1,930 kilograms (kgs/ha). This performance also placed Türkiye seventh in global cotton production and fourth in global cotton consumption (ICAC, 2022). The country produced 1.017.500 tons of fiber cotton in an area of 5.7 million decares, which was the result of processing 2.75 million tons of seed cotton in 2022. Most notably, 85.5% of Türkiye's cotton production in 2022 was concentrated in six provinces: Adana accounted for 40%, Şanlıurfa 14%, Diyarbakır 12%,

Aydın 9%, Hatay 7%, and İzmir 5% (TÜİK, 2022).

The Cicadellidae family is the largest within the Hemiptera order, encompassing over 40 subfamilies and more than 20,000 described species (Abdollahi et al., 2015; Demirel & Erbey, 2022; Tanyeri & Zeybekoğlu, 2022). These insects can feed on almost all vascular plants and are known to cause significant damage to crops. Most species, commonly referred to as leafhoppers, subsist on the phloem of plants, which can lead to both direct and indirect damage (Dietrich, 2013; Bayhan & Ölmez Bayhan, 2022). In Hatay province, species belonging to the Cicadellidae family are particularly harmful to cotton cultivation areas, with especially severe effects noted in hairless and broad-leaved cotton varieties (Delvare, 1996; Özgür et al., 1988; Bayhan & Ölmez Bayhan, 2022). Leafhoppers infest cotton fields from the emergence of seedlings and continue throughout the vegetation period. Severe infestations cause mottling on the leaves, are harmful to the development of seedlings in the early stages, and result in delayed growth, as well as reduced quality and quantity of the yield (Room & Wardhaugh, 1977; Forrester & Wilson, 1988). Studies and records indicate the presence of 476 Cicadellidae species in Türkiye (Demir, 2006a, 2006b, 2006c; Karavin et al., 2011; Uğur & Bayhan, 2023).

Within the cotton plantations of Türkiye, a complex consisting of the leafhopper species *Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* (Paoli) has been encountered, with both species inflicting considerable damage to the crops. Notably, *A. decedens* emerges as the dominant species in a variety of regional contexts (Başpınar, 1994; Göçmen et al., 1996;

Efil & Güçlü, 2004; Durusoy, 2005; Uğur & Bayhan, 2023). The cotton plant has been reported to be among the host plants for *Orosius orientalis* (Matsumura), a species commonly observed in certain cotton-growing regions within Türkiye, as evidenced by various studies (Efil & Güçlü, 2004; Mart & Sunulu, 2011; CABI, 2021). Additionally, *O. orientalis* holds economic significance in the country due to its role as a vector for the sesame phyllody phytoplasma disease (Sertkaya et al., 2007). It has been reported that *Psammotettix striatus* (Linnaeus) is a significant pest in Türkiye, affecting major crops such as maize and wheat (Mutlu et al., 2008), and attains substantial populations in cotton-growing areas (Mart & Sunulu, 2011). Additionally, this species has been identified as a vector for the wheat blue dwarf (WBD) phytoplasma disease (An et al., 1991). *Zyginidia sohrab* (Zachvatkin) has been recognized as a principal pest of maize in Türkiye, with reports indicating that its population density has reached serious levels in recent years (Atmaca et al., 2021). It is known that *Cicadulina bipunctella* (Matsumura) is a vector for Maize Stripe Virus (MSpV) (Kaya & Başpınar, 2019). Considering the capability of virus-carrying species to migrate long distances at night (Ossiannilsson, 1978), research on this family is of considerable significance.

Various studies have been conducted on leafhoppers in cotton production areas in Türkiye (Özgür et al., 1988;

Başpınar et al., 1996; Göçmen et al., 1996; Efil et al., 1999; Atakan et al., 2004; Efil & Güçlü, 2004; Demirel & Yildirim, 2008; Atakan, 2009; Mart & Sunulu, 2011; Dündar et al., 2012; Uğur & Bayhan, 2023), however, these studies are not sufficient to determine the presence and densities of leafhopper populations at different developmental stages of cotton. Additionally, a study elucidating the relationship between the population dynamics of leafhopper species and climatic parameters has not yet been conducted in Türkiye.

Therefore, this study aimed to determine the population dynamics of Cicadellidae species in organic cotton fields in Hatay province and to investigate the correlations between their population development and various climatic parameters.

## MATERIAL and METHOD

### Study Site

The study focused on an organic cotton field consisting of six parcels where *Gossypium hirsutum* L. was planted with 75 cm inter-row spacing (Table 1). The field is located in the 'Demirköprü' district of Hatay province (36°14'14"N, 36°20'21"E; 92 m elevation). The cotton variety called 'Lazer' was used for organic cotton production in the field, and it is a hairless, medium-tall, early cotton variety (ProGen, 2024). No chemical insecticide application was performed throughout the entire vegetation period of the cotton.

Table 1. The size of the parcels belonging to the field where the study was conducted (in daa) and locality information.

*Çizelge 1. Çalışmanın yürütüldüğü tarlaya ait parsellerin büyüklük (daa) ve lokalite bilgileri*

| Parcels | Size (decares) | Coordinates           |
|---------|----------------|-----------------------|
| A       | 68             | 36°14'12"N 36°19'59"E |
| B       | 127            | 36°14'14"N 36°20'21"E |
| C       | 161            | 36°14'06"N 36°20'23"E |
| D       | 74             | 36°13'56"N 36°20'25"E |
| E       | 131            | 36°14'03"N 36°20'42"E |
| F       | 89             | 36°14'20"N 36°20'49"E |

### Sampling Method

Field sampling was conducted weekly in June, July, August, and September, specifically on the dates 24 June, 1, 7, 15, 22, 29 July, 6, 12, 19, 26 August, 2, and 9 September 2022. Sampling focused on the middle and lower leaves of the cotton plant, utilizing a 45-cm diameter sweep net to collect leafhopper samples. The sweeping process was synchronized with a walking pace, with each step corresponding to one meter and one sweeping motion. To prevent the escape of captured insects, the net was swiftly twisted at a 180° angle at the end of each arc and at the beginning of the subsequent step.

Each sweep sample consisted of 100 step-sweeps, and separate sampling was conducted for each of the six parcels within the field. Throughout the entire vegetation period of the cotton, samples were collected

weekly and carefully labeled with information including the region, parcel, sampling number, date of collection, collector's name, and the field owner's name. The collected samples were then transported to the Entomology laboratory at Hatay Mustafa Kemal University and stored at -18°C.

Upon retrieval, frozen samples were delicately separated from soil and vegetation residues using a fine-tipped brush. These samples were examined under a stereo microscope and morphologically separated. However, due to the inability to distinguish between the species *A. decedens* and *E. decipiens* based on morphological characteristics, these two species have been considered as a single species complex. Adult cicadellids were counted among the examined samples, while nymphs, due to their extremely low numbers and inability to be identified,



were not evaluated.

Species codes were assigned to the morphologically separated adult samples, which were then placed in 1.5 ml Eppendorf tubes containing 70% alcohol. Each tube was labeled with the species code, date of collection, and the parcel code from which it was collected. The tubes were stored at 4 °C until the preparation process.

### Identification of Insect Samples

Genital preparations were made using male individuals of the samples obtained (Kaya & Başpınar, 2019). For the preparation process, the abdomens of male individuals were dissected and heated to boiling point in 10% KOH solution. The solution was boiled and allowed to cool to room temperature (25°C). The material in the cooled solution was placed in a coverslip containing glycerol and the genitalia were separated from the abdomen using a needle under a stereo microscope. Species identification was made by examining the genitalia separated from the abdomen under the same microscope, and the identified samples were placed in a 1.5 ml Eppendorf tube to be evaluated in the study and preserved.

### Meteorological Data

The weekly meteorological data for climate parameters were provided by the Hatay Meteorological Directorate (Anonymous, 2022).

### Statistical Analysis

In this study, the statistical analysis involved calculating the means and standard errors of data collected from field samples of leafhopper species in 2022 using Excel. These data were subsequently visually presented in conjunction with climate parameters. To elucidate the relationship between population development and climate parameters, Pearson correlation and linear regression analyses were performed utilizing IBM SPSS Statistics (Version 27) software (SPSS, 2020).

## RESULTS and DISCUSSION

### The Identified Leafhopper Species

In the study, 9 different species from 3 subfamilies within the Cicadellidae family have been identified and are presented in Table 2. Weekly sampling from these parcels yielded a total count of individual leafhoppers for each parcel, with species categorized under their corresponding subfamilies. Notably, the combined count of *A. decedens* and *E. decipiens* represented the most significant population within the Typhlocybinae subfamily, amassing a combined total of 60,620 individuals across all parcels. This suggests a robust presence of these species in the sampled area and potentially indicates their impact on the organic cotton ecosystem. *Zyginidia sohrab*, another member of the Typhlocybinae subfamily, registered the next highest population with 2,271 individuals collected, reinforcing its status as a common resident within the agricultural habitat.

Table 2. Cicadellidae species identified, and total sample numbers obtained from field samplings in Hatay province, 2022

Çizelge 2. Hatay ilinde 2022 yılında yapılan tarla örneklemelelerinde belirlenen Cicadellidae türleri ve elde edilen toplam örnek sayıları

| Subfamilies                                   | Number of samples collected |       |       |      |      |       | Total |
|---|-----------------------------|-------|-------|------|------|-------|-------|
|   | Parcels                     |       |       |      |      |       |       |
| Species                                       | A                           | B     | C     | D    | E    | F     |       |
| <b>Deltocephalinae</b>                        |                             |       |       |      |      |       |       |
| <i>Psammotettix striatus</i> (Linnaeus)       | 71                          | 36    | 39    | 53   | 39   | 39    | 277   |
| <i>Circulifer haematoceps</i> (Mulsant & Rey) | 5                           | 4     | 4     | 6    | 3    | 3     | 25    |
| <i>Orosius orientalis</i> (Matsumura)         | 29                          | 32    | 21    | 30   | 34   | 32    | 178   |
| <i>Cicadulina bipunctella</i> (Matsumura)     | 181                         | 138   | 103   | 155  | 108  | 115   | 800   |
| <i>Balclutha hebe</i> (Kirkaldy)              | 62                          | 74    | 91    | 79   | 76   | 75    | 457   |
| <b>Agalliinae</b>                             |                             |       |       |      |      |       |       |
| <i>Anaceratagallia laevis</i> (Ribaut)        | 14                          | 13    | 16    | 20   | 11   | 9     | 83    |
| <i>Anaceratagallia sinuata</i> (Mulsant&Rey)  | 33                          | 43    | 41    | 43   | 33   | 34    | 227   |
| <b>Typhlocybinae</b>                          |                             |       |       |      |      |       |       |
| <i>Asymmetrasca decedens</i> (Paoli) +        | 8140                        | 16388 | 12251 | 7540 | 6139 | 10162 | 60620 |
| <i>Empoasca decipiens</i> (Paoli)             |                             |       |       |      |      |       |       |
| <i>Zyginidia sohrab</i> (Zatchvakin)          | 408                         | 400   | 478   | 410  | 285  | 290   | 2271  |

Species of the Deltocephalinae subfamily also showcased in notable numbers, with *C. bipunctella* amassing the highest count of 800 individuals, followed by *Balclutha hebe* (Kirkaldy) with 457

individuals, and *P. striatus* with 277 individuals, further underscoring the diversity and prevalence of leafhopper species in this particular farming environment. The counts for *Circulifer haematoceps*

(Mulsant & Rey) and *O. orientalis* were lower in comparison but still notable with 25 and 178 individuals, respectively. Within the Agalliinae subfamily, *Anaceratagallia sinuata* (Mulsant & Rey) was more abundant than *A. laevis* (Ribaut), with respective totals of 227 and 83 individuals, pointing to the varied population distributions among the parcels

### Population Dynamics of Leafhoppers

Figure 1 illustrates the weekly seasonal population dynamics of *A. decedens* + *E. decipiens* from June 24th to September 9th, 2022. Upon examination of the data, it is evident that the population remained high throughout the entire vegetation period of cotton. A continuous increase in population density was observed starting from June 24th, with the peak for the vegetative phase being reached on July 22nd, recorded at  $1206.33 \pm 188.28$ . The transition into the reproductive phase was marked by a decline on July 29th, which was followed promptly by a sharp rise, reaching the highest population peak of the reproductive phase on August 6th, recorded at  $1268.83 \pm 485.77$ . This was then followed by a significant drop on August 26th, after which the population fluctuated and recorded the lowest density during the reproductive phase at  $510.17 \pm 81.00$ .

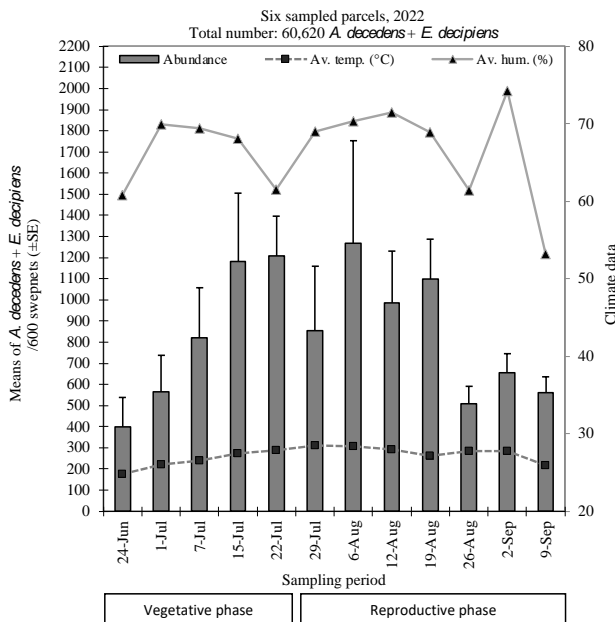


Figure 1. The weekly seasonal population dynamics of *Asymmetrasca decedens* + *Empoasca decipiens* on organic cotton

### Şekil 1. Organik pamuk üzerindeki *Asymmetrasca decedens* + *Empoasca decipiens*'in haftalık mevsimsel popülasyon dinamikleri

Figure 2 illustrates the weekly seasonal population dynamics of *Z. sohrab* from June 24th to September 9th, 2022. Upon analysis of the data, it was found that no individuals of *Z. sohrab* were detected during the first two weeks of the cotton plant's vegetative growth

phase. As the vegetative phase advanced, the population began to be observed from the third week ( $2.00 \pm 0.73$ ), and the number of individuals increased rapidly, reaching a peak during the second week of the reproductive phase on August 6th ( $69.67 \pm 9.37$ ). This peak was followed by a sharp decline to the lowest observed population size in the reproductive phase by August 19th ( $25.83 \pm 3.22$ ), then the population sharply increased again the following week ( $51.83 \pm 9.74$ ) and continued to show a fluctuating and elevated trend in the subsequent weeks.

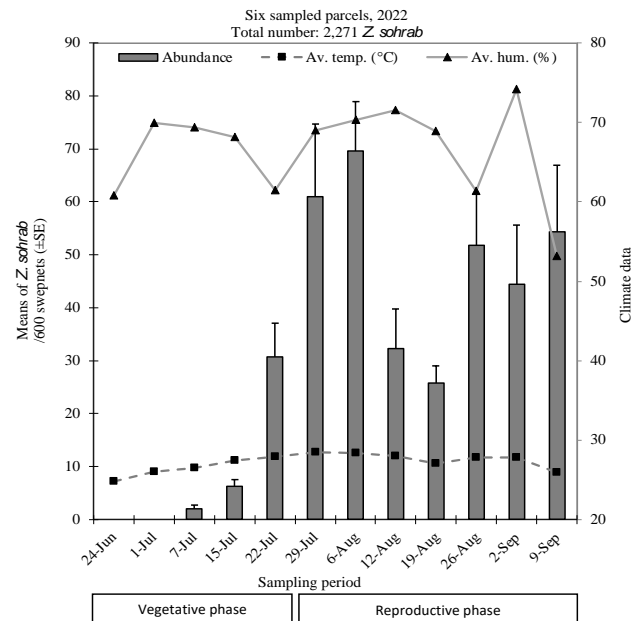


Figure 2. The weekly seasonal population dynamics of *Zyginidia sohrab* on organic cotton

### Şekil 2. Organik pamuk üzerindeki *Zyginidia sohrab*'in haftalık mevsimsel popülasyon dinamikleri

Figure 3 illustrates the weekly seasonal population dynamics of *C. bipunctella* from June 24th to September 9th, 2022. The data review reveals that during the vegetative phase of the cotton plant, no leafhopper individuals were detected. Population emergence, however, was first documented in the reproductive phase beginning from the second week (August 6th), with an average of  $2.50 \pm 0.67$  individuals. Subsequent weeks saw a gradual increase in population numbers, culminating in a peak at  $58.00 \pm 9.62$  individuals by September 9th.

Figure 4 illustrates the weekly seasonal population dynamics of *B. hebe* from June 24th to September 9th, 2022. Analysis of the data reveals that during the vegetative phase of the cotton plant, no individuals of this species were detected. With the commencement of the cotton plant's reproductive phase on August 6th, population presence was first recorded ( $0.50 \pm 0.34$ ) and subsequently, a sharp and sustained increase in population numbers was observed, culminating in the peak level on September 9th ( $27.00 \pm 4.36$ ).

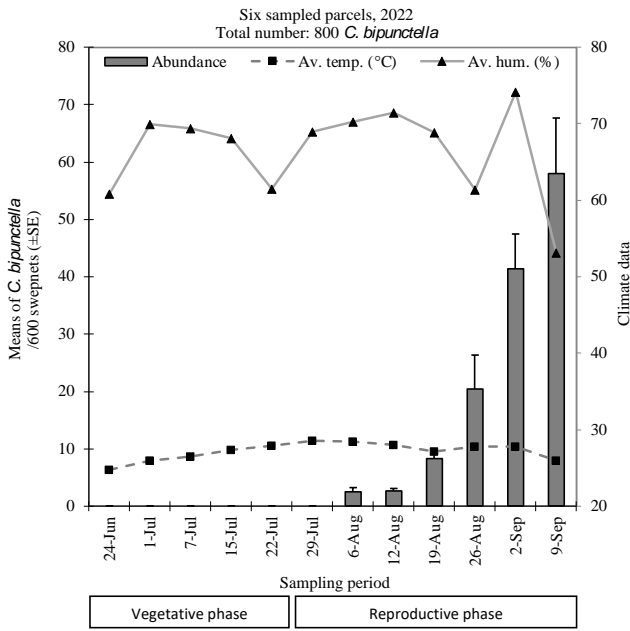


Figure 3. The weekly seasonal population dynamics of *Cicadulina bipunctella* on organic cotton  
 Şekil 3. Organik pamuk üzerindeki *Cicadulina bipunctella*'nin haftalık mevsimsel popülasyon dinamikleri

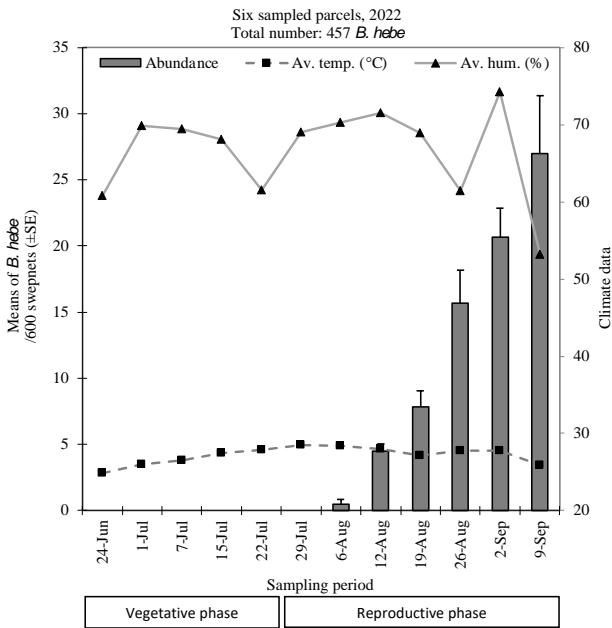


Figure 4. The weekly seasonal population dynamics of *Balclutha hebe* on organic cotton  
 Şekil 4. Organik pamuk üzerindeki *Balclutha hebe*'nin haftalık mevsimsel popülasyon dinamikleri

Figure 5 illustrates the weekly seasonal population dynamics of *P. striatus* from June 24th to September 9th, 2022. Analysis of the data reveals that the population density exhibited a fluctuating trend throughout the developmental period of the cotton plant. Observations of initial individuals commenced on June 24th during the vegetative phase, with the

population showing a steady increase in the ensuing weeks and reaching a peak in the vegetative phase marked by a high of  $4.33 \pm 0.80$  on both July 1st and 7th. Transitioning into the reproductive phase, the population underwent a decline, thereafter, fluctuating significantly during this phase, and ultimately attaining the highest recorded level on September 9th at  $14.17 \pm 2.10$ .

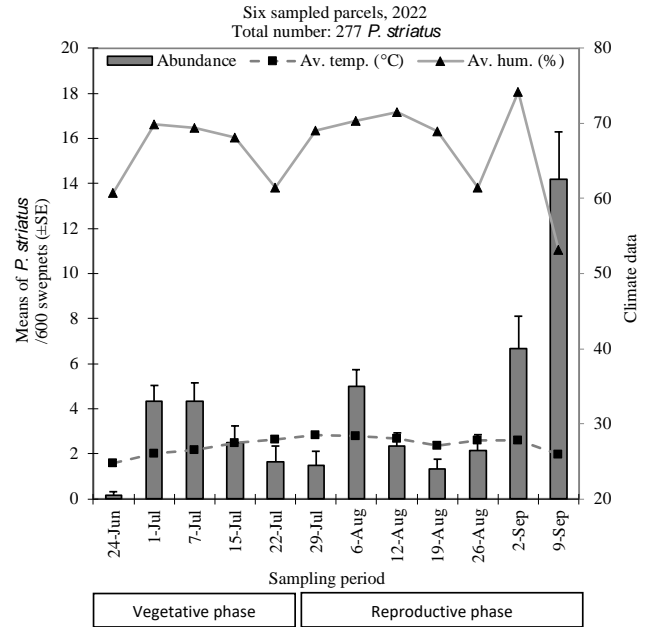


Figure 5. The weekly seasonal population dynamics of *Psammotettix striatus* on organic cotton  
 Şekil 5. Organik pamuk üzerindeki *Psammotettix striatus*'un haftalık mevsimsel popülasyon dinamikleri

Figure 6 illustrates the weekly seasonal population dynamics of *O. orientalis* from June 24th to September 9th, 2022. Upon examining the data, the population density is observed to have generally maintained a low level throughout the cotton plant's vegetative phase. The emergence of the first individuals was noted on July 1st ( $1.00 \pm 0.37$ ), with the peak count during this phase recorded on July 15th ( $3.00 \pm 0.58$ ), and the phase concluding with a relatively low population ( $1.17 \pm 0.31$ ). Although the population levels were low during the vegetative phase, a significant increase was evident in the first week of the cotton plant's reproductive phase (July 29th), with the population reaching its maximum ( $9.33 \pm 0.92$ ). The population experienced a sharp decline by August 6th ( $3.00 \pm 0.52$ ), and in the subsequent weeks, it displayed a higher and more variable pattern compared to the vegetative phase.

Figure 7 illustrates the weekly seasonal population dynamics of *C. haematoceps* from June 24th to September 9th, 2022. Upon examining the data, it is observed that the population density remained low during both cotton plant's phenological phases, with an initial presence of individuals on June 24th

(1.33±0.42). No observations were made the following week. A minor presence re-emerged on July 7th (0.67±0.21) and increased slightly by July 15th (1.33±0.33). The population then decreased to zero by July 22nd. As the cotton plant entered the reproductive phase, a very modest increase in population was recorded on July 29th (0.83±0.31). However, from the beginning of August, the population density dropped to zero and remained at this level throughout the reproductive phase.

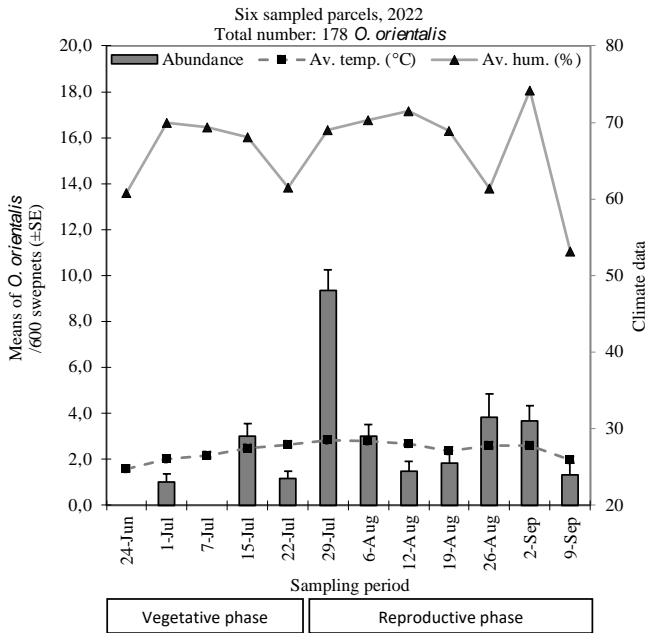


Figure 6. The weekly seasonal population dynamics of *Orosius orientalis* on organic cotton  
 Şekil 6. Organik pamuk üzerindeki *Orosius orientalis*'in haftalık mevsimsel popülasyon dinamikleri

Figure 8 illustrates the weekly seasonal population dynamics of *A. laevis* from June 24th to September 9th, 2022. Upon examining the data, it is observed that the population density throughout the monitoring period exhibited a fluctuating and low trend. In the first week of the vegetative phase (June 24th), no individuals were recorded, but the first sightings were noted on July 1st (1.50±0.43), which stood as the highest population level during the vegetative phase. In the subsequent weeks, there was a decrease observed on July 7th (0.50±0.22), a partial increase on July 15th (0.67±0.33), and a drop to zero by July 22nd, concluding the vegetative phase. With the start of the reproductive phase on July 29th, there was a significant increase in population density (3.67±0.76), peaking on August 12th (4.17±0.70). Following this, the population began to decline, dropping to zero on September 2nd and 9th.

Figure 9 illustrates the weekly seasonal population dynamics of *A. sinuata* from June 24th to September 9th, 2022. The data analysis indicates that no

individuals were present in the first week. Population density became prominent on July 1st (6.50±0.85), marking the highest average recorded during the vegetative phase.

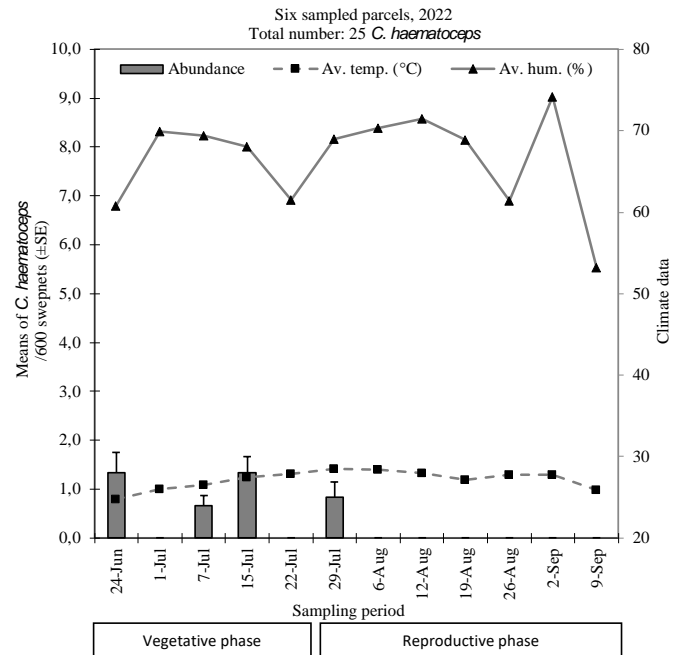


Figure 7. The weekly seasonal population dynamics of *Circulifer haematoceps* on organic cotton  
 Şekil 7. Organik pamuk üzerindeki *Circulifer haematoceps* 'in haftalık mevsimsel popülasyon dinamikleri

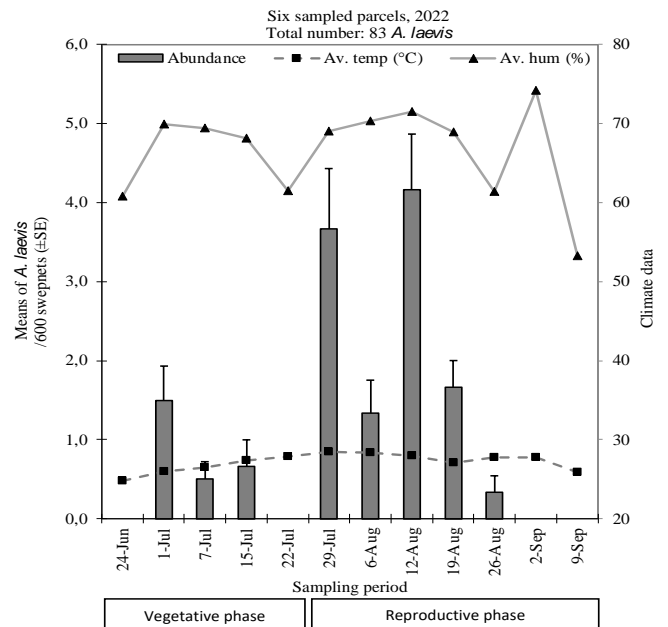


Figure 8. The weekly seasonal population dynamics of *Anaceratagallia laevis* on organic cotton  
 Şekil 8. Organik pamuk üzerindeki *Anaceratagallia laevis* 'in haftalık mevsimsel popülasyon dinamikleri



In the following weeks, the population exhibited variability, with a decrease on July 7th ( $4.67 \pm 0.72$ ), a further reduction on July 15th ( $2.83 \pm 0.54$ ), yet nearly returning to initial peak levels on July 22nd ( $5.83 \pm 1.17$ ). Throughout the vegetative phase, the population generally remained at higher levels compared to the subsequent reproductive phase.

As the cotton plant transitioned to the reproductive phase on July 29th, population levels partially declined ( $5.33 \pm 1.56$ ) but reached the highest level within this phase on August 6th ( $7.50 \pm 0.96$ ). The population began to drop after this peak, with a more pronounced decrease evident by August 12th ( $3.00 \pm 0.52$ ), continuing to dwindle in the weeks that followed, reaching a minimal presence by August 26th ( $0.50 \pm 0.22$ ), and disappearing completely on September 2nd and 9th.

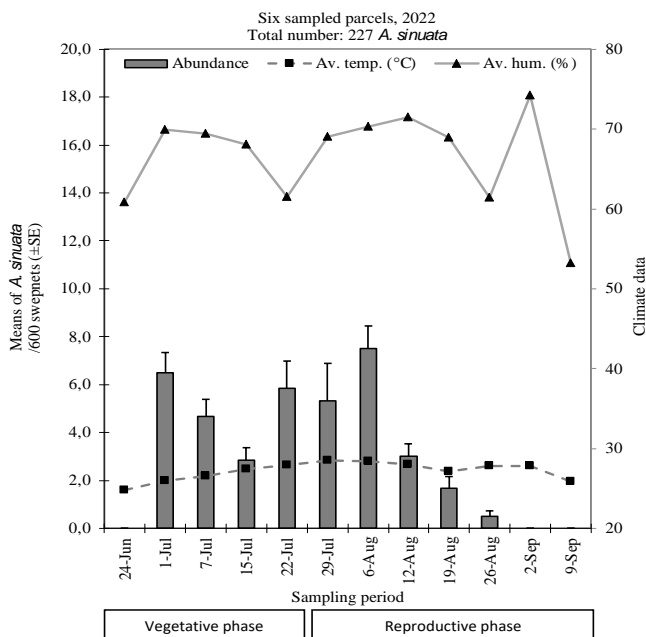


Figure 9. The weekly seasonal population dynamics of *Anaceratagallia sinuata* on organic cotton  
 Şekil 9. Organik pamuk üzerindeki *Anaceratagallia sinuata* 'nın haftalık mevsimsel popülasyon dinamikleri

### Correlation and Regression Analyses

The statistical analysis summarized in Table 3 reveals significant correlations between the population growth of various leafhopper species and climate parameters. For *A. decedens* + *E. decipiens*, moderate positive correlations with average ( $r=0.629$ ,  $p=0.014$ ) and minimum temperatures ( $r=0.578$ ,  $p=0.024$ ) suggest populations increase with warmer conditions, as reflected by regression models  $y=-3695.73+166.97x$  and  $y=-1027.63+78.79x$ . On the other hand, *Z. sohrab* shows a strong positive correlation with maximum temperature ( $r=0.790$ ,  $p=0.001$ ), and a moderate positive correlation with average temperature ( $r=0.645$ ,  $p=0.012$ ), indicating a significant rise in

numbers during warmer conditions, modeled by  $y=-475.90+15.87x$  and  $y=353.65-14.17x$ , respectively. Population growth for *C. bipunctella* displays a moderate correlation with maximum temperature as well ( $r=0.594$ ,  $p=0.021$ ), with the relationship described by  $y=-281.51+9.15x$ . Similarly, *B. hebe* exhibits a trend of increasing numbers with rising maximum temperatures ( $r=0.608$ ,  $p=0.018$ ), explained by  $y=-141.71+4.63x$ . *P. striatus*, however, has a moderate negative correlation with minimum temperature ( $r=-0.650$ ,  $p=0.011$ ), suggesting cooler temperatures might favor its growth, as shown by  $y=29.67-1.08x$ . Lastly, *O. orientalis* demonstrates moderate positive correlations with maximum ( $r=0.637$ ,  $p=0.013$ ) and average temperatures ( $r=0.647$ ,  $p=0.012$ ), as well as a strong positive correlation with maximum humidity ( $r=0.732$ ,  $p=0.003$ ), with respective models  $y=-38.49+1.28x$ ,  $y=-36.16+1.42x$ , and  $y=-18.90+0.255x$ , indicating that both warmer and more humid conditions are conducive to its population growth. However, no statistically significant correlation has been identified between the population growth of *C. haematoceps*, *A. laevis*, and *A. sinuata* and any climate parameters.

According to the data obtained from the study and the results of statistical analyses, the population distributions and densities of various leafhopper species exhibited variations in accordance with the vegetation stages of cotton. It was observed that certain species' population development could be influenced by different climatic parameters. The species complex comprising *A. decedens* + *E. decipiens* notably demonstrated the highest prevalence throughout the entire vegetation period of cotton. Similarly, *Z. sohrab*, *P. striatus*, *O. orientalis*, *A. laevis*, and *A. sinuata* exhibited presence during both vegetative and reproductive stages of cotton, with population developments following a fluctuating trajectory. Atmaca et al. (2021) reported that the population of *Z. sohrab* was high during the late August and September periods in cornfields. Likewise, in the current study, *Z. sohrab* was the second most abundant species and its high population density during these months could potentially be explained by the proximity of cornfields to the cotton fields. Conversely, *C. bipunctella* and *B. hebe* populations were only evident during the reproductive phase, with both species observing substantial increases and peaking towards the end of this period (September 9), whereas *C. haematoceps* did not exhibit substantial presence.

Previous studies mentioned that leafhopper population densities reach their maximum during the boll formation and maturation stages of cotton (Baloch & Soomro, 1980; Monsef, 1981; Lodos, 1982; Salem et al., 1988; Göçmen et al., 1996; Atakan et al., 2004, Başpınar et al., 1996; Mart & Sunulu, 2011).

Table 3. Significant correlation coefficient and R<sup>2</sup> values of Cicadellidae populations with respect to climate parameters  
*Çizelge 3. İklim parametreleri ile Cicadellidae popülasyonları arasındaki önemli korelasyon katsayısı ve R<sup>2</sup> değerleri*

| Climate parameters<br>(°C / %)   | Correlation<br>coefficient (r) | P value | F value | R <sup>2</sup> | Line equation      |
|--|--------------------------------|---------|---------|----------------|--------------------|
| <i>Asymmetrasca decedens</i> (Paoli) + <i>Empoasca decipiens</i> (Paoli) |                                |         |         |                |                    |
| Average temperature  | 0.629 <sup>b</sup>             | 0.014   | 6.56    | 0.396          | y=-3695.73+166.97x |
| Minimum temperature  | 0.578 <sup>b</sup>             | 0.024   | 5.03    | 0.335          | y=-1027.63+78.79x  |
| <i>Zyginidia sohrab</i> (Zatchvakın)                                     |                                |         |         |                |                    |
| Maximum temperature  | 0.790 <sup>a</sup>             | 0.001   | 16.58   | 0.624          | y=-475.90+15.87x   |
| Average temperature  | 0.645 <sup>b</sup>             | 0.012   | 7.13    | 0.416          | y=353.65-14.17x    |
| <i>Cicadulina bipunctella</i> (Matsumura)                                |                                |         |         |                |                    |
| Maximum temperature  | 0.594 <sup>b</sup>             | 0.021   | 5.44    | 0.352          | y=-281.51+9.15x    |
| <i>Balclutha hebe</i> (Kirkaldy)   |                                |         |         |                |                    |
| Maximum temperature  | 0.608 <sup>b</sup>             | 0.018   | 5.85    | 0.369          | y=-141.71+4.63x    |
| <i>Psammotettix striatus</i> (Linnaeus)                                  |                                |         |         |                |                    |
| Minimum temperature  | -0.650 <sup>b</sup>            | 0.011   | 7.30    | 0.422          | y=29.67-1.08x      |
| <i>Orosius orientalis</i> (Matsumura)                                    |                                |         |         |                |                    |
| Maximum temperature  | 0.637 <sup>b</sup>             | 0.013   | 6.83    | 0.406          | y=-38.49+1.28x     |
| Average temperature  | 0.647 <sup>b</sup>             | 0.012   | 7.18    | 0.418          | y=-36.16+1.42x     |
| Maximum humidity   | 0.732 <sup>a</sup>             | 0.003   | 11.55   | 0.536          | y=-18.90+0.255x    |

<sup>a</sup>High correlation (P<0.05)

<sup>b</sup>Moderate correlation (P<0.05)

In accordance, this study observed that *A. decedens* + *E. decipiens*, *Z. sohrab*, *O. orientalis*, *A. laevis* and *A. sinuata* achieved their highest population densities in the same period. In a study by Kaya and Başpınar (2019) conducted within an agroecosystem encompassing diverse plant parcels, including cotton, notable population densities of *C. bipunctella* using light trap data was reported, with the highest populations observed at the end of August and at the beginning of September. Similarly, in this study, the population of *C. bipunctella* reached a peak during comparable dates, achieving significantly high levels.

Statistical analyses conducted to determine the relationship between population development and climatic parameters have shown that, except for *C. haematoceps*, *A. laevis*, and *A. sinuata*, various temperature parameters (°C) have a statistically significant moderate to high positive effect (excluding *P. striatus*) on the population development of all other species (r=0.578-0.790, p<0.05). Unlike the others, it has also been observed that maximum humidity (%) may have a high and positive impact on the population development of *O. orientalis*, in addition to temperature (r=0.732, p<0.05). In alignment with these findings, Trebicki et al. (2010) similarly documented an increase in the activity of *O. orientalis* correlating with rising temperatures. These results generally suggest that increases in temperature may promote the population growth of leafhopper species.

## CONCLUSION

In this study, the population dynamics of leafhopper species in organically cultivated cotton fields in Hatay

province were determined, and the relationship between the population developments and climatic parameters was examined. Data obtained from the study indicate that the presence and densities of leafhopper populations varied within the vegetation stages of cotton. Furthermore, this study has provided insights into the potential influence of various climatic parameters on the population development of leafhopper species. These findings can assist in timely detection of pest presence in cotton cultivation areas and support the development of accurate and more cost-effective pest management strategies to suppress harmful leafhopper species.

## Contribution Rate Statement Summary of Researchers

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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## Contributions to *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 and *Uncopaederus* Korge, 1969 (Coleoptera, Staphylinidae, Paederinae) fauna of Türkiye

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### ABSTRACT

In this study, between 2009 and 2021, total of more than thousand specimens belonging to the genus *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 and *Uncopaederus* Korge, 1969 were collected in Türkiye. Most of the specimens examined were collected from the Marmara, Aegean, Central Anatolia, Western and Central Blacksea Regions. The specimens collected in this study were collected with aspirators and light traps. As a result of field studies, a total of eight species belonging to three genera were recorded. These are *Paederidus rubrothoracicus* (Goeze, 1777); *P. ruficollis* (Fabricius, 1777); *Paederus balcanicus* Koch, 1938, *P. fuscipes* Curtis, 1826, *P. riparius* Linnaeus, 1758, *P. littoralis* Gravenhorst, 1802, *P. mesopotamicus* Eppelsheim, 1889 and *Uncopaederus signiventris* (Smetana, 1962). Among these species, *P. rubrothoracicus* and *P. ruficollis* from the Marmara and Central Black Sea Regions, *P. fuscipes* from the Western Black Sea Region, and *P. riparius* from the Marmara Region of Turkey were reported for the first time. The presence of *P. balcanicus* in Türkiye has been confirmed. *U. signiventris* is endemic and distributed only in the Black Sea Region. In addition, *P. balcanicus* and *U. signiventris* are figured for the first time.

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## Türkiye *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 ve *Uncopaederus* Korge, 1969 (Coleoptera, Staphylinidae, Paederinae) Faunasına Katkılar

### ÖZET

Bu çalışmada, 2009-2021 yılları arasında Türkiye'den *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 ve *Uncopaederus* Korge, 1969 cinslerine bağlı toplam binden fazla örnek toplanmıştır. İncelenen örneklerin çoğu Marmara, Ege, İç Anadolu, Batı ve Orta Karadeniz Bölgelerinden toplanmıştır. Bu çalışmada toplanan örnekler aspiratör ve ışık tuzakları ile toplanmıştır. Arazi çalışmaları sonucunda sekiz tür kaydedilmiştir. Bunlar *Paederidus rubrothoracicus* (Goeze, 1777); *P. ruficollis* (Fabricius, 1777); *Paederus balcanicus* Koch, 1938, *P. fuscipes* Curtis, 1826, *P. riparius* Linnaeus, 1758, *P. littoralis* Gravenhorst, 1802, *P. mesopotamicus* Eppelsheim, 1889 ve *Uncopaederus signiventris* (Smetana, 1962) türleridir. Kaydedilen türlerden, *P. rubrothoracicus* ve *P. ruficollis* Marmara ve Orta Karadeniz; *P. fuscipes* Batı Karadeniz ve *P. riparius* türü ise Marmara Bölgelerinden ilk defa bildirilmiştir. Ayrıca, *P. balcanicus* türünün Türkiye'deki varlığı doğrulanmıştır. Kaydedilen türlerden *U. signiventris* endemik olup yalnızca Karadeniz Bölgesi'nde dağılım göstermektedir. Ek olarak, *P. balcanicus* ve *U. signiventris* türlerine ait resimler ilk defa verilmiştir.

### Entomoloji

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## INTRODUCTION

The family Staphylinidae Latreille, 1802 is the species-rich family within the Coleoptera order, is represented by 63,657 species (Irmler et al., 2018). There are around 7,000 members of the Paederinae subfamily, which is the fourth largest subfamily of the Staphylinidae family (Herman, 2001: updated).

The genus *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 and *Uncopaederus* Korge, 1969 (Coleoptera: Staphylinidae: Paederinae) are remarkable with their body colors and they have agricultural and public health importance. The species are generally active at night and they can be found in many habitats, but they can be seen mostly in agricultural areas, along rivers, streams, lakes and dams. Their light-oriented behavior allows them to be observed flying in large numbers around bright and strong light sources at night. Around 600 *Paederus* species are known in the world (Frank, 1988). The most common and best-known of the *Paederus* species is *Paederus fuscipes* Curtis, 1826. This species is frequently seen in residential areas and agricultural regions and is known to be beneficial because it feeds on some insects (e. g. *Corcyra* spp. *Heliothis* spp. *Aphis* spp.) that cause significant agricultural damage (Berglund et al., 1997; Krakerb et al., 2000; Komala et al., 2003; Nasir et al., 2012).

Species belonging to the genera *Paederus*, *Paederidus*, and possibly *Uncopaederus* (endemic to the Northern Anatolia) contain a very potent toxic substance called "pederin" or its derivatives in their body fluids (Pavan & Bo, 1953).

According to a recent study by Schülke & Smetana (2015), Anlaş (2018), Willers (2011, 2018), Assing (2017, 2020, 2022), the genus *Paederus* (129 species), *Paederidus* (13 species) and *Uncopaederus* (only one species) (Staphylinidae, Paederinae) contains 143 species in the Palaearctic region. Eight species of the genus *Paederus* (five species), *Paederidus* (two species), and *Uncopaederus* (one species) are known from Türkiye. *Uncopaederus signiventris* (Smetana, 1962) is an endemic species in Northern Anatolia.

The Turkish fauna of *Paederus*, *Paederidus* and *Uncopaederus* is more poorly known than some other genera e.g. *Achenium*, *Medon*, *Leptobium* etc. (Ayan & Anlaş, 2020; Orgel et al., 2021). The aim of this study is to contribute to the current knowledge of the *Paederus* Fabricius, *Paederidus* Mulsant & Rey, and *Uncopaederus* Korge fauna of Türkiye.

## MATERIAL and METHOD

The present paper is based on material obtained

during many field studies between 2009 and 2021 in Türkiye. Most of the specimens examined were collected from the Marmara, Aegean, Central Anatolia, Western, and Central Blacksea Regions, with a scope of three projects (TUBITAK: 112T907, 215Z080, and 119Z253) on the diversity and biogeography of the Paederinae in Türkiye. The specimens collected in this study were collected with aspirators and light traps by the authors and their colleagues. All specimens were identified by authors.

Specimens were studied with a Stemi 508 microscope (ZeissGermany). All photographs were taken using a Zeiss Axiocam 208 digital camera. Photographs were edited with Helicon Focus v. 8, and CorelDraw Graphics Suite 2023. All specimens are deposited in Alaşehir Zoological Museum, Manisa, Turkey (AZMM).

## RESULTS

In this study, eight species of the genus *Paederus* Fabricius, 1775, *Paederidus* Mulsant & Rey, 1878 and *Uncopaederus* Korge, 1969 belonging to the subfamily Paederinae are reported from Türkiye.

### *Paederidus* (s. str.) *rubrothoracicus* (Goeze, 1777)

**Material: Aksaray:** 1♂, 1♀, 22.III.2018, Melendiz Çayı, 38°12'00"N, 34°19'50"E, 1400 m, leg. Örgel & Yaman. **Ankara:** 1♂, 2♀, 28.IV.2019, Nallıhan, Cendere 2 km NW, 40°12'26"N, 31°06'08"E, 800 m, leg. Örgel & Köksal. **Balıkesir:** 1♂, 2♀, 10.VI.2018, Erdek, Kapıdağ Yarımadası, Kirazlı Manastırı Yolu, 40°27'36"N, 27°54'17"E, 375 m, leg. Sak, Yağmur & Bulut. **Eskişehir:** 2♀, 12.III.2018, Sündiken Dağları, 40°00'57"N, 30°57'14"E, 980 m, leg. Örgel & Yaman. **Kayseri:** 2♂, 16.V.2019, Develi, Yeniköy 7 km SE, 38°03'43"N, 35°43'13"E, 1740 m, leg. Örgel & Köksal. **Kırklareli:** 7♂, 8♀, 26.IV.2022, Demirköy, İğneada Longoz Ormanları Milli Parkı, Hamam Gölü, 41°49'16"N, 27°57'48"E, 42 m, leg. Kacar & Çelik. 8♂, 12♀, 26.IV.2022, Demirköy, İğneada Longoz Ormanları Milli Parkı, 41°50'59"N, 27°56'24"E, 1 m, leg. Kacar & Çelik. **Muğla:** 2♂♂, 3♀♀, 21.V.2015, Dalaman, 36°53'37"N, 28°53'37"E, 127 m, leg. Yağmur & Örgel. **Ordu:** 5♂, 7♀, 13.VI.2020, Aybastı, Uzundere 1 km N, 40°35'36"N, 37°24'56"E, 900 m, leg. Örgel & Kacar. **Tokat:** 2♂, 3♀, 07.IV.2022, Reşadiye, Yeşilyurt 2 km NE, 40°23'12"N, 37°12'56"E, 1120 m, leg. Örgel, Kacar & Çelik. **Yozgat:** 1♂, 28.VI.2016, Aydıncık, Kuşsaray, 40°05'07"N, 35°11'59"E, 1341 m, leg. Örgel & Yaman.

**Distribution in Türkiye:** Afyonkarahisar, Ankara, Antalya, Artvin, Aydın, Bolu, Denizli, Erzurum, Eskişehir, Elâzığ, İzmir, Konya, Manisa, Muğla, Sivas,

Trabzon, Tunceli, Uşak, Van (Anlaş, 2009, 2018; Anlaş & Rose, 2009; Sert et al., 2013; Çiftçi & Hasbenli, 2016; Özgen & Örgel, 2021; Daşdemir & Tozlu, 2022).

**Remarks:** This species is reported for the first time from the Marmara and Central Black Sea Region and Aksaray, Balıkesir, Kayseri, Kırklareli, Ordu, Tokat and Yozgat provinces.

**Distribution in the world:** This species occurs in Europe and Türkiye (Schülke & Smetana, 2015).

***Paederidus (s. str.) ruficollis*** (Fabricius, 1777)

**Material:** **Ankara:** 2♂, 10.III.2018, Mamak, Kutludüğün Yaylası, 39°52'11"N, 33°06'04"E, 1436 m, leg. Örgel & Yaman. **Balıkesir:** 1♂, 2♀, 10.VI.2018, Erdek, Kapıdağ Yarımadası, Kirazlı Manastırı Yolu, 40°27'36"N, 27°54'17"E, 375 m, leg. Sak, Yağmur & Bulut. **Ordu:** 5♂, 7♀, 13.VI.2020, Aybastı, Uzundere 1 km N, 40°35'36"N, 37°24'56"E, 900 m, leg. Örgel & Kacar. **Kütahya:** 1♂, 2♀♀, 28.VI.2014, Domanıç, Ortaca 1 km B, 39°49'55"N, 29°29'40"E, 700 m, leg. Yağmur & Örgel. **Yozgat:** 2♂, 07.IV.2018, Akdağmadeni, Aktaş 3 km W, 39°35'31"N, 35°49'23"E, 1475 m, leg. Yağmur & Örgel, Yaman.

**Distribution in Türkiye:** Afyonkarahisar, Ağrı, Ankara, Bolu, Denizli, Erzurum, İzmir, Kastamonu, Konya, Kütahya, Manisa, Muğla, Niğde, Sivas (Anlaş, 2009, 2018).

**Remarks:** This species is recorded for the first time from the Marmara and Central Black Sea Regions of Türkiye.

**Distribution in the world:** The species is widely distributed in Europe, North Africa, Iran, and Türkiye (Schülke & Smetana, 2015).

***Paederus (s. str.) balcanicus*** Koch, 1938 (Figure 1A-F)

**Material:** **Bursa:** 3♂, 11♀, 24.IV.2022, Ulubat Gölü, 40°09'15"N, 28°41'42"E, 5 m, leg. Kacar & Çelik.

**Distribution in Türkiye:** The species was known in only one locality from Balıkesir (Manyas Lake) province (Scheerpeltz, 1957; Horion, 1965; Coiffait, 1982).

**Remarks:** The species is recorded from Bursa (Ulubat Lake) in this study. The presence of *P. balcanicus* in Türkiye has been confirmed.

**Distribution in the world:** This species is known from southern and eastern Europe, Iran, and Türkiye (Schülke & Smetana, 2015).

***Paederus (Heteropaederus) fuscipes*** Curtis, 1826

**Material:** **Amasya:** 1♂, 7♀, 27.IV.2021, Hamamözü, Yemişen 1 km N, 40°45'50"N, 35°08'11"E, 1300 m, leg. Anlaş, Kacar & Çelik. **Bartın:** 1♂, 1♀, 10.VII.2020, Ulus, Kerpiçli 3 km SW, 41°43'13"N, 32°53'57"E, 580 m, leg. Örgel & Kacar. **Çanakkale:** 4♂, 7♀, 19.V.2022, Yenice, Örencik 3 km S, 39°48'20"N, 27°07'47"E, 300 m, leg. Kacar & Çelik. **Çankırı:** 1♂, 1♀, 20.V.2018,

Korgun, Çukurören 3 km SW 1390 m, 40°38'55"N, 33°22'05"E, leg. Örgel & Yaman. **Edirne:** 2♀, 10.IV.2021, Enez, Işıklı 2 km NW, 40°43'38"N, 26°17'45"E, 37 m, leg. Örgel, Kacar & Çelik. 1♀, 28.IV.2022, Enez, Gala Gölü Milli Parkı, 40°46'16"N, 26°14'04"E, 7 m, leg. Kacar & Çelik. **İstanbul:** 2♂, 5♀, Kurtköy, Aydos Ormanı, leg. Anlaş. **Kayseri:** 1♂, 1♀, 08.IV.2018, Tuzla Gölü, 39°00'55"N, 35°47'12"E, 1170 m, leg. Yağmur & Örgel, Yaman. **Kırklareli:** 7♂, 9♀, 26.IV.2022, Demirköy, İğneada Longoz Ormanları Milli Parkı, 41°49'44"N, 27°57'28"E, 1 m, leg. Kacar & Çelik. 10♂, 7♀, 28.VII.2001, 22.VI.2022, Demirköy, İğneada Longoz Ormanları Milli Parkı, 41° 50'59"N, 27°56'24"E, 1 m, leg. Kacar & Çelik. 9♂, 14♀, 28.VII.2021, 22.VI.2022, Demirköy, İğneada Longoz Ormanları Milli Parkı, Hamam Gölü, 41°49'16"N, 27 57'48"E, 42 m, leg. Kacar & Çelik. **Konya:** 1♂, 05.III.2018, Cihanbeyli, Kırkışla, 38°32'10"N, 32°51'05"E, 1025 m, leg. Örgel & Yaman; 1♀, 02.III.2018, Ilgın, Bulcuk, 38°06'52"N, 31°59'03"E, 1436 m, leg. Örgel & Yaman; 2♂, 7♀, 01.III.2018, Ilgın, Ilgın Gölü, 38°23'25"N, 31°53'21"E, 1068 m, leg. Örgel & Yaman. **Sivas:** 6♂, 7♀, 14.IV.2019, Doğanşar, 40°08'15"N, 37°29'40"E, 1695 m, leg. Anlaş, Örgel & Köksal. **Yozgat:** 2♂, 1♀, 07.IV.2018, Akdağmadeni, Aktaş 3 km N, 39°34'34"N, 35°48'57"E, 1800 m, leg. Yağmur & Örgel, Yaman.

**Distribution in Türkiye:** Afyonkarahisar, Adana, Ankara, Antalya, Artvin, Aydın, Balıkesir, Batman, Denizli, Diyarbakır, Edirne, Erzurum Eskişehir, Gaziantep, İstanbul, İzmir, Karaman, Kayseri, Kırıkkale, Kırklareli, Kütahya, Manisa, Mardin, Muğla, Muş, Niğde, Rize, Siirt, Sivas, Uşak, Trabzon, Van (Anlaş 2009, 2018, Anlaş & Rose 2009, Sert et al., 2013; Örgel & Tezcan, 2020; Özgen & Örgel, 2021; Kacar & Anlaş, 2022; Daşdemir & Tozlu, 2022).

**Remarks:** This species is recorded for the first time from the Western Black Sea Region and also Amasya, Bartın, Çanakkale, Edirne, İstanbul, Kırklareli provinces.

**Distribution in the world:** This species is very common and widely distributed in Palaearctic, Australian and Oriental Regions (Schülke & Smetana, 2015).

***Paederus (Poederomorphus) littoralis*** Gravenhorst, 1802

**Material:** **Aksaray:** 1♂, 1♀, 22.III.2018, Melendiz Çayı, 38°12'00"N, 34°19'50"E, 1400 m, leg. Örgel & Yaman; 1♂, 1♀, 22.III.2018, Ihlara Vadisi, 38°15'12"N, 34°18'06"E, 1300 m, leg. Örgel & Yaman. **Amasya:** 2♂, 2♀, 19.VI.2020, Merzifon, Aşağıbük 2 km SW, 40°49'56"N, 35°19'08"E, 1550 m, leg. Örgel & Kacar. **Bartın:** 16♂, 19♀, 07.IV.2021, Ulus, Uluyayla, 41°32'24"N, 32°48'16"E, 1000 m, leg. Örgel, Kacar & Çelik. 6♂, 8♀, 07.IV.2021, Ulus, Ahçlı, 41°40'58"N, 32°47'30"E, 450 m, leg. Örgel, Kacar & Çelik. 1♂, 07.IV.2021, Ulus, Kerpiçli 4 km SW, 41°42'54"N,



32°53'36"E, 570 m, leg. Örgel, Kacar & Çelik. **Bilecik:** 10♂, 12♀, 03.IV.2021, Bozhöyük, Yeşilçukurca 3 km S, 39°42'28"N, 29°49'59"E, 1425 m, leg. Örgel, Kacar & Çelik. 1♂, 03.IV.2021, Bozhöyük, Cihangazi 4 km E, 39°44'17"N, 29°52'32"E, 1376 m, leg. Örgel, Kacar & Çelik. 1♂, 11.XI.2021, Ulupınar, Selöz 3 km SW, 40°07'07"N, 29°54'26"E, 785 m, leg. Kacar & Çelik. **Bursa:** 1♂, 20.III.2021, Gemlik, Şükriye 1 km NW, 40°20'24"N, 29°16'01"E, 570 m, leg. Örgel & Kacar. 1♂, 1♀, 16.VI.2017, Bursa, Karacabey, Longoz Ormanları, leg. Yağmur. **Çanakkale:** 2♂, 2♀, 24.VI.2022, Lapseki, Çavuşköy 5 km NE, 40°19'53"N, 26°50'10"E, 190 m, leg. Anlaş, Kacar & Çelik. **Çankırı:** 1♂, 1♀, 27.IX.2017, Ilgaz Dağı, Göl kenarı, 41°00'09"N, 33°36'32"E, 1835 m, leg. Örgel & Yaman. **Edirne:** 1♀, 05.VI.2021, Enez, Hisarlı 2 km N, 40°42'18"N, 26°13'01"E, 78 m, leg. Kacar & Çelik. 1♀, 07.VI.2021, Uzunköprü, Kavacık, 41°11'14"N, 26°40'58"E, 68 m, leg. Kacar & Çelik. 1♂, 07.VI.2021, Lalapaşa, Küçünlü 3 km NW, 41°55'23"N, 26°47'26"E, 75 m, leg. Kacar & Çelik. **Eskişehir:** 3♂, 2♀, 27.IV.2019, Sarıcakaya, Beyyayla 2 km W, 40°08'04"N, 30°40'38"E, 1145 m, leg. Örgel & Köksal: 1♂, 1♀, 27.IV.2019, Sarıcakaya, Beyyayla 2 km W, 40°09'24"N, 30°42'55"E, 1320 m, leg. Örgel & Köksal: 4♂, 3♀, 29.III.2019, Tepebaşı, Sulukaraağaç 3 km N, 39°56'04"N, 30°29'28"E, 1140 m, leg. Örgel & Köksal. **Karabük:** 1♂, 2♀, 06.IV.2021, Keltepe Kayak Merkezi, 41°30'30"N, 32°27'59"E, 1474 m, leg. Örgel, Kacar & Çelik. 15♂, 20♀, 06.IV.2021, Eskipazar, Sallar 1 km SW, 40°57'41"N, 32°45'44"E, 1277 m, leg. Örgel, Kacar & Çelik. 2♂, 2♀, 06.IV.2021, Eskipazar, Tomuşlar 3 km S, 40°57'46"N, 32°39'03"E, 1237 m, leg. Örgel, Kacar & Çelik. **Karaman:** 1♂, 25.V.2016, Sarıveliler, Göktepe, Saçak Tepe, 36°38'22"N, 32°32'09"E, 1847 m, leg. Örgel & Yaman. **Kayseri:** 2♂, 1♀, 25.V.2018, Develi, Yaylacık, 1780 m, 38°03'49"N, 35°46'22"E, leg. Örgel & Yaman. **Kırklareli:** 1♂, 15.III.2021, Sarpdere 7 km NW, 41°52'07"N, 27°30'20"E, 370 m, leg. Anlaş & Kacar. **Ordu:** 1♂, 13.VI.2020, Aybastı, Uzundere 1 km N, 40°35'36"N, 37°24'56"E, 900 m, leg. Örgel & Kacar. 5♂, 7♀, 11.V.2022, Akkuş 3 km N, 40°46'13"N, 37°01'10"E, 1452 m, leg. Kacar & Çelik. **Kırşehir:** 1♀, 15.X.2018, Akçakent, Yetkili 2 km W, 39°38'18"N, 34°08'56"E, 1220 m, leg. Örgel & Yaman: 1♂, 3♀, 27.III.2019, Yağmurluarmutlu 1 km NW, 39°14'30"N, 33°57'04"E, 1300 m, leg. Örgel & Köksal: 1♂, 2♀, 01.V.2019, Akçakent, Yetkili 5 km NW, 39°40'09"N, 34°08'16"E, 988 m, leg. Örgel & Köksal: 1♂, 1♀, 27.III.2019, Kurtbeyliyeniyapan 2 km W, 39°15'34"N, 33°58'45"E, 1590 m, leg. Örgel & Köksal. **Konya:** 2♂, 6♀, 25.III.2019, Cihanbeyli, Böllük Gölü, 38°41'00"N, 32°58'52"E, 995 m, leg. Örgel & Köksal. **Neveşehir:** 1♂, 2♀, 25.III.2018, Ürgüp, Hodul Dağı, 38°30'43"N, 35°01'30"E, 1950 m, leg. Örgel & Yaman. **Sinop:** 4♂, 8♀, 02.IV.2011, 07.IV.2011, Nisi Gölü, Karakum, leg. Koç. 1♂, 17.V.2014, Sinop Merkez, 41°42'07"N, 35°10'53"E, 745 m, leg. Koç. 1♀, 31.V.2014, Sinop Merkez, 41°32'36"N, 36°09'24"E, 780 m, leg. Koç. 1♂,

2♀, 04.V.2021, Boyabat, Yeşilçam 2 km S, 41°40'38"N, 34°49'06"E, 1356 m, leg. Örgel, Kacar & Çelik. 1♂, 1♀, 02.V.2021, Durağan, Boyalıca 3 km SW, 41°30'06"N, 35°10'10"E, 1450 m, leg. Örgel, Kacar & Çelik. **Sivas:** 1♂, 18.IV.2018, İmranlı, Yürekaşı 2 km S, 39°35'31"N, 38°15'50"E, 1420 m, leg. Anlaş & Örgel, Yaman: 1♂, 16.IV.2018, Koyulhisar, 40°23'54"N, 37°58'03"E, 1930 m, leg. Anlaş & Örgel, Yaman. **Tekirdağ:** 1♂, 1♀, 09.IV.2021, Malkara, Karacahalil 4 km SW, 40°47'00"N, 26°55'02"E, 280 m, leg. Örgel, Kacar & Çelik. 1♀, 11.IV.2021, Malkara, Karacahalil, 40°48'09"N, 26°56'51"E, 185 m, leg. Örgel, Kacar & Çelik. 2♂, 3♀, 12.IV.2021, Şarköy, Güzelköy 5 km SE, 40°46'26"N, 27°15'41"E, 658 m, leg. Örgel, Kacar & Çelik. **Tokat:** 3♂, 2♀, 05.IV.2022, Çevreli 7 km N, 40°14'12"N, 36°52'08"E, 1280 m, leg. Örgel, Kacar & Çelik. 12♂, 10♀, 05.IV.2022, Almus, Teknecek 3 km N, 40°14'46"N, 36°49'44"E, 1380 m, leg. Örgel, Kacar & Çelik. 12♂, 10♀, 07.IV.2022, Reşadiye, Kuzbağı 4 km W, 40°26'19"N, 37°27'36"E, 1242 m, leg. Örgel, Kacar & Çelik. 4♂, 8♀, 14.V.2022, Reşadiye, Yağsıyah 1 km NE, 40°29'05"N, 37°33'48"E, 1416 m, leg. Kacar & Çelik. **Uşak:** 2♂♂, 02.VI.2014, Banaz, Susuz 3 km G, 928 m, 38°38'47"N, 29°43'17"E, leg. Yağmur & Örgel. **Yozgat:** 1♂, 1♀, 17.IV.2018, Çayıralan, Akdağmadeni Dağları, 1800m, 39°26'38"N, 35°49'53"E, m, leg. Yağmur & Örgel, Yaman. **Zonguldak:** 1♂, 1♀, 08.IV.2021, Ayvatlar 2 km SW, 41°17'54"N, 31°49'19"E, 364 m, leg. Örgel, Kacar & Çelik. 1♂, 05.IV.2021, Ereğli, Çevlik 2 km SW, 41°14'47"N, 31°40'45"E, 150 m, leg. Örgel, Kacar & Çelik.

**Distribution in Türkiye:** Adana, Afyonkarahisar, Aksaray, Amasya, Ankara, Antalya, Ardahan, Artvin, Bilecik, Denizli, Edirne, Erzurum, Eskişehir, Izmit, Kahramanmaraş, Karabük, Kars, Kastamonu, Kırşehir, Manisa, Mardin, Mersin-Karaman, Sakarya, Samsun, Sinop, Şanlıurfa, Trabzon, Uşak, Yozgat (Anlaş, 2009, 2018; Anlaş & Rose, 2009; Sert et al., 2013; Anlaş & Örgel, 2016; Çiftçi & Hasbenli, 2016; Altın & Yağmur, 2018; Kacar & Anlaş, 2022; Daşdemir & Tozlu, 2022).

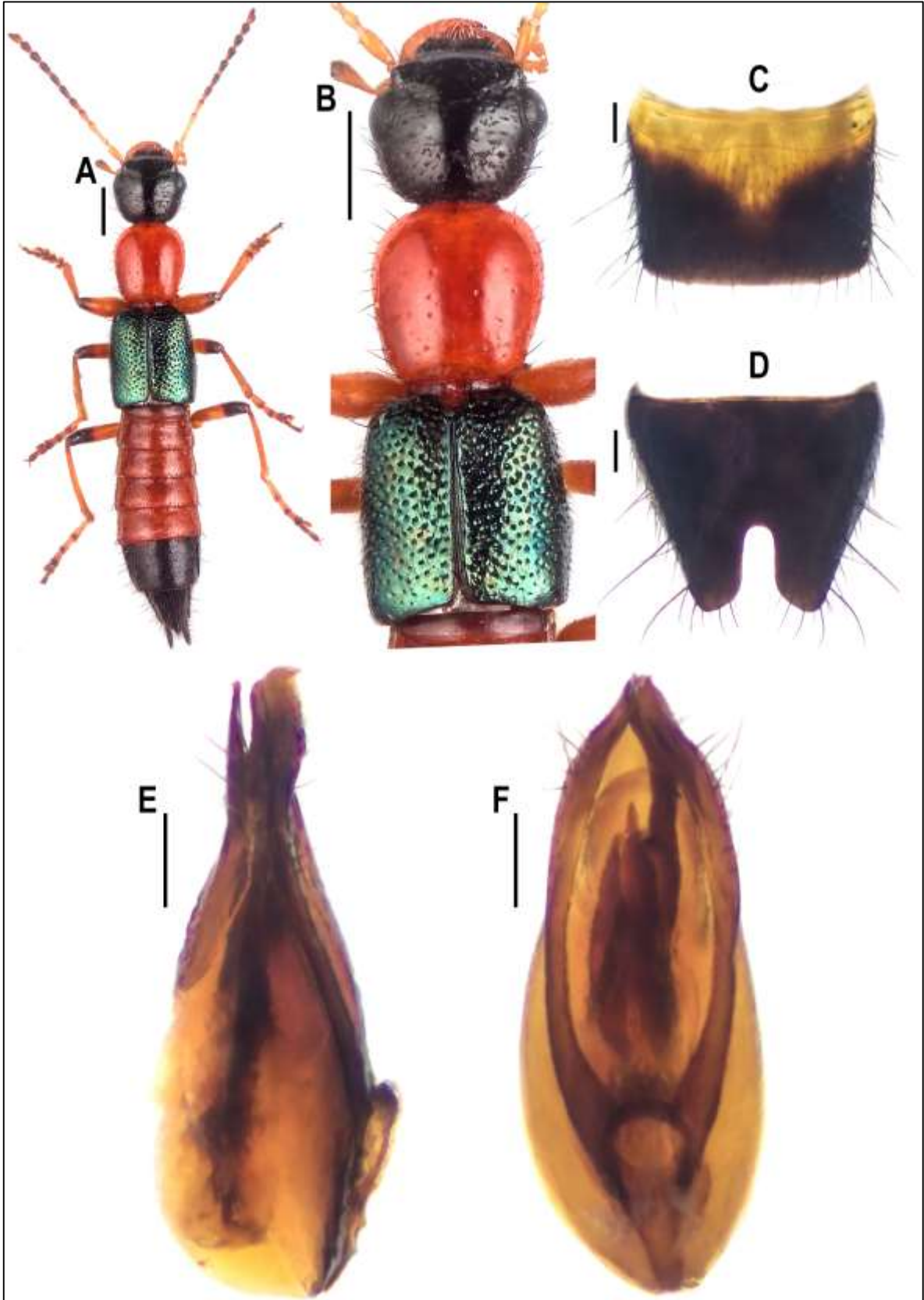
**Remarks:** This species is recorded for the first time from Bartın, Çanakkale, Kırklareli, Ordu, Tekirdağ, Tokat and Zonguldak provinces.

**Distribution in the world:** This species is known from Europe, Algeria, Iran, Cyprus, Türkiye and western Siberia (Schülke & Smetana, 2015; Anlaş, 2018).

**Paederus (Eopaederus) mesopotamicus** Eppelsheim, 1889

**Material:** **Sivas:** 8♂, 5♀, 18.IV.2018, Divriği, Çayözü, 39°35'42"N, 38°10'45"E, 1350 m, leg. Anlaş & Örgel, Yaman: 2♂, 1♀, 19.IV.2018, Divriği, Mursal 5 km W, Leke Dağı, 39°09'08"N, 37°55'43"E, 2080 m, leg. Yağmur & Örgel, Yaman: 1♂, 1♀, 20.IV.2019, Kangal, Karacaören 6 km E, 39°02'17"N, 37°44'32"E, 1460 m, leg. Anlaş, Örgel & Köksal.





**Figure 1A-F.** *Paederus balcanicus* Koch. A-habitus; B-forebody; C-male sternite VII; D-male sternite VIII; E-aedeagus, lateral; F-aedeagus, ventral. Scale bars: 1 mm (A-B); 0.2 mm (C-F).

**Şekil 1A-F.** *Paederus balcanicus* Koch. A-genel vücut; B-ön vücut; C-erkek sternit VII; D-erkek sternit VIII; E-aedeagus, yan görünüş; F-aedeagus, ön görünüş. Ölçek çubukları: 1 mm (A-B); 0.2 mm (C-F).

**Distribution in Türkiye:** Elâzığ, Erzincan, Diyarbakır, Gümüşhane, Siirt, Sivas and Tunceli (Anlaş, 2009, 2018).

**Distribution in the world:** The species is known from Iran, Iraq, Syria and Türkiye (Schülke & Smetana, 2015).

*Paederus (s. str.) riparius* (Linnaeus, 1758)

**Material:** **Aksaray:** 1♂, 22.III.2018, Ihlara Vadisi, 38°15'12"N, 34°18'06"E, 1300 m, leg. Örgel & Yaman. **Ankara:** 1♂, 2♀, 26.IX.2017, Kızılcahamam, Aluç Dağı, göl kenarı, 40°30'30"N, 32°34'59"E, 1482 m, leg. Örgel & Yaman. **Kırklareli:** 2♂, 3♀, 02.X.2009, Demirköy, İğneada, Hamam Gölü, 41°49'43"N, 27°57'31"E, leg. Kunt. 11♂, 14♀, 29.IX.2009, 23.V.2010, Demirköy, İğneada, Longoz Mert Gölü, ca. 41°49'N, 27°57'E, leg. Kunt. **Konya:** 7♂♂, 9♀♀, 28.V.2018, Seydişehir, Erenler Dağı, 1865 m, 37°36'13"N, 32°05'25"E, leg. Örgel & Yaman. **Samsun:** 1♂, 11.IV.2022, Bafra, Şahinkaya 8 km E, 41°19'01"N, 35°44'45"E, 1146 m, leg. Kacar & Çelik.

**Distribution in Türkiye:** Ankara, Denizli, İzmir, Konya, Manisa, Ordu (Anlaş, 2009, 2018; Sert et al., 2013).

**Remarks:** This species is recorded for the first time from the Marmara Region of Turkey and Kırklareli and Samsun provinces.

**Distribution in the world:** This species is widely distributed in Palaearctic and Nearctic Regions (Schülke & Smetana, 2015).

*Uncopaederus (s. str.) signiventris* (Smetana, 1962) (Figure 2A-F)

**Material:** **Amasya:** 2♂, 1♀, 17.VI.2020, Taşova, Borabay Gölü 8 km NW, 40°50'17"N, 36°05'46"E, 1620 m, leg. Örgel & Kacar. 4♂, 2♀, 17.VI.2020, Taşova, Borabay Gölü 9 km E, 40°49'48"N, 36°04'42"E, 1700 m, leg. Örgel & Kacar. 1♂, 18.VI.2020, Merkez, Seyfe 3 km W, 40°49'44"N, 35°55'33"E, 1600 m, leg. Örgel & Kacar. 4♂, 4♀, 24.IV.2021, Taşova, Borabay 9 km SE, 40°49'52"N, 36°04'58"E, 1720 m, leg. Anlaş, Kacar & Çelik. 1♂, 24.IV.2021, Taşova, Borabay 8 km SE, 40°50'16"N, 36°05'36"E, 1700 m, leg. Anlaş, Kacar & Çelik. 1♂, 1♀, 24.IV.2021, Taşova, Borabay 7 km SE, 40°50'52"N, 36°06'33"E, 1382 m, leg. Anlaş, Kacar & Çelik. **Ankara:** 2♂, 3♀, 18.V.2018, Kızılcahamam, Yukarıçanlı 8 km N, 1650 m, 40°43'43"N, 32°41'15"E, leg. Örgel & Yaman. **Bolu:** 1♂, 3♀, 04.VII.2020, Topardıç 4 km E, 40°34'02"N, 31°28'44"E, 1660 m, leg. Örgel & Kacar. 1♂, 1♀, 18.VI.2022, Mudurnu, Feruz 3 km N, 40°36'26"N, 31°20'58"E, 1572 m, leg. Kacar & Çelik. **Çankırı:** 2♂, 19.V.2018, Bayramören, Yazören 2 km N, 1570 m, 40°57'36"N, 33°06'21"E, leg. Örgel & Yaman; 4♂, 3♀, 21.V.2018, Ilgaz, Ilgaz Dağları, 1926 m, 41°02'49"N, 33°42'46"E, leg. Örgel & Yaman; 3♂,

3♀, 21.V.2018, Ilgaz, Ilgaz Dağları, 1750 m, 41°00'37"N, 33°36'14"E, leg. Örgel & Yaman. **Çorum:** 2♂, 22.VI.2020, Kargı, Tepelice 3 km W, 41°12'41"N, 34°27'30"E, 1870 m, leg. Örgel & Kacar. 1♂, 03.V.2021, Kargı, Cihadiye 5 km NE, 41°13'29"N, 34°33'30"E, 1570 m, leg. Örgel, Kacar & Çelik. **Karabük:** 2♂, 4♀, 13.XI.2021, Karaağaç 2 km SW, 41°03'16"N, 32°28'16"E, 1405 m, leg. Kacar & Çelik. **Kastamonu:** 1♂, 12♀, 24.VI.2020, Taşköprü, Kayadibi 2 km NE, 41°44'22"N, 34°12'58"E, 1720 m, leg. Örgel & Kacar. 1♂, 24.VI.2020, Çatalzeytin, Mamatlar 5 km S, 41°45'20"N, 34°06'22"E, 1710 m, leg. Örgel & Kacar. 2♂, 1♀, 07.V.2021, Daday, Çamlıbel 5 km NE, 41°22'04"N, 33°15'09"E, 1486 m, leg. Örgel, Kacar & Çelik. 6♂, 8♀, 06.V.2021, Küre, Alınca 2 km S, 41°43'23"N, 33°38'29"E, 1260 m, leg. Örgel, Kacar & Çelik. 9♂, 12♀, 05.V.2021, Taşköprü, Kayadibi 3 km SW, 41°44'50"N, 34°13'16"E, 1530 m, leg. Örgel, Kacar & Çelik. 1♂, 13.VI.2022, Daday, Akılçalman 2 km S, 41°24'37"N, 33°21'51"E, 977 m, leg. Kacar & Çelik. **Ordu:** 1♂, 2♀, 08.IV.2022, Kabadüz, Çambaşı Yaylası, 40°37'08"N, 37°58'36"E, 1700 m, leg. Örgel, Kacar & Çelik. 1♂, 1♀, 14.V.2022, Mesudiye, Mahmudiye 1 km SE, 40°35'35"N, 37°39'31"E, 1252 m, leg. Kacar & Çelik. 1♂, 11.V.2022, Akkuş, Gedikli 4 km NW, 40°44'11"N, 36°59'07"E, 1280 m, leg. Kacar & Çelik. **Sinop:** 2♂, 4♀, 05.V.2021, Türkeli, Çatak 8 km NW, 41°44'53"N, 34°18'42"E, 1720m, leg. Örgel, Kacar & Çelik. 2♂, 4♀, 02.V.2021, Durağan, Boyalıca 3 km SW, 41°30'06"N, 35°10'10"E, 1450 m, leg. Örgel, Kacar & Çelik. 1♂, 1♀, 04.V.2021, Boyabat, Günpınar 2 km E, 41°39'48"N, 34°44'46"E, 1360 m, leg. Örgel, Kacar & Çelik. **Tokat:** 1♂, 1♀, 12.VI.2020, Başçiftlik 3 km N, 40°34'24"N, 37°10'45"E, 1700 m, leg. Örgel & Kacar.

**Distribution in Türkiye:** Çankırı, Kastamonu, Rize, Samsun, Sinop (Smetana, 1962; Anlaş, 2009, 2018).

**Remarks:** This species is recorded for the first time from Amasya, Bolu, Çorum, Karabük, Ordu and Tokat provinces.

**Distribution in the world:** This species is only known from Türkiye. Endemic.

## ACKNOWLEDGEMENTS

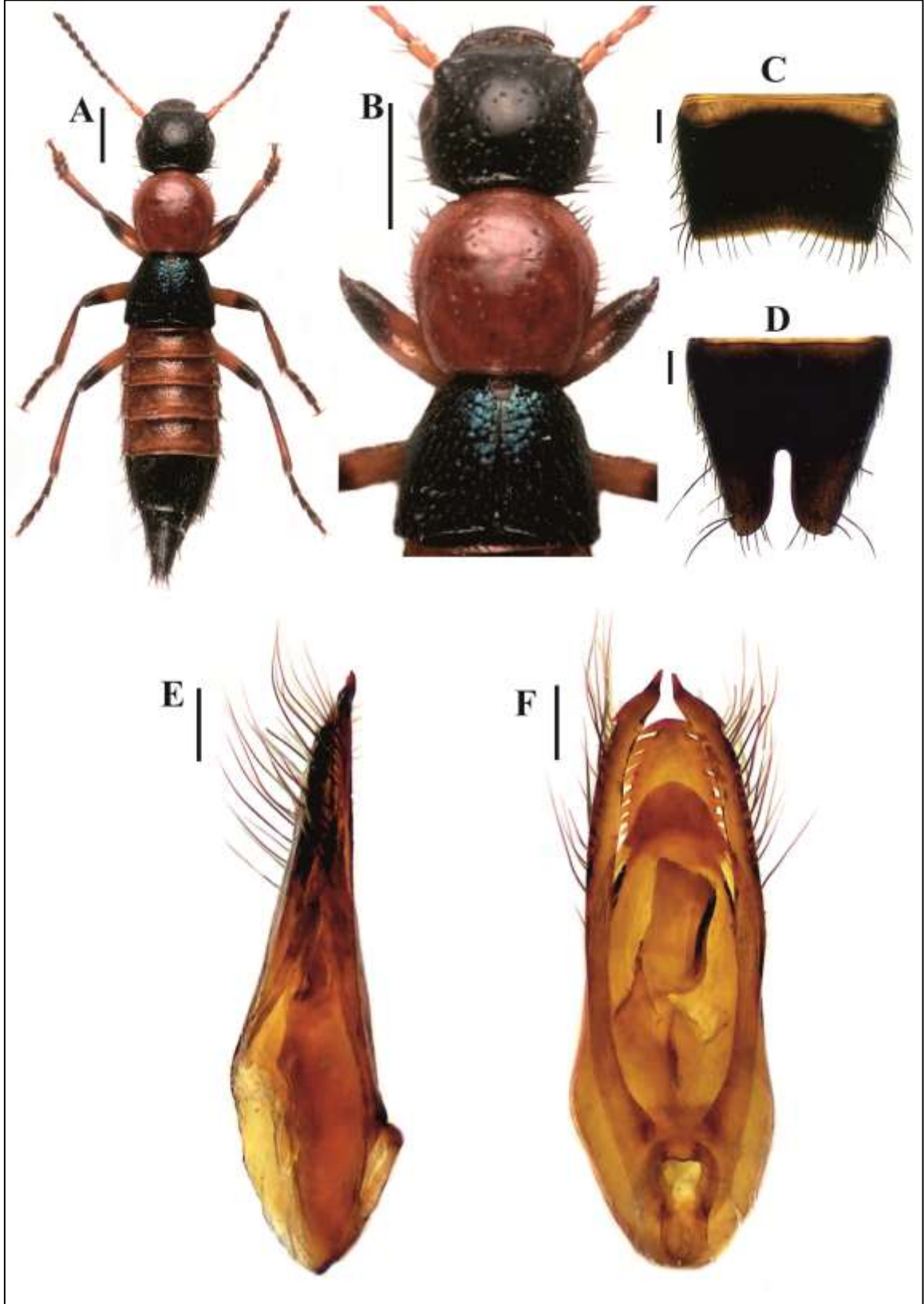
This study is prepared from part of a master thesis approved by the Institute of Natural Sciences of Manisa Celal Bayar University in December 2023. This study was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project nos: 112T907, 215Z080 and 119Z253).

## Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

## Statement of Conflict of Interest

Authors have declared no conflict of interest.



**Figure 2A-F.** *Uncopaederus signiventris* (Smetana). A-habitus; B-forebody; C-male sternite VII; D-male sternite VIII; E-aedeagus, lateral; F-aedeagus, ventral. Scale bars: 1 mm (A-B); 0.2 mm (C-F).

**Şekil 2A-F.** *Uncopaederus signiventris* (Smetana). A-genel vücut; B-ön vücut; C-erkek sternit VII; D-erkek sternit VIII; E-aedeagus, yan görünüş; F-aedeagus, ön görünüş. Ölçek çubukları: 1 mm (A-B); 0.2 mm (C-F).



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## Endofit ve Epifit Bakteri İzolatlarının Bazı Turunçgil Fungal Hastalık Etmenlerine Karşı *in vitro* Biyokontrol Etkinlik ve Etki Mekanizmalarının Belirlenmesi

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### ÖZET

Bu çalışmada, sağlıklı turunçgil çeşitlerinden izole edilen 48 farklı antagonist biyolojik mücadele etmeni (BCA) ve bitki gelişimini teşvik eden (PGP) endofit ve epifit bakteri izolatının turunçgillerde sorun bazı fungal hastalık etmenlerine (*Colletotrichum gloeosporioides*, *Fusarium solani*, *Geotrichum citri-aurantii*) karşı *in vitro* antagonistik etkinlikleri ve etkinlik mekanizmalarının belirlenmesi amaçlanmıştır. MALDI-TOF tanımlama çalışmaları sonucunda 33 izolat Gram-pozitif (*Bacillus*, *Lysinibacillus*, *Cronobacter*, *Staphylococcus*) 15 izolat ise Gram-negatif (*Acinetobacter*, *Pseudomonas*, *Kosakonia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pantoea*, *Rahnella*, *Raoultella*, *Rhizobium*, *Siccibacter*) bakteri cinslerine ait türler olarak belirlenmiştir. İkili kültür testlerinde *Bacillus vallismortis* YGL73ep, *B. thuringiensis* YGT22en, *B. subtilis* YGS5en, *B. cereus* YGK25en ve *Pseudomonas chlororapsis* YGM82ep izolatları *G. citri-aurantii*, *C. gloeosporioides* ve *F. solani*'nin misel gelişimini %65.5-77.2 oranlarında engelleyen en etkili antagonist bakteri izolatları olarak belirlenmiştir. Temsili olarak seçilen *P. chlororapsis* YGM82ep, *B. vallismortis* 73YGep, *B. thuringiensis* 22YGen ve *B. cereris* 25YGen izolatları tarafından üretilen uçucu organik bileşikler (VOC's) fungal etmenlerin *in vitro* misel gelişimlerini önemli ölçüde engellemiştir. Test edilen 25 bakteri izolatının, 20 tanesi siderofor, 24 tanesi amonyak, 19 tanesi protease, 5 tanesi ise hidrojen siyanür üretiminde pozitif etkinlik göstermiştir. Bakteri izolatlarından 21 tanesi IAA hormonu üretmiş, 7 izolatın ise fosforu çözebilmeye yeteneğinde olduğu belirlenmiştir. Elde edilen sonuçlar, yüksek düzeyde antagonist ve PGP etkinliği gösteren BCA izolatlarının, turunçgil fungal hastalıkların baskılanmasında biyopreparat, yetiştiriciliğinde ise biyogübre olarak kullanılma potansiyeline sahip olduğunu göstermiştir.

### Bitki Koruma

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

Antagonist  
Biyolojik mücadele  
Solgunluk,  
Antraknoz,  
Ekşi çürüklük

## Determination of *in vitro* Biocontrol Efficacy and Mechanisms of Action of Endophytic and Epiphytic Bacterial Isolates against Some Citrus Fungal Disease Agents

### ABSTRACT

In this study, it was aimed to determine the *in vitro* antagonistic activities and mechanisms of activity of 48 different antagonist biological control agents (BCA) and plant growth promoting (PGP) endophyte and epiphyte bacterial isolates against some fungal disease agents (*Colletotrichum gloeosporioides*, *Fusarium solani*, *Geotrichum citri-aurantii*) in citrus fruits. As a result of MALDI-TOF identification studies, 33 isolates were identified as Gram-positive (*Bacillus*, *Lysinibacillus*, *Cronobacter*, *Staphylococcus*) and 15 isolates were identified as Gram-negative (*Acinetobacter*, *Pseudomonas*, *Kosakonia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pantoea*, *Rahnella*, *Raoultella*, *Rhizobium*, *Siccibacter*). *Bacillus vallismortis* YGL73ep, *B. thuringiensis* YGT22en, *B. subtilis* YGS5en, *B. cereus* YGK25en and *Pseudomonas chlororapsis* YGM82ep isolates were found to be the most effective antagonist isolates suppressing mycelial growth of *G. citri-aurantii*, *C. gloeosporioides* and *F. solani* by 65.5-77.2% in *in vitro* dual culture tests. Volatile organic compounds (VOCs) produced by representative isolates

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*P. chlororapsis* YGM82ep, *B. vallismortis* 73YGep, *B. thuringiensis* 22YGen, and *B. cererus* 25YGen significantly inhibited *in vitro* mycelial growth of fungal agents. Of the 25 bacterial isolates tested, 20 isolates were positive for siderophore, 24 isolates for ammonia, 19 isolates for protease, and 5 isolates for hydrogen cyanide production. Of the bacterial isolates tested, 21 isolates produced the hormone IAA and 7 isolates were found to be capable of solubilizing phosphorus. The results showed that the BCA isolates with high levels of antagonistic and PGP activities have the potential to be used as biopreparat for the suppression of citrus fungal diseases and also as biofertilizer in citrus cultivation.

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## GİRİŞ

Anavatanı Çin, Güneydoğu Asya ve Hindistan olan turunçgil günümüzde subtropik iklimlere sahip neredeyse tüm ülkelerde yetiştirilmektedir. Turunçgiller, dünyada en fazla yetiştirilen ve tüketilen meyve grubu olup, 9.8 milyon ha alanda 103 milyon ton üretim gerçekleştirilmiştir. Turunçgil üretiminde ilk sırayı 41 milyon tonla Çin almakta olup, bu ülkeyi 18 milyon tonla Brezilya ve 15 milyon ton ile Kuzey Amerika izlemektedir. Türkiye, 5 milyon ton ile turunçgil üretiminde yedinci sırada yer almaktadır (FAO, 2021).

Turunçgiller yetiştirme süresince olduğu kadar depolanma koşulları sırasında da birçok toprak ve hava kökenli fungal hastalıklar tarafından etkilenir. Dünyada ve Türkiye'nin önde gelen turunçgil üretim bölgelerinde sıkça görülen fungal hastalıklar arasında antraknoz (*Colletotrichum* spp) (Guarnaccia ve ark., 2017; Uysal ve ark., 2022.), Fusarium solgunluğu ve kök çürüklüğü (*Fusarium* spp.) (Kurt ve ark., 2020), turunçgil meyvelerinde kahverengi çürüklük ve gövde zamklanması (*Phytophthora citrophthora*) (Kurbetli ve ark., 2016), uçkurutan (*Plenodomus tracheiphilus*) (Krasnov ve ark., 2023), Alternaria kahverengi leke hastalığı (*Alternaria citri*) (Güney ve ark., 2023) bulunmaktadır. Bahçe koşullarında ortaya çıkan hastalıkların yanı sıra, depolama sürecinde de ekşi çürüklük (*Geotrichum citri-aurantii*), yeşil küf (*Penicillium digitatum*) ve mavi küf (*Penicillium italicum*) hastalıkları turunçgil üretiminde önemli ekonomik kayıplara neden olan diğer önemli hastalıklardır (Uysal & Kurt, 2019;).

*Colletotrichum* türleri genellikle antraknoz hastalığına neden olur ve fungus alemi içinde Ascomycota şubesine, Sordariomycetes sınıfına, Phyllachorales takımına ve Phyllachoraceae familyasına aittir (Shivas ve ark., 2016). Antraknoz hastalığı, turunçgillerin sürgünlerinde geriye doğru kuruma, yapraklarda lekeler, erken dönemde yaprak ve meyve dökülmeleri gibi belirtilerle kendini gösterir.

Hastalık belirtileri, bitkilerin tüm vegetatif ve generatif organlarında görülebilir (Wang ve ark., 2021).

Son yıllarda Türkiye'nin turunçgil üretiminin yapıldığı alanlarda *Fusarium solanini*'nin sıklıkla görülmeye başlamasıyla birlikte bu bölgelerdeki ağaçlarda farklı kök çürüklükleri ve solgunluk belirtileri ortaya çıkmıştır. Yeni tesis edilen bahçelerde ve yetişkin ağaçlardan oluşan bahçelerde yoğun kurumalara ve solgunluklara rastlanmakta, ileri düzeyde bu tür hastalıklardan dolayı ağaçların sökümü ile sonuçlanan kayıplar ortaya çıkmaktadır (Kurt ve ark., 2020). Fungal hastalık etmeni, solgunluk, sararma, nekrozlaşma ve yaprakların dökümü ile birlikte enfekteli ağaçların hızla ölümüne neden olur (Leslie & Summerell, 2006; Yaseen & D'Onghia, 2012; Al-Sadi ve ark., 2014; Bueno ve ark., 2014).

*Geotrichum citri-aurantii*'nin yol açtığı ekşi çürüklük hastalığı, bol yağışlı mevsimlerde meyve ürünlerinde büyük verim kayıplarına sebep olabilir. Bu hastalık, olgunlaşmış veya aşırı olgunlaşmış meyvelerde, yeşil ve olgunlaşmamışlara kıyasla daha fazla etki gösterebilir. Özellikle nemli iklimlerde uzun süren dönemlerde ve depolama süresi uzun olan meyvelerde bu hastalık ciddi sorunlara yol açabilir (Kara & Soylu, 2020; Soylu ve ark., 2022).

Toprak kökenli patojenlere karşı bazı fungusitlerin ya tamamen etkisiz ya da yetersiz kaldığı görülmektedir (Kara ve ark., 2024). Bazı patojenlere karşıda hiç ruhsatlı fungusit bulunmamaktadır. Bu sebeple bilim dünyası son yıllarda biyolojik mücadele etmenlerinin devreye girmesini hedeflemeleri nedeniyle mevcut konu üzerine kurgulanmış çalışmalara yoğunlaşmıştır (Soliman & Badeaa, 2002; Kara ve ark., 2020; Kara & Soylu, 2022; Soylu ve ark., 2022).

Bitkilerde sorun olan fungal ve bakteriyel kökenli hastalıklarla kimyasal mücadeleye alternatif yollardan biri ise sağlıklı bitki veya yetiştirdikleri topraklardan izole edilen antagonist ve bitki gelişimini teşvik eden (Plant Growth Promoting Bacteria, PGPB)

bakteriyel biyokontrol etmenlerinin kullanıldığı biyolojik mücadeledir. Son yıllarda, hastalıklarla biyolojik mücadele kapsamında PGPR, epifitik bakteriler ve endofit bakterilerin kullanımı artmaktadır. Daha önce yapılan çalışmalarda, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Chryseobacterium*, *Clavibacter*, *Curtobacterium*, *Enterobacter*, *Micrococcus*, *Paenibacillus*, *Phyllobacterium*, *Pseudomonas*, *Serratia* ve *Stenotrophomonas* gibi çeşitli bakteriyel cinslere ait endofit ve epifit karakterli antagonistik bakteri türleri farklı kültür bitkileri ve yabancı bitkilerden izole edilerek biyolojik mücadele ve bitki gelişimini teşvik etme amaçlı çalışmalarda incelenmiştir (Tekiner ve ark., 2019; Sturz ve ark., 2000; Duman & Soylu, 2019; Aktan & Soylu, 2020; Soylu ve ark., 2020).

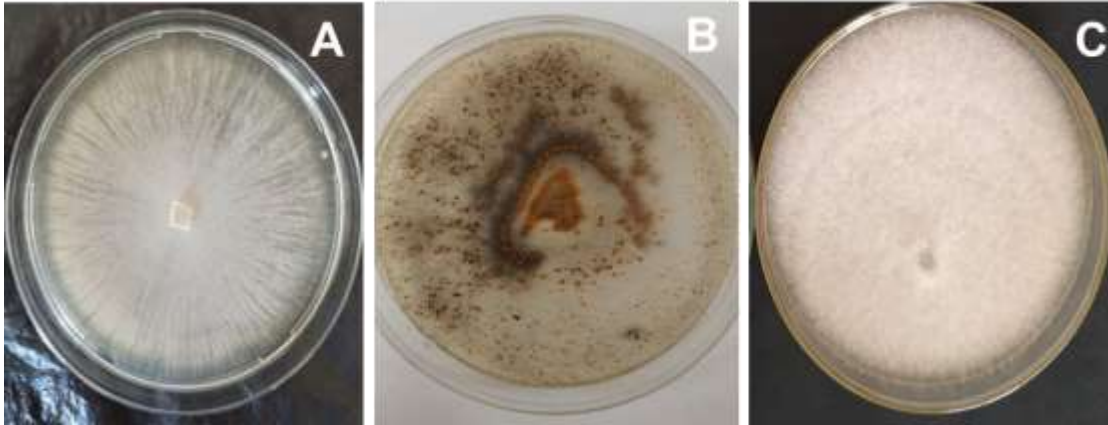
Yapılan bu çalışmada, farklı turuncgöl türlerine ait sağlıklı ağaçların farklı kısımlarından (i) endofit ve epifit bakteri izolatların izolasyonu, tanılanması; (ii) turuncgöllerde sorun olan fungal hastalık etmenlerinden *Geotrichum citri-aurantii*, *Colletotrichum gloeosporioides* ve *Fusarium solani*'nin misel gelişimleri üzerinde *in vitro* biyokontrol

etkinliklerinin belirlenmesi; (iii) fungal etmenlerin misel gelişiminin engellenmesinde ve bitki büyümesinin gelişiminde (PGP) rol oynayan olası etki mekanizmalarının karakterize edilmesi amaçlanmıştır.

## MATERYAL ve METOD

### Fungal Hastalık Etmenleri

Biyoeftinlik çalışmalarında kullanılan ve çalışmanın ana materyalini oluşturan fungal hastalık etmenleri *Fusarium solani* (Cfs8), *Colletotrichum gloeosporioides* (LC5) ve *Geotrichum citri-aurantii* (MKUGca2), HMKÜ Bitki Sağlığı Kliniği Uygulama ve Araştırma Merkezi kültür koleksiyonundan temin edilmiştir. Bu patojenler Hatay ilinin en fazla turuncgöl yetiştiriciliğinin yapıldığı Erzincan ilindeki farklı hastalık belirtisi gösteren portakal (Washington Navel), mandarin (Owari Satsuma) ve limon (Meyer) ağaçlarının kök, gövde, sürgün, yaprak ve meyvelerinden Patates Dekstroza Agar (PDA) besi yerinde izole edilmiş (Şekil 1), morfolojik ve moleküler olarak tanımlanmıştır (Kurt ve ark., 2019; Kara ve ark., 2020; Kurt ve ark., 2020).



Şekil 1. Çalışmada kullanılan *Geotrichum citri-aurantii* (A), *Colletotrichum gloeosporioides* (B) ve *Fusarium solani* (C) izolatlarının tek spor kültürleri

Figure 1. Single spore cultures of *Geotrichum citri-aurantii* (A), *Colletotrichum gloeosporioides* (B) and *Fusarium solani* (C) isolates used in the study

### Endofit ve Epifit Bakteri İzolatlarının İzolasyonu ve Seçimi

Farklı turuncgöl çeşitlerinden alınan sağlıklı bitki örnekleri bahçeden getirildikten sonra kök, gövde, sürgün, yaprak ve gövde olarak gruplandırılmış ve her gruba ayrı bir kod verilmiştir. Endofit bakteri izolasyonu için, bitki örneklerinin yüzeyleri muhtemel fungal/bakteriyel epifit kontaminantların uzaklaştırılması amacıyla temiz çeşme suyu altında yıkanmış, daha sonra %70'lik etanol (3 dak.) ve %2'lik sodyum hipoklorit solüsyonunda (2 dak.) bekletilmek suretiyle yüzey dezenfeksiyonları tamamlanmıştır. Gerek etanol gerekse sodyum hipoklorit solüsyonunun dokulardan uzaklaştırılmak amacıyla dokular 3 kez steril su içinde (her defasında 2 dak.) durulanmıştır.

Son durulama suyundan alınan 200 µl su örneği besi yerine yayılarak yüzey dezenfeksiyonun kontrolü yapılmıştır. Yüzey dezenfeksiyonu tamamlanan sağlıklı bitki dokuları 0.05 mM MgCl<sub>2</sub> tampon çözeltisi bulunan steril havan içerisinde iyice ezildikten sonra 5 dak. bekletilmiştir. Buradan alınan bakteri süspansiyonu seçici King B (KB) besi yeri üzerine bagetle yayılmak suretiyle ekimleri yapılmıştır. Epifit bakterilerin izolasyonunda sağlıklı bitki kısımlarına ön dezenfeksiyon amaçlı herhangi işlem yapılmamıştır. Sağlıklı bitki dokuları 1-2 cm boyunda kesildikten sonra bitki dokuları veya toprak-kök karışımı (5 gr) doğrudan steril 0.05 mM MgCl<sub>2</sub> tampon çözeltisi (yaklaşık 50 ml) içine konulup, 30 dak. 200 rpm orbital çalkalayıcı içerisinde çalkalamaya



birakılmış, daha sonra buradan alınan süspansiyonlar seri olarak sulandırıldıktan ( $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$  ve  $10^6$ ) sonra KB besi içeren petri kaplarına bağıtle yayılmıştır. Bazı petriler içerisindeki besiyerleri üzerine sağlıklı kök dokuları doğrudan konularak kök yüzeyinden bakterilerin izolasyonları gerçekleştirilmiştir.

Aday epifit ve endofit bakteri izolatların bitki patojeni olup olmadığının belirlenmesinde tütün bitkisinde aşırı duyarlılık testi (HR) ile patates yumuşak çürüklük testleri yapılmıştır (Lelliot ve Stead, 1987). Her iki test sonucunda "negatif" olarak değerlendirilen aday bakteri izolatları TSA besi yerine çizildikten sonra 37 °C en az 48-72 saat inkübasyona bırakılmış, burada "gelişmeyen" insan patojeni olmadığı kabul edilen bakteri izolatları gerek tanılama gerekse *in vitro* biyoetkinlik çalışmalarında "aday" biyolojik mücadele etmeni izolatlar olarak değerlendirilmeye alınmıştır.

### Bakteri İzolatlarının Tanılanması

Aday epifit ve endofit bakteri izolatlarının tanılanması çalışmalarında tütün yapraklarında HR, patates dilimleri üzerinde yumuşak çürüklük oluşturmeyen, aynı zamanda 37 °C gelişme göstermeyen aday bakteri izolatları tanılanmıştır. Saf kültürde gelişen tek koloni izolatların tanılanması MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany) yöntemi kullanılarak yapılmıştır. Saf kültürden elde edilen ve TSA besi yerinde 24-36 saat içinde gelişen saf bakteri kolonileri, etanol-formik asit yöntemiyle ekstrakte edilmiş ve her örnek noktasına 1 µl HCCA Matrix solüsyonu eklenmiş ve kurutulmuştur. Daha sonra, bakteriler MALDI-TOF MS cihazına yüklendikten sonra bakteri izolatlarının tür teşhisleri, BIOTYPER™ 3.0 (Bruker Daltonics GmbH, Bremen, Germany) yazılımı kullanılarak, cihazın mikroorganizma kütüphanesindeki standart türlerin spektrumları ile eşleştirilerek yapılmıştır (Aktan & Soylu, 2020).

### Bakteri İzolatlarının *in vitro* Koşullarda Antagonistik Etkileri

Elde edilen bakteri izolatlarının *in vitro* biyokontrol etkinlik potansiyelleri *G. citri-aurantii*, *C. gloeosporioides* ve *F. solani*ye karşı belirlenmiştir. Bakteri izolatlarının fungal etmenlerin misel gelişimini engelleme (antagonistik) etkinlikleri 9 cm çapında, içerisinde TSA+PDA (1:1) besi yeri içeren petri kaplarında "ikili kültür" testleriyle belirlenmiştir. İkili kültür testlerinde petrinin üst noktasından 2 cm gerisine çizgi ekim yöntemi ile çizilmiş bakteri izolatları 25 °C'de 48 saat ön inkübasyona bırakılmıştır. Bu sürenin sonunda 5 günlük fungal kültürlerinden alınan 6 mm çaplı misel diskleri gelişen bakteri kolonisinden 4 cm gerisine yerleştirilmiş ve tekrar gelişmeye bırakılmıştır.

TSA+PDA besiyerlerine herhangi bir bakteri izolatu çizilmemiş, sadece fungal izolatu yerleştirildiği petriler "kontrol" olarak kullanılmıştır. İkili kültür testlerinde, "kontrol" petrilerinde bulunan fungal hastalık etmeni izolatlar petriyi tamamen kapladıkları zaman (fungal etmenlere göre değişmekle birlikte genelde inokulasyondan 4-5 gün sonra), tüm petrilerde fungal etmenlerin bakteriye doğru yönelen misel gelişim (MG<sub>u</sub>) mesafeleri cetvelle ölçülmüştür. Bakterilerin antagonistik (engelleme) etkinlikleri, kontrol petrilerdeki misel gelişim uzunlukları (MG<sub>k</sub>) baz alınarak her bir bakteri izolatu misel gelişimini engelleme oranları %Abbott formülü kullanılmak suretiyle hesaplanmıştır (Soylu ve ark., 2020).

$$\%Engelleme = ((MG_k - MG_u) / MG_k) * 100$$

### Bakteri İzolatlarının Siderofor ve Proteaz Üretme Potansiyellerinin Belirlenmesi

Bakteri izolatlarının siderofor ve proteaz üretme potansiyelleri uygun besi yerleri ve yöntemlere göre belirlenmiştir. Siderofor etkinliği Chrom Azurol-S Agar (CAS) besi yeri üzerinde (Schwyn ve Neilands, 1987), proteaz enzimi üretim etkinliği %2 yağı alınmış süt tozu (Skimmed Milk Powder, Merck, Darmstadt, Germany) bulunan Luria Broth Agar (SMLBA) besi yeri üzerinde belirlenmiştir (Perneel ve ark., 2007). Bakteri kolonileri, steril kürdan yardımıyla seçilerek her bir izolat için üç farklı noktaya inokule edilmiştir. İnokulasyon işleminden sonra, bakteri izolatları 26 °C'de 2 gün boyunca inkübasyona alınmıştır. İnokulasyon süresinin sonunda, CAS besiyerinde "sarı-turuncu" renkteki engelleme bölgeleri ve SMLBA besiyerinde "şeffaf" renkteki engelleme bölgeleri gözlemlenmiştir. Bu engelleme bölgeleri, test edilen bakterilerin siderofor ve proteolitik aktivitesi olduğunu göstermektedir. Engelleme bölgelerinin ve bakteri kolonilerinin çapları ayrı ayrı ölçülmüştür. Siderofor (Sid-indeks) ve proteolitik (Pro-indeks) etkinlik indeksleri, engelleme zon çapının bakteri koloni çapına oranlanmasıyla hesaplanmıştır (Vazquez ve ark., 2000). Her bir bakteri izolatu için ölçümler üç ayrı petri kabında gerçekleştirilmiş ve deneme iki farklı zamanda tekrarlanmıştır.

### Bakteri İzolatlarının Fosfor Çözme Potansiyellerinin Belirlenmesi

Bakterilerin fosforu çözme yetenekleri Pikovskaya Agar (PVK) besi yerinde tri kalsiyum fosfat Ca<sub>3</sub>(PO<sub>4</sub>) bileşiğinin çözülme şiddetine göre petrilerde belirlenmiştir (Kumar ve ark., 2012). PVK besi yeri üzerine taze bakteri kültürlerinden steril kürdan ile alınıp 3 farklı noktaya aşılandıktan sonra, petriler 26 °C'de 7 gün inkübasyona bırakılmıştır. İnokulasyondan 5 gün sonra bakteri izolatlarının geliştiği noktadaki kolonilerin etrafında gelişen şeffaf engelleme bölgeleri, test edilen bakteri izolatu fosforu çözme yeteneğinde olduğunu göstermiştir. Bakteri

izolatlarının fosfor çözme etkinliği, ölçülen zon çapının gelişen bakteri kolonisinin çapına oranlanması ile fosfor çözme İndeks değerleri (Fos-İndeks) belirlenmiştir (Vazquez ve ark., 2000). Söz konusu testlerde bakteri izolatu için ölçümler ve değerlendirmeler 3 ayrı petri içerisinde gerçekleştirilmiş olup, deneme 2 farklı zamanda tekrarlanmıştır.

### **Bakteri İzolatlarının İndol Asetik Asit (IAA) Üretme Potansiyellerinin Belirlenmesi**

Bakterilerin IAA üretme yetenekleri Glickman ve Dessaux, (1995) tarafından tarif edildiği şekilde Salkowski yöntemi kullanılarak tespit edilmiştir. Seçilmiş bakteri izolatların 48 saatlik taze kültürlerinden bakteri süspansiyonu ( $10^8$  hücre/ml) hazırlandıktan sonra, 500 µl hacmindeki süspansiyon L-tryptophan ( $3 \text{ mg ml}^{-1}$ ) içeren 5 ml steril LB besi yeri bulunan cam tüplere (10x120 mm) aşılanmıştır. Bakteri izolatlarının aşılandığı besi yeri içeren tüpler 26 °C ayarlı inkübatörlü orbital çalkalayıcı (200 rpm) üzerinde 4 gün süreyle tekrar inkübasyona bırakılmıştır. Tüplerdeki sıvı besi yerinde gelişen bakteri süspansiyonundan alınan 2 ml süspansiyon soğutmalı santrifüjde 5000 rpm'de 30 dak. santrifüj edilmiştir. Sonrasında, tüplerin üst kısmından alınan 1 ml süspansiyon steril eppendorf tüplere aktarılmış ve üzerlerine yaklaşık 40 µl fosforik asit eklenmiştir. Bu karışımın tamamı 2 ml Salkowski çözeltisi (150 ml %98'lik  $\text{H}_2\text{SO}_4$ , 250 ml of distile  $\text{H}_2\text{O}$ , 7.5 ml of 0.5 M  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) içeren steril cam tüplere aktarılmıştır. Tüpler, inkübatörde 25 °C'de 30 dak. karanlıkta inkübasyona bırakılmıştır. İnkübasyon sonrasında süspansiyon renginin "açık sarı"dan "kırmızı-pembe" renge dönüşmesi, test edilen bakteri izolatının besiyeri içeriğinde IAA ürettiğini göstermiştir. Üretilen IAA miktarları UV-vis spektrofotometre (Perkin Elmer, Lamda 25, USA) cihazı ile 535 nm dalga boyunda ölçülmüştür (Patten & Glick, 2002). IAA miktarları standart IAA (Merck, Darmstadt, Germany) çözeltisi ile hazırlanan "standart konsantrasyon eğrisiyle" karşılaştırılmak suretiyle µg/ml (ppm) düzeyinde belirlenmiştir.

### **Bakteri İzolatlarının Amonyak ( $\text{NH}_3$ ) Üretme Potansiyellerinin Belirlenmesi**

Bakterilerin amonyak ( $\text{NH}_3$ ) üretme etkinlikleri Cappuccino ve Sherman, (2013) tarafından bildirildiği şekilde Pepton Water (PW) besiyelerine Nessler's çözeltisi ilave edilmek suretiyle belirlenmiştir. İçerisinde steril 5 ml PW içeren cam tüplere steril öze yardımıyla 2 günlük taze test edilen bakteri izolatlarının kültüründen alınan bakteri kolonisi bulaştırılmıştır. İnokulasyonların yapıldığı tüpler 150 rpm'e ayarlı inkübatörlü orbital çalkalayıcıda 26 °C'de 4 gün süreyle inkübasyona bırakılmıştır. Bulaştırmaların yapıldığı tüplerde gelişen bakteri

süspansiyonlarından 1 ml alınmış, santrifüj (13000 rpm 5 dak) yapılarak süpernatant solusyonu elde edilmiştir. Elde edilen süpernatant solusyonu üzerine 50 µl hacminde Nessler's çözeltisi ilave edildikten hemen sonra (2-4 saniye) besi yeri içerisinde üretilen "amonyak" birikiminin olup olmadığı, tüplerdeki besi yeri renginin "açık sarı"dan "kahverengi veya koyu sarı" renge dönüşmesiyle kalitatif (gözlemlenerek) olarak tespit edilmiştir.

### **Bakteri İzolatlarının Hidrojen Siyanür (HCN) Üretim Potansiyellerinin Belirlenmesi**

Castric (1977) tarafından bildirildiği üzere, 4.4 gr/L glycine eklenmiş TSA besi yerinde gelişen bakteri hücreleri tarafından üretilen uçucu HCN bileşeni petri kapağında bulunan ve pikrik asit/sodyum karbonat ile ıslatılmış (%0.5 pikrik asit (PA) + 2% sodyum karbonat ( $\text{Na}_2\text{CO}_3$ ): 100 ml steril su+0.5 gr PA+2 gr  $\text{Na}_2\text{CO}_3$ ) filtre kâğıdı renginin sarı renkten kahverengi veya koyu kırmızıya dönüşmesiyle kalitatif olarak belirlenmiştir.

### **Bakteri İzolatlarının Uçucu Bileşen(ler)inin *in vitro* Antagonistik Etkinliğinin Belirlenmesi**

Bakteri izolatları tarafından üretilen uçucu bileşen(ler)inin *in vitro* koşullarda antifungal etkinlikleri daha önceden bildirilmiş "kapalı ikili kültür (sealed plate)" testleri ile belirlenmiştir (Chaves-Lopez ve ark. 2015). Antagonist bakteri izolatu, içinde TSA besiyeri içeren petrilere çizilmiş, fungus izolatu (6 mm disk) ise içerisinde PDA besiyeri olan diğer petriye yerleştirilmiştir. Daha sonra her iki kapak birbirlerine bakacak şekilde kapatılmış, oluşan uçucu bileşenlerin ortamdan uzaklaşması engellemek amacıyla petri kapaklarının kenarları parafilm ile sarılmıştır. Kontrol uygulaması olarak TSA besiyeri üzerine herhangi bir bakteri izolatu çizilmemiş, PDA içeren petriye sadece fungus misel diski yerleştirilmiştir. Uygulama sonrası petrilere 25 °C de inkübasyona bırakılmıştır. Kontrol petrilere bulunan fungus PDA besi yeri üzerinde gelişimi tamamlandıktan sonra, fungal izolatların misel gelişimi (MGu) çapları ölçülmüş ve kontrol petrilere misel (MGk) gelişim çaplarına kıyaslanarak % engelleme oranları hesaplanmıştır. Her bakteri izolatu için ölçümler 3 ayrı petri kabında gerçekleştirilmiş olup, denemenin tekerrürü 2 farklı zamanda yapılmıştır.

### **Deneme Deseni ve İstatistik Analizler**

Bakterilerin fungal etmenlere karşı *in vitro* antagonistik etkinlikleri ve etki mekanizmalarının belirlenmesi çalışmaları tesadüfi parseller deneme desenine göre kurulmuş, her bir uygulama için 3 tekerrür kullanılmıştır. Denemelerde elde edilen veriler, SPSS istatistik programı for Windows Version 17 (SPSS, 2008) kullanılarak tek yönlü varyans analizi

yapılmış ve uygulamalar arasındaki farklılıklar Duncan Çoklu Karşılaştırma Testi ( $P \leq 0.05$ ) ile analiz edilmiştir.

## BULGULAR ve TARTIŞMA

### Endofit ve Epifit Bakterilerin İzolasyonu

Hatay ilindeki turuncgil bahçelerinden, ağaçların sağlıklı toprak altı (kök, kök boğazı ve köklere yakın topraklar) ve toprak üstü (sürgün, yaprak ve meyve) aksamlarından, biyokontrol etkinlik çalışmalarında kullanılmak üzere toplamda 53 epifit ve 32 endofit olmak üzere 85 bakteri izolatu elde edilmiştir. Yapılan testler sonucunda bitkide ve insanda hastalık etmeni olmadığı sonucuna varılan 26 endofit, 45'i ise epifit olmak üzere toplam 71 aday bakteri izolatu elde edilmiştir. MALDI-TOF tanılama çalışmaları sonucunda indeks değeri 1.7 ve üzerinde olan toplam 71 bakteri izolattan, 51 izolat Gram-pozitif, 20 izolat ise Gram-negatif olarak belirlenmiştir. Tür düzeyinde kesin teşhisi yapılan ve literatüre göre insan/hayvan/gıda patojenleri olduğu tespit edilen (*Bordetella petrii*, *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp. ve *Staphylococcus* spp.) türlere ait izolatlara çalışmalara dahil edilmemiş olup, bu izolatlarda dışında kalan 48 aday bakteri izolatlara ile detaylı çalışmalar yapılmaya karar verilmiştir.

### Endofit ve Epifit Bakterilerin Tanılanması

İzolasyon çalışmaları sonucunda bitki ve insan patojeni olmadığı belirlenen 48 adet bakteri izolatu elde edilmiştir. Elde edilen 48 izolatu MALDI TOF MS analizleri sonucunda 22 izolat *Bacillus* türü, 7 izolat *Lysinibacillus*, 3 izolat *Acinetobacter*, 2'şer izolat *Pseudomonas* ve *Kosakonia*, 1'er izolat *Burkholderia*, *Cronobacter*, *Curtobacterium*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pantoea*, *Rahnella*, *Raoultella*, *Rhizobium*, *Siccibacter* ve *Staphylococcus* cinslerine ait olduğu belirlenmiştir.

### Bakteri İzolatlarının *in vitro* Koşullarda Fungal Etmenlere Karşı Antagonistik Etkileri

Fungal hastalık etmenlerinin misel gelişimleri farklı antagonist bakteriler tarafından engellenme potansiyelleri *in vitro* ikili kültür testlerinde misel gelişim çapları ölçülerek belirlenmiş, elde edilen sonuçlar Çizelge 1 ve Şekil 1'de sunulmuştur. Test edilen fungal hastalık etmenlerinden *G. citri-aurantii*'nin misel gelişimini en yüksek düzeyde engelleyen izolatlara sırasıyla, %77.2 ile *B. vallismortis* YGL73ep, %76.6 ile *B. cereus* YGK25en, %73.3 ile *Pseudomonas chlororaphis* YGM82ep olarak belirlenmiştir (Şekil 2A). Antraknoz hastalık etmeni *C. gloeosporioides*'in misel gelişimini en yüksek düzeyde engelleyen izolatlara sırasıyla %72.7 ile *B. thuringiensis* YGT22en, %71.1 ile *B. vallismortis* YGL73ep ve *B. cereus* YGK25en, %65.56 ile *P.*

*chlororaphis* YGM82ep olarak belirlenmiştir (Şekil 1B). Bir diğer hastalık etmeni *F. solani*'nin misel gelişimini en yüksek düzeyde engelleyen izolatlara ise sırasıyla, %71.1 engelleme oranı ile *B. subtilis* YGS5en olduğu belirlenirken, bu izolatu %70.0 engelleme oranı ile *B. cereus* YGK25en ve %66.1 engelleme oranı ile *P. chlororaphis* YGM82ep izolatlara izlenmiştir (Şekil 2C). Misel gelişimini engellemede yüksek düzeyde etkinlik gösteren izolatlara arasında olduğu kadar aynı türe ait izolatlara da misel gelişimini engellemesi arasında istatistiksel olarak önemli düzeylerde farklılıklar görülmüştür (Çizelge 1). Bu çalışmada kullanılan 3 farklı fungal etmene karşı  $\geq 50$  antagonistik etkinlik gösteren 11 endofit ve epifit bakteri izolat arasında Gram negatif *P. chlororaphis* YGM82ep dışında kalan bakteri izolatlara tamamının Gram pozitif *Bacillus* spp. ait izolatlara olduğu belirlenmiştir. İzolatlar arasında *G. citri-aurantii* etmenine karşı  $> 50$  antagonistik etkinlik gösteren toplam 21 izolattan 14 tanesi, *C. gloeosporioides*'e karşı toplam 17 izolattan 15 tanesinin, *F. solani*'ye karşı ise 17 izolattan 13 tanesinin benzer şekilde *Bacillus* spp. ait izolatlara olduğu tespit edilmiştir (Çizelge 1).

Yapılan *in vitro* ikili kültür çalışmalarında antagonistik etkinliği yüksek olarak belirlenen *Bacillus cereus*, *B. mojavensis*, *B. vallismortis*, *B. subtilis*, ve *B. thuringiensis* gibi epifit ve endofit kökenli *Bacillus* spp. türlerinin antimikrobiyal etkinliğe sahip farklı bileşikler üretme potansiyeline sahip olmaları nedeniyle biyolojik mücadele çalışmalarında en fazla irdelenen bakteriyel türlerdir (Tariq ve ark., 2020). Soylu ve ark. (2022) tarafından yakın zamanda yapılan araştırmada, sağlıklı turuncgil ağaçlarından elde edilen 16 farklı bakteri izolatu'nun *G. citri-aurantii*'nin misel gelişiminin engellenmesi *in vitro* ikili kültür testleriyle ortaya konulmuştur. Test edilen bakteri izolatlara arasında, *Bacillus* spp.'ye ait izolatlara misel gelişimini %59.5 ile %78.6 aralığında engellerken, *B. subtilis* CM8 (%78.6) izolatu en yüksek antagonistik etkiyi gösteren izolat olarak belirlenmiştir. Chenniappan ve ark. (2019) tarafından yapılan çalışma ile zerdeçal bitkisinin kök ve rizomlarından izole edilen bakteriler 7 farklı rizom kök çürüklüğü hastalık etmeni olan *Rhizoctonia solani*, *Schizophyllum commune*, *Macrophomina phaseolina*, *Fusarium graminearum* ve *F. solani*'ye karşı antagonistik etkinlikleri araştırılmıştır. Söz konusu fungal patojenlere karşı *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *B. tequilensis*, *B. cereus*, *B. subtilis* türlerine ait 16 izolatu'nun değişen oranlarda antagonistik etkinlik gösterdikleri bildirilmiştir. Yapılan benzer çalışmalarda *Bacillus* spp. ait bazı izolatlara *in vitro* ve *in vivo* koşullarda turuncgillerde depo hastalık etmenlerinden *P. digitatum*, *P. crustosum*, *P. italicum* ve *G. citri-aurantii*'ye karşı antifungal etkinlik gösterdiği bildirilmiştir (Hong ve ark., 2014; Chen ve ark., 2018; Tian ve ark., 2020).



Çizelge 1. Antagonist bakteri izolatlarının *in vitro* ikili kültür testlerinde 3 farklı fungal etmenin misel gelişimini (mm)<sup>a</sup> engellemede gösterdikleri antagonistik etkinlikleri

Table 1. Antagonistic activity of antagonist bacterial isolates in inhibiting mycelial growth (mm)<sup>a</sup> of 3 different fungal agents in *in vitro* dual culture tests

| Bakteri İzolatu                              | Fungal etmenler          |         |                          |         |                        |         |
|--|--------------------------|---------|--------------------------|---------|------------------------|---------|
|  | <i>G. citri-aurantii</i> |         | <i>C. gloesporioides</i> |         | <i>F. solani</i>       |         |
|  | MG (mm)                  | MGE (%) | MG (mm)                  | MGE (%) | MG (mm)                | MGE (%) |
| <i>Bacillus vallismortis</i> YGL73ep         | 13,67 <sup>aA</sup>      | 77,22   | 17,33 <sup>abA</sup>     | 71,12   | 26,00 <sup>cFB</sup>   | 56,67   |
| <i>Bacillus cereus</i> YGK25en               | 14,00 <sup>abA</sup>     | 76,67   | 17,33 <sup>abA</sup>     | 71,12   | 18,00 <sup>aA</sup>    | 70,00   |
| <i>Pseudomonas clororapsis</i> YGM82ep       | 16,00 <sup>acA</sup>     | 73,33   | 20,67 <sup>bcB</sup>     | 65,55   | 20,33 <sup>abB</sup>   | 66,12   |
| <i>Bacillus cereus</i> YGK28ep               | 17,33 <sup>b-dA</sup>    | 71,12   | 26,33 <sup>c-gB</sup>    | 56,12   | 27,00 <sup>d-gB</sup>  | 55,00   |
| <i>Bacillus cereus</i> YGM3ep                | 19,33 <sup>c-eA</sup>    | 67,78   | 25,33 <sup>d-gB</sup>    | 57,78   | 21,33 <sup>a-eA</sup>  | 64,45   |
| <i>Bacillus cereus</i> YGS13ep               | 20,00 <sup>d-fA</sup>    | 66,67   | 24,00 <sup>c-fA</sup>    | 60,00   | 34,00 <sup>j-IB</sup>  | 43,33   |
| <i>Bacillus cereus</i> YGK31en               | 22,00 <sup>e-gA</sup>    | 63,33   | 30,67 <sup>h-jB</sup>    | 48,88   | 31,33 <sup>g-kB</sup>  | 47,78   |
| <i>Bacillus thuringiensis</i> YGT22en        | 22,00 <sup>e-gB</sup>    | 63,33   | 16,33 <sup>aA</sup>      | 72,78   | 28,33 <sup>e-iC</sup>  | 52,78   |
| <i>Bacillus cereus</i> YGP6en                | 23,00 <sup>f-hA</sup>    | 61,67   | 21,00 <sup>b-dA</sup>    | 65,00   | 26,00 <sup>c-fA</sup>  | 56,67   |
| <i>Rahnella aquatilis</i> YGP63ep            | 23,00 <sup>f-hAB</sup>   | 61,67   | 22,33 <sup>c-eA</sup>    | 62,78   | 25,33 <sup>b-eB</sup>  | 57,78   |
| <i>Bacillus cereus</i> YGKL16ep              | 23,33 <sup>f-hA</sup>    | 61,12   | 26,00 <sup>e-gB</sup>    | 56,67   | 25,33 <sup>b-eAB</sup> | 57,78   |
| <i>Bacillus cereus</i> YGP1en                | 23,33 <sup>f-hA</sup>    | 61,12   | 25,33 <sup>d-gA</sup>    | 57,78   | 31,33 <sup>g-kA</sup>  | 47,78   |
| <i>Bacillus mojavensis</i> YGM15en           | 23,33 <sup>f-hA</sup>    | 61,12   | 25,67 <sup>e-gB</sup>    | 57,22   | 29,33 <sup>e-jC</sup>  | 51,12   |
| <i>Burkholderia cepacia</i> YGM75ep          | 23,33 <sup>f-hA</sup>    | 61,12   | 33,33 <sup>j-nB</sup>    | 44,45   | 52,00 <sup>prC</sup>   | 13,33   |
| <i>Bacillus mojavensis</i> YGL69ep           | 24,67 <sup>ghA</sup>     | 58,88   | 30,67 <sup>h-jB</sup>    | 48,88   | 24,00 <sup>b-eA</sup>  | 60,00   |
| <i>Acinetobacter johnsonii</i> YGM76en       | 25,00 <sup>ghA</sup>     | 58,33   | 35,67 <sup>l-oB</sup>    | 40,55   | 58,33 <sup>tC</sup>    | 2,78    |
| <i>Bacillus simplex</i> YGS12ep              | 26,00 <sup>hiA</sup>     | 56,67   | 22,00 <sup>c-eA</sup>    | 63,33   | 35,33 <sup>k-mB</sup>  | 41,12   |
| <i>Enterobacter xiangfangensis</i> YGM80ep   | 26,33 <sup>hiA</sup>     | 56,12   | 32,33 <sup>i-mB</sup>    | 46,12   | 32,00 <sup>g-kB</sup>  | 46,67   |
| <i>Rhizobium radiobacter</i> YGM77ep         | 26,33 <sup>hiA</sup>     | 56,12   | 31,33 <sup>h-lA</sup>    | 47,78   | 49,33 <sup>opB</sup>   | 17,78   |
| <i>Bacillus cereus</i> YGM9en                | 29,00 <sup>ijA</sup>     | 51,67   | 26,33 <sup>e-gA</sup>    | 56,12   | 21,00 <sup>a-eA</sup>  | 65,00   |
| <i>Herbaspirillum aquaticum</i> YGM78ep      | 29,33 <sup>k-A</sup>     | 51,12   | 31,00 <sup>h-kA</sup>    | 48,33   | 59,67 <sup>tB</sup>    | 0,55    |
| <i>Bacillus endophyticus</i> YGL74en         | 31,33 <sup>j-lA</sup>    | 47,78   | 33,33 <sup>j-nA</sup>    | 44,45   | 59,00 <sup>tB</sup>    | 1,67    |
| <i>Klebsiella oxycota</i> YGP62en            | 31,67 <sup>j-lA</sup>    | 47,22   | 45,00 <sup>prB</sup>     | 25,00   | 32,67 <sup>h-kA</sup>  | 45,55   |
| <i>Lysinibacillus sphaericus</i> YGM10en     | 32,67 <sup>k-mA</sup>    | 45,55   | 36,67 <sup>m-oB</sup>    | 38,88   | 53,00 <sup>p-sC</sup>  | 11,67   |
| <i>Bacillus subtilis</i> YGS5en              | 32,67 <sup>k-mC</sup>    | 45,55   | 27,33 <sup>f-hB</sup>    | 54,45   | 17,33 <sup>aA</sup>    | 71,12   |
| <i>Lysinibacillus sphaericus</i> YGM4ep      | 33,00 <sup>lmA</sup>     | 45,00   | 35,00 <sup>j-oA</sup>    | 41,67   | 38,33 <sup>l-nB</sup>  | 36,12   |
| <i>Bacillus safaeensis</i> YGM83ep           | 33,00 <sup>lmB</sup>     | 45,00   | 22,33 <sup>c-eA</sup>    | 62,78   | 55,67 <sup>r-tC</sup>  | 7,22    |
| <i>Lysinibacillus sphaericus</i> YGS35ep     | 34,33 <sup>l-nA</sup>    | 42,78   | 34,00 <sup>j-rO</sup>    | 43,33   | 60,00 <sup>tB</sup>    | 0,00    |
| <i>Lysinibacillus sphaericus</i> YGK24en     | 36,00 <sup>m-oA</sup>    | 40,00   | 34,00 <sup>j-rO</sup>    | 43,33   | 33,33 <sup>i-lA</sup>  | 44,45   |
| <i>Lysinibacillus sphaericus</i> YGKL15en    | 37,00 <sup>noA</sup>     | 38,33   | 37,67 <sup>noA</sup>     | 37,22   | 40,00 <sup>mnA</sup>   | 33,33   |
| <i>Lysinibacillus sphaericus</i> YGP4en      | 38,33 <sup>oA</sup>      | 36,12   | 50,00 <sup>stB</sup>     | 16,67   | 57,67 <sup>stC</sup>   | 3,88    |
| <i>Kosakonia cowanii</i> YGP7en              | 38,33 <sup>oB</sup>      | 36,12   | 53,67 <sup>tuC</sup>     | 10,55   | 25,33 <sup>b-eA</sup>  | 57,78   |
| <i>Pantoea agglomerans</i> YGM81ep           | 39,33 <sup>oB</sup>      | 34,45   | 37,67 <sup>noB</sup>     | 37,22   | 31,00 <sup>f-kA</sup>  | 48,33   |
| <i>Lysinibacillus sphaericus</i> YGP3en      | 44,67 <sup>pB</sup>      | 25,55   | 47,33 <sup>rsB</sup>     | 21,12   | 28,67 <sup>e-iA</sup>  | 52,22   |
| <i>Bacillus megaterium</i> YGT18ep           | 48,67 <sup>rB</sup>      | 18,88   | 38,00 <sup>oA</sup>      | 36,67   | 35,00 <sup>klA</sup>   | 41,67   |
| <i>Pseudomonas koreensis</i> YGL72ep         | 48,67 <sup>rB</sup>      | 18,88   | 35,33 <sup>k-oA</sup>    | 41,12   | 36,00 <sup>k-nA</sup>  | 40,00   |
| <i>Siccibacter colletis</i> YGP67ep          | 55,33 <sup>sC</sup>      | 7,78    | 42,67 <sup>pB</sup>      | 28,88   | 27,67 <sup>e-hA</sup>  | 53,88   |
| <i>Bacillus megaterium</i> YGM12en           | 56,67 <sup>stA</sup>     | 5,55    | 49,67 <sup>stA</sup>     | 17,22   | 59,00 <sup>tA</sup>    | 1,67    |
| <i>Bacillus megaterium</i> YGL68ep           | 56,67 <sup>stB</sup>     | 5,55    | 35,33 <sup>koA</sup>     | 41,12   | 59,33 <sup>tC</sup>    | 1,12    |
| <i>Cronobacter sakazakii</i> YGKL12ep        | 57,00 <sup>stB</sup>     | 5,00    | 60,00 <sup>vC</sup>      | 0,00    | 40,67 <sup>nA</sup>    | 32,22   |
| <i>Acinetobacter lwoffii</i> YGL60en         | 60,00 <sup>stA</sup>     | 0,00    | 59,33 <sup>vA</sup>      | 1,12    | 59,67 <sup>tA</sup>    | 0,55    |
| <i>Raoultella ornithinolytica</i> YGP64en    | 60,00 <sup>stA</sup>     | 0,00    | 60,00 <sup>vA</sup>      | 0,00    | 59,33 <sup>tA</sup>    | 1,12    |
| <i>Acinetobacter lwoffii</i> YGS37en         | 60,00 <sup>stB</sup>     | 0,00    | 43,67 <sup>prA</sup>     | 27,22   | 45,33 <sup>oA</sup>    | 24,45   |
| <i>Curtobacterium flaccumfaciens</i> YGS25en | 60,00 <sup>stB</sup>     | 0,00    | 56,67 <sup>uvA</sup>     | 5,55    | 57,67 <sup>stA</sup>   | 3,88    |
| <i>Kosakonia cowanii</i> YGKL17en            | 60,00 <sup>stB</sup>     | 0,00    | 60,00 <sup>vB</sup>      | 0,00    | 49,33 <sup>opA</sup>   | 17,78   |
| <i>Staphylococcus succinus</i> YGL55ep       | 60,00 <sup>stB</sup>     | 0,00    | 51,67 <sup>tA</sup>      | 13,88   | 57,33 <sup>stB</sup>   | 4,45    |
| <i>Bacillus megaterium</i> YGM5ep            | 60,00 <sup>stC</sup>     | 0,00    | 43,33 <sup>prA</sup>     | 27,78   | 51,67 <sup>prB</sup>   | 13,88   |
| <i>Bacillus subtilis</i> YGS19ep             | 60,00 <sup>stC</sup>     | 0,00    | 28,67 <sup>giB</sup>     | 52,22   | 22,33 <sup>adA</sup>   | 62,78   |
| <b>Kontrol</b>                               | 60,00 <sup>x</sup>       | 0,00    | 60,00 <sup>v</sup>       | 0,00    | 60,00 <sup>z</sup>     | 0,00    |

Misel gelişimi (mm); MGE: Misel gelişiminin engellenme oranı (%)

<sup>a</sup> Aynı sütun içerisinde yer alan misel gelişim çaplarının ortalama değerlerin yanındaki benzer küçük harfler ile aynı satır içerisinde yer alan ortalama değerlerin yanındaki benzer büyük harfler izolatlar arasındaki farkın istatistiksel olarak önemli olmadığını gösterir (Duncan Çoklu Karşılaştırma Testi, P<0.05). Koyu renkli olarak yazılmış izolatlar testlerde yüksek etkinlik gösteren izolatlardır

MG:





Şekil 2. Bakteri izolatlarının ikili kültür testlerinde *G. citri-aurantii* (A), *C. gloeosporioides* (B) ve *F. solani* (C) etmenlerinin misel gelişimlerinin engellenmesi üzerine antagonistik etkinlikleri  
Figure 2. Antagonistic activities of bacterial isolates on inhibition of mycelial growth of *G. citri-aurantii* (A), *C. gloeosporioides* (B) and *F. solani* (C) in dual culture tests

Yapılan biyokimyasal ve moleküler analizlerde *Bacillus* spp. ve *Pseudomonas* spp. ait antagonist BCA izolatların fungal hastalık etmenlerine karşı antagonistik etkinliklerinin 2,4-diacetylphloroglucinol, pyrrolnitrin, pyoluteorin, bacillomycin D, fengycin, hydrogen cyanide, ve litik enzim gibi antifungal bileşiklerden kaynaklandığı belirlenmiştir (Tariq ve ark., 2020).

#### Bakterilerin Fungal Patojenin Misel Gelişimini Engellemede Rol Oyanayan (Antagonistik) Mekanizmaların Belirlenmesi

**Siderofor üretim potansiyelleri:** Siderofor üretimi CAS besi yeri kullanılarak belirlenmiştir. CAS besi yeri üzerinde bakteriler tarafından oluşturulan engelleme zonunun bakteri çapına oranlanmasıyla elde edilen siderofor etkinlik indeks değerlerine bakılmıştır (Şekil 2). Test edilen tüm izolatlar arasından en fazla siderofor üretimi 3.78 indeks değeri ile *Pseudomonas koreensis* YGL72ep tarafından gösterilmiş olup bu izolatı sırasıyla 2.33 ve 2.30 indeks değerleri ile sırasıyla *Pseudomonas koreensis* YGL72ep, *Bacillus simplex* YGS12ep ve *Burkholderia cepacia* YGM75ep izolatları izlemiştir (Çizelge 2; Şekil 3A).

**Proteaz üretim potansiyelleri:** Mikolitik bir enzim olan proteaz, bazı biyokontrol etmeni antagonist bakteriler tarafından üretilebilen, fungal hastalık etmenlerinin hücre duvarı yapısında bozulmalara ve erimelere neden olan proteolitik enzimlerden biridir (Şekil 3B). Test edilen izolatlar arasında en yüksek proteaz aktivitesi 1.78 indeks değeri ile *Bacillus mojavensis* YGM15en izolatı tarafından gösterilmiştir. Bu izolatı sırasıyla 1.61 indek değeri ile *B. simplex* YGS12ep, 1.59 indeks değeri ile *B. cereus* YGM3ep izolatları takip edilmiştir (Çizelge 2; Şekil 3B).

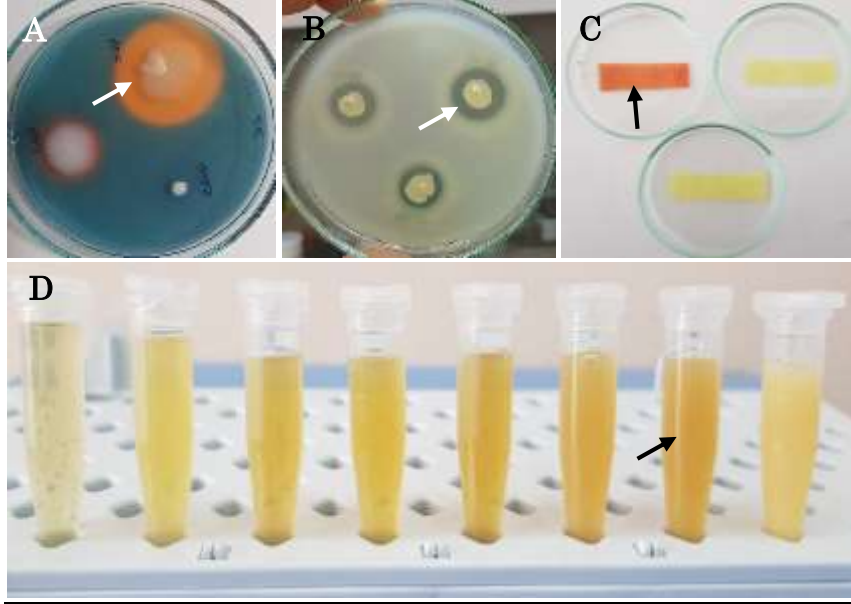
#### Hidrojen siyanür (HCN) üretim potansiyelleri:

Yapılan gözlemlere göre 25 izolat arasında sadece 5 izolatın değişen oranlarda HCN ürettiği belirlenmiştir. En yüksek HCN üretimi *Bacillus megaterium* YGT18ep tarafından üretilirken, bu izolatı *Pseudomonas chlororaphis* YGM82ep ile *Raoultella ornithinolytica* YGP64en izolatları izlemiştir. Bu bakterileri nispeten daha düşük HCN üretimine sahip olan *B. vallismortis* YGL73ep ve *P. koreensis* YGL72ep izolatları takip etmiştir. Aralarında yüksek oranda antagonistik etkinlik gösteren *B. cereus* YGM3ep ve *B. thuringiensis* YGT22en gibi birçok izolatın HCN üretme

potansiyelinin bulunmadığı tespit edilmiştir (Çizelge 2; Şekil 3C).

**Amonyak üretim potansiyelleri:** Yapılan gözlemlere göre test edilen 25 izolattan *Acinetobacter johnsonii* YGM76en dışında tamamı pepton içeren besiyerinde

değişen şiddetlerde amonyak üretmiştir. En yüksek amonyak üretimi *Burkholderia cepacia* YGM75ep izolatinca üretilirken, antagonistik etkiye sahip birçok türün yüksek oranlarda amonyak ürettiği tespit edilmiştir (Çizelge 2; Şekil 3D).



Şekil 3. Bakterilerin (A) CAS Agar üzerinde siderofor, (B) SMLBA besi yeri üzerinde protease enzim, (C) TSA besi yerinde hidrojen siyanür, (D) Pepton Water besi yerinde amonyak üretme etkinlikleri. Etkinlikler bakteri kolonisi etrafında ortaya çıkan zonlar (ok), filtre kâğıdının turuncuya, besi yerinin koyu sarı renge dönüşmesiyle (ok) karakterize edilmiştir.

**Figure 3.** Potential of bacteria to produce siderophore on CAS agar (A), protease enzyme on SMLBA medium (B), production of hydrogen cyanide on TSA medium (C), production of ammonia on Pepton Water medium. Efficiencies of bacterial isolates were evident as inhibition zone around bacterial colony (arrow), changes of colour on filter paper to orange, changes of colour of medium to dark yellow (arrow).

### Bakterilerin Bitki Gelişimini Teşvik Etmede (PGP) Rol Oyanayan Mekanizmalarının Belirlenmesi

**Fosfor çözme potansiyelleri:** Bakteri izolatların PVK besiyeri üzerinde fosfor çözme etkinlikleri tüm izolatlar açısından değerlendirildiğinde Gram negatif bakteri izolatlarının Gram pozitif izolatlara oranla daha yüksek etkinlik gösterdiği belirlenmiştir. Test edilen izolatlar arasında en yüksek fosfor çözme etkinliği 2.57 fosfor çözme indeks değeri ile *Rahnella aquatilis* YGP63ep izolatı tarafından gösterilmiş olup, bu izolatı 2.50 indeks değeri ile *Raoultella ornithinolytica* YGP64en, 2.27 indeks değeri ile *Pseudomonas koreensis* YGL72ep izolatları izlemiştir (Çizelge 2; Şekil 4A). İkili kültür testlerinde yüksek antagonistik etkinliğe sahip olan *R. aquatilis* YGP63ep izolatının aynı zamanda yüksek düzeyde fosfor çözme yeteneğininde (2.57) olduğu belirlenmiştir. Diğer yandan *R. ornithinolytica* YGP64en izolatının ikili kültür testlerinde antagonistik etkinliğini çok düşük seviyede olmasına rağmen, fosfor çözme yeteneğinin yüksek düzeyde (2.27) olduğu belirlenmiştir. Bu durum antagonistik etkinliği yüksek olan tür/izolatların aynı anda bitki gelişimini teşvik etmede etkin olamayabileceğini göstermiştir (Çizelge 2; Şekil

4A). Kakao ağaçlarının kök bölgesinden izole edilen bakterilerin, kök çürüklüğü hastalığı etmeni olan *Phytophthora palmivora*'ya karşı antagonistik etkinlikleri araştırıldığı çalışmalarda, *Pseudomonas chlororaphis* olarak teşhisi yapılan 3 izolatın fungal etmene karşı yüksek antagonistik etkinlik gösterdiği, gösterilen antagonistik etkinliklerinin, siderofor ve HCN'den kaynaklandığı bildirilmiştir (Acebo-Guerrero ve ark., 2015). Ghazy ve ark., (2021) Mısır bitkisinde *Cephalosporium maydis*'in neden olduğu geç solgunluk hastalığına karşı PGP kök bakterilerinin potansiyelini araştırmışlardır. Altı izolatın (*Bacillus subtilis*, *B. circulance*, *B. coagulans*, *B. licheniformis*, *Pseudomonas fluorescens* ve *P. koreensis*) hem *in vitro* hem de *in vivo* koşullarda antagonistik etkinlikleri ile bakterilerin siderofor üretme potansiyelleri belirlemişlerdir. Sonuçta, en yüksek antagonistik etki ve siderofor üretimini yapan bakterilerin *B. subtilis* ve *P. koreensis* olduğunu bildirmişlerdir. Söz konusu çalışmada belirlenen *P. koreensis* izolatının siderofor üretiminin yüksek seviyede olması mevcut çalışmadaki sonuçları destekler nitelikte olmuştur.

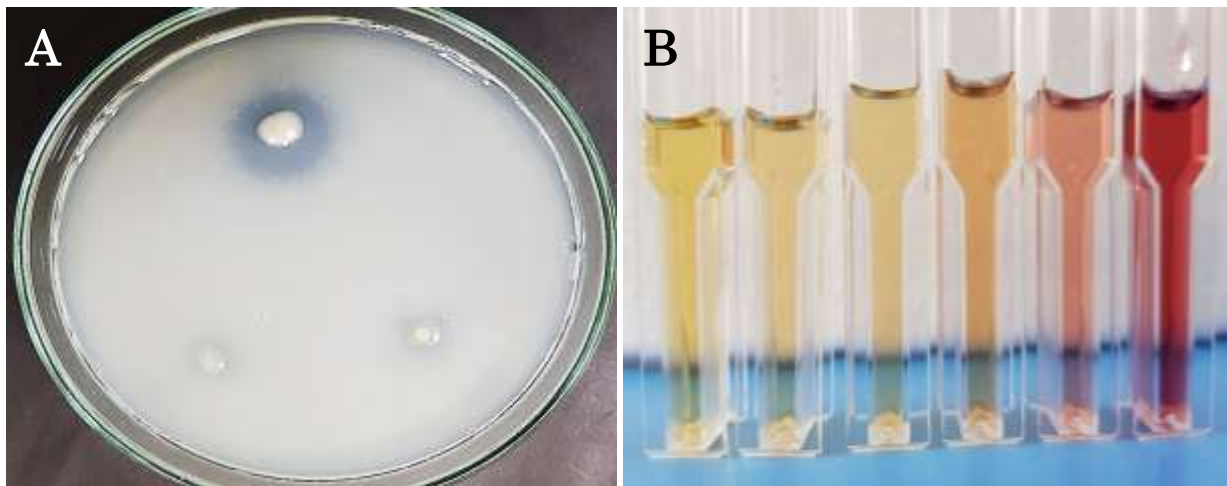
Çizelge 2. Seçilmiş antagonist bakteri izolatların fungal etmenlere karşı gösterdikleri olası biyokontrol ve bitki gelişimini teşvik eden etkinlik mekanizmalarının belirlenmesi<sup>a</sup>

Table 2. Determination of possible mechanisms of biocontrol and plant growth promoting activities of selected antagonist bacterial isolates against fungal agents<sup>a</sup>

| Bakteri izolatı                              | Misel gelişimi engelleyici antagonist mekanizmalar |                     |     |                 | Bitki gelişimini teşvik eden mekanizmalar |                     |
|--|--|---------------------|-----|-----------------|---|---------------------|
|  | Sid-İnd  | Pro-İnd             | HCN | NH <sub>3</sub> | Fosfor Çözme İndeksi                      | IAA (µg/ml)         |
| <i>Pseudomonas koreensis</i> YGL72ep         | 3.78 <sup>g</sup>                                  | 1.23 <sup>b-d</sup> | +   | +               | 2.27 <sup>c</sup>                         | 69.68 <sup>o</sup>  |
| <i>Bacillus simplex</i> YGS12ep              | 2.33 <sup>f</sup>                                  | 1.61 <sup>f-g</sup> | ++  | -               | 0.00 <sup>a</sup>                         | 16.88 <sup>m</sup>  |
| <i>Burkholderia cepacia</i> YGM75ep          | 2.30 <sup>f</sup>                                  | 1.09 <sup>b</sup>   | +++ | -               | 1.19 <sup>b</sup>                         | 4.46 <sup>d</sup>   |
| <i>Pseudomonas chlororaphis</i> YGM82ep      | 2.15 <sup>f</sup>                                  | 1.31 <sup>b-e</sup> | ++  | ++              | 0.00 <sup>a</sup>                         | 49.83 <sup>n</sup>  |
| <i>Raoultella ornithinolytica</i> YGP64en    | 1.73 <sup>d-e</sup>                                | 1.51 <sup>d-g</sup> | ++  | ++              | 2.50 <sup>d</sup>                         | 0.01 <sup>a</sup>   |
| <i>Lysinibacillus sphaericus</i> YGP3en      | 1.56 <sup>c-e</sup>                                | 0.00 <sup>a</sup>   | ++  | -               | 0.00 <sup>a</sup>                         | 10.97 <sup>k</sup>  |
| <i>Bacillus cereus</i> YGM3ep                | 1.55 <sup>c-e</sup>                                | 1.59 <sup>e-g</sup> | +   | -               | 0.00 <sup>a</sup>                         | 10.51 <sup>jk</sup> |
| <i>Bacillus mojavensis</i> YGM15en           | 1.55 <sup>c-e</sup>                                | 1.78 <sup>g</sup>   | ++  | -               | 0.00 <sup>a</sup>                         | 11.36 <sup>k</sup>  |
| <i>Bacillus cereus</i> YGK25en               | 1.49 <sup>b-d</sup>                                | 1.47 <sup>c-f</sup> | +   | -               | 0.00 <sup>a</sup>                         | 10.31 <sup>jk</sup> |
| <i>Bacillus megaterium</i> YGT18ep           | 1.45 <sup>b-d</sup>                                | 1.22 <sup>b-d</sup> | +   | +++             | 0.00 <sup>a</sup>                         | 6.96 <sup>sh</sup>  |
| <i>Rahnella aquatilis</i> YGP63ep            | 1.42 <sup>b-d</sup>                                | 1.19 <sup>b-c</sup> | ++  | -               | 2.57 <sup>d</sup>                         | 9.16 <sup>j</sup>   |
| <i>Curtobacterium flaccumfaciens</i> YGS25en | 1.34 <sup>b-d</sup>                                | 1.09 <sup>b</sup>   | +   | -               | 0.00 <sup>a</sup>                         | 0.59 <sup>ab</sup>  |
| <i>Rhizobium radiobacter</i> YGM77ep         | 1.24 <sup>bc</sup>                                 | 0.00 <sup>a</sup>   | ++  | -               | 0.00 <sup>a</sup>                         | 1.84 <sup>bc</sup>  |
| <i>Pantoea agglomerans</i> YGM81ep           | 1.22 <sup>b-c</sup>                                | 1.20 <sup>b-c</sup> | +   | -               | 0.00 <sup>a</sup>                         | 13.93 <sup>l</sup>  |
| <i>Bacillus vallismortis</i> YGL73ep         | 1.20 <sup>b-c</sup>                                | 1.08 <sup>b</sup>   | ++  | +               | 0.00 <sup>a</sup>                         | 2.39 <sup>c</sup>   |
| <i>Bacillus subtilis</i> YGS5en              | 1.16 <sup>b-c</sup>                                | 1.15 <sup>b</sup>   | +   | -               | 0.00 <sup>a</sup>                         | 7.93 <sup>hi</sup>  |
| <i>Staphylococcus succinus</i> YGL55ep       | 1.14 <sup>b-c</sup>                                | 1.22 <sup>b-d</sup> | ++  | -               | 0.00 <sup>a</sup>                         | 13.53 <sup>l</sup>  |
| <i>Bacillus cereus</i> YGP1en                | 1.10 <sup>b-c</sup>                                | 1.36 <sup>b-f</sup> | ++  | -               | 0.00 <sup>a</sup>                         | 5.96 <sup>e-g</sup> |
| <i>Cronobacter sakazakii</i> YGKL12ep        | 1.05 <sup>b</sup>                                  | 0.00 <sup>a</sup>   | ++  | -               | 1.09 <sup>b</sup>                         | 7.46 <sup>h</sup>   |
| <i>Acinetobacter johnsonii</i> YGM76en       | 0.00 <sup>a</sup>                                  | 0.00 <sup>a</sup>   | -   | -               | 0.00 <sup>a</sup>                         | 5.38 <sup>d-f</sup> |
| <i>Acinetobacter lwoffii</i> YGS37en         | 0.00 <sup>a</sup>                                  | 0.00 <sup>a</sup>   | +   | -               | 0.00 <sup>a</sup>                         | 7.73 <sup>hi</sup>  |
| <i>Bacillus thuringiensis</i> YGT22en        | 0.00 <sup>a</sup>                                  | 1.47 <sup>c-f</sup> | ++  | -               | 0.00 <sup>a</sup>                         | 6.75 <sup>f-h</sup> |
| <i>Klebsiella oxycota</i> YGP62en            | 0.00 <sup>a</sup>                                  | 0.00 <sup>a</sup>   | +   | -               | 0.00 <sup>a</sup>                         | 4.87 <sup>de</sup>  |
| <i>Siccibacter colletis</i> YGP67ep          | 0.00 <sup>a</sup>                                  | 0.00 <sup>a</sup>   | ++  | -               | 1.14 <sup>b</sup>                         | 1.45 <sup>a-c</sup> |
| <i>Kosakonia cowanii</i> YGP7en              | 1.93 <sup>e-f</sup>                                | 1.13 <sup>b</sup>   | ++  | -               | 1.16 <sup>b</sup>                         | 0.02 <sup>a</sup>   |

**Sid-İnd:** Siderofor; **Pro-İnd:** Protease; **NH<sub>3</sub>:** Amonyak; **HCN:** Hidrojen Siyanür; -, renk değişimi olmadığını, ±, +, ++, +++ ise HCN veya amonyak konsantrasyonunun artışına paralel renk değişim şiddetindeki artışı gösterir. Kalın olarak yazılmış izolatlar testlerde yüksek etkinlik gösteren izolatlardır.

<sup>a</sup> Aynı sütun içerisinde yer alan ortalama değerlerin yanındaki aynı harfler izolatlar arasındaki farkın istatistiksel olarak önemli olmadığını gösterir (Duncan Çoklu Karşılaştırma Testi, P<0.05)



Şekil 4. Antagonist bakteri izolatlarının (A) PVK besi yerinde fosforu çözme, (B) L-tryptophane içeren LB besi yerinde IAA üretim potansiyelleri (ok).

Figure 4. Potentials of bacterial isolates to solubilise of phosphorus on PVK media (A) and production of IAA in LB medium containing L-tryptophane (arrow)



Gusain ve ark., (2015) Himalaya dağlarındaki yağmur ormanlarından izole ettikleri ve *Pseudomonas koreensis*, *Arthrobacter nitroguajacolicus* YB4 ve *Klebsiella oxytoca* olarak tanımladıkları bakteri izolatlarının fosforu çözme, IAA ve siderofor üretme potansiyellerinin belirlenmesine yönelik yaptıkları çalışmalarında, 4 izolatında yüksek düzeylerde siderofor ve IAA üretme yeteneğinde olduklarını, *P. koreensis*, *A. nitroguajacolicus* ve *K. oxytoca* izolatlarının fosforu çözme etkinliğinde bulunduğunu, 5 izolatın uygulandığı bitkilerde fosfor alımının arttığı, bitki biyokütlelerinde (yaş ve kuru ağırlık olarak) ciddi ağırlık artışlarının olduğu belirlenmiştir. Aralarında *P. chloropsis* ve *Pseudomonas* spp., ait izolatların fungal hastalık etmenlerinin misel gelişimlerini yüksek düzeyde engellediği, engellemede özellikle fenazin, HCN, siderofor ve IAA ürettikleri, ancak topraktaki bağlı fosforu çözmede etkili olmadıkları, fakat aralarından 5 izolatın toprakta bağlı çinkoyu çözebildiklerini böylece bitki gelişimini önemli düzeyde teşvik ettikleri bildirilmiştir (Shahid ve ark., 2017; Nandi ve ark., 2017). Türkiye’de farklı konukçu-patojen ilişkilerin irdelendiği benzer biyolojik mücadele çalışmalarında *Pseudomonas* ve *Bacillus* türlerine ait olan PGPB ve PGPR izolatlarının başta toprak kökenli olmak üzere bir çok önemli fungal hastalık etmenlerinin engellenmesinde ve bitki gelişimini teşvik etmede siderofor, proteaz, amonyak, HCN gibi antagonistik; fosforu çözme, IAA üretme gibi mekanizmalar ile etkili olduğuna yönelik çalışmalar bulunmaktadır (Soylu ve ark., 2005; Yıldız ve ark. 2012; İmriz ve ark., 2014; Atay ve ark., 2020; Aktan ve Soylu, 2020; Kara ve ark., 2020; Soylu ve ark., 2020; Soylu ve ark., 2021; Soylu ve ark., 2022; Kara ve Soylu, 2022).

### Bakteri İzolatlarının Uçucu Bileşen(ler)inin *in vitro* Antagonistik Etkinliğinin Belirlenmesi

Bakteri izolatlarının uçucu bileşen(ler)inin *in vitro* antagonistik etkinliğinin belirlenmesine yönelik yapılan çalışmalarda ikili kültür testlerinde 3 farklı fungal etmene karşı “yüksek” etkinlik gösteren *B. cereus* YGK25en, *B. thuringiensis* YGT22en, *B. vallismortis* YGL73ep, ve *P. chlororaphis* YGM82ep izolatları ile çalışmalar yapılmıştır (Çizelge 3, Şekil 5). Yapılan kapalı ikili kültür test sonuçlarına göre 3 farklı fungal etmene karşı en yüksek antifungal etkinlik *P. chlororaphis* YGM82ep izolatı tarafından gösterilmiştir. *P. chlororaphis* YGM82ep izolatı tarafından üretilen uçucu bileşenler *G. citri-aurantii*, *C. gloeosporioides* ve *F. solani*’nin misel gelişimini sırasıyla %89.2, %82.6, %57.1 oranında engellediği belirlenmiştir. Test edilen *Bacillus thuringiensis* YGT22en izolatı ise *G. citri-aurantii*’nin misel gelişimini %77.7 gibi yüksek oranda engellediği gözlenmiştir (Şekil 5). Çizelge 3’de sunulan izolatların uçucu bileşenlerinin antagonistik etkinlikleri, Çizelge 2’de sunulan *in vitro* ikili kültür test sonuçlarıyla karşılaştırıldığında, *Pseudomonas chlororaphis* YGM82ep izolatının uçucu bileşenlerinin *G. citri-aurantii* ve *C. gloeosporioides*’e, *Bacillus thuringiensis* YGT22en izolatının uçucu bileşenlerinin *G. citri-aurantii*’ye, *Bacillus vallismortis* YGL73ep izolatının uçucu bileşenlerinin ise *F. solani*’ye karşı daha yüksek düzeylerde etkinlik gösterdiği tespit edilmiştir. Diğer izolatların uçucu bileşenlerinin antifungal etkinliklerinde *in vitro* ikili kültür test sonuçlarıyla kıyaslandığında daha düşük etkinlik göstermiştir (Çizelge 3).

Çizelge 3. Antagonist etkinliği yüksek izolatlar tarafından üretilen uçucu organik bileşenlerinin *in vitro* ikili kültür testlerinde fungal etmenlerin misel gelişiminin engellenmesi üzerine olan antifungal etkinlikleri  
Table 3. Antifungal activity of volatile organic compounds produced by isolates with high antagonist activity on inhibition of mycelial growth of fungal agents in vitro dual culture tests

| Antagonist bakteri izolatları           | Fungal etmenler    |                    |                          |                    |                           |                    |
|---|--------------------|--------------------|--------------------------|--------------------|---------------------------|--------------------|
|   | <i>F. solani</i>   |                    | <i>G. citri-aurantii</i> |                    | <i>C. gloeosporioides</i> |                    |
|   | MG<br>(mm)         | MGE<br>(%)         | MG<br>(mm)               | MGE<br>(%)         | MG<br>(mm)                | MGE<br>(%)         |
| <i>Kontrol</i>                          | 81.67 <sup>d</sup> | -                  | 90.00 <sup>e</sup>       |                    | 86.33 <sup>e</sup>        |                    |
| <i>Pseudomonas chlororaphis</i> YGM82ep | 35.00 <sup>a</sup> | 57.15 <sup>D</sup> | 9.67 <sup>a</sup>        | 89.26 <sup>Y</sup> | 15.00 <sup>a</sup>        | 82.63 <sup>Y</sup> |
| <i>Bacillus vallismortis</i> YGL73ep    | 34.33 <sup>a</sup> | 57.97 <sup>Y</sup> | 46.67 <sup>c</sup>       | 48.14 <sup>D</sup> | 73.67 <sup>c</sup>        | 14.67 <sup>D</sup> |
| <i>Bacillus thuringiensis</i> YGT22en   | 60.00 <sup>c</sup> | 26.54 <sup>D</sup> | 20.00 <sup>b</sup>       | 77.78 <sup>Y</sup> | 79.67 <sup>d</sup>        | 7.72 <sup>D</sup>  |
| <i>Bacillus cereus</i> YGK25en          | 50.00 <sup>b</sup> | 38.78 <sup>D</sup> | 63.33 <sup>d</sup>       | 29.63 <sup>D</sup> | 58.33 <sup>b</sup>        | 32.44 <sup>D</sup> |

MG: Misel gelişimi (mm); MGE: Misel gelişiminin engellenme oranı (%)

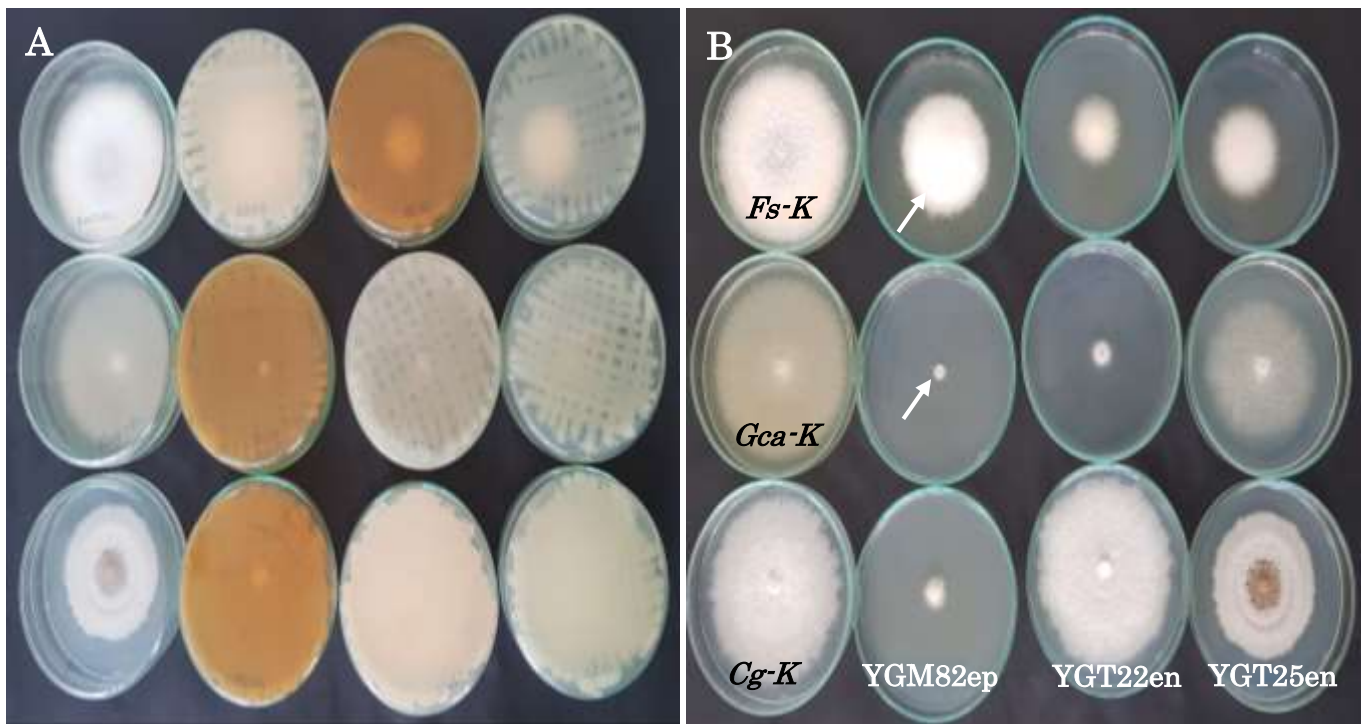
Sütun içinde yer alan ortalama değerlerin yanındaki farklı küçük harfler uygulamalar arasındaki farkın istatistiksel olarak Duncan Çoklu Karşılaştırma testine göre önemli olduğunu gösterir (P<0.05).

D ve Y: Uçucu bileşen(ler)in antagonist etkinliğinin ikili kültür testlemesine göre daha düşük (D) veya daha yüksek (Y) düzeyde olduğunu gösterir.



Farklı *Pseudomonas* ve *Bacillus* türlerine ait antagonist bakteri izolatları tarafından besiyerlerine difüze olabilen (2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, iturin, surfactin, fengycin vb.) antimikrobiyal etkinliğe sahip bileşenlerin yanısıra uçucu organik metabolitler (VOCs, Volatile Organic Compounds) üreterek patojen gelişimini engellediği/baskıladığına yönelik bir çok çalışma bulunmaktadır (Raio & Puopolo, 2021; Dimkic ve ark., 2022). Çalışmalarda etkili olarak belirlenen *P. chlororaphis* izolatının ürettiği oldukları 3-Methyl-1-butanol, Methanethiol, ve Butanediol gibi uçucu bileşenlerin sedir ağaçlarında fungal etmenlerden *S.*

*cardinale*, tatlı patatesten *Ceratocystis fimbriata*, tütün bitkisinde *E. caratovora*'ya karşı yüksek düzeyde antifungal ve antibakteriyel etkinlik gösterdiği, *Arabidopsis thaliana* bitkisinde ise kuraklığa karşı bitkinin dayanıklılığını artırdığı bildirilmiştir (Han ve ark., 2006; Cho ve ark., 2008; Zhang ve ark., 2019; Raio ve ark., 2020). Benzer şekilde, farklı *Bacillus* spp. ait antagonist izolatlar tarafından üretilen uçucu organik metabolitlerin yapılan *in vitro* ve *in vivo* testlerle farklı bitkilerinde hastalık etmeni olan *Fusarium* spp. karşı antifungal etkiye sahip oldukları bildirilmiştir (Khalaf ve Raizada, 2018; Baez-Vallejo ve ark., 2020; Toloza-Moreno ve ark., 2020; Ezrari ve ark., 2021).



Şekil 5. Farklı antagonist bakteri izolatların ait uçucu bileşenlerin fungal etmenlerin misel gelişimi üzerine antagonistik etkinlikleri. (A) Kapalı ikili kültür testinde üst petri kapağında bakteri, (B) alt kapaktaki besiyerinde uçucu bileşenlere maruz kalmış fungal etmenlerin misel gelişimi (ok).

Figure 5. Antagonistic activity of volatile components produced by different antagonist bacterial isolates on mycelial growth of fungal agents. Mycelial growth of fungal agents (arrow) exposed to bacterial isolates in the upper petri dish (A) and volatile components in the lower dish in a closed dual culture test (B).

## SONUÇ ve ÖNERİLER

Sağlıklı turuncu ağaçlarının kök, gövde, sürgün ve meyvelerinde izole edilen endofit ve epifit BCA izolatlarının *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii* ve *Fusarium solani*ye karşı antifungal etkinliğinin yanısıra, BCA izolatların antagonistik ve bitki gelişimini teşvik eden olası bazı mekanizmaları yapılan çalışmalarla ortaya konulmuştur. Test edilen bakteri izolatlarının sahip oldukları antagonistik ve bitki gelişimini teşvik eden mekanizmaları değerlendirildiğinde *Bacillus* ve bazı *Pseudomonas* spp. ait BCA izolatların oldukça etkili

olduğu görülmüştür. İzolatların test edildikleri mekanizmalardaki etkinlikleri türlere veya izolatlara bağlı değişiklik göstermiştir. Antagonistik etkinliği yüksek olan izolatların göstermiş oldukları biyoetkinliklerinde birden fazla antagonistik mekanizmaların rol oynadığı tespit edilmiştir. Gelecekte bitki hastalıklarıyla mücadelede biyopreparat ve/veya biyogübre olarak kullanılacak ticari tarımsal üründe yüksek etkinlik gösteren bu izolatların karışım halinde biyoförmüle edilmesinin en uygun seçenek olduğu değerlendirilmiştir.

## 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.

## Teşekkür

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## Mortality Effects of *Beauveria bassiana* and *Purpureocillium lilacinum* Isolates and Efficacy of a Wettable Formulation on *Palemona prasina* (Hemiptera: Pentatomidae) Nymphs

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### ABSTRACT

In this study, the mortality effect of two *Beauveria bassiana* and two *Purpureocillium lilacinum* isolates on the 4th stage nymphs of *Palemona prassiana* was determined. The most lethal isolate was formulated as wettable powder and tested on the pest. Furthermore, Y-tube olfactometry tests were conducted to detect behavioral response of the nymphs in presence of the fungus. All the experiments were carried out under controlled conditions. The mortality varied depending on the isolates between 28.51% and 82.14% on the 12<sup>th</sup> day. *Beauveria bassiana* FAI-38 caused the highest mortality (82.14% at  $1 \times 10^8$  conidia ml<sup>-1</sup>) with LC<sub>50</sub> and LT<sub>50</sub> estimations of  $3.3 \times 10^6$  conidia ml<sup>-1</sup> and 8.4 days, respectively. According to data taken 6 and 12 days after application, the wettable powder formulation was found to be significantly more effective (89.65% at  $1 \times 10^7$  conidia ml<sup>-1</sup>, LT<sub>50</sub> 6.08 days). According to the Y-tube olfactometry tests, the nymphs exhibited avoidance from unformulated *B. bassiana* spores; however, once the spores were formulated as wettable powder, the behavior of the insects changed to neutral. It is concluded that *Beauveria bassiana* FAI-38 presents a potential as a control agent, and the wettable powder formulation of the fungus improves its effectiveness by increasing mortality and removing repellency effect of the fungal spores.

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## *Beauveria bassiana* ve *Purpureocillium lilacinum* İzolatlarının *Palemona prasina* (Hemiptera: Pentatomidae) Nimflerine Ölüm Etkisi ve Islanabilir Toz Formülasyonun Etkinliği

### ABSTRACT

Bu çalışmada, *Palemona prassiana*'nın dördüncü dönem nimflerine iki *Beauveria bassiana* ve iki *Purpureocillium lilacinum* izolatının ölümcül etkileri belirlenmiş, en yüksek ölüm etkisi gösteren izolatın bir ıslanabilir toz formülasyonu hazırlanarak test edilmiştir. Ayrıca, zararlının fungusa gösterdiği davranış tepkisi Y-tüpü olfaktometre testleri ile ortaya konmuştur. Tüm denemeler kontrollü şartlar altında gerçekleştirilmiştir. Ölüm oranları izolata bağlı olarak 12. günde %28.51 ile %82.14 arasında farklılık göstermiştir. En yüksek ölüm oranı *Beauveria bassiana* FAI-38 izolatında gözlenmiştir ( $1 \times 10^8$  konidi/ml konsantrasyonda %82.14) ve LC<sub>50</sub>, LT<sub>50</sub> değerleri sırasıyla  $3.3 \times 10^6$  konidi/ml ve 8.4 gün olarak hesaplanmıştır. Uygulamadan 6 ve 12 gün sonra alınan verilere göre, ıslanabilir toz formülasyonu önemli derecede daha etkili bulunmuştur ( $1 \times 10^7$  konidi ml<sup>-1</sup>'de %89.65, LT<sub>50</sub> 6.08 gün). Y-tüpü olfaktometre testlerine göre, nimfler, formüle edilmemiş *B. bassiana* sporlarından uzaklaşma tepkisi sergilemiştir. Fungus sporlar ıslanabilir toz olarak formüle edildiğinde böcekler herhangi bir davranış göstermemişlerdir. *Beauveria bassiana* FAI-38'in mücadele etmeni olarak potansiyel gösterdiği; ölüm oranını artırması ve sporların uzaklaştırıcı etkisini ortadan kaldırması nedeniyle ıslanabilir toz formülasyonun fungusun etkinliğini geliştirdiği sonucuna varılmıştır.

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## INTRODUCTION

Green shield bug, *Palomena prasina* L. (Hemiptera: Pentatomidae) is an important pest of hazelnuts causing yield and quality losses in Turkey and other hazelnut-producing countries. Exporters' associations complain about poor quality kernels mostly due to green shield bugs. This pest causes yield reduction and reduced quality (shriveled and/or spotted kernels) (Ak et al., 2017), the latter of which becomes quite significant, especially for those produced for export (Kurt, 1975). Currently, two pesticide applications are required to control this pest in hazelnut orchards in Turkey. The first is in the second half of April against overwintered adults, and the second one in the first half of July if the nymphs are detected in orchards. Unfortunately, the only available control method for this pest is spraying chemical insecticides. Appropriate selection of plant protection products is of great importance because the second application is very close to harvest imposing risks for chemical residues in export products. Besides, although chemical insecticides are effective, concerns about their negative side effects on the environment and people directed researchers to seek alternatives suitable for sustainable agriculture, organic farming, and protecting biological diversity. As an alternative approach, biological control has been used or evaluated for various insect pests. It is considered to be widespread all over the world in the future due to pest resistance to effective chemicals, difficulties in developing new ones, public concerns about food safety and genetically modified agricultural products, and increasing demand for organic products (Birişik, 2015). The International Organization for Biological Control (IOBC) estimated that the share of biological control products in the total plant protection product market will be 30-35% in 2050. Today, around 800 chemical agents are widely used in the world to control pest organisms.

There are a few studies on the biological control of hazelnut green shield bug using entomopathogenic fungi. Erper et al. (2016) tested isolates of *Beauveria bassiana*, *Isaria fumosorosea*, *Simplicillium lamellicola*, *Lecanicillium muscarium* to the nymphs in controlled conditions, and found that the *B. bassiana* and one *L. muscarium* were the most virulent with 95 and 98% mortality, respectively, in 12 days. Özdemir (2021) obtained even higher mortalities in a shorter time using isolates from seven species including *B. bassiana*, *B. pseudobassiana*, *I. fumosorosea*. Yiğit (2022) applied *B. bassiana*, *Metarhizium anisopliae*

and *I. fumosorosea* against nymphs and adults under controlled conditions and in the field. They found one of the *B. bassiana* isolates as the most promising. The isolate caused 75-80% mortality in 14 days in field trials. Studies were conducted under controlled conditions with the exception of a field trial by Yiğit (2022), where fungal spores were applied without formulating. For the success of *B. bassiana* applications, the fungus should have persistence on foliage as much as high pathogenicity to the targeted pest. Once a potential entomopathogenic fungus is obtained, formulation of the fungus can help to fulfill this requirement with an even higher potency as a biological control agent. There are studies on testing formulated entomopathogenic fungi on other pest species in the family Pentatomidae. Parker et al. (2015) used wettable powder and emulsifiable suspension formulations of *B. bassiana* against nymphs of brown marmorated sting bug, wettable powder formulation being more efficacious. Sosa-Gomez et al (1998) tested kaolin-based powder formulations of *M. anisopliae* and *B. bassiana* for the control of *Nezara viridula*, *Piezodorus guildinii*, and *Euschistus heros* in soybean plots.

One other issue for the success of a fungus is concerned with the behavior of the pest insect towards the fungus to be applied. For infection to occur, the fungus should come in contact with the insect's body during its foraging activity. Recognition of the pathogen and avoidance by the insect can hinder the success of the application of the fungus as a biological control agent (Doğan et al., 2017). The level of such behavioral response was found to depend on insect-fungus interaction and spore concentration (İncir, 2018). It is mostly demonstrated that insects show avoidance from entomopathogenic fungi (Baverstock et al., 2010; Wei et al., 2020; Avery et al., 2021; Daisy 2022; Geedi et al., 2022). However, fungal spores mixed with some compounds or ingredients for combined applications or to formulate the spores did not induce such avoidance behavior (Wang & Powell 2004; Fernandez-Grandon et al. 2020). Therefore, formulating entomopathogenic fungi at appropriate concentrations can increase the efficacy (Wang & Powell, 2004).

In this study, a potential entomopathogenic fungus was selected amongst isolates obtained from hazelnut green shield bugs, and it was formulated for higher pathogenicity. The avoidance behavior of the pest was also tested for its suitability for later field applications.

## MATERIAL and METHODS

### Insect Culture

Overwintered *Palomena prasina* adults were collected from hazelnut orchards to start a culture. Adults were maintained at  $25\pm 2^{\circ}\text{C}$  and  $75\pm 5\%$  relative humidity in 16:8 (L:D) photoperiod in a climate room. To obtain their eggs, food, water, and filter paper were placed inside glass jars. Beans that were grown in a greenhouse in the hazelnut research institution were used as food. Before using the beans were cleaned by dipping in 3% sodium bicarbonate solution for 5 minutes. As a supplement, 5-10 pieces of unsalted sunflower seeds or half a slice of apple were also provided. The green beans, apples, and water were changed every other day, while sunflower seeds were changed every 4-5 days. Egg clusters were transferred to a 150 ml plastic cup and the insect-rearing room was kept until hatching. Once they reached the second stage, the nymphs were provided with water and green beans. The nymphs at the 4<sup>th</sup> stage were used in the experiments.

### Fungi

In this study, two *Beauveria bassiana* and two *Purpureocillium lilacinum* isolates were tested to evaluate their pathogenicity against *P. prasina*. The fungi were previously isolated from *Palomena prasina* adults and identified with morphological and molecular studies (unpublished data). *B. bassiana* FAI38 and FAI22 were isolated from hosts collected from the Central District of Giresun Province and Perşembe District of Ordu Province, respectively. *P. lilacinum* FAI1 and FAI70 were obtained from hosts gathered from the Central District of Giresun Province and Gülyalı District of Ordu Province, respectively.

### Preparing conidial suspensions

First, the fungi were inoculated on PDA and kept in the dark at  $26\pm 2^{\circ}\text{C}$  for 4 weeks. The conidia on the surface were collected with a sterile polystyrene spreader within 0.02% tween80 solution. This suspension was passed through sterile gauze after vortexing and the concentration was determined by using a hemocytometer. The density was adjusted by diluting to the concentration to be used in the test. Conidia viability was determined by germination test. 100  $\mu\text{l}$  suspension prepared at  $1\times 10^5$  conidia  $\text{ml}^{-1}$  was spread on 1.5% aqueous agar and incubated in a closed Petri dish at  $26\pm 2^{\circ}\text{C}$  in the dark for 24 hours. During examination under a light microscope, conidia with germ tubes equal to or longer than spore length or diameter were considered germinated.

### Fungus Screening Bioassay

Each experimental unit was set by using two 155 ml plastic cups, one upside down on top of the other one,

forming 310 ml space for the insects. The bottom of the cup on the top was cut off and covered with tulle for aeration prior to the setup. In each experimental unit, a piece of twig with a three-leaved fruit peel was used as nutrition for the insects and 10 ml of distilled water was added to keep the plant alive. The husk fruits with leaves were collected from hazelnut trees where foliar plant protection products had not been applied in Giresun Hazelnut Research Institute. In order to keep the coniferous leaf fresh, 1 mm was cut from the petiole every other day. Ten 4<sup>th</sup> instar hazelnut green shield bug nymphs were placed in each unit. Two ml conidial suspension ( $1\times 10^8$  conidia  $\text{ml}^{-1}$ ) of designated fungus was applied to the nymphs by a hand sprayer. The nymphs in control units were sprayed with sterile %0.02 tween80 without fungal spores. The experiment had three replications and was carried out at  $26\pm 2^{\circ}\text{C}$  temperature,  $75\pm 5\%$  relative humidity in a climate cabinet with 16 h light / 8 h dark photoperiod. The insects were checked on the 6<sup>th</sup> and 12<sup>th</sup> days, dead insects were counted, recorded, and removed from the experimental units. The Abbott formula was used to arrange the control measurements. The arcsine transformation was applied to the mortality rates. They were subjected to one-way ANOVA and Duncan multiple comparison tests using IBM SPSS statistics 23 program.

### Preparation and efficacy of *Beauveria bassiana* FAI38 Formulation

A wettable powder formulation of *Beauveria bassiana* FAI38 was prepared and tested against *P. prasina*. The fungus conidia were obtained using the solid fermentation method described by Barış & Er (2021). Rice was used as a substrate for the fermentation process. The spores obtained in powder form were stored at  $+4^{\circ}\text{C}$  until the formulation was prepared. The formulation included 68% rice flour, 20% conidia ( $4\times 10^{11}$  conidia  $\text{kg}^{-1}$ ), and 12% adjuvants and surfactant. The formulation was used at the ratio of 2.5 gr per 1 lt water for applications. The experiment was conducted and the data were analyzed as described above for Fungus Screening Bioassays. The formulation was used at the above-mentioned ratio (1X) and its 10-fold (10X) together with two corresponding concentrations ( $1\times 10^6$  and  $1\times 10^7$  conidia  $\text{ml}^{-1}$ ) of the fungal conidia without formulating for comparison. The nymphs in control units were sprayed with %0.02 tween, sterile distilled water, and without fungal spores. The insects were checked on 6<sup>th</sup> and 12<sup>th</sup> days, dead insects were counted, recorded, and removed from the experimental units. An additional experiment was carried out to calculate the  $\text{LT}_{50}$  of the formulation for 10X application according to the method described above for the estimation of  $\text{LT}_{50}$  value of unformulated spores.



## Olfactometer Bioassays

In order to determine the behavioral response of *P. prasina* nymphs to the presence of *B. bassiana* spores, four sets of Y-tube olfactometry tests were carried out. In each test, the insects were presented with two choices of food (3 fruit husks and one hazelnut leaf) with different treatments. All the treatments were achieved by spraying 2 ml of designated suspension onto the food while the controls received the same amount of %0.02 tween and distilled water. Four sets of Y-tube olfactometry tests were (1) unformulated FAI-38 spores vs control, (2) FAI-38 formulation vs control, (3) FAI-38 formulation vs formulation ingredients without spores, and (4) unformulated FAI-38 vs FAI-38 formulation. Unformulated spores were applied at  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  concentration and the formulation at 10X application ratios.

The lengths of the main arm and the side arms of the olfactometer were 10 cm and 24 cm, respectively. It was made of glass with 0.4 cm thickness. The air pressure was kept constant and was monitored with barometers connected to the entrance and exit of the olfactometer. The air was cleaned before entering the olfactometer by passing it through a glass balloon containing activated charcoal and then through a microfilter. The experiment was conducted under constant light at  $26 \pm 2$  °C temperature in a room. Between each trial, the Y-tube olfactometer assembly was cleaned with acetone, washed with distilled water, and dried in an oven at 150 °C for 40 minutes. In each test, thirty 4th instar nymphs were used individually. The nymphs were kept without food for 10 hours prior to the tests. Fifteen minutes were given for each insect to choose one of two arms after placing the insect at the entrance. Those that moved at least 5 cm into one arm were considered to make a decision. When an individual did not enter either of the arms before the end of the period, it was considered with no choice. Each experiment was repeated after the choices were replaced on opposite arms of the Y-tube olfactometer to eliminate any possible effects of the placement.

The chi-square test was applied to data once the preferences. The t-test was used to determine whether changing places of choice in the olfactometer makes a difference.

## RESULTS and DISCUSSION

For fungus screening test, two *Beauveria bassiana* and two *Purpureocillium lilacinum* isolates were tested against 4<sup>th</sup> instar hazelnut green shield bug nymphs. Mortality data 6 and 12 days after treatment are presented in Figure 1.

Variation amongst 6 days post-treatment mortalities were statistically insignificant except for *P. lilacinum* FAI-1 causing lower nymph mortality than the rest. The mortality rate at the end of the 12<sup>th</sup> day varied

between 28.51% and 82.14%. *Beauveria bassiana* FAI-38 provided the highest mortality  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  (82.14%) while the other isolates resulted in significantly lower nymphal mortalities. Therefore, *B. bassiana* FAI-38 was chosen as the most promising isolate and used in later experiments. Additionally, the mortality effect of *B. bassiana* FAI-38 was investigated further by concentration and time dependent probit analyses. Probit line equation for concentration-mortality relation was  $y = -4.434 + 0.680x$  (Figure 2) with  $LC_{50}$ ,  $LC_{95}$  estimations of  $3.3 \times 10^6$  conidia/ml (95% c.i.:  $1 \times 10^6 - 8.53 \times 10^6$ ) and  $8.66 \times 10^9$  conidia  $\text{ml}^{-1}$  (95% c.i.:  $2.18 \times 10^8 - 9.61 \times 10^9$ ), respectively. The equation for time-mortality relation was  $y = -2.039 + 0.243x$  (Figure 3) with  $LT_{50}$  and  $LT_{95}$  values 8.403 days (95% c.i.: 7.781 - 9.117) and 15.183 days (95% c.i.: 13.750 - 17.280), respectively.

Erper et al. (2016) achieved 95% nymphal mortality at the end of the 12<sup>th</sup> day in an experiment where they applied *B. bassiana* at  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  concentration at 25°C temperature and  $90 \pm 5\%$  relative humidity. Ozdemir (2021), Yigit (2022) reached up to 100% mortality at the end of the 14 days after spraying *B. bassiana* at  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  at 25°C temperature. Since Ozdemir (2021) carried the experiment in sealed petri dishes and Yigit (2022) in closed cups, the air humidity was most likely to be rather high favoring initiation of fungal infection faster and by more spores. Similarly, the experiment of Erper et al. (2016) was conducted at high ambient humidity. Ambient temperature and humidity, ventilation, light, air and the condition of the host itself have important effects on the pathogenicity of entomopathogenic fungi (Padmini & Padmaja, 2010). As many *Beauveria* isolates show host specificity (Thakur & Sandhu, 2010), at least some of the differences in mortality levels could be due to the isolates themselves.

The wettable powder formulation of *B. bassiana* FAI38 was tested against *P. prasina* nymphs at 1X and 10X and the results are given in Figure 4 along with the results of corresponding unformulated concentrations of the spores. Variation in mortality was significant. The efficacy of the formulations was significantly higher than their corresponding unformulated conidia treatments both on the 6<sup>th</sup> and 12<sup>th</sup> days post application. The formulation at 1X delivered statistically the same result with 10 fold unformulated conidia, indicating a requirement of about 10-fold less conidia with formulation to reach the same mortality level. The equation for the time-mortality relation was  $y = -1.139 + 0.187x$  (Figure 5) with  $LT_{50}$  and  $LT_{95}$  values 6.076 days (95% c.i.: 5.287 - 6.825) and 14.849 days (95% c.i.: 13.131 - 17.518), respectively. Both  $LT_{50}$  and  $LT_{95}$  values for formulation were lower than those for unformulated conidia even though unformulated conidia were used at higher spore concentration (10X formulation application corresponds to  $1 \times 10^7$  conidia

ml<sup>-1</sup>, unformulated spore concentration was 1×10<sup>8</sup> conidia ml<sup>-1</sup>). While the confidence intervals of the LT<sub>95</sub> values coincide, the confidence intervals for the LT<sub>50</sub>

values are well separated, showing faster effect of the formulation comparing to unformulated conidia.

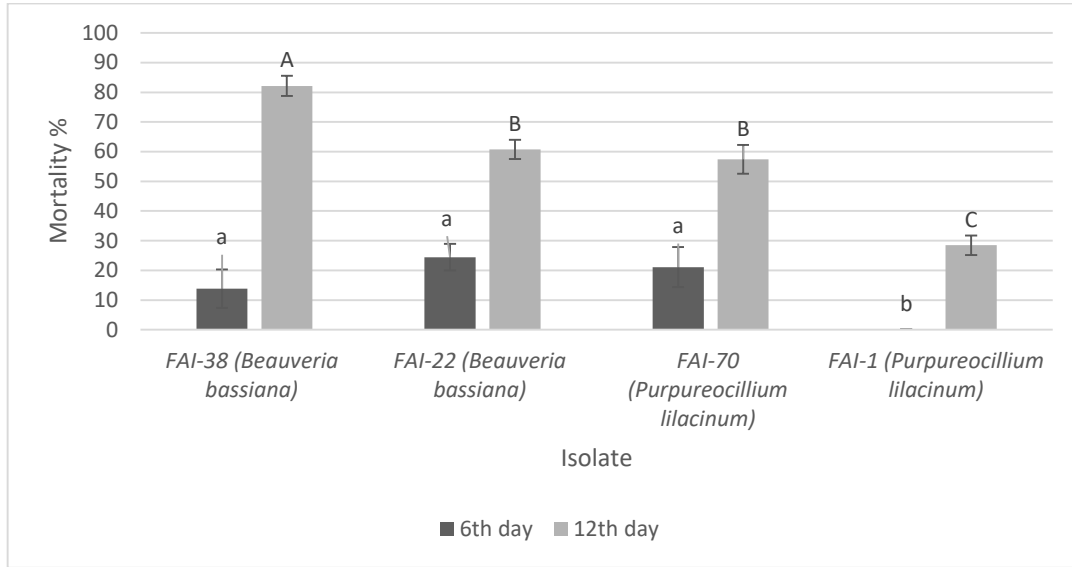


Figure 1. Corrected mortality of *Palomena prasina* nymphs after application of entomopathogenic fungi at the concentration of 1×10<sup>8</sup> conidia/ml (Data were subjected to Abbott's correction formula; control mortalities were 0-6,66%, one-way ANOVA was applied to the data and the differences between treatments were determined by Duncan test (n=3), error bars indicate standard errors, different letters within each time indicate statistically important differences, 6th day: F<sub>3,8</sub>=13.383 P=0.002 and 12th day: F<sub>3,8</sub>=31.374 P=0.000).

Şekil 1. Entomopatojen fungus izolatlarının 1×10<sup>8</sup> konidi ml<sup>-1</sup> konsantrasyonda uygulandıktan sonra *Palemona prasina* nimflerinin düzeltilmiş ölüm oranları (Verilere Abbott'un düzeltme formülüne uygulanmıştır; kontrol ölümleri %0-6,66 arasında değişmektedir; verilere tek yönlü ANOVA uygulanmış ve uygulamalar arasındaki farklar Duncan testi ile belirlenmiştir (n=3), hata çubukları standart hataları göstermektedir, aynı gündeki farklı harfler istatistiksel olarak önemli farklılıkları göstermektedir, 6th day: F<sub>3,8</sub>=13.383 P=0.002 and 12th day: F<sub>3,8</sub>=31.374 P=0.000)

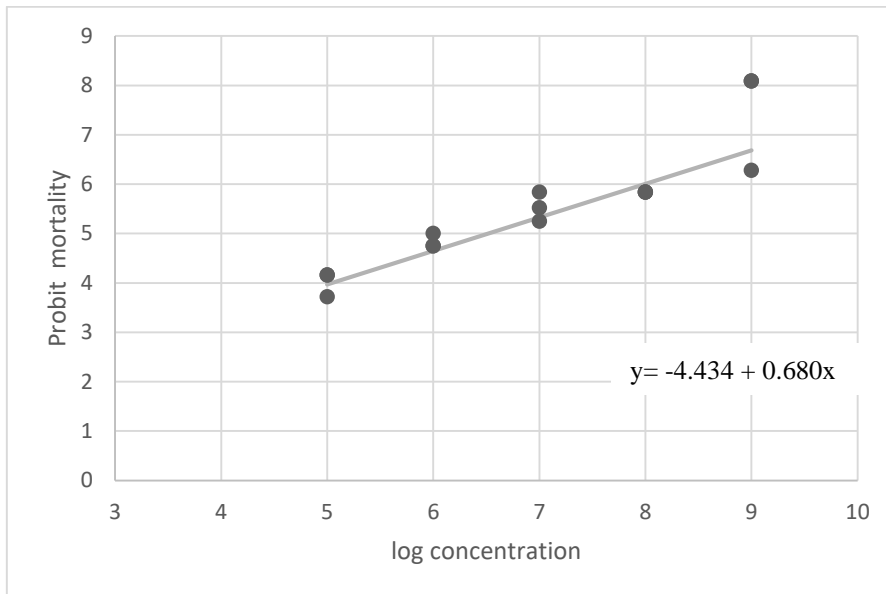


Figure 2. Concentration-dependent mortality of *Palomena prasina* nymphs 12 days after *Beauveria bassiana* FAI-38 applications

Şekil 2. *Beauveria bassiana* FAI-38 uygulamalarından 12 gün sonra *Palomena prasina* nimflerinin konsantrasyona bağlı ölümleri

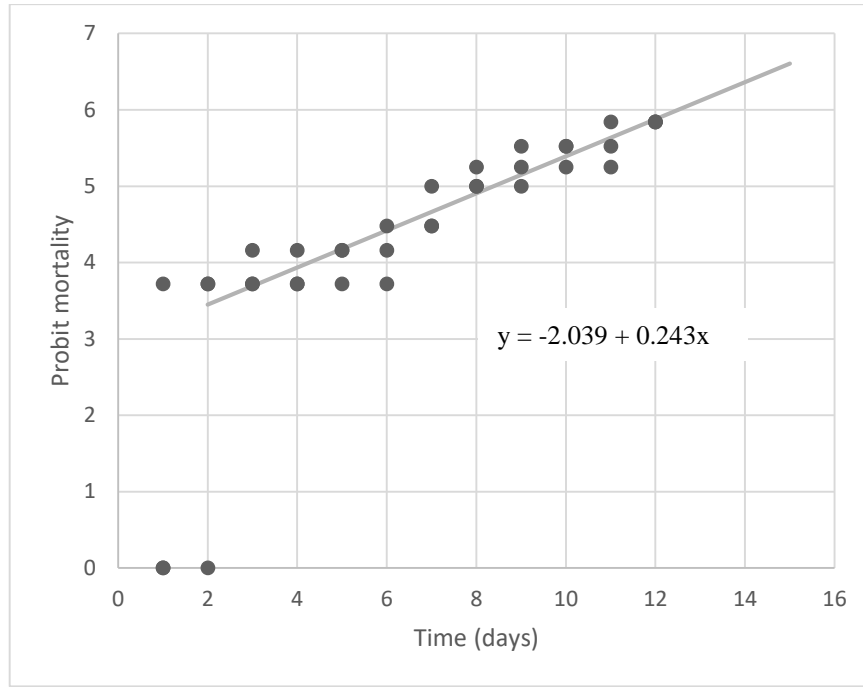


Figure 3. Time-dependent mortality of *Palomena prasina* nymphs due to *Beauveria bassiana* FAI-38 application at the concentration of  $1 \times 10^8$  conidia  $ml^{-1}$ .

Şekil 3.  $1 \times 10^8$  spor  $ml^{-1}$  konsantrasyonunda *Beauveria bassiana* FAI-38 uygulaması sonrasında *Palomena prasina* nimflerinin zamana bağlı ölümleri

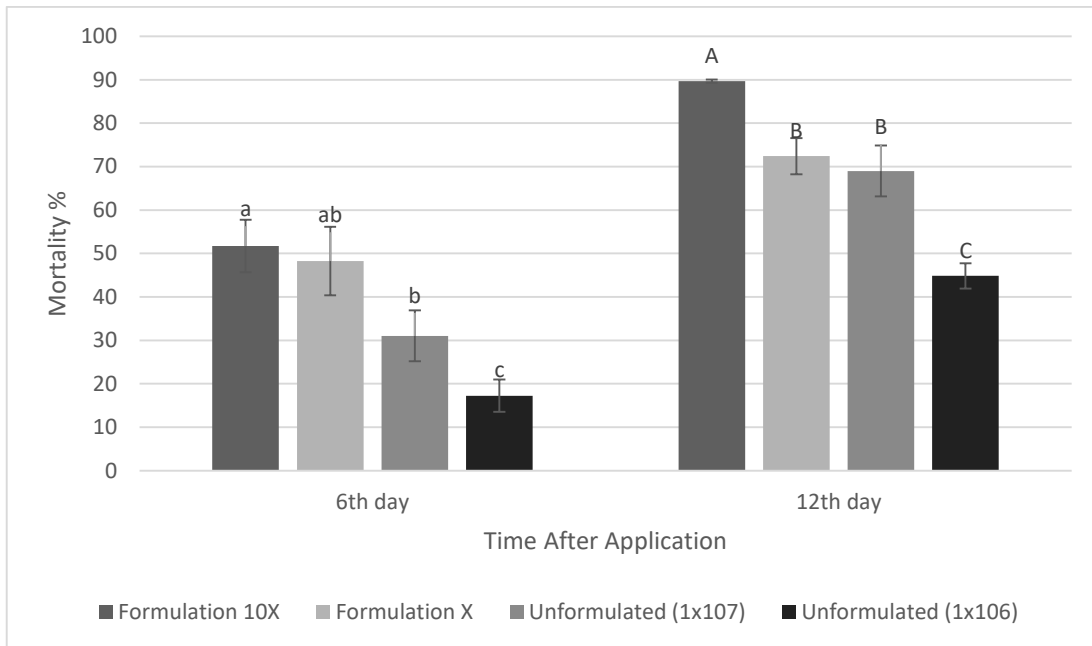


Figure 4. Mortality of *Palomena prasina* nymphs after application of WP formulation at 1X and 10X and two corresponding concentrations ( $1 \times 10^6$  and  $1 \times 10^7$  conidia  $ml^{-1}$ ) of the fungal conidia without formulating, (Data were subjected to Abbott's correction formula; error bars indicate standard errors control mortalities: 0-3.33%,  $n=3$ , different lowercase and capital letters indicate statistical differences on the 6th and 12th days respectively according to Duncan multiple comparison test 6th day:  $F_{3,8}=7.094$   $P=0.012$  and 12th day:  $F_{3,8}=23.743$   $P=0.000$ )

Şekil 4. *Palomena prasina* nimflerinin 1X ve 10X WP formülasyonu ve konidinin formülasyonsuz karşılık gelen iki konsantrasyonu ( $1 \times 10^6$  ve  $1 \times 10^7$  konidi  $ml^{-1}$ ) uygulanmasından sonraki ölümleri. (Verilere Abbott'un düzeltme formülüne uygulanmıştır; hata çubukları standart hataları göstermektedir, kontrol ölümleri: %0-3.33,  $n=3$ , Duncan çoklu karşılaştırma testine göre farklı küçük ve büyük harfler sırasıyla 6. ve 12. günlerdeki istatistiksel farklılıkları göstermektedir, 6th day:  $F_{3,8}=7.094$   $P=0.012$  and 12th day:  $F_{3,8}=23.743$   $P=0.000$ )

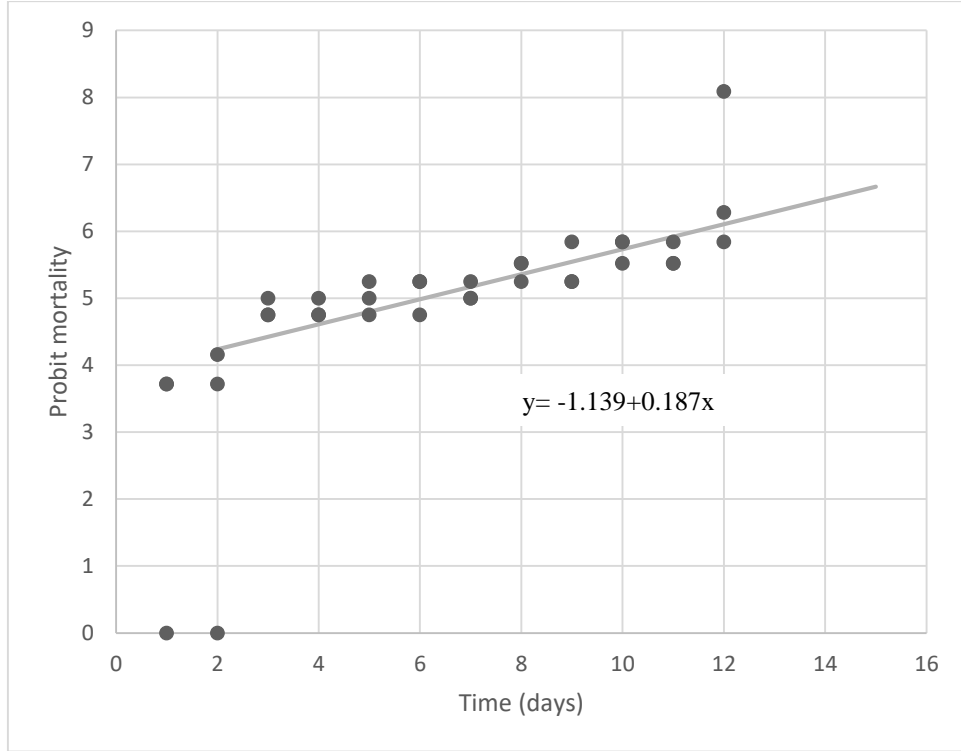


Figure 5. Time-dependent mortality of *Palomena prasina* nymphs due to applications of *Beauveria bassiana* FAI-38 WP formulation at 10X

Şekil 5. *Beauveria bassiana* FAI-38 WP formülasyonunun 10X uygulaması sonucu *Palomena prasina* nimflerinin zamana bağlı ölümleri

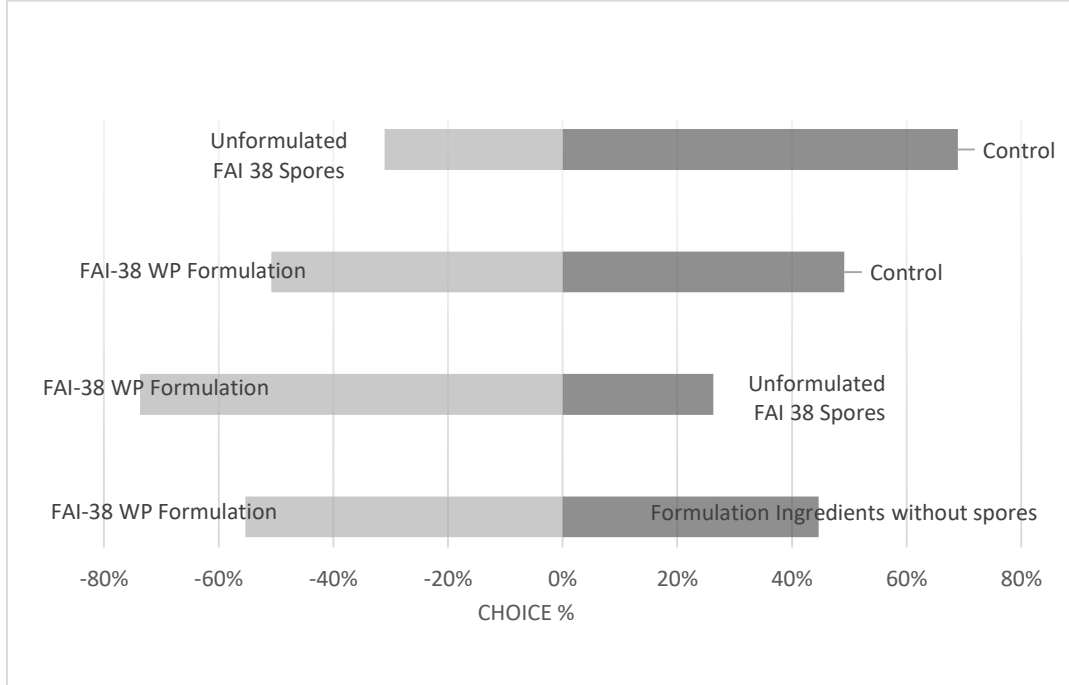


Figure 6. Results of the Y-tube olfactometer bioassays showing the behavioral response of *Palomena prasina* nymphs to the presence of *Beauveria bassiana* conidia (Formulations were used at 10X and unformulated spores at  $1 \times 10^7$  spores  $ml^{-1}$  concentration)

Şekil 6. *Palomena prasina* nimflerinin *Beauveria bassiana* sporlarına karşı davranışsal tepkisini gösteren Y tüpü olfaktometre testlerinin sonuçları (Formülasyon 10X ve formüle edilmemiş spor  $1 \times 10^7$  spor  $ml^{-1}$  konsantrasyonunda kullanılmıştır)



Formulation of *B. bassiana* has not been tested before on *P. prasina*; therefore, there are not any records to compare these results. However, there are few studies on formulated entomopathogenic fungi against other pest species in the family Pentatomidae. Parker et al. (2015) tested two formulations (wetable powder and emulsifiable suspension) of *B. bassiana* against the brown marmorated stink bug, *Halyomorpha halys*. They found both formulations effective at  $1 \times 10^7$  conidia  $\text{ml}^{-1}$  with 67–80% and 95–100% mortality 9 and 12 days post treatment, respectively. The wettable powder formulation was concluded as slightly more efficacious. Kaolin powder formulations of *B. bassiana* and *M. anisopliae* were used against three species of stink bugs (*Nezara viridula* (L.), *Euschistus heros* (F.) and *Piezodorus guildinii* (Westwood)) by Sosa-Gomez et al (1998). *M. anisopliae* formulation was found better and the  $\text{LT}_{50}$  value was  $4.3 \pm 0.2$  days for *P. guildinii*,  $4.6 \pm 0.2$  days for *N. viridula* and  $7.4 \pm 0.5$  days for *E. heros*. In field trials, up to 48% mortality was recorded.

Behavioral responses of *P. prasina* nymphs in Y-tube olfactometer tests are presented in Figure 6. When one of the choices was unformulated spores, significantly more nymphs preferred the alternative choice. In one test the preferred alternative was control (39 nymphs vs 19 nymphs; no choice=2 nymphs;  $X^2=6.897$ ,  $P=0.009$ ), indication of avoidance from fungal spores. In another test, the alternative to unformulated spores was FAI-38 formulation, and the nymphs still showed avoidance and preferred the formulation (45 nymphs vs 14 nymphs; no choice=1 nymph  $X^2=16.288$ ,  $P=0.000$ ). The nymphs did not show a significant preference between FAI-38 formulation (30 nymphs) and control (29 nymphs) (no choice=1 nymphs;  $X^2=0.017$ ,  $P=0.8960$ ), demonstrating that the nymphs stopped avoiding the spores when formulated. Preferences between FAI-38 formulation (26 nymphs) and formulation ingredients without spores (21 nymphs) were not significantly different either (no choice=13 nymphs;  $X^2=0.532$ ,  $P=0.466$ ). Changing the places of two choices in Y-tube olfactometer in all four tests did not cause significant change in the preferences [unformulated FAI-38 spores vs control ( $t=0.271$ ,  $P=0.849$ ), FAI-38 formulation vs control ( $t=13.024$ ,  $P=0.646$ ), FAI-38 formulation vs formulation ingredients without spores ( $t=1.103$ ,  $P=0.646$ ), unformulated FAI-38 vs FAI-38 formulation ( $t=0.302$ ,  $P=0.533$ )].

It is quite commonly reported that insects can recognize and stay away from entomopathogenic fungi (Baverstock et al., 2010; Wei et al., 2020; Avery et al., 2021; Geedi et al., 2022; Daisy, 2022). A similar reaction was evident from the results when *P. prasina* nymphs were set to choose between unformulated spores and control. However, such reaction was not

seen when the choices were FAI-38 formulation and control. Similar phenomenon was reported previously. Wang & Powell (2004) demonstrated that *Reticulitermes flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) avoided *M. anisopliae* conidia but its bait formulation did not repel the insects. Fernandez-Grandon et al. (2020) showed that *Aphidius colemani* was deterred by plants treated with *M. anisopliae* but not by plants treated with a combination of *M. anisopliae* and pyrethrum.

## CONCLUSION

This study shows that *Beauveria bassiana* FAI-38 provides a high level of *P. prasina* nymphal mortality comparable to those in literature, presenting the potential for further studies for developing as a control agent. Furthermore, formulating *B. bassiana* FAI-38 as wettable powder increases and accelerates the mortality with an additional advantage of removing the avoidance reaction of *P. prasina* nymphs.

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## Author's Contributions

The contribution of the authors is equal.

## Conflict of Interest

The author declares that there is no conflict of interest in the study.

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## Turunçgil Bahçelerinde Meyve Dökümüne Neden Olan Fungal Patojenlerin Tanısı ve Bazı Bileşiklerinin Antifungal Etkileri

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### ÖZET

Bu çalışmada, Hatay ilinde turunçgil bahçelerinde meyve dökümüne neden olan fungal etmenlerin belirlenmesi ve bu patojenlere karşı bor bileşikleri ve pirolignöz asitin (PA) *in vitro* antifungal etkileri araştırılmıştır. Hatay'ın Erzin, Dört Yol, Arsuz ve Samandağ ilçelerinde yer alan portakal, mandarin, limon, greyfurt bahçelerinde yere dökülen meyvelerden toplam 30 adet izolat elde edilmiştir. Elde edilen izolatlardan PDA besi yerinde hastalık izolasyonu yapılmıştır. Sonra bu izolatlardan mikroskop incelemeleri, DNA izolasyonu, PCR ve sekanslama çalışmaları ile teşhisleri yapılmıştır. Morfolojik ve moleküler tanımlama çalışmaları sonucunda *Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *C. karsti*, *Diaporthe foeniculina* meyve dökümlerinden sorumlu hastalık etmenleri olarak teşhis edilmiştir. Yapılan patojenisite denemeleri sonucunda tüm fungal izolatlar patojen olarak belirlenmiştir. Elde edilen fungal etmenler içerisinde en yüksek virülenslik %92.6-88.9 ile *Colletotrichum* spp., tarafından gösterilmiş olup bu türleri %66.7 ile *D. foeniculina*, %55.6 ile *F. oxysporum* ve %44.4 ile *A. alternata* türleri takip etmiştir. Bor bileşikleri ve PA'nın farklı konsantrasyonları (%0.03, 0.05, 0.07, 0.09, 0.1, 0.12, 0.15, 0.3, 0.5, 0.7, 1.0, 1.5; w/v) elde edilen 5 fungal patojen izolatın misel gelişimi ve konidi çimlenmesi üzerindeki antifungal etkileri *in vitro* koşullarda araştırılmıştır. Fungal türlerin misel gelişimi, farklı dozlarda borik asit (%0.12), boraks (%0.1), etidot-67 (%0.1) ve PA (%1.5) uygulamaları tarafından tamamen engellenmiştir. Öte yandan borik asit (%0.15), boraks (%0.12), Etidot-67 (%0.12) ve PA (%1.5) uygulamaları, izolatların konidi çimlenmesini önemli ölçüde engellemiştir. Elde edilen fungal türlere karşı EC<sub>50</sub> değerleri, misel gelişimi için %0.059-0.69 ve konidi çimlenme için %0.065-0.82 arasında bulunmuştur. Bu çalışma, Türkiye'de turunçgil meyve dökümlerine neden olan fungal hastalık etmenlerine karşı bor bileşikleri ve PA'nın antifungal etkinliğini araştıran ilk çalışmadır.

### Bitki Koruma

### Araştırma Makalesi

### Makale Tarihiçesi

Geliş Tarihi : 23.03.2024

Kabul Tarihi : 11.06.2024

### Anahtar Kelimeler

Citrus

Alternatif mücadele

Meyve dökümü

Bor bileşikleri

Pirolignöz asit

## Identification of Fungal Pathogens Causing Fruit Drop in Citrus Orchards and Antifungal Effects of Some Compounds

### ABSTRACT

This study identified the fungal agents causing fruit drop in citrus orchards in Hatay province and investigated the *in vitro* antifungal effects of boron compounds and pyroligneous acid (PA) against these pathogens. Thirty isolates were obtained from orange, mandarin, lemon, and grapefruit orchards in the Erzin, Dört Yol, Arsuz, and Samandağ districts of Hatay. Pathogen isolation was performed on the PDA medium. The isolates were then identified through microscopic examinations, DNA isolation, PCR, and sequencing studies. Morphological and molecular identification revealed *Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Colletotrichum karsti*, and *Diaporthe foeniculina* as the disease agents responsible for fruit drop. Pathogenicity tests confirmed all isolates as

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Boron compounds

Pyroligneous acid



pathogenic. *Colletotrichum* spp. exhibited the highest virulence with 92.6-88.9%, followed by *D. foeniculina* at 66.7%, *F. oxysporum* at 55.6%, and *A. alternata* at 44.4%. The antifungal effects of boron compounds and various concentrations of PA (0.03%, 0.05%, 0.07%, 0.09%, 0.1%, 0.12%, 0.15%, 0.3%, 0.5%, 0.7%, 1.0%, 1.5%; w/v) on the mycelial growth and conidial germination of five fungal pathogen isolates were investigated in vitro. Mycelial growth was completely inhibited by boric acid (0.12%), borax (0.1%), ethidote-67 (0.1%), and PA (1.5%). Additionally, boric acid (0.15%), borax (0.12%), Etidot-67 (0.12%), and PA (1.5%) significantly inhibited conidial germination. The EC<sub>50</sub> values for mycelial growth ranged from 0.059% to 0.69%, and for conidial germination, from 0.065% to 0.82%. This is the first study investigating the antifungal activity of boron compounds and PA against fungal pathogens causing citrus fruit drop in Türkiye.

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## GİRİŞ

Türkiye'de tarım ve kırsal kalkınma planlarında stratejik bir ürün olarak kabul edilen turunçgiller (*Citrus* spp.), ekonomik ihracat değerleri ve yerel tüketimleri göz önüne alındığında, önemli bir meyve grubunu oluşturmaktadır (Uysal ve ark., 2022). Türkiye turunçgil üretiminin türlere göre dağılımı, %41' i mandarin, % 28' i portakal, %24' ü limon, %7' si altıntop şeklinde gerçekleşmiştir. Türkiye'de son yıllarda turunçgil üretimi, 2 milyon 311 bin ton portakal, 2 milyon 953 bin ton mandarin, 2 milyon 325 bin ton limon, yaklaşık 285 bin ton altıntop ve 3 bin 500 ton turunç olmak üzere yaklaşık 7 milyon 878 bin ton olarak kaydedilmiştir. Turunçgil meyve türlerinin üretiminde bir önceki yıla göre mandarinde %58.3, portakalda %74.8 ve limonda %75.8 oranında bir artış sağlanmıştır (Anonim, 2023).

Turunçgil üretim alanlarında verim, kalite ve ihracatı sınırlayan en önemli faktörlerden birisi, bitki hastalıklarından kaynaklanan ekonomik kayıplardır. Dünya genelinde ve Türkiye'de turunçgil üretim sürecinde, fungus ve fungus benzeri organizmalar tarafından oluşturulan çeşitli hastalıklar arasında öne çıkanlar; kahverengi leke (*Alternaria alternata* f.sp. *citri*), uçkurutan (*Plenodomus tracheiphilus* (Petri) L.A. Gruyter, Aveskamp&Verkley.), kahverengi çürüklük ve gövde zamklanması (*Phytophthora citrophthora* (Smith and Smith) Leonian ve son yıllarda artan öneme sahip olan *Colletotrichum* türlerinin neden olduğu antraknoz hastalığıdır (Guarnaccia ve ark., 2017a; Avcı, 2018; Uysal ve ark., 2019; Uysal ve ark., 2022a). Son zamanlarda, turunçgillerde *Diaportheaceae* ve *Botryosphaeriaceae* familyasında yer alan türlerin neden olduğu sakızlanma, geriye doğru ölüm ve kanser hastalıkları ön plana çıkmakta ve ciddi ağaç kayıplarının yaşandığı bildirilmektedir (Batista ve ark., 2021; Bezerra ve ark.,

2021). Türkiye'de limonda meyve çürüklüğüne neden olan *Diaporthe foeniculina*'nın ilk kaydı Tekiner ve ark., (2020) tarafından bildirmiştir.

Turunçgil ağaçlarında meyve dökümü hem iç hem de dış birçok faktöre bağlı olarak gerçekleşmektedir. Bu faktörler arasında büyüme düzenleyicilerinin dengesizliği, hastalık ve zararlılar, aşırı sıcak hava, rüzgâr, su stresi, yüksek nem, sel vb. unsurlar sayılabilir. Genel olarak turunçgil ağaçları ilkbaharda bol çiçek açarken, açan çiçeklerin çok azı meyveye dönüşmekte ve hasada kadar olgunluğa erişmektedir. Meyveye dönüşen çiçeklerin dökülmesi büyümenin farklı aşamalarında oluşmaktadır. Turunçgilde dökümler çiçeklenme sonrası döküm (i), Haziran dökümü (ii), ve hasat öncesi dökümü (iii), olmak üzere üç farklı dönemde meydana gelmektedir (Sezer ve ark., 2019).

Meyve dökümü çiçeklenmeden hemen sonra başlar ve çok küçük meyvelerin düşmesine neden olur. Bu dökümün, aşırı tutumdan kaynaklandığı bilinmektedir. Bu meyve dökümü çiçeklenmeden aylar sonra, haziran ayında aşırı meyve tutumu sırasında oluşur. Toplam döküm, meyvelerin yaklaşık yüzde 10'unu oluşturmaktadır. iii) Olgunlaşma öncesi ve hasat öncesi meyveleri içeren üçüncü döküm neredeyse olgunlaşmış ve hasat edilebilir meyvelerin dökülmesidir. Ağustos ayından olgunlaşma öncesi meyve dökümü olarak başlar ve hasat öncesine kadar devam eder. Bu döküm üretici için ekonomik öneme sahiptir. Çünkü, bu dökümde tamamen büyümüş meyveler dökülmekte ve ağır kayıplara neden olmaktadır (Bishnoi ve ark., 2023).

Dökümlerin en önemli sebeplerinden biri patojenlerden kaynaklı meyve dökümü, genellikle ağustos ayında başlar, hasada kadar devam eder ve eylül ortasından ekim ortasına kadar zirveye ulaşır.



Patojen kaynaklı meyve dökülmesine, farklı fungal etmenler sebep olmaktadır. Bunlardan bilinen en yaygın olanları *Colletotrichum gloeosporioides*, *C. karsti*, *Diplodia* sp., *Alternaria* sp. ve *Botrytis cinerea* türleri olarak bildirilmektedir (Rattanpal ve ark., 2019).

Turunçgillerin patolojik meyve dökümünün mücadelesinde; hastalıklı, ölü, çürüyen dalların çıkarılarak ağaçların budanması sonrası bordo bulamacı veya bakır oksiklorür 50 WP (3g litre<sup>-1</sup> su) uygulaması ayrıca, ağaçlardaki mumyalanmış hastalık etmeni taşıyan meyvelerin toplanıp toprağa gömülerek imha edilmesi önerilmektedir (Rattanpal ve ark., 2019; Uysal ve ark., 2022b).

Fungisitlerin insan ve çevre sağlığı üzerindeki potansiyel olumsuz etkilerinin bilinmesi ve söz konusu sentetik fungisitlere karşı bitki fungal etmenlerinin direnç geliştirme sorunu, bitki hastalıklarının yönetiminde daha güvenli, kalıcı ve çevre dostu alternatiflerin araştırılmasını zorunlu kılmıştır (Conway ve ark., 2005).

Odunun kömüre dönüştürülmesinde kullanılan Retort adı verilen fırınlarda, proliz sürecinde ortaya çıkan yan ürünlerden biri, aynı zamanda “odun sirkesi” olarak da bilinen Pirolignöz asittir (Fengel & Wegener, 1984). Pirolignöz asit (PA), asetik asit, formik asit, metanol, fenol, keton gibi 200’den fazla bileşik içermektedir (Mu ve ark., 2003; Kadota & Nimii, 2004). Son yıllarda yapılan çalışmalar ile PA’nın toprak kalitesini iyileştirdiği, bitki büyümesini düzenleyici etkisinin bulunduğu, hastalık ve zararlıları kontrol ettiği bildirilmiştir (Apai & Thongdeethae, 2002; Mu ve ark., 2003; Chen ve ark., 2020; Uysal, 2024; Kara ve ark., 2024). Bu ürünün, *Pythium* sp., *Rhizoctonia solani*, *Fusarium oxysporum* f.sp. *lycopersici*, *P. aphanidermatum*, *S. homoeocarpa*, *Penicillium* spp., *Phytophthora capsici*, (Numata ve ark., 1994), *Penicillium griseofulvum* (Baimark & Niamsa, 2009); *Sclerotium oryzae*, *Helminthosporium maydis*, *R. solani*, *Colletotrichum gloeosporioides* *Choanephora cucurbitarum* (Chalermnan & Peerapan, 2009); *Pestalotiopsis microspore*, *F. oxysporum*, *R. solanacearum* (Baharom ve ark. 2020), *Trametes versicolor*, *Fomitopsis palustris* (Oramahi & Yoshimura, 2013) ve *Botrytis cinerea* (Chen ve ark. 2020), gibi çeşitli fungal etmenlere karşı etkili olduğu bildirilmiştir.

Pirolignöz asit, Uzakdoğu Asya ülkelerinde temel doğal tarım girdisi olarak hızla kabul görmektedir. Çevre dostu ve bitkilerde büyümeyi teşvik eden bir madde olarak kullanımı ile pek çok fungal hastalıklara karşı biyosit olarak kullanılmasından dolayı tarımsal üretimde fungal hastalıklarla mücadelede pestisitlere karşı alternatif olması önem arz etmektedir. Bunun yanında en önemli özelliklerinden biri, üreticiler açısından kullanım kolaylığı ve girdi maliyetinin düşük olması ürünü cazip kılmaktadır.

Bor, doğada her yerde bulunan, az miktarda bile olsa bitki büyümesini destekleyen ve eksikliği durumunda bitkilerde çeşitli belirtilere ve bozukluklara neden olabilen temel bir mikro elementtir (Lieten, 2002; Shi ve ark., 2011; Dibek ve ark., 2020). Türkiye, 3.3 milyon tonluk üretimle dünyanın en yüksek bor rezervine sahip olup, ülkemizi Rusya, Güney Amerika ve ABD takip etmektedir (Eti Maden, 2021). Bor bileşikleri arasında boraks veya sodyum tetraborat dekahidrat, disodyum oktaborat tetrahidrat, borik asit, bor oksit, bor karbür, bor nitrür, bor triklorür, bor tribromür ve bor triflorür (Tenmak-Boren) bulunmaktadır. Bor, çeşitli formlarda (borik asit, boraks, etidot vb.), şeftalilerde kahverengi çürüklüğün (*M. laxa*) neden olduğu meyve çürüklüğü (Thomidis & Exadaktylo, 2010) ve nektarin gibi sert çekirdekli meyve türlerinde fungal meyve hastalıklarına (Thomidis ve ark. 2015), elmada mavi küfe neden olan *Penicillium expansum*’a (Erper ve ark. 2019a), kabaklarda kök ve kök boğazı çürüklüğü oluşturan *R. solani*’ye (Erper ve ark., 2019b), bor ürünlerinin ve pirolignöz asit bileşiğinin kayısı ve bademde çiçek ve sürgün yanıklığı etmeni *Monilinia laxa*’ya karşı etkili olduğu saptanmıştır (Uysal, 2024).

Türkiye’de yetiştirilen turunçgil ağaçlarında zaman zaman şiddetli verim kayıplarına ve meyve dökümlerine sebep olan biyotik faktörler konusunda yeterli araştırma bulunmamaktadır. Bu faktörler arasında fungal enfeksiyonlara bağlı meyve dökümlerinin nedeni ve etmenleri ile bunların çözümü noktasında yapılacak olan bir çalışma, kaliteli ve verimli bir turunçgil üretimi için kaçınılmaz bir durum arz etmektedir. Bu nedenle bu çalışmada, turunçgil üretim alanlarında gözlenen patolojik meyve dökümlerine sebep olan fungal etmenler, morfolojik ve moleküler yöntemlerle belirlendikten sonra patojen olan fungal türler ile mücadelede alternatif yöntemlerden PA ve bor bileşiklerinin etkinlikleri *in vitro* koşullarda araştırılmıştır.

## MATERYAL ve METOD

### Fungal izolatlar

**Fungal izolasyon:** Hatay’ın Erzin, Dörtüyük, Arsuz ve Samandağ ilçelerindeki turunçgil bahçelerindeki portakal, mandarin ve altıntop (greyfurt) türlerinde Ekim-Kasım 2021 ve Mart-Ağustos 2022 aylarında ağaçlar, çiçek ve meyve dökümleri yönünden kontrol edilerek toplam 30 meyve bahçesinde tesadüfi örneklemeler yapılmıştır (Uysal ve ark., 2022). Enfeksiyon nedeni ile oluşmuş klorotik veya nekrotik dokuları içeren bitki parçaları, steril bir bistüri ile kesilmiştir. 1-2 mm büyüklüğündeki doku parçaları, %75’lik etanol içerisinde 1 dakika yüzey dezenfeksiyonu yapılmıştır (Uysal & Kurt, 2019). Bu doku parçaları, steril distile suda çalkalanıp steril kurutma kağıtlarında 15-30 dk kurumaya bırakılmıştır. Kuruyan parçalar, genel besi ortamı

olan Patates Dekstroz Agar (PDA, Merck KGaA, Darmstadt, Germany) içeren Petri kaplarına (90 mm) transfer edilmiştir. Besi ortamlarına bakteriyel bulaşmaları önlemek için streptomisin sülfat ( $100 \mu\text{g mL}^{-1}$ ) eklenmiştir. Petri kapları,  $25^\circ\text{C}$ 'de 5 gün boyunca inkübe edilmiş ve gelişen kolonilerden PDA ortamına saflaştırmalar yapılmıştır (Uysal, 2019; Uysal & Kurt, 2019; Kurt ve ark., 2020a).

**Morfolojik tanılama:** Morfolojik özellikler kullanılarak hastalıklara neden olduğu düşünülen fungal izolatların ön teşhisleri yapılmıştır. Bu amaçla, inkübasyondan 10 gün sonra koloni özellikleri (misel gelişiminin üst ve alt yüzeylerinin renkleri), büyüme hızı (mm), konidial boyutlar ve PDA üzerindeki pigment oluşumları değerlendirilmiştir (Guarnaccia & Crous, 2017; Uysal ve ark., 2022; Güler Güney ve ark., 2023; Soylu ve ark., 2024).

**Moleküler tanılama:** Tek sporlu kültürlerden geliştirilen temsili 10 izolatın 5-7 günlük fungus kültürlerinden QIAGEN DNeasy (50) Plant mini kit (Qiagen Inc., Valencia, CA) kullanılarak genomik DNA ekstraksiyonu yapılmıştır. Tüm DNA çözeltilerinin konsantrasyonları Qubit 2.0 Florometre (Thermo Fisher Scientific, Witham, MA, ABD) kullanılarak belirlenmiştir. ITS-rDNA bölgesi, evrensel primerler ITS1 (CTTGGTCATTTAGAGGAAGTAA) ve ITS4 (TCCTCCGCTTATTGATATGC) kullanılarak (White ve ark., 1990; Soylu ve ark., 2024) termal döngü işlemleriyle desteklenmiştir. PCR, 25  $\mu\text{L}$  reaksiyon toplam hacmi, 1,25 U Taq DNA polimeraz (Thermo Fisher Scientific), 5  $\mu\text{L}$  10x tampon, 0,5  $\mu\text{L}$  50 mM MgCl, 0,75  $\mu\text{L}$  10 mM dNTP, her primerden 10 pmol, 10-20 ng genomik DNA ile gerçekleştirilmiştir. Amplifikasyonlar 3 dak. süreyle  $95^\circ\text{C}$ , ardından 30 sn. süreyle  $95^\circ\text{C}$ 'lik 35 döngü,  $55^\circ\text{C}$  ve  $72^\circ\text{C}$ 'de 1 dak. süreyle 45 sn. ve 10 dak. süreyle  $72^\circ\text{C}$ 'lik son uzama aşaması şeklinde gerçekleştirilmiştir. PCR ürünlerinden gen dizilemesi yaptırılmış ve nükleotid dizileri elde edilmiştir. Tüm diziler, standart nükleotid Basic Local Alignment Search Tool (BLAST) programı (Boratyn ve ark. 2013) kullanılarak National Centre for Biotechnology Information (NCBI) tarafından alınan GenBank Nükleotid Veri Tabanında saklanan dizilerle karşılaştırılarak tür tanıları gerçekleştirilmiştir. Bu fungal izolatlar, Hatay Mustafa Kemal Üniversitesi Bitki Sağlığı Kliniği Uygulama ve Araştırma Merkezi'nde kullanılıncaya kadar daha uzun süreli depolama için gliserollü PDA içeren 1,5 mL'lik tüplerde  $-80^\circ\text{C}$ 'de üç seri halinde muhafaza edilmiştir (Kurt ve ark., 2020b).

### Patojenisite testleri

Fungal izolatların patojenisitesi için, portakal (washington çeşidi) mandarin (satsuma çeşidi) ve limon (interdonato çeşidi) meyveleri kullanılmıştır. Meyve dökümüne neden olduğundan kuşkulanan fungus türlerini temsil eden 5 izolat (MFo15, OAa11,

OCg8, MCk6, LDf5), Hatay turuncgil bahçelerinden toplanan meyveler, Aiello ve ark. (2015) ve Guarnaccia ve ark. (2017)'dan uyarlanmış yara/damla yöntemine göre ve her bir izolat için 9 meyve olacak şekilde inokule edilmiştir. Meyveler %70'lik etanole daldırılarak dezenfekte edilmiş ve iki kez steril suyla durulanmıştır. Steril bir pipet ucu ile meyvelerin ekvatorial bölgesinden 3'er adet (2-3 mm çap) yaralar açılmıştır. Fungal izolatların PDA ortamında  $25^\circ\text{C}$ 'de 10 gün boyunca geliştirilen kültürlerinden  $1 \times 10^6$  konidi  $\text{mL}^{-1}$  konsantrasyonda spor süspansiyonu hazırlanmıştır. Meyvedeki her bir inokülasyon noktasına, hazırlanan spor süspansiyonundan 10  $\mu\text{L}$  enjekte edilmiştir. Kontrol grubu meyvelere ise sadece saf su uygulaması yapılmıştır. İnoküle edilmiş ve kontrol meyveleri, plastik kaplara yerleştirilmiş ve plastik polietilen torbalar içerisinde inkübe edilmiştir. Hastalık değerlendirilmesi, inokülasyondan 10 gün sonra gerçekleştirilen hastalık değerlendirmelerinde, her izolat için enfekteli inokülasyon noktalarının yüzdesi hesaplanmıştır (Tekiner ve ark., 2020). Bu yüzde değer  $[(\%) = (\text{enfekte olmuş inokülasyon noktaları} / \text{inokülasyon noktaları}) \times 100]$  formülü ile değerlendirilmiştir

### Bor ve PA bileşiklerinin fungal patojenlerin misel gelişimi üzerine etkileri

Bor bileşikleri (Borik asit-  $\text{H}_3\text{BO}_3$ , Boraks-disodyum tetraborat dekahidrat-  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  ve Etidot 67 disodyum oktaborat tetrahidrat-  $\text{Na}_2\text{B}_8\text{O}_{13} \cdot 4\text{H}_2\text{O}$ ) Eti Maden işletmesinden temin edilmiştir. PA (Pirolognoz asit-odun sirkesi), okaliptüs ağacı odunlarının  $450^\circ\text{C}$ 'de gazlaştırma teknolojisi ile fırınlarda yakılmasıyla biyokömür ve pirolignöz asit ürünleri özel bir firmadan (Düzce, Türkiye) temin edilmiştir (Uysal, 2024; Kara ve ark., 2024). Bor bileşikleri borik asit, boraks-disodyum tetraborat dekahidrat, Etidot-disodyum oktaborat tetrahidratın ve PA tekli ve kombinasyon halinde farklı tür, lokasyon ve konukçuyu temsil eden fungal patojenlerin misel gelişimi üzerine etkileri, PDA besisi yerinde *in vitro* koşullardan incelenmiştir. Misel büyümesi çeşitli konsantrasyonlarda, borik asit (0.03, 0.05, 0.07, 0.1 ve 0.12 w/v), boraks (0.03, 0.05, 0.07, 0.09 ve 0.1), Etidot (0.03, 0.05, 0.07, 0.09 ve 0.1 w/v), PA (0.3, 0.5, 0.7, 1.0, 1.2  $\mu\text{g} \cdot \text{mL}^{-1}$ ) (Spiegel & Stammler 2006; Hrustić ve ark. 2018; Durak ve ark. 2021) ve BA+Borax (0.07+0.05) BA+PA (0.08+0.8), Boraks+PA (0.05+0.8), Etidot+PA (0.06+0.8) kombinasyonlarını içeren PDA besisi yerinde fungusların radyal büyümesinin ölçülmesiyle belirlenmiştir (Uysal, 2024). Bu bileşikler, otoklav edilmiş ve  $50^\circ\text{C}$ 'ye soğutulmuş PDA ortamına eklenmiş ve Petri kaplarına (15x90mm) dökülmüştür. Bileşik eklenmeyen PDA kontrol olarak kullanılmıştır. PDA üzerinde 5 gün boyunca aktif olarak gelişen fungus kolonilerinin kenarlarından mantar delici yardımıyla kesilerek çıkarılan misel diskleri (5 mm

çapında) yaklaşık 10 mL PDA içeren Petri kaplarının ortasına miseliyal kısım üste gelecek şekilde yerleştirilmiştir. Farklı fungal izolatlarla ait olan kültürler, karanlıkta 25°C'de 7 -10 gün boyunca inkübe edilmiştir. Deneme her bir konsantrasyon ve izolat kombinasyonu için 3 tekrerrür oluşturulmuş ve her bir Petri kabı bir tekrerrür olarak kabul edilmiştir. Denemeye alınan bileşiklerin etkinliğini belirlemek için fungus kolonilerinin gelişim çapları (mm) cetvel yardımıyla ölçülmüş ve aritmetik ortalamaları alınarak hesaplanmıştır (Uysal, 2024).

### Bor ve PA bileşiklerinin fungal patojenlerin konidial çimlenme üzerine etkileri

Ele alınan Bor ve PA bileşiklerinin fungal izolatların konidial çimlenmesine etkileri, borik asit (0.05, 0.07, 0.1, 0.12 ve 0.15 w/v), boraks (0.05, 0.07, 0.09, 0.1 ve 0.12), Etidot (0.05, 0.07, 0.09, 0.1 ve 0.12 w/v), PA (0.5, 0.7, 0.9, 1.2, 1.5 µg mL<sup>-1</sup>) tekli ve BA+Borax (0.07+0.07) BA+PA (0.09+0.8), Boraks+PA (0.07+0.8), Etidot 67+PA (0.07+0.8) kombinasyonlarını içeren PDA ortamındaki fungal konidi sayımları yapılarak belirlenmiştir. Bu amaçla, thoma lamı kullanılarak 7-10 günlük kültürlerden steril distile su içinde 1x10<sup>6</sup> konidi ml<sup>-1</sup> konsantrasyonda spor süspansiyonu hazırlanmıştır. Daha sonra farklı ürünler içeren 5 mL WA besi yerine 50 µL spor süspansiyonu aktarılmıştır. Her bir uygulama, tekli ve kombinasyon halinde üç kez

tekrarlanmıştır. Fungal izolatların, 25°C'de karanlıkta 20 saat inkübasyondan sonra, konidial çimlenme yüzdesi (her tedavi için ortalama 100 konidi) ışık mikroskobu (Nikon Eclipse Ni-U, Japonya) altında sayılarak hesaplanmıştır.

### Veri analizi

*In vitro* koşullarda elde edilen veriler, SPSS istatistik programı for Windows Version 17 (SPSS, 2008) kullanılarak varyans analizine (ANOVA) tabi tutulmuş ve uygulamalar arasındaki farklılık düzeyi, Duncan Çoklu Aralık Testi (P≤0.05) ile belirlenmiştir. Bor ve PA bileşiklerinin farklı dozlarının misel gelişimini % 50 düzeyinde engelleyen etkili konsantrasyonları (EC<sub>50</sub>) farklı konsantrasyonlarda elde edilen engelleme değerleri kullanılarak SPSS paket istatistik programı Windows Version 17 (SPSS, 2008) yardımı ile Probit analizi yapılarak belirlenmiştir.

## BULGULAR ve TARTIŞMA

### Fungal izolatlar

Hatay ili turunçgil üretim alanlarında yürütülen sorveyler sırasında ağaçlardan toplanan meyvelerin sapa bağlanan kısımlarda kahverengileşme-siyahlaşma şeklinde renk değişimleri ve meyve sapında kurumalar şeklinde belirtilerin ortaya çıktığı gözlenmiştir (Şekil 1).



Şekil 1. Turunçgil bahçelerinde gözlenen meyve dökümleri ve enfeksiyon belirtileri  
Figure 1. Fruit drops and infection symptoms observed in the citrus orchards

Hatay ili Erzin, Dörtüyl, Arsuz ve Samandağ ilçelerinden hastalık belirtisi gösteren portakal, mandarin, limon ağaçlarından alınan meyve örneklerinden toplam 30 izolat elde edilmiştir. İlçeler bazında incelendiğinde, en fazla izolatin Dörtüyl (10 izolat) ve Arsuz (9 izolat)'da en az izolat Samandağ (4 izolat)'da olduğu belirlenmiştir. Bu izolatlardan 6'sı çiçek dökümlerinden 24'ü ise meyve dökümlerinden elde edilmiştir (Çizelge 1). Ayrıca hastalık etmeni

patojen elde edilemeyen çiçek ve meyvelerde fizyolojik sebeplerden ya da böcek zararlarından dolayı döküm gerçekleştiği saptanmıştır (Çizelge 1).

### Morfolojik tanılama

Hastalık izolasyonları sonrasında elde edilen funguslar PDA besi yerinde 10 gün boyunca gelişmeleri sağlanarak ön teşhisleri yapılmıştır.



Çizelge 1. Hatay ilindeki meyve dökümüne neden olan fungal izolatların turunçgil tür ve çeşitleri ile ilişkili bitki dokularına göre dağılımları

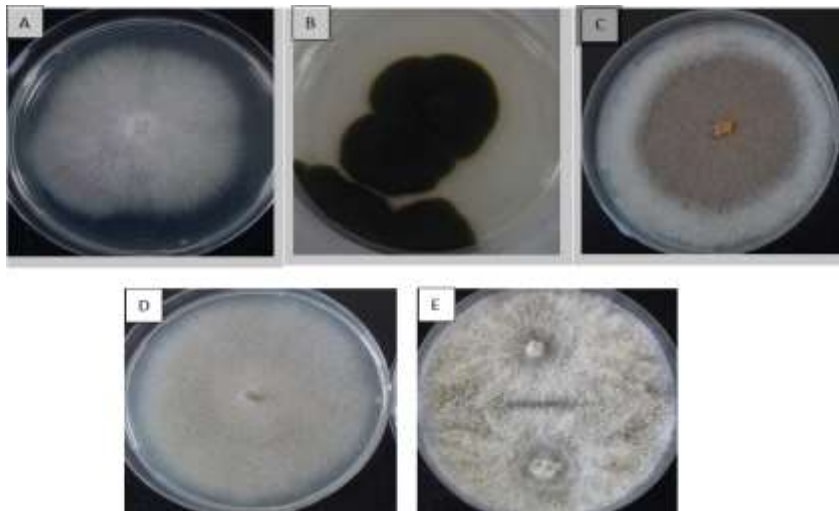
Table 1. Distribution of plant tissues associated citrus species and varieties and of isolates causing fruit drop in Hatay province

| Lokasyon      | Turunçgil türleri | İzolat    | Bitki dokusu |           | Toplam |
|---------------|-------------------|-----------|--------------|-----------|--------|
|               |                   |           | Çiçek        | Meyve     |        |
| Erzin         | Portakal          | 3         |              | 3         | 7      |
|               | Mandarin          | 4         | 1            | 3         |        |
| Dörtyol       | Portakal          | 3         |              | 3         | 10     |
|               | Altıntop          | 4         | 2            | 2         |        |
|               | Mandarin          | 3         | 1            | 2         |        |
| Arsuz         | Portakal          | 4         |              | 4         | 9      |
|               | Mandarin          | 2         |              | 2         |        |
|               | Limon             | 3         | 1            | 2         |        |
| Samandağ      | Mandarin          | 4         | 1            | 3         | 4      |
| <b>Toplam</b> |                   | <b>30</b> | <b>6</b>     | <b>24</b> |        |

Yapılan gözlemler neticesinde, *Fusarium*'un genellikle seyrek bir yapıda olduğu ve krem beyaz renkte miselyal gelişim sergilediği belirlenmiştir. *Alternaria*'nın hifleri kalın ve pamuksu bir yapıda olup miseller ilk başta renksizken daha sonra soluk griden koyu renk yeşile dönmüştür. Konidioforlar tek tek ayrılıp soluk kahverengi olarak gözlemlenmiştir. *Colletotrichum* türleri arasında morfolojik farklılıklar olsa da genel olarak, PDA üzerinde beyaz bir hif gelişimi gözlemlenmiş, ardından gri renge dönüşerek havai miselyumlar oluşturmuştur. Bu miselyumlar arasında turuncu renkli konidiomata yapıları ve siyah renkte eşeysiz üreme organları olan aservuluslar tespit edilmiştir (Uysal ve ark., 2022). *Diaporthe*

olarak tanımlanan koloniler, PDA ortamında beyazdan açık kahverengiye dönüşen bir miselyum meydana getirmiş ve zamanla koyu kahverengiye evrilmiştir. Bununla birlikte, koloni kenarlarında belirgin büyüme halkaları oluşmuş ve koloniler yaşlandıkça siyah renge dönüşmüştür (Tekiner ve ark., 2020).

Bu amaçla, fungusların koloni gelişimleri ve mikroskop altında gözlenen eşeyli ve eşeysiz üreme yapılarının değerlendirilmesi sonucunda bu fungal etmenlerin, *Fusarium*, *Alternaria*, *Colletotrichum*, *Diaporthe* cinsine ait türler olduğu belirlenmiştir (Şekil 2).



Şekil 2. Fungal patojenler: *F. oxysporum* (A), *A. alternata* (B), *C. gloeosporioides* (C), *C. karsti* (D), *D. foeniculina* (E)  
Figure 2. Fungal pathogens: *F. oxysporum* (A), *A. alternata* (B), *C. gloeosporioides* (C), *C. karsti* (D), *D. foeniculina* (E)

### Moleküler tanılama

Fungal DNA izolasyonları sonucunda, genomik DNA miktarları 20-30 ng  $\mu\text{L}^{-1}$  arasında değişkenlik göstermiştir. ITS (Internal Transcribed Spacer) evrensel primer çiftleri kullanılarak yapılan PCR

amplifikasyonu sonucunda, genellikle 450-550 baz çifti büyüklüğünde bantlar elde edilmiştir. Farklı fungal izolatlar arasında %98-100 arasında değişen bir nükleotid benzerlik oranı tespit edilmiş ve bu izolatlar için NCBI GenBank'ta erişim numaraları alınmıştır. (Çizelge 2).



Çizelge 2. Hatay ili turunçgil bahçelerinde meyve dökümüne neden olan temsili fungal izolatlar  
Table 2. Representative fungal isolates causing fruit drop in citrus orchards of Hatay province

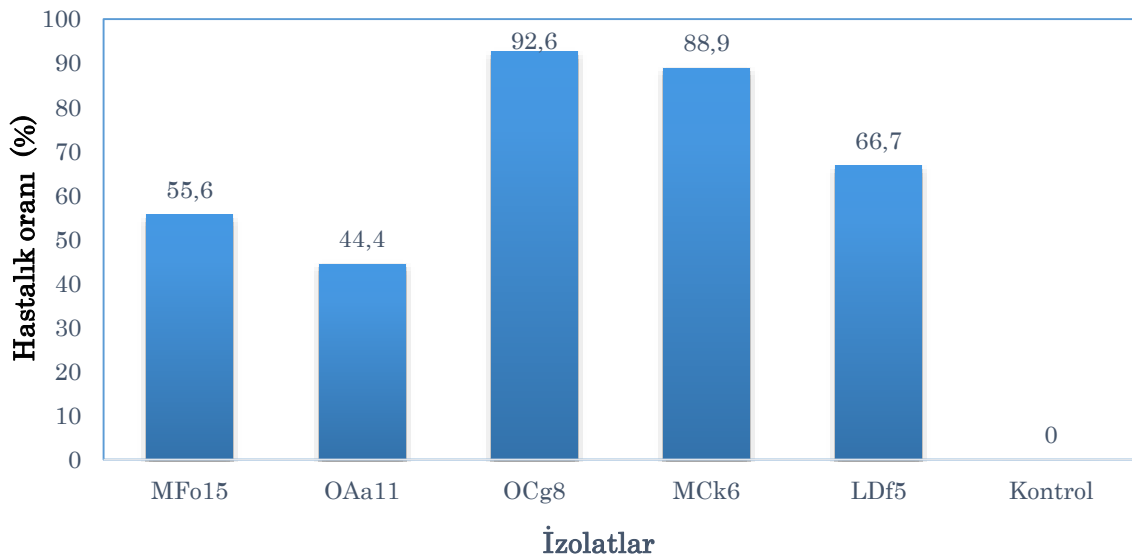
| Fungal izolatlar                      | İzolat No | Lokasyon | Yıl  | Konukçu  | Genbank Erişim numarası |
|---------------------------------------|-----------|----------|------|----------|-------------------------|
| <i>Fusarium oxysporum</i>             | MFo15     | Erzin    | 2021 | Portakal | PP109412                |
| <i>Alternaria alternata</i>           | OAA11     | Erzin    | 2021 | Portakal | PP109410                |
| <i>Colletotrichum gloeosporioides</i> | OCg8      | Dörtyol  | 2020 | Portakal | PP109411                |
| <i>Colletotrichum karsti</i>          | MCK6      | Dörtyol  | 2021 | Mandarin | MH156748                |
| <i>Diaporthe foeniculina</i>          | LDf5      | Arsuz    | 2021 | Limon    | PP109413                |

Moleküler çalışmalar sonucunda fungal izolatların, *F. oxysporum* tür kompleksi, *A. alternata* tür kompleksi, *C. gloeosporioides* tür kompleksi, *C. karsti* ve *D. foeniculina* türlerine ait olduğu kesin olarak tanımlanmıştır.

### Patojenisite testleri

Portakal (washington çeşidi) mandarin (satsuma çeşidi) ve limon (interdonat çeşidi) meyvelerinde gerçekleştirilen patojenisite testlerine göre, tüm fungusların meyvelerde enfeksiyon oluşturduğu gözlenmiştir. Kontrol olarak kullanılan meyvelerde ise herhangi bir enfeksiyon tespit edilmemiştir. Elde edilen sonuçlara göre, en fazla hastalık oranı, *C. gloeosporioides* OCg8 ve *C. karsti* MCK6 türleri tarafından oluşturulmuştur. Uysal ve ark. (2022) tarafından yapılmış bir çalışmada, *Colletotrichum* türlerinin turunçgil meyve dökümlerinde en etkin

fungal türler olduğunu bildirmişlerdir. *D. foeniculina* LDf5 fungal etmeni, %66.7 hastalık oranı ile *Colletotrichum* türlerini takip etmiştir (Şekil 3). Benzer şekilde Tekiner ve ark., 2020' de *D. foeniculina*'nın limonda meyve çürüklüğüne neden olduğunu bildirmişlerdir. Benzer şekilde, *Diaporthe* cinsine ait türlerinde turunçgil ağaçlarında sürgün yanıklığı, kanser, geriye doğru ölüm şeklinde farklı hastalık tipleri de oluşturduğu daha önceki çalışmalarda bildirilmiştir (Huang ve ark. 2013; Guarnaccia & Crous, 2017). Bu çalışma ile söz konusu tespit edilen fungal türlerin turunçgil ağaçlarının tüm aksamalarında enfeksiyona neden olmalarının yanı sıra, meyve sapında enfeksiyon oluşturarak meyve dökümüne neden olduğu bu çalışma ile bir kez daha desteklenmiştir. *F. oxysporum* MFo15 ve *A. alternata* OAA11 türlerinin en az hastalık oranı oluşturduğu saptanmıştır. (Şekil 3).



Şekil 3. Meyve dökümüne neden olan fungal türlerin patojenisite testi; meyvelerdeki inokulasyon noktaları yüzdesi [MFo15: *F. oxysporum*, OAA11: *A. alternata*, OCg8: *C. gloeosporioides*, MCK6: *C. karsti*, LDf5: *D. foeniculina*]  
Figure 3. Pathogenicity testing of fungal species that cause fruit drop; percentage of inoculation points on fruits [MFo15: *F. oxysporum*, OAA11: *A. alternata*, OCg8: *C. gloeosporioides*, MCK6: *C. karsti*, LDf5: *D. foeniculina*]

Bu türlerin, bitkiler zayıf düştüğü ve hasat geciktiği zaman enfeksiyon gerçekleştirdiği, yapılan arazi sörveyleri sonucundaki gözlemler ile örtüşmektedir.

### Bor ve PA bileşiklerinin fungal patojenlerinin misel gelişimi üzerine etkileri

Bu çalışmada, bor bileşiklerinin ve PA'nın tekli ve kombinasyonları, turunçgil ağaçlarında meyve dökümüne neden olan patojenlerin misel gelişimi

üzerindeki antimikrobiyal etkisi araştırılmıştır. Kullanılan bileşiklerin patojenlerin miseliyal gelişimi (mm) üzerindeki antifungal etkileri Çizelge 3'te gösterilmiştir. Farklı dozlar içeren borik asit (%0.12), boraks (%0.1), Etidot 67 (%0.1) ve PA (%1.5) uygulamalarında patojenlerin miseliyal gelişiminin tamamen engellendiği tespit edilmiştir. Bor ürünlerinden Boraks ve Etidot 67, borik asit uygulamasına göre daha düşük konsantrasyonda fungal miseliyal gelişimi engellediği gözlenmiştir. Bor bileşiklerinin PA ile kombinasyonları incelendiğinde ise, her bir bileşiğin konsantrasyon değerleri daha düşük kullanılarak miseliyal gelişimin engellendiği bulunmuştur. Elde edilen bulgulara göre, Etidot 67+PA ve Borax+PA kombinasyonları, en yüksek engelleme oranına sahip uygulamalar olarak belirlenmiştir. Bu durum, üzerinde çalışılan ürünleri bahçe koşullarında kullanırken kombinasyon şeklinde uygulamalara yer verilmesinin daha etkili sonuçlar doğurabileceğini ortaya koymaktadır. (Uysal, 2024). Bor bileşikleri için EC<sub>50</sub> değerleri incelendiğinde, temsili 5 farklı fungal izolatın, %0.059- 0.067 arasında değişen seviyelerde EC<sub>50</sub> değerlerine sahip olduğu belirlenmiştir. PA'nın fungal izolatlar için ortaya koyduğu EC<sub>50</sub> değerleri %0.62-0.69 arasında değişkenlik göstermiştir (Çizelge 3). Denemede, iki bağımsız deneyden elde edilen veriler ayrı ayrı analiz edilmiş ancak önemli ölçüde farklı değildir (P=0.041). Probit analizi kullanılarak her bileşik için EC<sub>50</sub> değeri (%50 inhibisyon için gereken konsantrasyon) hesaplanmıştır.

### **Bor ve PA bileşiklerinin fungal patojenlerinin konidial çimlenme üzerine etkileri**

Bor ürünleri ve PA'nın tekli ve kombinasyon halindeki uygulamaları, 5 farklı turuncgil meyve döküm patojeninin konidial çimlenmesine antimikrobiyal etkinliklerini araştırmak için gerçekleştirilmiştir (Çizelge 4). Elde edilen bulgulara göre farklı konsantrasyonlardaki borik asit (%0.15), Boraks (%0.12) ve Etidot-67 (%0.12) bileşikleri yüksek konsantrasyonlarında, *C. karsti* (MCK6) ve *D. foeniculina* (LDf5)'yı tamamen engellemesine rağmen denemeye alınan diğer patojen fungusların konidial çimlenmesinde çok yüksek etki gözlenmemiştir. Öte yandan PA'nın en yüksek konsantrasyonunda (%1.5) tüm fungal türlerde tamamen engelleme saptanmıştır. Bor bileşiklerinin kombinasyon halinde kullanımı ile düşük konsantrasyonlar da bile yüksek engelleme oranı saptanmıştır. Bor bileşiklerde, 5 fungal patojen izolatın %0.065-0.095 arasında değişen EC<sub>50</sub> değerlerine sahip olduğu görülmüştür. PA'nın EC<sub>50</sub> değerleri %0.66-0.82 arasında değişkenlik göstermiştir (Çizelge 3). Çalışmada kullanılan alternatif bileşiklerde miseliyal gelişim için hesaplanan değerlere göre konidial çimlenmeyi engellemede belirlenen EC<sub>50</sub> değerlerinin daha yüksek olduğu

saptanmıştır. Elde edilen bu bulgular, Uysal, (2024) tarafından *Monilinia laxa*'ya karşı yapılan uygulamalardan elde edilen EC<sub>50</sub> değerleri ve diğer bulguları ile uyumluluk göstermektedir. Bununla birlikte kış kabaklarında kök ve kökboğazı çürüklüğüne neden olan *R. solani*'ye karşı bazı bor bileşiklerinin belirgin bir şekilde yararlı olabileceğini ortaya koyan Erper ve ark. (2019b)'nın bulguları, bu çalışmada elde edilen verileri destekler niteliktedir. Benzer bir çalışmada (Baharom ve ark., 2020), hindistan cevizi kabuğu, karambola ve mangodan hazırlanan PA'nın, *F. oxysporum*, *C. gloeosporioides*, *Pestalotiopsis microspora* ve *Ralstonia solanacearum*'a karşı önemli antimikrobiyal etki gösterdiği bildirilmiştir. Bu çalışmanın sonuçlarının, PA'nın potansiyel yönü ile bağlantılı bir şekilde mevcut araştırmanın verilerini doğrulayarak uyum içerisinde olduğu gözlenmiştir. Üç farklı üründen elde edilen PA'ların, soğan patojeni *Fusarium proliferatum*'a karşı yapılan antifungal etkinlik çalışmasında, kayısı çekirdeğinden üretilen PA'nın misel büyümesi için 3.5 µL mL<sup>-1</sup> ve konidial çimlenme için 1.0 µL mL<sup>-1</sup> konsantrasyon değerlerinde en etkili PA olarak bildirilmiştir (Kara ve ark., 2024). Denemede, iki bağımsız deneyden elde edilen veriler ayrı ayrı analiz edilmiş ancak önemli ölçüde farklılık görülmemiştir (P = 0.037). Probit analizi kullanılarak her bileşik için EC<sub>50</sub> değeri (%50 inhibisyon için gereken konsantrasyon) hesaplanmıştır.

Yakın zamanda yapılan bir çalışmada kayısı çekirdeği, kermes meşe ağacı ve fındık kabuğu gibi 3 farklı bitki atıklarından elde edilen PA'nın antifungal etkinliği soğanlarda önemli toprak kökenli fungal hastalık etmeni *F. proliferatum*'a karşı araştırılmıştır (Kara ve ark., 2024). Yapılan GC-MS analizleri sonucunda farklı bitkilerden elde edilen PA'ların ana bileşenlerinin o-guaiacol (%18.98–26.18), creosol (%11.08–16.38), syringol (%6.35–8.67), p-ethylguaiacol (%5.53–10.04), o-creosol (%4.0–10.33) ve fenol (%2.34–7.90) olduğu belirlenmiştir. Mevcut çalışmalarda kullanılan okaliptüs PA'nın ana kimyasal bileşenlerinin ise yakın zamanda yapılmış bir diğer çalışmada 2-methoxy-4-methylphenol (%18.78), fenol, 2-methoxy (%27.68) ve kaempferol (%3.12) olduğu bildirilmiştir (Uysal, 2024). PA' lardaki isli durumun genelde içeriğinde yüksek oranda bulunan fenolik bileşenlerden kaynaklanmaktadır (Grewal ve ark., 2018). Farklı bitkisel kaynaklı PA ve kimyasal ana bileşenlerinden farklı fenoliklerin bitki patojeni fungal ve bakteriyel hastalık etmenlerine karşı *in vitro* ve *in vivo* antimikrobiyal etkinlikten sorumlu olduğu yapılmış önceki bir çok çalışmada bildirilmiştir (Gao ve ark., 2021; Bouket ve ark., 2022; Sivaram ve ark., 2022; Pertile & Frac, 2023; Kara ve ark., 2024).

### **SONUÇ ve ÖNERİLER**

Mevcut çalışma, turuncgil ağaçlarında gözlenen

patolojik meyve dökümlerine sebep olan fungal etmenler, bunların morfolojik ve moleküler yöntemlerle tanısı ile mücadelede alternatif yöntemlerden PA ve bor bileşiklerinin fungal patojenlere karşı etkinlikleri ortaya koymaktadır. Tarımsal üretimde karşılaşılan bitki sağlığı sorunlarının çözümünde sıklıkla başvuru olan yöntemlerin başında gelen kimyasal pestisit kullanımının insan sağlığına ve çevreye olan zararlı etkileri göz önüne alınarak çevre dostu ve organik kökenli PA ve bor bileşiklerinin sürdürülebilir kalkınmaya katkıda bulunmak amacıyla kullanımına

yönelik çalışmalara ihtiyaç duyulmuştur. Bu kapsamda değerlendirildiğinde çalışmanın, turuncgil patojenlerine karşı dünyada ve Türkiye’de ilk kez yürütülmesi sebebiyle özgün ve etkin nitelikler taşıdığı aşikardır. Kimyasal pestisitlerin tarımda yaygın kullanımı, halk sağlığı endişelerini artırırken, bazı fungusitler Dünya Sağlık Örgütü (WHO) tarafından tehlikeli olarak sınıflandırılmış ve Avrupa Birliği’nde yasaklanmıştır. Bu nedenle, organik ürünler, bitki hastalıklarını kontrol etmek için kimyasal olmayan, çevre dostu bir alternatif olarak önerilmektedir.

Çizelge 3. Farklı konsantrasyonlarda seçilmiş olan bileşiklerin, fungal izolatların miseliyal gelişimi üzerine *in vitro* antifungal etkileri

Table 3. *In vitro* antifungal effects of compounds selected at different concentrations on mycelial growth of fungal isolates

| Bileşikler                    | Kons.            | Fungal patojenlerin miseliyal gelişimi (mm)* |                             |                            |                            |                             |
|-------------------------------|------------------|--|-----------------------------|----------------------------|----------------------------|-----------------------------|
|                               |                  | MFo15  | OAA11                       | OCg8                       | MCK6                       | LDf5                        |
| <b>Borik asit</b><br>(%, w/v) | <b>0.0</b>       | 80.0 (±0.0) <sup>f</sup>                     | 80.0 (±0.0) <sup>f</sup>    | 80.0 (±0.0) <sup>f</sup>   | 80.0 (±0.0) <sup>f</sup>   | 80.0 (±0.0) <sup>f</sup>    |
|                               | <b>0.03</b>      | 64.0 (±0.5) <sup>eB</sup>                    | 61.4 (±0.2) <sup>eA</sup>   | 61.5 (±0.2) <sup>eA</sup>  | 60.6 (±0.1) <sup>eA</sup>  | 57.5 (±0.2) <sup>eA</sup>   |
|                               | <b>0.05</b>      | 48.0 (±0.0) <sup>dA</sup>                    | 50.7 (±0.1) <sup>dA</sup>   | 50.7 (±0.3) <sup>dA</sup>  | 52.2 (±0.1) <sup>dB</sup>  | 48.7 (±0.1) <sup>dA</sup>   |
|                               | <b>0.07</b>      | 33.0 (±0.5) <sup>cB</sup>                    | 34.7 (±0.0) <sup>cC</sup>   | 29.5 (±0.1) <sup>cA</sup>  | 35.6 (±0.2) <sup>cC</sup>  | 25.5 (±0.1) <sup>cA</sup>   |
|                               | <b>0.1</b>       | 18.7 (±0.2) <sup>bC</sup>                    | 17.8 (±0.1) <sup>bC</sup>   | 13.6 (±0.2) <sup>bA</sup>  | 15.3 (±0.3) <sup>b</sup>   | 12.9 (±0.4) <sup>bA</sup>   |
|                               | <b>0.12</b>      | 0.0(±0.0) <sup>a</sup>                       | 0.0(±0.0) <sup>a</sup>      | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>      |
| <b>EC<sub>50</sub></b>        | -                | <b>0.062±0.5</b>                             | <b>0.062±0.5</b>            | <b>0.060±0.6</b>           | <b>0.062±0.5</b>           | <b>0.059±0.6</b>            |
| <b>Boraks</b><br>(%, w/v)     | <b>0.0</b>       | 80.0 (±0.0) <sup>f</sup>                     | 80.0 (±0.0) <sup>d</sup>    | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>    |
|                               | <b>0.03</b>      | 68.7 (±0.3) <sup>eC</sup>                    | 66.5 (±0.3) <sup>cdB</sup>  | 65.0 (±0.0) <sup>cdA</sup> | 69.4 (±0.3) <sup>cdD</sup> | 65.1 (±0.3) <sup>cdA</sup>  |
|                               | <b>0.05</b>      | 58.9 (±0.2) <sup>dD</sup>                    | 55.6 (±0.4) <sup>bcB</sup>  | 56.7 (±0.3) <sup>bcC</sup> | 52.6 (±0.5) <sup>eA</sup>  | 52.6 (±0.4) <sup>eA</sup>   |
|                               | <b>0.07</b>      | 35.3 (±0.3) <sup>cCD</sup>                   | 36.1 (±0.5) <sup>bcD</sup>  | 32.7 (±0.3) <sup>bcA</sup> | 34.0 (±0.5) <sup>bcB</sup> | 34.8 (±0.1) <sup>bcBC</sup> |
|                               | <b>0.09</b>      | 19.0 (±0.5) <sup>bB</sup>                    | 21.0 (±0.0) <sup>abC</sup>  | 19.3 (±0.2) <sup>abB</sup> | 11.3 (±0.2) <sup>abA</sup> | 11.9 (±0.3) <sup>abA</sup>  |
|                               | <b>0.1</b>       | 0.0(±0.0) <sup>a</sup>                       | 0.0(±0.0) <sup>a</sup>      | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>      |
| <b>EC<sub>50</sub></b>        | -                | <b>0.064±0.7</b>                             | <b>0.063±0.5</b>            | <b>0.063±0.6</b>           | <b>0.060±0.7</b>           | <b>0.061±0.7</b>            |
| <b>Etidot-67</b><br>(%, w/v)  | <b>0.0</b>       | 80.0 (±0.0) <sup>d</sup>                     | 80.0 (±0.0) <sup>d</sup>    | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>    |
|                               | <b>0.03</b>      | 73.9 (±0.1) <sup>cdD</sup>                   | 70.7 (±0.8) <sup>cdC</sup>  | 70.1 (±0.0) <sup>cdC</sup> | 67.5 (±0.3) <sup>bcA</sup> | 68.6 (±0.5) <sup>bcB</sup>  |
|                               | <b>0.05</b>      | 57.3 (±0.5) <sup>bcdB</sup>                  | 58.3 (±0.2) <sup>bcB</sup>  | 58.6 (±0.4) <sup>bcB</sup> | 53.0 (±0.1) <sup>bA</sup>  | 54.2 (±0.5) <sup>bA</sup>   |
|                               | <b>0.07</b>      | 34.0 (±0.5) <sup>bcB</sup>                   | 37.9 (±0.5) <sup>bcC</sup>  | 34.9 (±0.5) <sup>bcB</sup> | 29.0 (±0.2) <sup>abA</sup> | 28.3 (±0.3) <sup>abA</sup>  |
|                               | <b>0.09</b>      | 19.0 (±0.0) <sup>abBC</sup>                  | 20.0 (±0.0) <sup>abC</sup>  | 18.3 (±0.2) <sup>abB</sup> | 13.6 (±0.1) <sup>aA</sup>  | 13.2 (±0.1) <sup>aA</sup>   |
|                               | <b>0.1</b>       | 0.0(±0.0) <sup>a</sup>                       | 0.0(±0.0) <sup>a</sup>      | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>      |
| <b>EC<sub>50</sub></b>        | -                | <b>0.065±0.6</b>                             | <b>0.067±0.6</b>            | <b>0.066±0.6</b>           | <b>0.060±0.6</b>           | <b>0.061±0.6</b>            |
| <b>PA</b><br>(%, w/v)         | <b>0.0</b>       | 80.0 (±0.0) <sup>d</sup>                     | 80.0 (±0.0) <sup>d</sup>    | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>   | 80.0 (±0.0) <sup>d</sup>    |
|                               | <b>0.3</b>       | 70.1 (±0.1) <sup>cdC</sup>                   | 73.9 (±0.3) <sup>cdD</sup>  | 67.0 (±0.5) <sup>cdA</sup> | 69.3 (±0.1) <sup>cdB</sup> | 67.0 (±0.3) <sup>cdA</sup>  |
|                               | <b>0.5</b>       | 57.0 (±0.1) <sup>bcB</sup>                   | 59.6 (±0.1) <sup>bcdC</sup> | 58.0 (±0.6) <sup>bcB</sup> | 54.7 (±0.3) <sup>bcA</sup> | 54.6 (±0.5) <sup>bcA</sup>  |
|                               | <b>0.7</b>       | 40.0 (±0.3) <sup>bcD</sup>                   | 39.0 (±0.5) <sup>bcD</sup>  | 36.7 (±0.3) <sup>bcC</sup> | 32.3 (±0.1) <sup>bcB</sup> | 31.0 (±0.7) <sup>bcA</sup>  |
|                               | <b>1.0</b>       | 22.6 (±0.1) <sup>abC</sup>                   | 23.0 (±0.0) <sup>abC</sup>  | 20.6 (±0.1) <sup>abB</sup> | 15.9 (±0.2) <sup>abA</sup> | 16.4 (±0.2) <sup>abA</sup>  |
|                               | <b>1.5</b>       | 0.0(±0.0) <sup>a</sup>                       | 0.0(±0.0) <sup>a</sup>      | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>     | 0.0(±0.0) <sup>a</sup>      |
| <b>EC<sub>50</sub></b>        | -                | <b>0.68±0.5</b>                              | <b>0.69±0.5</b>             | <b>0.67±0.5</b>            | <b>0.63±0.5</b>            | <b>0.62±0.5</b>             |
| <b>BA+</b>                    | <b>0.07+0.05</b> | 5.0 (±0.0) <sup>BC</sup>                     | 7.3 (±1.4) <sup>C</sup>     | 6.0 (±1.0) <sup>C</sup>    | 1.6 (±1.5) <sup>AB</sup>   | 0.0 (±0.0) <sup>A</sup>     |
| <b>Boraks</b>                 |                  |  |                             |                            |                            |                             |
| <b>BA+PA</b>                  | <b>0.08+0.8</b>  | 7.3 (±1.4) <sup>B</sup>                      | 7.0 (±1.0) <sup>B</sup>     | 3.3 (±1.6) <sup>A</sup>    | 0.0 (±0.0) <sup>A</sup>    | 0.0 (±0.01) <sup>A</sup>    |
| <b>Boraks+</b>                | <b>0.05+0.8</b>  | 5.6 (±0.6) <sup>B</sup>                      | 4.6 (±2.0) <sup>B</sup>     | 5.0 (±0.0) <sup>B</sup>    | 0.0 (±0.0) <sup>A</sup>    | 0.0 (±0.0) <sup>A</sup>     |
| <b>PA</b>                     |                  |  |                             |                            |                            |                             |
| <b>Etidot</b>                 | <b>0.06+0.8</b>  | 1.6 (±1.6) <sup>A</sup>                      | 3.6 (±1.8) <sup>A</sup>     | 0.0 (±0.0) <sup>A</sup>    | 0.0 (±0.0) <sup>A</sup>    | 0.0 (±0.0) <sup>A</sup>     |
| <b>67+PA</b>                  |                  |  |                             |                            |                            |                             |

[MFo15: *F. oxysporum*, OAA11: *A. alternata*, OCg8: *C. gloeosporioides*, MCK6: *C. karsti*, LDf5: *D. foeniculina*]

Boraks (Disodium tetraborate decahydrate); Etidot-67 (Disodium octaborate tetrahydrate); BA (Borik asit); PA (Pirrolignöz asit)

\*Her sütun veya satırda sırasıyla farklı küçük veya büyük harflerle takip edilen ortalama değerler (n=3), Duncan Çoklu Aralık Testine göre anlamlı derecede farklıdır (P<0.05)

Çizelge 4 Farklı konsantrasyonlarda kullanılan bileşiklerin fungal izolatların konidi çimlenmesi üzerine *in vitro* etkileri

Table 4. *In vitro* effects of compounds used at different concentrations on conidial germination of fungal isolates

| Bileşikler                    | Kons.            | Fungal patojenlerin konidi çimlenmesi |                            |                            |                           |                           |
|-------------------------------|------------------|---------------------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
|                               |                  | MFo15                                 | OAa11                      | OCg8                       | MCK6                      | LDf5                      |
| <b>Borik asit</b><br>(%. w/v) | <b>0.0</b>       | 100.0(±0.0) <sup>f</sup>              | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>  | 100.0(±0.0) <sup>f</sup>  |
|                               | <b>0.05</b>      | 88.6 (±0.6) <sup>eD</sup>             | 90.0 (±0.9) <sup>eD</sup>  | 81.0 (±0.2) <sup>eC</sup>  | 77.0 (±0.8) <sup>eB</sup> | 71.6 (±1.4) <sup>eA</sup> |
|                               | <b>0.07</b>      | 70.0 (±0.0) <sup>dBC</sup>            | 72.6 (±0.6) <sup>dC</sup>  | 67.6 (±1.2) <sup>dB</sup>  | 62.6 (±1.2) <sup>dA</sup> | 60.3 (±0.3) <sup>dA</sup> |
|                               | <b>0.1</b>       | 50.3 (±0.3) <sup>cC</sup>             | 50.6 (±1.0) <sup>cC</sup>  | 48.3 (±0.3) <sup>cB</sup>  | 43.6 (±0.8) <sup>cA</sup> | 42.3 (±0.3) <sup>cA</sup> |
|                               | <b>0.12</b>      | 30.6 (±0.6) <sup>bB</sup>             | 32.0 (±0.0) <sup>bB</sup>  | 30.3 (±0.3) <sup>bB</sup>  | 24.6 (±0.5) <sup>bA</sup> | 23.0 (±1.5) <sup>bA</sup> |
|                               | <b>0.15</b>      | 13.3(±0.9) <sup>aC</sup>              | 15.0 (±0.0) <sup>aC</sup>  | 6.0(±1.2) <sup>aB</sup>    | 0.0(±0.0) <sup>aA</sup>   | 0.0(±0.0) <sup>aA</sup>   |
| <b>EC<sub>50</sub></b>        | -                | <b>0.093±0.4</b>                      | <b>0.095±0.4</b>           | <b>0.087±0.3</b>           | <b>0.080±0.4</b>          | <b>0.076±0.3</b>          |
| <b>Boraks</b><br>(%. w/v)     | <b>0.0</b>       | 100.0(±0.0) <sup>f</sup>              | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>  | 100.0(±0.0) <sup>f</sup>  |
|                               | <b>0.05</b>      | 91.6 (±1.6) <sup>eC</sup>             | 91.6 (±1.6) <sup>eC</sup>  | 85.0 (±0.0) <sup>eB</sup>  | 75.3 (±0.3) <sup>eA</sup> | 75.0 (±0.0) <sup>eA</sup> |
|                               | <b>0.07</b>      | 76.3 (±0.6) <sup>dC</sup>             | 78.3 (±1.4) <sup>dC</sup>  | 70.0 (±0.0) <sup>dB</sup>  | 62.6 (±1.4) <sup>dA</sup> | 60.3 (±0.3) <sup>dA</sup> |
|                               | <b>0.09</b>      | 53.6 (±1.3) <sup>cC</sup>             | 50.3 (±0.3) <sup>cB</sup>  | 52.6 (±0.3) <sup>cC</sup>  | 48.3 (±0.3) <sup>cB</sup> | 40.3 (±0.3) <sup>cA</sup> |
|                               | <b>0.1</b>       | 31.6 (±0.8) <sup>bD</sup>             | 27.6 (±2.6) <sup>bCD</sup> | 24.0 (±0.5) <sup>bBC</sup> | 21.6 (±0.3) <sup>bB</sup> | 11.9 (±0.1) <sup>bA</sup> |
|                               | <b>0.12</b>      | 10.0 (±0.0) <sup>aB</sup>             | 11.6 (±1.6) <sup>aB</sup>  | 0.0 (±0.0) <sup>aA</sup>   | 0.0 (±0.0) <sup>aA</sup>  | 0.0 (±0.0) <sup>aA</sup>  |
| <b>EC<sub>50</sub></b>        | -                | <b>0.086±0.5</b>                      | <b>0.085±0.5</b>           | <b>0.080±0.5</b>           | <b>0.074±0.5</b>          | <b>0.071±0.5</b>          |
| <b>Etidot-67</b><br>(%. w/v)  | <b>0.0</b>       | 100.0(±0.0) <sup>f</sup>              | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>  | 100.0(±0.0) <sup>f</sup>  |
|                               | <b>0.05</b>      | 90.0 (±0.0) <sup>eD</sup>             | 89.3 (±0.8) <sup>eD</sup>  | 80.6 (±0.6) <sup>eC</sup>  | 75.3 (±0.3) <sup>eB</sup> | 70.0 (±0.0) <sup>eA</sup> |
|                               | <b>0.07</b>      | 70.6 (±0.6) <sup>dC</sup>             | 72.6 (±1.2) <sup>dC</sup>  | 63.0 (±1.5) <sup>dB</sup>  | 50.0 (±0.0) <sup>dA</sup> | 47.6 (±0.3) <sup>dA</sup> |
|                               | <b>0.09</b>      | 46.0 (±1.0) <sup>cD</sup>             | 45.3 (±0.3) <sup>cCD</sup> | 43.6 (±0.3) <sup>cC</sup>  | 30.0 (±0.0) <sup>cB</sup> | 24.3 (±0.6) <sup>cA</sup> |
|                               | <b>0.1</b>       | 23.6 (±0.3) <sup>bD</sup>             | 24.6 (±0.3) <sup>bD</sup>  | 17.6 (±1.3) <sup>bB</sup>  | 20.0 (±0.0) <sup>bC</sup> | 13.2 (±0.1) <sup>bA</sup> |
|                               | <b>0.12</b>      | 8.3 (±0.8) <sup>aC</sup>              | 8.3 (±0.8) <sup>aC</sup>   | 2.6 (±0.3) <sup>aB</sup>   | 0.0 (±0.0) <sup>aA</sup>  | 0.0 (±0.0) <sup>aA</sup>  |
| <b>EC<sub>50</sub></b>        | -                | <b>0.081±0.5</b>                      | <b>0.082±0.5</b>           | <b>0.075±0.5</b>           | <b>0.068±0.5</b>          | <b>0.065±0.5</b>          |
| <b>PA</b><br>(%. w/v)         | <b>0.0</b>       | 100.0(±0.0) <sup>f</sup>              | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>   | 100.0(±0.0) <sup>f</sup>  | 100.0(±0.0) <sup>f</sup>  |
|                               | <b>0.5</b>       | 86.0 (±1.0) <sup>eD</sup>             | 79.6 (±0.8) <sup>eC</sup>  | 79.6 (±0.3) <sup>eC</sup>  | 75.3 (±1.4) <sup>eB</sup> | 68.0 (±0.9) <sup>eA</sup> |
|                               | <b>0.7</b>       | 65.3 (±0.3) <sup>dD</sup>             | 63.6 (±0.6) <sup>dD</sup>  | 60.0 (±0.0) <sup>dC</sup>  | 52.3 (±1.5) <sup>dB</sup> | 48.6 (±0.6) <sup>dA</sup> |
|                               | <b>1.0</b>       | 43.6 (±0.8) <sup>cC</sup>             | 46.0 (±1.0) <sup>cD</sup>  | 44.0 (±0.5) <sup>cC</sup>  | 30.0 (±0.0) <sup>cB</sup> | 27.3 (±0.6) <sup>cA</sup> |
|                               | <b>1.2</b>       | 16.6 (±1.6) <sup>bBC</sup>            | 19.3 (±0.3) <sup>bC</sup>  | 15.3 (±0.3) <sup>bB</sup>  | 7.6 (±1.3) <sup>bA</sup>  | 5.0 (±0.0) <sup>bA</sup>  |
|                               | <b>1.5</b>       | 0.0 (±0.8) <sup>a</sup>               | 0.0 (±0.0) <sup>a</sup>    | 5.3 (±2.8) <sup>a</sup>    | 0.0 (±0.8) <sup>a</sup>   | 0.0 (±0.0) <sup>a</sup>   |
| <b>EC<sub>50</sub></b>        | -                | <b>0.82±0.4</b>                       | <b>0.80±0.4</b>            | <b>0.78±0.4</b>            | <b>0.70±0.4</b>           | <b>0.66±0.4</b>           |
| <b>BA+</b><br><b>Boraks</b>   | <b>0.07+0.07</b> | 18.3 (±1.6) <sup>B</sup>              | 15.0 (±2.8) <sup>B</sup>   | 5.0 (±2.8) <sup>A</sup>    | 3.3 (±1.6) <sup>A</sup>   | 1.6 (±1.6) <sup>A</sup>   |
| <b>BA+PA</b>                  | <b>0.09+0.8</b>  | 6.6 (±1.6) <sup>AB</sup>              | 10.0 (±2.0) <sup>B</sup>   | 9.3 (±0.6) <sup>B</sup>    | 1.6 (±1.6) <sup>A</sup>   | 1.0 (±1.0) <sup>A</sup>   |
| <b>Boraks+PA</b>              | <b>0.07+0.8</b>  | 3.3 (±1.2) <sup>A</sup>               | 8.3 (±1.6) <sup>B</sup>    | 3.3 (±1.5) <sup>A</sup>    | 1.2 (±1.2) <sup>A</sup>   | 0.0 (±0.0) <sup>aA</sup>  |
| <b>Etidot</b><br><b>67+PA</b> | <b>0.07+0.8</b>  | 10.0 (±0.0) <sup>B</sup>              | 10.6 (±0.6) <sup>B</sup>   | 1.0 (±1.0) <sup>A</sup>    | 0.0 (±0.0) <sup>aA</sup>  | 0.0 (±0.0) <sup>aA</sup>  |

[MFo15: *F. oxysporum*, OAa11: *A. alternata*, OCg8: *C. gloeosporioides*, MCK6: *C. karsti*, LDf5: *D. foeniculina*]

Boraks (Disodium tetraborate decahydrate); Etidot-67 (Disodium octaborate tetrahydrate); BA (Borik asit); PA (Piroloignöz asit)

\*Her sütun veya satırda sırasıyla farklı küçük veya büyük harflerle takip edilen ortalama değerler (n=3), Duncan Çoklu Aralık Testine göre anlamlı derecede farklıdır (P<0.05)

Bu sebeple, turuncgillerde meyve dökümüne neden olan fungal hastalık etmenleri ve bunların mücadelesi üzerine araştırmaların sürdürülmesi kaçınılmaz bir durumdur. Bu bağlamda, turuncgil üreticiliğinde ciddi kayıplara sebep olan meyve döküm sorunları tespit edilerek buna neden olan fungal hastalık etmenlerinin kesin tanıları yapılmış ve bunlara karşı *in vitro* düzeyde organik içerikli ürünlerin etkinlikleri belirlenmiştir. Ayrıca bor elementi, bitki gelişimi için gerekli ve zorunlu bir mikroelement olup, yüksek miktarda kullanıldığında bitkilerde toksik etkilere yol açabilmektedir. Bu nedenle, PA ile kombine bor bileşikleri ile yapılan çalışmadan elde edilen bulgular doğrultusunda araştırmanın, bahçe koşullarında

meyve dökümü yaşanan alanlarda geniş ölçekli olarak sürdürülmesi zorunluluk arz etmektedir.

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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.

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## Kahramanmaraş İlinde Belirlenen Küsküt Türleri (*Cuscuta* spp.): Bu Türlerin Morfolojik Yapıları ve Konakçaya Bağlı Yoğunluklarının Belirlenmesi

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### ÖZET

Bu çalışma Kahramanmaraş iline bağlı merkez ilçeler (Dulkadiroğlu ve Onikişubat) dâhil toplam 11 ilçede tarım ve tarım dışı alanlarda sorun oluşturan tam parazit küsküt (*Cuscuta* spp.) türlerini, yoğunluk ve konukçularını belirlemek amacı ile 2018-2021 yıllarında yürütülmüştür. Yapılan çalışmalar değerlendirildiğinde; il genelinde tüm ilçeler dikkate alındığında toplam altı küsküt taksonu belirlenmiştir. Tespit edilen taksonlar; *Cuscuta campestris* Yunck., *C. approximata* Bab., *C. monogyna* subsp. *monogyna* Vahl., *C. lupuliformis* Krock., *C. pedicellata* Ledeb ve *C. planiflora* Ten. Küsküt taksonlarının beş farklı bitki grubuna göre konukçu olduğu bitki türleri incelendiğinde; tarla bitkisi (23), meyve ağacı (7), süs bitkisi (7), orman (9) ve yabancı ot (62) türü olmak üzere 35 familyaya ait 108 takson tespit edilmiştir. Yoğunluk değerlerine göre; tarla, süs bitkileri ve yabancı otlar üzerinde *C. campestris*, yonca'da *C. approximata* ve odunsu bitkilerde ise *C. lupuliformis* çok yoğun olarak ( $m^2/küsküt\ dal > 10$ ) belirlenmiştir. Bu çalışmadan elde edilen tüm veriler polikültür tarım yapılan Kahramanmaraş il genelinde ilgili taksonların varlığına ve muhtemel risklerine yönelik önemli veriler sağlar. Ayrıca, odunsu bitkilerin de ciddi bir konak olabildiğine yönelik ilk bulguları sunar.

### Herboloji

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

Cuscuta

Morfoloji

Konukçu

Yoğunluk

Kahramanmaraş

## Dodder Species (*Cuscuta* spp.) Identified in Kahramanmaraş Province: Morphological Structures of These Species and Determination of Their Densities Depending on the Host

### ABSTRACT

This study was conducted in 2018 - 2021 in 11 districts, including the central districts (Dulkadiroglu and Onikisubat) of Kahramanmaraş province located in the Mediterranean Region of Turkey. The aim of this study was to determine the species, density, and hosts of the obligate parasitic weed (*Cuscuta* spp.) that causes issues in both agricultural and non-agricultural areas. Based on the studies that were conducted, it was identified that 6 species of dodder have been spread throughout all districts of this province. The species identified are *Cuscuta campestris* Yunck., *C. approximata* Bab., and *C. monogyna* subsp. *monogyna* Vahl., *C. lupuliformis* Krock., *C. pedicellata* Ledeb, and *C. planiflora* Ten. Through the investigation, it was found that the plant species that constitute dodder hosts are divided into five different plant groups: 108 species belonging to 35 families were identified, including 23 field plants, 7 fruit trees, 7 ornamental plants, 9 forest species, and 62 weed species. According to density values; *C. campestris* on the field, ornamental plants and weeds, *C. approximata* on clover, and *C. lupuliformis* on woody plants were determined very densely ( $m^2/dodder\ branch > 10$ ). The results obtained from this study provide important information regarding the presence and potential risks of related species throughout Kahramanmaraş province, where many plant species are cultivated. In addition, the results obtained demonstrate that woody plants are a host for these parasitic species.

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## GİRİŞ

Hayatlarının tamamını veya bir dönemini belli bir konukçuya bağlı olarak sürdüren bitkiler parazit bitki olarak tanımlanmaktadır. Parazit bitkiler konakladığı bitkinin su ve mineral madde ve organik bileşiklerini alarak bitkilerin zayıflamasına ve ileri dönemde ölmesine de neden olabilir. Parazit bitkiler tam ve yarı parazit bitkiler olmak üzere iki büyük gruba ayrılır. Tam parazit bitkiler; kök ve yaprak gibi organları olmayan, klorofil içermeyen tüm besin ihtiyaçlarını houstoriumları vasıtasıyla konak bitkiden karşılayan çiçekli bitkilerdir (Üstüner & Aksoy, 2021). Küsküt türleri (*Cuscuta* spp.) bir veya çok yıllık konukçuların üzerinde klorofilsiz ve tam parazit olarak yaşayan asalak bitkilerdir. Parazit olarak yaşamasından dolayı kültür bitkisinde gelişim bozukluğuna, vejetatif kısımlarda büyümenin yavaşlamasına- durmasına, hatta bitkinin ölümüne (Lubenov, 1985; Kadioğlu, 1992) ve ayrıca konaklarını çepeçevre sararak fotosentez yapmasına engel olur.

Türkçe Bitki Adları Sözlüğü (Baytop, 1997)'ne göre; Türkiye'de küsküt bitkisine verilen yöresel isimler; Bağbozan, Bostanbozan, Canavarotu, Cinsaçı, Gelinsaçı, Eftimon, Kızıl sarmaşık, Küşüt ve Şeytansaçı'dır. Kahramanmaraş ilinde ise küsküt türleri ilçelere göre; Afşin ilçesinde "sevda otu", Elbistan ilçesinde "yılan tuzacı", Çağlayancerit ilçesinde "sarmaşık", Pazarcık ilçesinde "verem otu" ve Göksun ilçesinde "kanser otu" gibi isimler ile anılır.

Küsküt türleri; Convolvulaceae familyası, *Cuscuta* L. cinsine ait tek yıllık ve tam parazit bir bitkidir (Dawson ve ark., 1994). Küsküt gövdesi ipliksi ve silindirik yapıda olup konukçu gövdesine sarılcı, yapraksız ve klorofilsizdir. Küsküt türleri çift çenekli (geniş yapraklı) ve tek çenekli (dar yapraklı) bitkilerin vejetatif kısımlarında zarar yapmaktadır (Üstüner & Öztürk, 2018; Üstüner ve ark., 2019; Dal & Üstüner, 2020; Üstüner, 2022; Almhedmed & Üstüner, 2023). Tohumla ve bitki parçaları (vejetatif yolla) ile çoğalma yeteneğinde olup, tek bir olgun küsküt bitkisi toprakta 10-20 yıl boyunca canlı kalabilen binlerce tohum üretebilmektedir (Garcia-Torres, 1993). Her ne kadar Davis (1978)'e göre Türkiye'de *Cuscuta* L. 16 taksonu bildirilmiş olsa da, 2020 yılında Yazlık ve Albayrak (2020) tarafından yapılan revizyon çalışmasıyla ilgili takson sayısının 23 adet olduğu bildirilmiştir. Kahramanmaraş'ta ise iki taksonun (*C. palaestina* subsp. *balansae* ve *C. planiflora*) doğal olarak yayılış gösterdiği bilinmektedir. Anadolu'da kültür alanlarında bulunan küsküt türlerinin yayılışları ve konukçuları üzerinde yapılan araştırmaya göre; kültür

bitkilerinde parazit olarak bulunan üç farklı küsküt taksonu (*C. campestris*, *C. approximata* ve *C. monogyna*) belirlenmiştir. Bunların yanında *C. campestris* ise özellikle şekerpancarı, soğan, yonca ve yazlık sebzeler üzerinde olduğu tespit edilmiştir (Nemli, 1986). Küsküt (*Cuscuta approximata*) türü yoncannın da gelişimini yavaşlatıp verimini düşüren en önemli sorunlardan biridir (Uygur, 1991; Kondap & Kumar, 1993). Tarla küskütü'nün (*C. campestris*) şeker pancarı bitkisinde yaprak verimini ve yumru boyutundaki olumsuz etkisi; ortalama yaprak verimi (enfekte olmayanlar için 32.943.6 kg/ha ve enfekte olanlar için 18.451.4 kg/ha) ve yumru boyutları (enfekte olmayanlar için 28.116×8.244 cm ve enfekte olanlar için 18.984×6.269 cm) tespit edilmiştir (Üstüner & Öztürk, 2018). Kahramanmaraş ilinde *C. campestris*'in mercimek, nohut, biber, maydanoz ve patlıcanda ciddi verim ve kalite kaybına neden olduğu bildirilmiştir (Üstüner & Öztürk, 2018; Üstüner ve ark., 2019; Dal & Üstüner, 2020; Üstüner, 2020; Üstüner, 2022; Almhedmed & Üstüner, 2023; Elsekran ve ark., 2024). Tarla küskütü'nün (*C. campestris*) kültür bitkilerinde meydana getirdiği verim kaybı %50-90 arasında değiştiği bildirilmektedir (Lanini & Kogan, 2005; Üstüner, 2018; Güvenç ve ark., 2021).

Küsküt türlerinin dünya genelinde kültür bitkilerinde özellikle yonca, üçgül, domates, havuç, soğan ve biberde verim kaybına yol açarak ciddi anlamda ekonomik kayıplara neden olduğu belirtilmektedir (Nagy ve ark., 1983; Dawson ve ark., 1994).

Nohut ve mercimek tarlasında küsküt türlerinin tohumları çimlendikten sonra 6-9 gün boyunca canlılığını sürdürebilmek için yeterli besin rezervine sahip olduklarından dolayı bu süre içerisinde uygun bir konukçu bitkiye tutunabilmektedir (Üstüner ve ark., 2019). Tarım ürünlerinde üretimi artırmanın teknik olarak birçok yolu bulunmaktadır. Bunlardan bir tanesi de modern bitki koruma yöntemleriyle yabancı otlarla savaşımının çevresel ve ekolojik dengeleri gözeterek yapılabilmesidir. Bunun için yabancı otların biyolojilerinin, ekolojilerinin, kültür bitkileriyle ilişkilerinin, kontrol altına alınma yöntemlerinin, yoğunluk ve yaygınlık düzeylerinin çok iyi bilinmesi gerekir (Ekim & Yıldırım, 1993; Özer, 1993; Özer ve ark., 1998; Türe & Köse, 2000). Küsküt'ün çok geniş bir konukçu yelpazesi bulunmaktadır, bunların başlıcaları; Asteraceae, Convolvulaceae, Solanaceae, Fabaceae ve Brassicaceae gibi bazı familya üyeleridir (Lian ve ark., 2006). Küskütün zarar verdiği bazı önemli kültür

bitkileri arasında; domates, şeker pancarı, yonca, patates, baklagiller, soğan, sarımsak, karpuz, havuç, biber ve aspir türleri sayılabilir (Nadler-Hassar & Rubin, 2003).

Kahramanmaraş bölgesinde patlıcan, nohut, süs bitkileri ve elma gibi birçok kültür alanlarında ve kültür alanları dışında küsküt türlerinin konukçu ve yoğunluğunun bilinmesiyle gerekli tedbirlerin kısa sürede ve daha etkili bir şekilde alınması mümkün olabilir. Bu nedenle; küsküt'ten dolayı verim ve kalite kayıplarının aza indirgenmesi için Kahramanmaraş'ta tarımsal alanlarda küsküt türleri ve yoğunluğunun belirlenmesi ve mevcut duruma yönelik mücadele önerilenini geliştirilmesi amaçlanmıştır.

### MATERYAL ve METOD

Küsküt türleri ve küsküt'ün infekte ettiği kültür bitkileri ve yabancı ot türleri bu çalışmanın ana materyallerini oluşturmuştur. Vejetasyon dönemi boyunca belirli periyotlar ile incelenen alanlarına

gidilmiş, bitki örnekleri toplanarak herbaryum materyali haline getirilmiş ve numaralandırılmıştır. Toplanan küsküt türleri ve konukçu bitki türlerinin teşhisinde temel olarak Davis editörlüğünde yayınlanan Türkiye Florası ile çeşitli komşu ülke ve Avrupa florasına ait eserlerin yanında küsküt türleri hakkında yayınlanan farklı eserler de incelenmiştir (Post, 1932; Yuncker, 1932; Komarow, 1945; Davis, 1965-1985; Tutin ve ark., 1976; Nemli, 1978; Güner ve ark., 2000; Yazlık & Albayrak, 2020). Ayrıca teşhisi yapılan türlerin kontrolleri için KSÜ Fen-Edebiyat Fakültesi Biyoloji Bölümü (KSUH) Herbaryumu ile Türkiye'de bulunan farklı herbaryumlardaki örneklerden de yararlanılmıştır.

Herbaryum örnekleri hazırlanan küsküt taksonlarına ait çiçek ve tohum fotoğrafları Olympus SZX16 marka stereo zoom ayarlı mikroskopta incelenmiş, fotoğrafları çekilmiş ve çalışmaya eklenmiştir (Çizelge 1). Türlerin polen fotoğrafları Hamed, (2005) ile Demir ve ark., (2017)'den alınmıştır.

Çizelge 1. Araştırmada tespit edilen küsküt taksonlarına ait teşhis anahtarı

Table 1. Identification key for dodder species identified in the research

| Türlere ait özellikler<br><i>Characteristics of the species</i>   | Takson adı<br><i>Taxa</i>                    |
|---|--|
| 1-Stilus 1 (birleşik), sert-kaba gövdeli, gövde (0.7), 1,1.5, (2) mm çapında, ağaç ya da çalı parazitleri   |  |
| 2-Korolla tüpü belirsizce kaliksten uzun, pulsu yapraklar hemen hemen anterlere kadar uzanmış, stilus boyu meyvedeki stigmaya neredeyse eşit ...  | <i>C. monogyna</i><br>subsp. <i>monogyna</i> |
| 2-Korolla tüpü kaliksten 2 kat uzun, pulsu yapraklar anterlerlerden kısa, stilus boyu meyvedeki stigmadan 2-3 kat uzun.....   | <i>C. lupuliformis</i>                       |
| 1-Stilus ve stigma 2, ince ipliksi gövdeli, gövde (0.1), 0.5, (0.6) mm çapında, ot ya da çalı parazitleri   |  |
| 3-Stigma başçık şeklinde  |  |
| 4-Korolla lobları içeri doğru kıvrık, üçgensel, sivri uçlu, pulsu yapraklar iki yarıklı değil, çoğunlukla saçaklı ve boyu stamenlere ulaşır; stilusların boyu kapsül boyunun yarısı kadar, üstten basık küre şeklindeki kapsülün çapı (1.5), 2-3 mm., çiçekler 4-5 parçalı.....   | <i>C. campestris</i>                         |
| 3-Stigmanın boyu eninden uzun   |  |
| 5-Stigma uzun; kapsül düzensiz şekilde açılır.  |  |
| 6-Çiçekler çoğunlukla 4 parçalı, çiçek sapı belirgin, çoğunlukla şemsiye şeklinde veya az çiçekli simöz, 2-3 mm. çapında, papilla taşımaz; kaliks lobları üçgensel ya da küt uçlu .....   | <i>C. pedicellata</i>                        |
| 5-Stigmanın boyu stilusa eşit ya da az uzun, stilus + stigma boyu çoğunlukla ovaryum boyuna eşit ya da uzun, loblar hemen hemen sivri uçlu; tohumlar genellikle uzun en az 0.7 mm.  |  |
| 7- Çiçekler çoğunlukla 5 parçalı; stilus + stigma boyu çoğunlukla ovaryum boyuna eşit ya da uzun  |  |
| 8- Çiçekler 1.5-2.5 mm, sapları var; çiçek kümesinin çapı 6 (7) mm.'den az; kaliks etli ve loblarının boyu tüpten uzun, belirgin şekilde korolla tüpünden uzun ve uca doğru şişkin; korolla lobları etli ve uca doğru şişkin; kapsül boyu 1.5 mm.'ye kadar uzar; tohumlar 0.7-0-9 mm. gövde çok ince ipliksi.....   | <i>C. planiflora</i>                         |
| 8- Çiçekler 2.5-4 mm, sapları var; çiçek kümesinin çapı (3.5-)5- 13 (-15) mm kaliks loblarının boyu kaliks tüpü kadar, korolla tüpünden uzun ve uç kısmı kalın, bir kısmı zarsı yapıda ve şişkin, altın sarısı veya kahverengi-parlak; korolla lobları zarsı veya uca doğru belirsizce kalın; stamenler korolla tüpünden uzun, kapsül boyu 1.5 mm den fazla ve üstten basık küre; tohumlar 1-1.3 mm; gövde orta incelikte ..... | <i>C. approximata</i>                        |

Örnekleme yapılan tarım alanları ve tarım dışı alanlar bölgeyi temsil edecek şekilde seçilerek, Ağustos-Ekim aylarında bir defa olmak üzere alanı 1m<sup>2</sup> olan çerçeve (kadrat) rastgele atılarak ölçümler yapılmıştır. Örnek alınan alanlar arasında en az 2 km mesafe olmasına dikkat edilmiş, alanın büyüklüğüne göre 5 dekara kadar 3, 5-10 dekar arasında 5 ve 10 dekardan daha büyük tarlalar için en az 8 çerçeve atılarak gerçekleştirilmiştir (Bora & Karaca, 1970).

Birim alandaki küsküt bulaşık kültür bitkisi sürgün sayısının belirlenmesi: Yabancı ot yoğunluğu; metrekarede bulunan küsküt ile infekteli bitki sürgünlerin (dal) sayımı yapılarak yoğunluk hesaplaması yapılmıştır. Yabancı ot yoğunluğu aşağıdaki formül ile hesaplanmıştır (Güncan, 2001).

Yoğunluk= B/n

B= Alınan örnekte toplam birey sayısı

n= Alınan örnek sayısı

Küsküt örnekleri Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Bitki Koruma Bölüm Herbariumuna getirilmiş ve türler Flora of Turkey and the East Aegean Islands (Davis, 1978)'e göre teşhis edilmiştir. Küsküt yoğunluğunun ölçek yardımıyla belirlenmesinde yoncanın küsküt'le parazitik oranı Tepe (1997) ve küsküt'ün şekerpancarında parazitik oranı Üstüner ve Öztürk (2018)'ün kullandığı küsküt yoğunluk skalası esas alınarak hesaplanmıştır. Bunun için aşağıda verilen ölçütlere göre arazide gözleme dayalı yoğunluk değerlendirmeleri yapılmıştır: Yoğunluk skalası:

- (1) Küsküt yok
- (2) Az bulaşık (Tepe (1997), Üstüner ve Öztürk (2018) skalasına göre)
- (3) Orta seviyede bulaşık (Tepe (1997), Üstüner ve Öztürk (2018) skalasına göre)
- (4) Bulaşık (Tepe (1997), Üstüner ve Öztürk (2018) skalasına göre)
- (5) Ağır bulaşık (Tepe (1997), Üstüner ve Öztürk (2018) skalasına göre)

## BULGULAR ve TARTIŞMA

Bu çalışmada, Kahramanmaraş ilinde 11 ilçe düzeyinde ayrı ayrı küsküt sürveyi yapılmıştır. Kahramanmaraş bölgesi (Afşin, Andırın, Çağlayancerit, Dulkadiroğlu, Ekinözü, Elbistan, Göksun, Nurhak, Pazarcık Onikişubat ve Türkoğlu) ilçelerinde 108 farklı bitkiyi parazitleyen 6 adet küsküt taksonu Çizelge 1'de verilmiştir. Kahramanmaraş ilçelerine göre belirlenen küsküt türleri; Çağlayancerit, Ekinözü ve Nurhak ilçelerinde *C. campestris*, *C. approximata*, *C. monogyna* subsp. *monogyna* ve *C. lupuliformis*; Dulkadiroğlu ve Onikişubat ilçelerinde *C. campestris*, *C. approximata* ve *C. pedicellata*; Afşin, Elbistan ve Göksun ilçelerinde

*C. campestris*, *C. approximata*, *C. monogyna* subsp. *monogyna* ve *C. planiflora*; Andırın, Türkoğlu ve Pazarcık ilçelerinde ise *C. campestris* ve *C. approximata* türlerine rastlanmıştır. Davis'e (1978) göre Kahramanmaraş ilinde iki tür tespit edilirken bu araştırma ile 6 küsküt türü belirlenmiştir. Bu farklılığın nedenleri Türkiye Flora'sının hazırlanmasında yetersiz örnekle çalışılması ve zaman içerisinde küsküt tohumlarının insan, hayvan, böcek, sulama suyu, rüzgâr, yağmur, alet ve ekipman gibi faktörlerle hızla yayılabilmesi olabilir.

Kahramanmaraş ili ve ilçelerinde yapılan sürvey çalışması sonucunda tespit edilen küsküt türleri ve morfolojik özellikleri de belirlenmiştir. Buna göre;

### *Cuscuta campestris* Yunck. (Tarla küskütü)

Gövde genellikle ipliksi yapıda ve beyazımsı-sarımsı renklidir. Çiçek kümesi küremsi olup 3-12 mm çapında ve yoğun çiçeklidir. Çiçek sapları çoğunlukla çiçekten daha kısadır. Çiçekler 2-3 mm uzunluğunda, beyaz veya krem renkli olup 5 parçalı ve salgı keseleri taşır. Çanak neredeyse taç tüpü ile aynı boydadır, taç lobları oval, dairemsi şekildedir. Pullar yumurtamsı şekilde ve kenarları derince yırtık görünümlüdür. Erkek organ; taç loblarından, iplikçikler ise başçıklardan daha kısa veya eşittir. Meyve küremsi 1-5 mm çapındadır. Tohumlar; yumurtamsı, 1-2 mm, yüzeyi düz, sarımsı ya da açık kahverengindedir. Çiçeklenme Mayıs-ekim ayları arasındadır. Polen prolat, subprolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 1).

### *Cuscuta approximata* Bab. (Bağboğan otu)

Gövdesi ince veya orta kalınlıkta, parlak sarı veya kırmızı renklidir. Çiçek kümesi yoğun ve 4-15 mm. çapındadır. Çiçekler; 2-5 mm. uzunluğunda, sapsız, beyaz renkli ve düz yüzeyli, 5 parçalı. Çanak lobları üçgensiyumurtamsı kısmen sert uçlu, etli yapıda belirsiz bir şekilde şişkindir. Taç; çan şeklindedir, fakat meyve olgunlaştığında küre şeklini alır ve kalıcıdır. Taç lobları üçgensiy ya da yumurtamsı, küt uçludur. Pullar; dikdörtgensiy, düz veya ayırık, uçları kısmen derince yırtıklıdır. Başçıklar tüpten dışarı uzanır ve boyu iplikçikler kadardır. Meyve basık küre şeklindedir. Tohumlar; 1-1.5 mm. uzunluğunda, yüzeyi pürüzlü, sarımsiy ya da açık kahverengindedir. Çiçeklenme Mayıs-ekim ayları arasındadır. Polen prolat, subprolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 2).

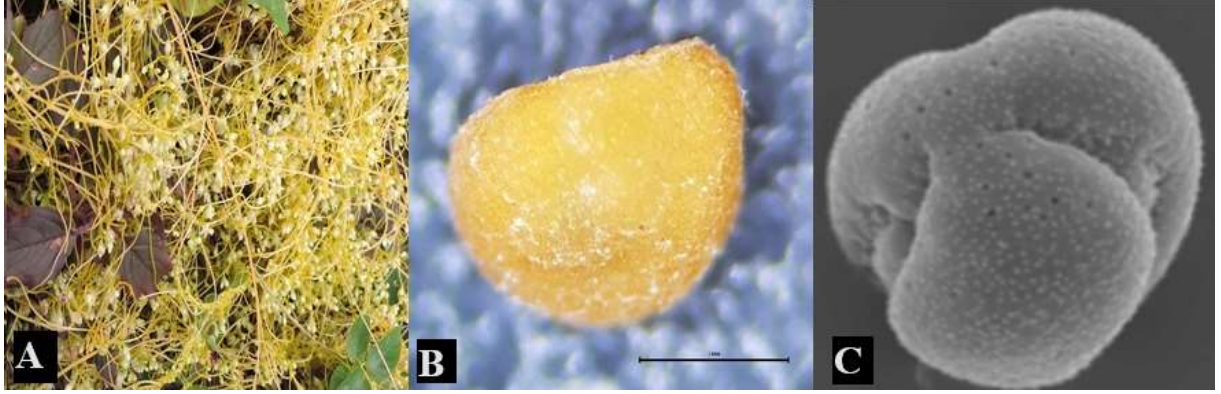
### *Cuscuta pedicellata* Ledeb. (Boğmaca otu)

Dallanma gösteren zayıf yapılu pürüzsüz gövde sarımsiy kırmızı renktedir. Çiçek kümesi, şemsiye veya hemen hemen başçık şeklinde ve 3-9 mm. Çapında olup genellikle az çiçeklidir. Çiçekler 2-3 mm, uzun saplı, beyaz renkli, 4 parçalıdır. Taç biraz etli yapıdadır ve lobları üçgensiy ve küt uçludur. Taç beyaz veya parlak



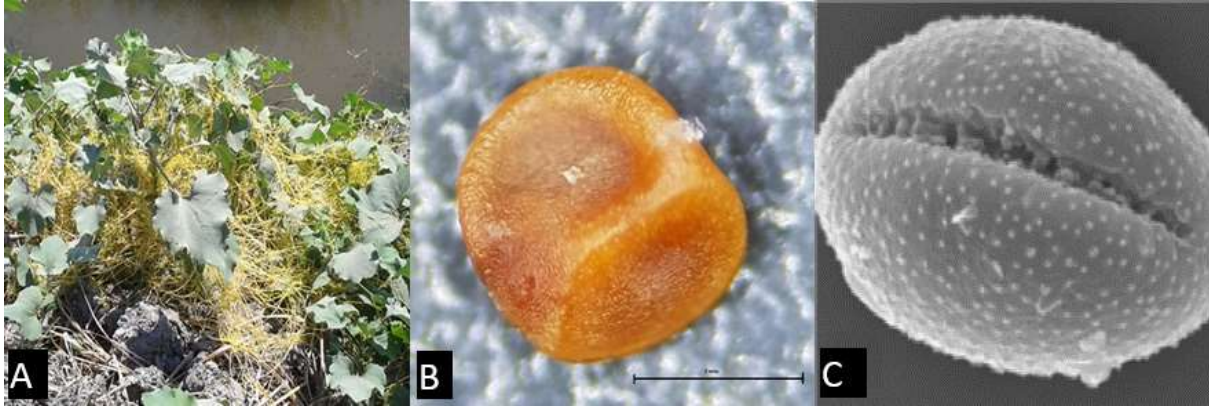
pembe renkli, hemen hemen küresel şekildedir. Pullar; dikdörtgensi, düz veya iki parçalı ve gagalıdır, kısa püsküllüdür. Erkek organ iplikçiklerden kısadır. Tohum yaklaşık 1-1.5 mm çapında ve küresel, yüzeyi

pürüzlü, sarımsı ya da açık kahverenkli. Çiçeklenme mart-temmuz ayları arasındadır. Polen subprolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 3).



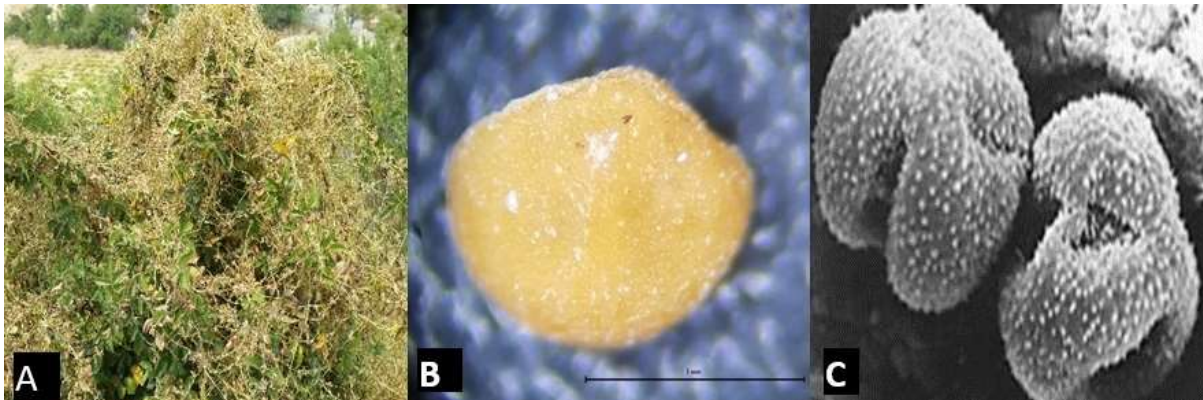
Şekil.1. *C. campestris* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orjinal fotoğraf; C, © Demir ve ark., (2017)'den alınmıştır.

Figure 1. *C. campestris* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, from © Demir et al., (2017).



Şekil.2. *C. approximata* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orjinal fotoğraf; C, © Demir ve ark., (2017)'den alınmıştır.

Figure 2. *C. approximata* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, from © Demir et al., (2017).



Şekil.3. *C. pedicellata* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orjinal fotoğraf; C, © Hamed, (2005)'den alınmıştır.

Figure 3. *C. pedicellata* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, © from Hamed, (2005).



***Cuscuta monogyna* subsp. *monogyna* Vahl. (Kızilkurt otu)**

Bitki gövdesi orta kalınlıkta, beyaz veya kırmızımsı renkli ve dallanma vardır. Çiçek kümesi birleşik 1-6 çiçeklidir. Çiçekler 2-6 mm uzunluğunda ve etli görünümde olup beyaz-pembe renkli, sapsız veya nadiren kısa saplıdır. Çanak lobları üçgeni-ovat

şekildedir. Taç lobları oval-ovat, küt uçlu, tırtıklı ve çanak organdan uzundur. Erkek organ sapsız, tabanda kısmen oyukludur. Pullar küt uçlu, kısa dişlidir. Tohumlar; yaklaşık 1.5-3.5 mm çapında yumurtamsı, yüzeyi yivli, sarı renkli olup olgunlaştığında koyu kahverengine dönüşmektedir. Çiçeklenme haziran-ekim ayları arasındadır. Polen prolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 4).



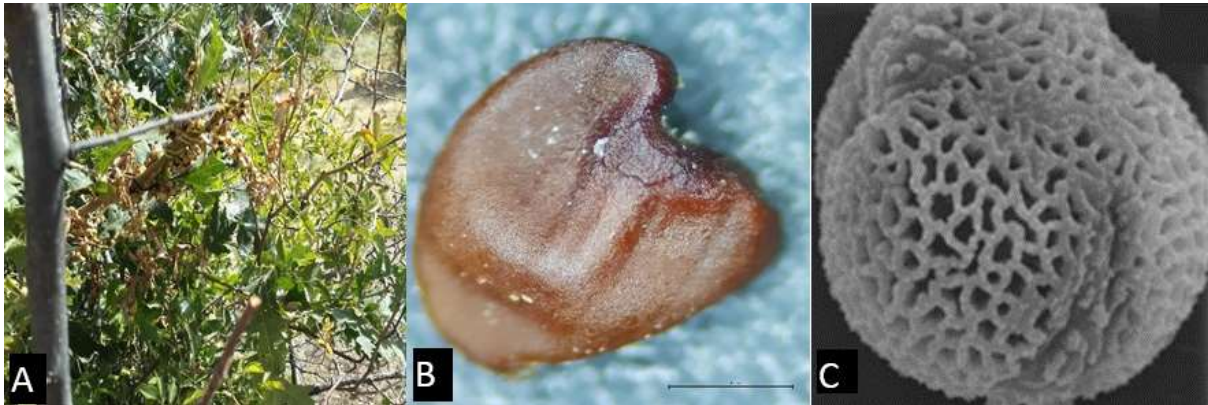
Şekil.4. *C. monogyna* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orjinal fotoğraf; C, © Demir ve ark., (2017)'dan alınmıştır.

Figure 4 *C. monogyna* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, from © Demir et al., (2017).

***Cuscuta lupuliformis* Krockner**

Gövde belirgince kalındır ve kırmızımsı renklidir. Çiçek kümesi 2-10 çiçekli ve başak şeklindedir. Çiçekler 3-5 mm uzunluğunda, saplı, beyaz ya da pembe renkli, 5 parçalıdır. Çanak lobları tüpten uzun, küt uçludur. Taç tüpü çanağın iki katı ve silindirik, dik

yapılı, küt uçludur. Erkek organ sapsız ve tabanda oyukludur. Pullar saçaklıdır. Tohumlar; yaklaşık 2-3 mm uzunluğunda yumurtamsı, yüzeyi pürüzlü, sarımsı kahverenkli. Çiçeklenme temmuz-eylül ayları arasındadır. Polen prolat, subprolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 5).



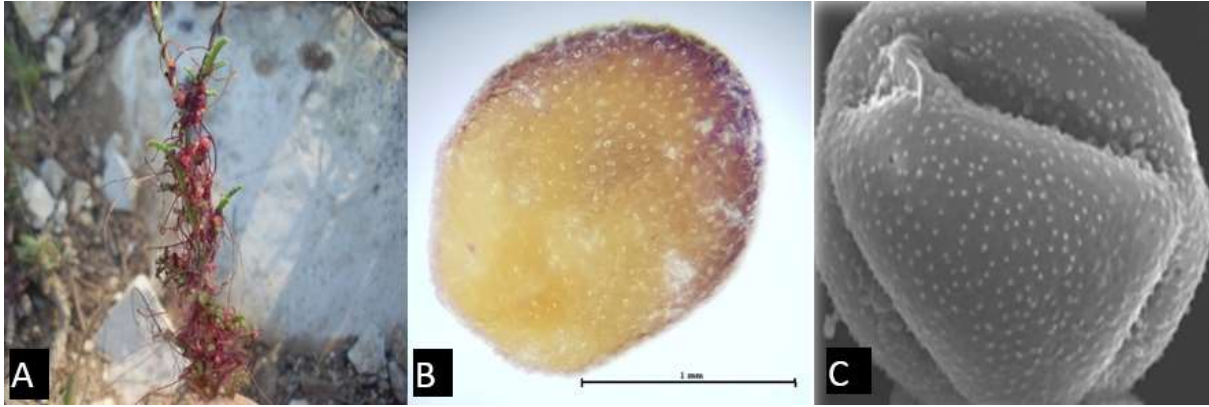
Şekil.5. *C. lupuliformis* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orjinal, fotoğraf C, © Demir ve ark., (2017)'dan alınmıştır.

Figure 5. *C. lupuliformis* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, from © Demir et al., (2017).

***Cuscuta planiflora* Ten. (Göktenyağan)**

Bu küsküt türünün gövdesi (dal) ince ve kırmızımsı renktedir. Çiçek kümesi 3-7 mm çapında birkaç veya çok çiçekli, küresel ve yoğundur. Çiçekler 1.5-2.5 mm uzunluğunda, sapsız çoğunlukla beyaz renkli, kalın ve 5 parçalıdır. Çanak yaprak çoğunlukla taç tüplerinden uzun. Loblar tüpten daha uzun, dikdörtgensiz mızraklı,

yumurtamsı ya da üçgeni, tepede sivri şekildedir. Başçıklar küçük ve tüpü aşar. Kapsül küresel şekildedir. Tohumlar; yaklaşık 0.7-1 mm çapında, yumurtamsı, yüzeyi pürüzlü, sarımsı kahverenkli. Çiçeklenme mart-ağustos ayları arasındadır. Polen prolat şekildedir (Davis, 1978; Yuncker, 1932), (Şekil 6).



Şekil 6. *C. planiflora* A) Genel görünüm B) Tohum C) Polen (A ve B yazarlar tarafından çekilen orijinal fotoğraf; C, © Demir ve ark., (2017)'dan alınmıştır.  
Figure 6. *C. planiflora* A) General view B) Seed C) Pollen (A and B original photograph by authors; C, from © Demir et al., (2017).

Kahramanmaraş ilinde yapılan bu çalışmada; *Cuscuta pedicellata*, *Cuscuta monogyna* subsp. *monogyna*, *Cuscuta lupuliformis* ve *Cuscuta planiflora* türleri ilk kez tespit edilmiştir. Aynı zamanda morfolojik taksonomisi ile ilgili yapılan çalışmalar da ilk kez yapılmıştır. Türkiye'de *Cuscuta* L. türlerinin morfolojik taksonomisi ile ilgili yapılan çalışmalar yok denecek kadar azdır. Nemli (1978)'ye göre, Boissier (1879) ve Yuncker (1932) Anadolu'daki *Cuscuta* türlerine değinmelerine rağmen, konu yüzeysel olarak ele alınmıştır.

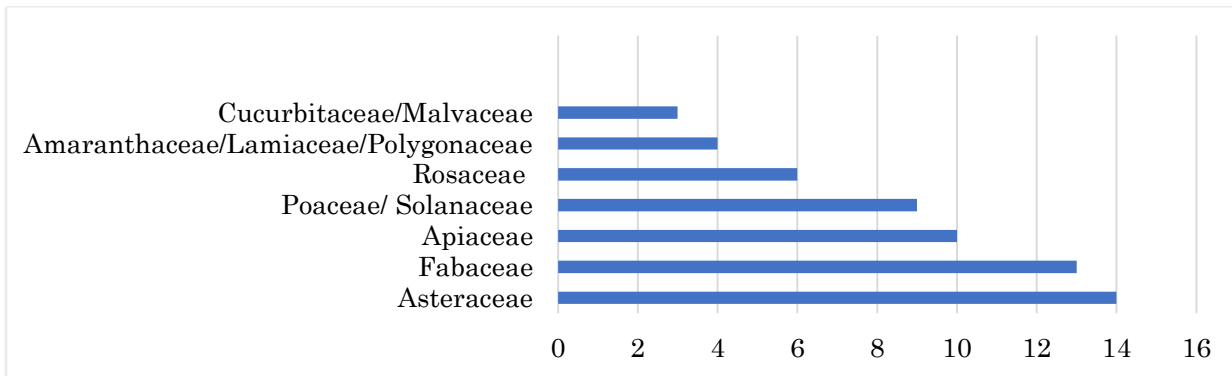
#### Kahramanmaraş ili ve ilçelerinde rastlanan konukçu (konak) türleri

Tarım alanlarında ve dışında tespit edilen küsküt (*Cuscuta* spp.) türlerinin tam parazit bitki olarak üzerinde yaşadığı konukçuları araziden toplanarak herbaryum örneği haline getirilmiş ve tür teşhisleri yapılmıştır. Çalışma sonucunda; küsküt türlerinin

parazitlediği tarla bitkileri, süs bitkileri, meyve ağaçları, orman/park ağaçları ve yabancı otlar olmak üzere 5 farklı grupta konukçusu saptanmıştır. Bu konaklardan 23'ü tarla bitkileri, 7'si meyve ağacı, 7'si süs bitkisi, 9'u orman/park ağacı, 62'si yabancı ot türü olmak üzere 35 familyaya ait 108 farklı takson tespit edilmiştir (Şekil 7).

Küsküt (*Cuscuta* spp.) türlerinin konukçuları 5 ana gruba ayrılmıştır;

**Tarımsal bitkiler;** şeker pancarı (*Beta vulgaris*), nohut (*Cicer arietinum*), mercimek (*Lens culinaris*), patates (*Solanum tuberosum*), soğan (*Allium cepa*), biber (*Capsicum annuum*), karpuz (*Citrullus lanatus*), kavun (*Cucumis melo*), hıyar (*Cucumis sativus*), domates (*Lycopersicon esculentum*), patlıcan (*Solanum melongena*), nane (*Mentha x piperita*), maydanoz (*Petroselinum crispum*), mülhüye (*Corchorus olitorius*) gibi türler konak olarak tespit edilmiştir (Çizelge 2).



Şekil 7. Küsküt türlerinin tespit edildiği konaklara ait familyalar  
Figure 7. Families of hosts where dodder species were detected

Çizelge 2. Küsküt türlerinin görüldüğü tarla bitkileri

Table 2. Field plants with dodder species

| Familiya adı<br><i>Family</i> | Tür adı<br><i>Species</i>                                 | Türkçe adı<br><i>Turkish name</i> |
|-------------------------------|---|-----------------------------------|
| Apiaceae                      | <i>Petroselinum crispum</i> (Mill.) Fuss                  | Maydanoz                          |
| Asteraceae                    | <i>Helianthus annuus</i> L.                               | Ayçiçeği                          |
| Amaranthaceae                 | <i>Beta vulgaris</i> L.                                   | Şeker pancarı                     |
| Cucurbitaceae                 | <i>Cucumis sativus</i> L.                                 | Hıyar                             |
|                               | <i>Citrullus lanatus</i> (Thumb.) Matsum. et Nakai        | Karpuz                            |
|                               | <i>Cucumis melo</i> L.                                    | Kavun                             |
| Fabaceae                      | <i>Cicer arietinum</i> L.                                 | Nohut                             |
|                               | <i>Phaseolus vulgaris</i> L.                              | Fasulye                           |
|                               | <i>Lens culinaris</i> Medik.                              | Mercimek                          |
|                               | <i>Medicago sativa</i> L.                                 | Yonca                             |
|                               | <i>Vicia faba</i> L.                                      | Bakla                             |
| Lamiaceae                     | <i>Vigna unguiculata</i> (L.) Walp.                       | Börülce                           |
|                               | <i>Mentha x piperita</i> L.                               | Nane                              |
|                               | <i>Ocimum basilicum</i> L.                                | Fesleğen                          |
| Liliacea                      | <i>Allium cepa</i> L.                                     | Soğan                             |
| Poaceae                       | <i>Zea mays</i> L.  | Mısır                             |
| Solanaceae                    | <i>Capsicum annuum</i> var. <i>frutescens</i> (L.) Kuntze | Süs biberi                        |
|                               | <i>C. annuum</i> var. <i>grossum</i> (Willd.) Sendtn.     | Dolmalık biber                    |
|                               | <i>C. annum</i> L.  | Maraş biberi                      |
|                               | <i>Solanum melongena</i> L.                               | Patlıcan                          |
|                               | <i>S. tuberosum</i> L.                                    | Patates                           |
| Tiliaceae                     | <i>Lycopersicon esculentum</i> Mill.                      | Domates                           |
|                               | <i>Corchorus olitorius</i> L.                             | Mühliye                           |

**Meyve türleri;** elma (*Malus pumila*), erik (*Prunus x domestica*), kayısı (*Armeniaca vulgaris*), üzüm (*Vitis vinifera*), ığde (*Elaeagnus angustifolia*), kuşburnu

(*Rosa canina*) gibi türler konak olarak tespit edilmiştir (Çizelge 3).

Çizelge 3. Küsküt türlerinin görüldüğü meyve türleri

Table 3. Fruit plants with dodder species

| Familiya adı<br><i>Family</i> | Tür adı<br><i>Species</i>        | Türkçe adı<br><i>Turkish name</i> |
|-------------------------------|----------------------------------|-----------------------------------|
| Elaeagnaceae                  | <i>Elaeagnus angustifolia</i> L. | İğde                              |
|                               | <i>Malus pumila</i> Mill.        | Elma                              |
| Rosaceae                      | <i>Rubus sanctus</i> Schreb.     | Böğürtlen                         |
|                               | <i>Prunus x domestica</i> L.     | Erik                              |
|                               | <i>Armeniaca vulgaris</i> Lam.   | Kayısı                            |
|                               | <i>Rosa canina</i> L.            | Kuşburnu                          |
| Vitaceae                      | <i>Vitis vinifera</i> L.         | Üzüm                              |

**Süs bitkileri;** petunya (*Petunia* spp.), hasır otu (*Typha* spp.), yabani gül (*Rosa canina*), kadife çiçeği (*Tagetes erecta*), gavura çiçeği (*Gaura lindheimeri*) gibi türler konak olarak tespit edilmiştir (Çizelge 4).

**Park ve Orman ağaçları;** akasya (*Acacia karroo*), selvi kavak (*Populus afghanica*), söğüt (*Salix alba*), saçlı meşe (*Quercus cerris*), çınar (*Platanus orientalis*), melez karaağaç (*Ulmus x hollandica*), ılgın (*Tamarix smyrnensis*) gibi türler konak olarak tespit edilmiştir (Çizelge 5).

**Yabancı otlar;** ebe gümece (*Malva sylvestris*), pıtrak (*Xanthium strumarium*), sirken (*Chenopodium album*), geliç (*Sorghum halepense*), kamış (*Phragmites australis*), bozot (*Heliotropium europaeum*), zincir pıtrağı (*Xanthium spinosum*) gibi türler konak olarak tespit edilmiştir (Çizelge 6).

Bu araştırmada küsküt türlerin konak olarak tercih ettiği bitkilerden *Populus afghanica*, *Salix alba*, *Acacia karroo*, *Fagus orientalis*, *Quercus cerris*, *Platanus orientalis*, *Paliurus spina-christi*, *Tamarix smyrnensis* ve *Corchorus olitorius* Türkiye'de ilk kez tespit edilmiştir. Türkiye'de küsküt ile yapılan önceki



araştırmalarda bu türlere rastlanılmamıştır. ABD Wisconsin eyaletinde *Cuscuta coryli* türüne *Corylus*

spp., *Asclepias syriaca*, *Solidago canadensis* ve *Helianthus* spp.'de görülmüştür (Marh, 2024).

Çizelge 4. Küsküt türlerinin görüldüğü süs bitkileri

Table 4. Ornamental plants with dodder species

| Familiya adı (Family) | Tür adı (Species)                              | Türkçe adı (Turkish name) |
|-----------------------|--|---------------------------|
| Asteraceae            | <i>Tagetes erecta</i> L.                       | Kadife çiçeği             |
| Geraniaceae           | <i>Pelargonium zonale</i> (L.) L'Hér. ex Aiton | Sardunya                  |
| Lamiaceae             | <i>Plectranthus scutellarioides</i> (L.) R.Br. | Kolyoz-yaprağı güzel      |
| Onagraceae            | <i>Gaura lindheimeri</i> Engelm. & A.Gray      | Gavura çiçeği             |
| Rosaceae              | <i>Rosa canina</i> L.                          | Yabani gül                |
| Solanaceae            | <i>Petunia</i> spp.                            | Petunya çiçeği            |
| Typhaceae             | <i>Typha</i> spp.                              | Hasır otu                 |

Çizelge 5. Küsküt türlerinin görüldüğü park ve orman ağaçları

Table 5. Garden and forest trees with dodder species

| Familiya adı<br>Family | Tür adı<br>Species  | Türkçe adı<br>Turkish name |
|------------------------|---|----------------------------|
| Salicaceae             | <i>Populus afghanica</i> (Aitch. & Hemsl.) C.K.Schneid.<br><i>Salix alba</i> L. | Selvi kavak<br>Söğüt       |
| Fabaceae               | <i>Acacia karroo</i> Hayne  | Akasya                     |
| Fagaceae               | <i>Fagus orientalis</i> Lipsky<br><i>Quercus cerris</i> L.                      | Doğu kayını<br>Saçlı meşe  |
| Platanaceae            | <i>Platanus orientalis</i> L.   | Çınar                      |
| Rhamnaceae             | <i>Paliurus spina-christi</i> P. Mill.  | Karaçalı                   |
| Tamaricaceae           | <i>Tamarix smyrnensis</i> Bunge   | İlgın                      |
| Ulmaceae               | <i>Ulmus × hollandica</i> Mill.   | Melez Karaağaç             |

Kahramanmaraş ilçelerine göre belirlenen küsküt türleri; Çağlayancerit, Ekinözü ve Nurhak ilçelerinde *Cuscuta campestris*, *C. approximata*, *C. monogyna* subsp. *monogyna* ve *C. lupuliformis*; Dulkadiroğlu ve Onikişubat ilçelerinde *C. campestris*, *C. approximata* ve *C. pedicellata*; Afşin, Elbistan ve Göksun ilçelerinde *C. campestris*, *C. approximata*, *C. monogyna* subsp. *monogyna* ve *C. planiflora*; Andırın, Türkoğlu ve Pazarcık ilçelerinde ise *C. campestris* ve *C. approximata* türlerine rastlanmıştır.

Kahramanmaraş ili ve ilçelerinde birçok tarla bitkileri, süs bitkileri ve yabancı ot türlerini infekte ettiği belirlenen en yaygın küsküt türünün *C. campestris* olduğu tespit edilmiştir. *C. campestris* Türkiye'de geniş bir yayılış alanına sahip olup deniz seviyesinden 1500 m. yüksekliğe kadar görülebilir. Bu türün özellikle kültür bitkisi ve yabancı otlar olmak üzere çok sayıda konukçusu bulunmaktadır. Biber, nohut, yonca, şeker pancarı, soğan, tütün, anason ve kimyon *C. campestris*'in en yaygın konukçularıdır. Anadolu'da *C. campestris*'in 55 konukçusu saptanmıştır. Çoğunlukla otsu formda olan bu bitkilerin 27'sinin tarım bitkisi olduğu belirlenmiş en yaygın olarak bulunduğu tür ise *Beta vulgaris* L. (pancar) olmuştur. Bunu *Medicago sativa* (yonca), *Trifolium* spp. (üçgül), *Vicia faba* (bakla), *Capsium annuum* (biber), *Allium cepa* (soğan), *Daucus carota* (havuç), *Pimpinella anisum* (anason), *Carum carvi* (kimyon), *Nicotiana tabacum* (tütün), *Vicia sativa* (fiğ),

*Solanum melongena* (patlıcan), *Cicer arietinum* (nohut), *Asparagus officinalis* (kuşkonmaz), *Vitis vinifera* (asma), *Cucumis melo* (kavun), *Solanum tuberosum* (patates), *Lycopersicon esculentum* (domates) ve bazı süs bitkilerinin olduğu farklı çalışmalarda bildirilmiştir (Nemli, 1978; Kadioğlu, 1992; Parker & Riches, 1993; Dawson ve ark., 1994; Nemli ve ark., 2015). Bu literatürlerdeki veriler elde ettiğimiz konak türlerin bazılarıyla benzer olup bazı türler ise bu çalışma ile ilk kez tespit edilmiştir.

İlk kez bu araştırma ile *C. monogyna* subsp. *monogyna* türü; ılgın, söğüt ve çınar gibi park ve orman ağaçlarında, asma, elma ve çeşitli meyve fidanları gibi odunsu bitkilerde tespit edilmiştir. Kahramanmaraş ilinde yonca alanlarında sadece *C. approximata* yaygın olarak gözlenmiş olup yoncada *C. approximata* yaygınlığı konusunda Yıldırım & Tepe (2014), Tepe ve ark. (2017), Kaya ve ark. (2018) ile paralellik göstermiştir.

İlk kez bu çalışma ile *C. lupuliformis* türüne meyve, park ve orman ağaçlarında özellikle de sulama kanalları boyunca kavak, söğüt, kuşburnu gibi türlerde ve bahçe kenarlarında rastlanmıştır. Aynı zamanda Aksu nehir yatağı ve kıyısında kendiliğinden yetişen, çınar, kavak, söğüt, ılgın, meşe gibi orman ağaçlarında da yaygın olarak saptanmıştır. Acatay (1966) tarafından Bingöl, Siirt, Elazığ orman bölgelerinde mazı meşesi türlerinde *C. lupuliformis*'in görüldüğü paraleldir.



Çizelge 6. Küsküt türlerinin görüldüğü yabancı otlar  
Table 6. Weeds with dodder species

| Familiya adı (Family) | Tür adı (Species)                                  | Türkçe adı (Turkish name)  |
|-----------------------|--|----------------------------|
| Amaranthaceae         | <i>Amaranthus retroflexus</i> L.                   | Kırmızı köklü tilkikuyruğu |
|                       | <i>A. cruentus</i> L.                              | Horoz ibiği                |
|                       | <i>Chenopodium album</i> L.                        | Sirken                     |
| Apiaceae              | <i>Bifora radians</i> M.Bieb.                      | Kokarot                    |
|                       | <i>Eryngium creticum</i> Lam.                      | Göz dikenli                |
|                       | <i>E. kotschy</i> Boiss.                           | Deve elması                |
|                       | <i>Turgenia latifolia</i> (L.) Hoffm.              | Karaheci                   |
|                       | <i>Ammi visnaga</i> (L.) Lam.                      | Diş otu                    |
|                       | <i>Echinophora tenuifolia</i> L.                   | Sarı çördük                |
|                       | <i>Torilis arvensis</i> (Huds.) Link               | Dercikotu                  |
|                       | <i>Oenanthe aquatica</i> (L.) Poir.                | Su rezenesi                |
| Asteraceae            | <i>Scandix australis</i> L.                        | Kışkiş                     |
|                       | <i>Centaurea virgata</i> Lam.                      | Acı süpürge                |
|                       | <i>C. carduiiformis</i> DC.                        | Peygamber çiçeği           |
|                       | <i>C. solstitialis</i> L.                          | Çakırdikeni                |
|                       | <i>C. hyalolepis</i> Boiss.                        | Belhok                     |
|                       | <i>C. calcitrapa</i> L.                            | Çobankaldıran              |
|                       | <i>Chondrilla juncea</i> L.                        | Karakavuk                  |
|                       | <i>Cichorium intybus</i> L.                        | Yabani hindiba             |
|                       | <i>Cirsium arvense</i> (L.) Scop.                  | Köy göçüren                |
|                       | <i>Lactuca serriola</i> L.                         | Dikenli yabani marul       |
|                       | <i>Senecio vernalis</i> Waldst. & Kit.             | Kanarya otu                |
| Boraginaceae          | <i>Xanthium spinosum</i> L.                        | Dikenli pıtrak             |
|                       | <i>X. strumarium</i> L.                            | Pıtrak                     |
| Boraginaceae          | <i>Heliotropium europaeum</i> L.                   | Bozot                      |
| Convolvulaceae        | <i>Convolvulus arvensis</i> L.                     | Tarla Sarmaşığı            |
|                       | <i>C. betonicifolius</i> Mill.                     | Büyük yayılğan             |
| Equisetaceae          | <i>Equisetum arvense</i> L.                        | Atkuyruğu                  |
| Euphorbiaceae         | <i>Euphorbia falcata</i> L.                        | Eğri sütleşen              |
|                       | <i>Chrozophora tinctoria</i> (L.) A.Juss.          | Siğil otu                  |
| Fabaceae              | <i>Astragalus barbeyanus</i> Post                  | Geven                      |
|                       | <i>Securigera libanotica</i> (Boiss.) Lassen       | Kıvrık otu                 |
|                       | <i>Trifolium</i> spp.                              | Üçgül                      |
|                       | <i>Vicia sativa</i> L.                             | Fığ                        |
|                       | <i>Prosopis farcta</i> (Banks & Sol.) J.F.Macbr.   | Çeditotu                   |
| Hypericaceae          | <i>Alhagi maurorum</i> Medik.                      | Devedikeni                 |
|                       | <i>Hypericum retusum</i> Aucher ex Jaub. & Spach   | Kantaron                   |
| Lamiaceae             | <i>Mentha longifolia</i> (L.) L.                   | Yabani nane                |
| Lythraceae            | <i>Lythrum salicaria</i> L.                        | Kırmızı kan çiçeği         |
| Malvaceae             | <i>Malva neglecta</i> Wallr.                       | Ebegümece                  |
|                       | <i>Hibiscus trionum</i> L.                         | Yabani bamyası             |
|                       | <i>Alcea digitata</i> Alef.                        | Boylu hatmi                |
| Onagraceae            | <i>Epilobium parviflorum</i> Schreb.               | Yakı otu                   |
| Papaveraceae          | <i>Fumaria officinalis</i> L.                      | Şahtere otu                |
|                       | <i>Elymus repens</i> (L.) Gould                    | Ayrık otu                  |
| Poaceae               | <i>E. repens</i> (L.) Gould                        | Ayrık otu                  |
|                       | <i>Alopecurus myosuroides</i> Huds.                | Tilkikuyruğu               |
|                       | <i>Bromus arvensis</i> L.                          | Brom otu                   |
|                       | <i>Cynodon dactylon</i> (L.) Pers.                 | Köpek dişi ayrığı          |
|                       | <i>Digitaria sanguinalis</i> (L.) Scop             | Çatal otu                  |
|                       | <i>Sorghum halepense</i> (L.) Pers.                | Kanyaş                     |
|                       | <i>Setaria viridis</i> (L.) P.Beauv.               | Yeşil kirpi darı           |
| Polygalaceae          | <i>Phragmites australis</i> (Cav.) Trin. ex Steud. | Kamış                      |
|                       | <i>Polygala pruinosa</i> Boiss.                    | Puslu sütünotu             |
| Polygonaceae          | <i>Polygonum aviculare</i> L.                      | Kuş madımağı               |
|                       | <i>P. arenastrum</i> Bor.                          | Madımak                    |
|                       | <i>P. persicaria</i> L.                            | Söğüt otu                  |
|                       | <i>Rumex acetosella</i> L.                         | Kuzukulağı                 |
| Portulacaceae         | <i>Portulaca oleracea</i> L.                       | Semizotu                   |
| Solanaceae            | <i>Physalis alkekengi</i> L.                       | Güney feneri               |
|                       | <i>Solanum nigrum</i> L.                           | Köpek üzümü                |
| Zygophyllaceae        | <i>Tribulus terrestris</i> L.                      | Demir dikenli              |

Bu çalışma bulgularından edile edilen sonuçlar Türkiye'de farklı bölge ve kültürlerde yapılan çalışmalar (Kondap & Kumar, 1993; Üstüner & Öztürk, 2018; Üstüner ve ark., 2019; Dal & Üstüner, 2020; Almhedmed & Üstüner, 2023; Demir ve ark., 2024; Elsekran ve ark., 2024) ile uyumludur. Bu paralellikler dikkate alındığında küsküt taksonlarının bitkisel özelliklerinin bir alandan farklı bir alana taşınımında etkili olan faktörler konusunda dikkatli olunması gerektiğini ortaya koyar.

## SONUÇ ve ÖNERİLER

Kahramanmaraş ilinde tarım alanı ve dışında 6 küsküt türü (*C. campestris*, *C. approximata*, *C. lupuliformis*, *C. pedicellata*, *C. monogyna* subsp. *monogyna* ve *C. planiflora*) belirlenmiştir. Bu türlerden *C. campestris* özellikle tarla, süs bitkileri ve yabancı otlar üzerinde çok yaygın ve yoğun olarak tespit edilmiştir. Yonca tarlalarında *C. approximata* orman ve fidanlıklarda ise *C. lupuliformis* türü çok yoğun olarak belirlenmiştir.

Kahramanmaraş ilinde küsküt konukçuları değişkenlik göstermekle beraber küsküt'ün biyolojik özelliklerine göre de farklılık gösterebilir. Bu ilde 6 küsküt türü belirlenirken bazı konaklar da ilk kez bu araştırma ile ortaya konmuştur. *C. monogyna* subsp. *monogyna* konakları arasında; *Acacia karroo*, *Populus afghanica*, *Salix alba*, *Quercus cerris*, *Platanus orientalis* ve *Tamarix smyrnensis* türler konak olarak ilk kez tespit edilmiştir. Bu araştırma ile ilk kez meyve ağaçlarından; *Malus pumila*, *Prunus domestica*, *Armeniaca vulgaris*, *Vitis vinifera*, *Elaeagnus angustifolia*, *Rosa canina* türler küsküt'ün konakları olarak tespit edilmiştir.

Kahramanmaraş ili genelinde küsküt türlerin yoğunluğu dikkate alınmalıdır. Özellikle tarım alanlarında küsküt türleriyle mücadele edilirken tarla kenarlarında veya yol kenarlarında görülen küsküt türleriyle de mücadele edilmesi gerekir. Yol, tarla ve kanal kenarlarında küsküt türleriyle mücadele edilmediği gözlenmiştir. Tarım alanlarında küsküt çıkışı hava sıcaklığına bağlı olarak değişmekle beraber erken ilkbahar döneminde elle yolma, el çapa veya çikış öncesi herbisitler ile mücadele edilmelidir (Üstüner, 2020; Üstüner & Aksoy, 2021). Küsküt'ün çimlenme döneminde veya kültür bitkisine sarılmadan önceki dönemde 400 g/l Propyzamide herbisit uygulaması sonucunda etkili olduğu bildirilmiştir (Günçan & Karaca, 2018). Küsküt türleri muhakkak kontrol altında tutulması gereken önemli tam parazit bitkidir.

## 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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## A new genus record for Türkiye: *Sesbania* Adanson (*Fabaceae*)

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### ABSTRACT

The genus *Sesbania* Adanson (*Fabaceae*) is recorded for the first time in the flora of Türkiye. Collected specimens of this genus from Tarsus/Mersin are described here as a new record, *Sesbania herbacea* (Mill.) McVaugh which is an alien weed. Its detailed morphological features, Turkish name and photographs in its natural habitat, and the finding location are given in this study.

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## Türkiye İçin Yeni Bir Cins Kaydı: *Sesbania* Adanson (*Fabaceae*)

### ÖZET

*Sesbania* Adanson (*Fabaceae*) cinsi Türkiye’de ilk kez kaydedilmiştir. Bu cinse ait örnekler Mersin ilinden toplanmış ve yabancı orjinli bir yabancı ot türü olarak *Sesbania herbacea* (Mill.) McVaugh. adıyla yeni bir tür olarak tanımlanmıştır. Türe ait örneğin morfolojik özellikleri, Türkçe ismi, doğal yayılım alanından alınan fotoğrafları ve bulunduğu lokasyon bu çalışmada verilmiştir.

### Bitki Koruma

### Araştırma Makalesi

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### Anahtar Kelimeler

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*Fabaceae*

Yabancı Ot

Yeni kayıt

Türkiye

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## INTRODUCTION

The family *Fabaceae* Lindl. includes 793 genera worldwide (POWO, 2024). The genus *Sesbania* Adanson, one of its members, is annual or perennial (trees, shrubs) and is widely distributed throughout the tropics and subtropics of the world (Evans, 1990; Lavin & Schrire, 2005). It is a taxonomic group in the subfamily of *Papilionoideae*, and it is one of the largest genera in these three subfamilies (*Mimosoideae*, *Papilionoideae*, and *Caesalpinioideae*) of the family *Fabaceae*, which contains the highest proportion of species noduled by nitrogen fixative rhizobial bacteria (Sprent, 2001). *Sesbania* species which are tolerant of

both drought and waterlogged conditions, prefer riparian or wetland habitats, and their ecological preference is the most unusual compared to especially their closest relatives (tribes *Loteae* and *Robinieae*) (Evans, 1990; Schrire et al., 2005a, b). *Sesbania herbacea* (Mill.) McVaugh. is native to North America and occurs naturally in the United States from New York to the Southeast, and southwest to Texas and California. It is also present in Mexico and Central America (Sheahan, 2013; POWO, 2024).

There are seventy-two *Fabaceae* genera in Türkiye (Güner et al., 2012). Although there are 60 accepted species of *Sesbania* genus in the world, this genus does not exist in Türkiye (POWO, 2024).

The genus *Sesbania* Adanson was found for the first time in Türkiye as a result of the identification of *Sesbania herbacea*. The species is described in detail, supported by photographs. Additionally, a scientific Turkish name is suggested for the *S. herbacea* species.

## MATERIAL and METHOD

The living plant samples of *Sesbania herbacea* were collected in Mersin (S. Tünk, collector number CUBK-1LEGF-10) during a weed survey in soybean fields at Adana, Mersin, Osmaniye, and Hatay provinces in Türkiye in September 2021 (Figure 1).

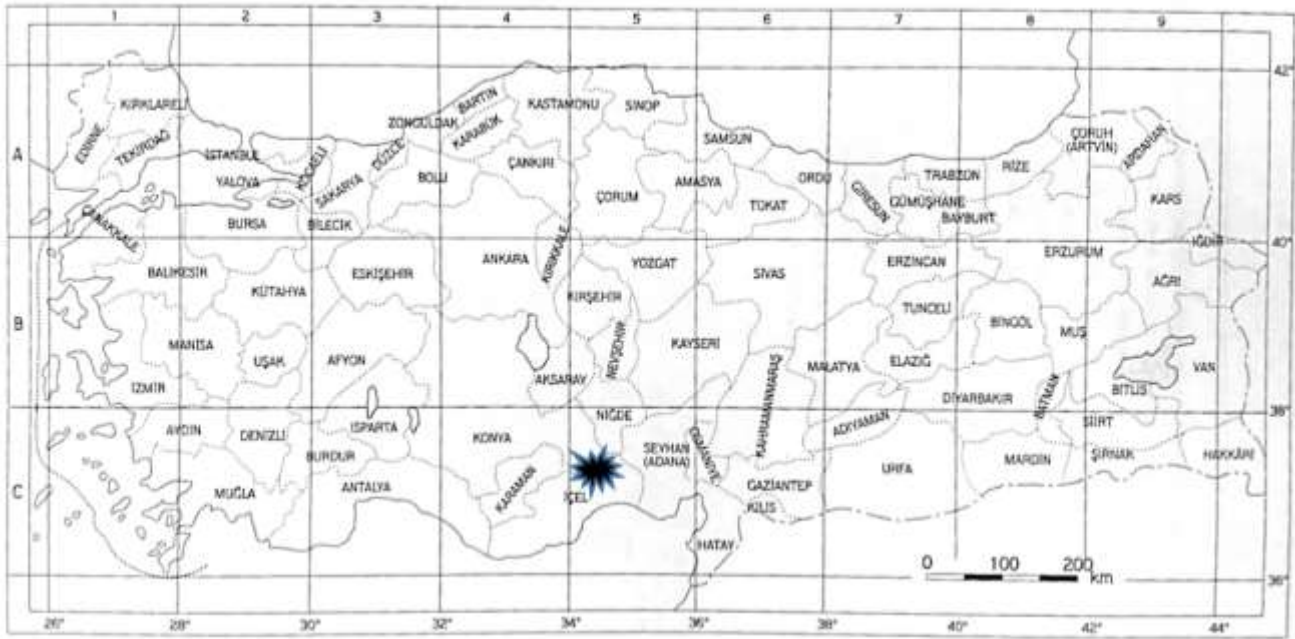


Figure 1. Collecting site of *hemp sesbania* (*Sesbania herbacea*) in Türkiye [The map of Türkiye according to the grid system of P. H. Davis (1965)].

Şekil 1. Akasya Otu (*Sesbania herbacea*)'nun Türkiye'de görüldüğü yer (P. H. Davis (1965)'de kareleme sistemine göre Türkiye haritası).

The specimens of this species were photographed and dried according to the herbarium techniques, and then the necessary morphological examinations were made on them. The voucher specimens were kept at the Herbarium of the Plant Protection Department of Çukurova University. When we checked the Flora of Turkey, we found that plant taxa were not found in the flora. On top of this, the species was identified and determined using the diagnostic key of the possible land of origin (North America) (Anonymous, 2024; Farruggia, 2009). The description of the species was written according to the data obtained in this study. This material was photographed under the DMSZ7P Digital Microscope to determine the seed and dissected flower properties.

## RESULTS

### *Sesbania* Adanson

Annual or perennial herbs, shrubs, or small trees. Stems and twigs unarmed or with prickles. Indumentum simple. Stipules are usually present. Leaves alternate, once paripinnate, leaflets opposite or subopposite. Inflorescences racemes or panicles,

ascending, lax or pendant, axillary; bracts and paired bracteoles early deciduous. Flowers 1-12; calyx campanulate; teeth subequal, shorter than the tube; corolla glabrous, pale yellow, orange to orange-red; stamens 9+1, diadelphous, anthers dorsifixed; pistil glabrous or style with spreading hairs; stigma capitate or slightly elongate, at the same position as anthers; ovules 1-many. The pod is usually long, dehiscent, beaked, sometimes winged, and transversely septate (Gillett, 1963; Brummitt et al., 2007).

Farruggia et al. (2018) mentioned the variation among the fruits of *Sesbania* in their study based on different research (Rydberg, 1923; Gillett, 1963; Hutchinson, 1964; Lavin & Sousa, 1995). It was grouped as;

- 1) Tardily dehiscent, linear, and many-seeded (e.g. *S. sect. Sesbania*);
- 2) Tardily dehiscent, torulose, and few- to several-seeded (*S. sect. Daubentoniopsis* (Rydb.) Lavin);
- 3) Tardily dehiscent, bladderly-inflated, and 2-seeded (*S. sect. Glottidium* (Desv.) Lavin) and 4) Sometimes indehiscent, quadrangular to 4-winged, and several seeded (*S. sect. Daubentonia* (DC.) Benth.).

This newly recorded genus of *Sesbania* taxonomically takes part in the clade of Robinioids. This clade could also comprise the tribes of *Sesbanieae*, *Robinieae*, and *Lotaeeae*. This new record is represented by these three tribes in the Flora of Turkey. There is no close genera to *Sesbania* in the flora of Türkiye.

### *Sesbania herbacea* (Mill.) McVaugh

**Turkish Name:** Akasya Otu

Annual or semi-woody perennial herbs. **Stems**, erect, glabrous, mostly herbaceous, become woody when it gets older. The plant can grow up to 3.5 m in height (Figure 2). Its leaves are alternate, pinnately compound, 7-20 cm; stipules 10.0-10.3 mm. The leaves have 20 to 72 oppositely arranged leaflets. **Leaflets**, oblong to linear, entire, mostly glabrous, (9.0-)15-24(-30) mm long, (1.0-)3.0-5.0(-6.0) mm wide. Inflorescences racemes, in axial, glabrous; peduncle (3-) 13.0-17.0(-43) mm (Figure 2). **Flowers**, hermaphrodite, papilionaceous and zygomorphic. Flower size (10.0-)15.0-17.0(-20.0) mm long; calyx radial, (4.0-)5.0-6.0(-8.0) mm long, teeth 5, subulate-acuminate, (0.6-)1.4-1.6(-2.6) mm long; calyx tube rim with short hairs, the

banner of petals yellow-orange with purplish spots on the outer surface, ovate to obovate, (7.0-)12.0-13.0(-16.0) mm long, (6-)13.0-14.0(-17.0) mm wide; wings of petals yellow-orange, (7.0-)11.0-12.0(-14.0) mm long, (2.5-)4.0-5.0(-6.0) mm wide; keel yellow-orange, purple or maroon at apex, apex rounded-acute, (7.2-)10.0-11.0(-13) mm long, (4.2-)6.0-7.5(-8.0) mm wide; stamen strongly curved inward within keel; style curved back towards banner (Figure 3). **Roots** contain nodules that fix nitrogen on both below-ground roots and laterally on above-ground stems (Figure 4). **Legumes**, a linear pod, brown with maroon-red mottling, (82-)180.0-220.0 (-254.0) mm long, (2.0)2.3-3.0(4.0) mm wide; stipe (2.5-)4.0-6.0(-8.5) mm in fruit; beak (3-)5.0-6.0(-11.0) mm, narrowly tapering (Figure 5). **Seeds** are oblong, green-brown, brownish, generally with purple-black mottling, 3.0-4.0 mm long (Figure 6).

**Flowering/Fruiting season:** July-October

**Examined material:** South of Türkiye, Mersin, Tarsus, a soybean field, S. Tünk, CUBK-1LEGF-10 (Çukurova University, Agricultural Faculty, Dept. of Plant Protection Herbarium)



✓ Figure 2. Hemp sesbania (*Sesbania herbacea*) in soybean field (S. Tünk, CUBK-1LEGF-10)  
Şekil 2. Soya tarlasında Akasya Otu (*Sesbania herbacea*) (S. Tünk, CUBK-1LEGF-10).

As a conclusion, the number of *Fabaceae* genus increased by seventy-two due to the new record of genus *Sesbania* in Türkiye. Also, *Sesbania herbacea* is the first species of the genus. The increase in plant

diversity research in Türkiye leads to the definition of new taxa and the determination of new record plant taxa for Türkiye (Behçet & Altınsoy, 2023).



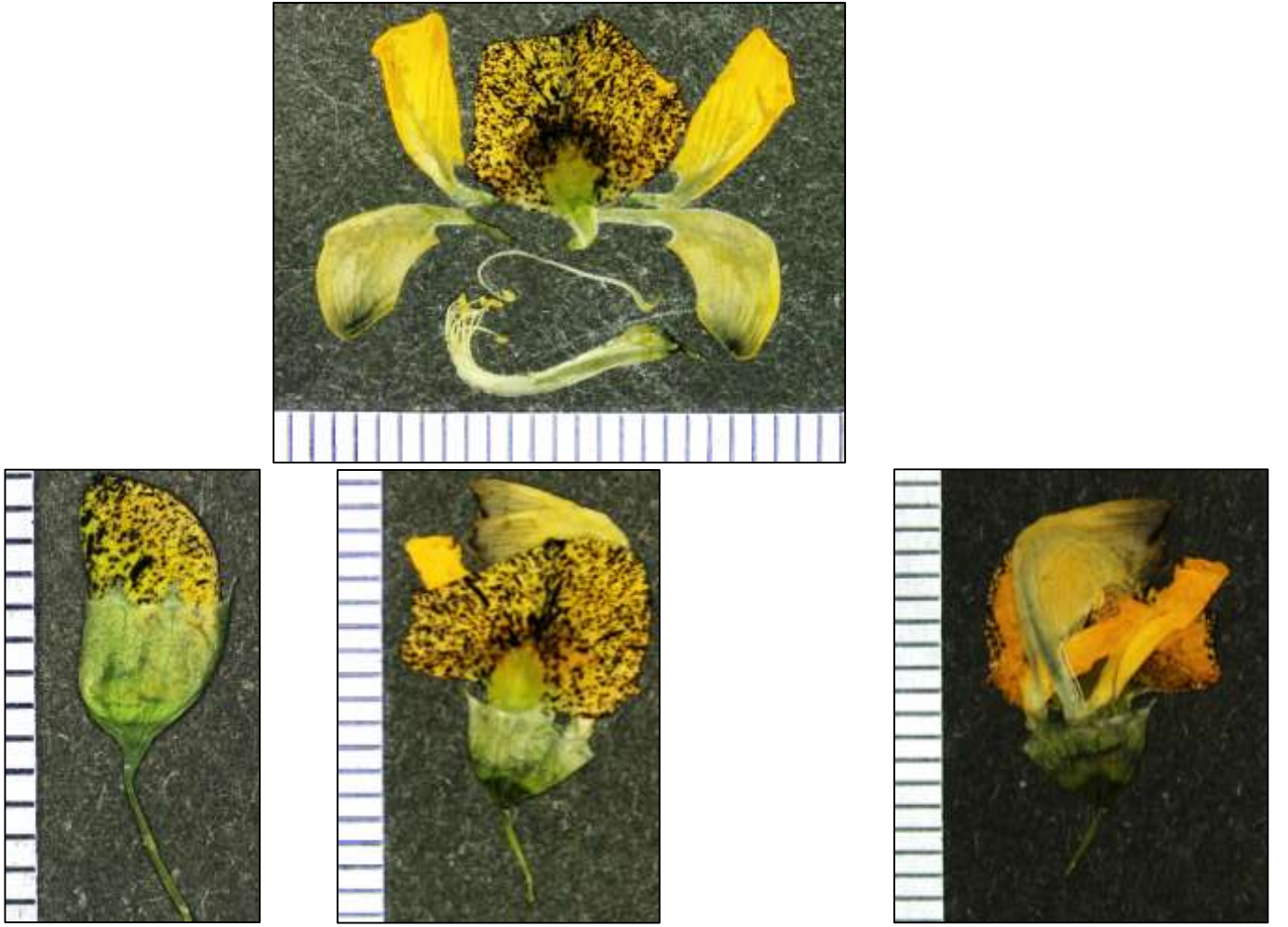


Figure 3. Flowers of hemp sesbania (*Sesbania herbacea*) (Scale 1 mm) (S. Tünk, CUBK-1LEGF-10).

Şekil 3. Akasya Otu (*Sesbania herbacea*)'nun çiçekleri (Ölçek 1mm) (S. Tünk, CUBK-1LEGF-10).



Figure 4. Root and root nodules of hemp sesbania (*Sesbania herbacea*) (S. Tünk, CUBK-1LEGF-10).

Şekil 4. Akasya Otu (*Sesbania herbacea*)'nun kök ve kök nodülleri (S. Tünk, CUBK-1LEGF-10).



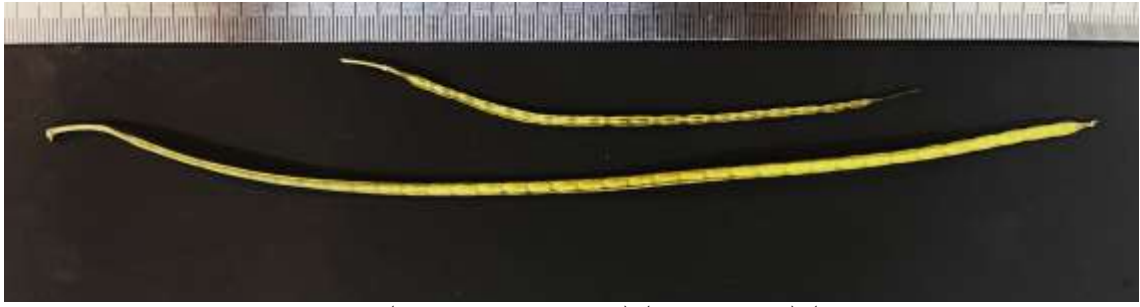


Figure 5. Fruits of hemp sesbania (*Sesbania herbacea*) (Scale 1 mm) (S. Tünk, CUBK-1LEGF-10).  
Şekil 5. Akasya Otu (*Sesbania herbacea*)'nun meyveleri (Ölçek 1mm) (S. Tünk, CUBK-1LEGF-10).



Figure 6. Seeds of hemp sesbania (*Sesbania herbacea*) (Scale 1 mm) (S. Tünk, CUBK-1LEGF-10).  
Şekil 6. Akasya Otu (*Sesbania herbacea*)'nun tohumları (Ölçek 1mm) (S. Tünk, CUBK-1LEGF-10).

## DISCUSSION

*Sesbania herbacea* species belonging to the genus *Sesbania* were observed as seeds in the soybean harvested material at Tarsus/Mersin in 2020. During the weed surveys carried out in Tarsus in August-September 2021, the plant was found in the soybean field. This species determined that it is not native to the flora of Türkiye like *Sida spinosa* belonging to the new record genus *Sida* (Tünk et al., 2024).

*S. herbacea* is found on sandy soils, shallow flooded areas, disturbed habitats, and cultivated fields. It is a semi-woody plant and can be grown as a perennial legume in frost-free regions, and as an annual warm-season legume in frost regions because it dies from frost (Sheahan, 2013). This weed has symbiotic associations with *Rhizobium* bacteria that perform nitrogen fixation in its roots and mycorrhizal fungi (Aziz et al., 1995; Wang & Martínez-Romero, 2000).

It prefers clay and heavy loamy soils but grows poorly in sandy soils (Johnston et al., 1979, McWhorter & Anderson 1979).

*Sesbania* species have alkaloids called saponins, which

are poisonous to cattle and can cause death (Allen & Allen, 1981; Powell et al., 1990). However, the leaves and seeds of *S. herbacea* are non-toxic and its saponins are not harmful to ruminants (Evans & Rotar, 1987). Also, it does not contain toxic sesbanimides (Powell et al., 1990); but it can have low toxicity in domestic animals (Burrows & Tyrl, 2001).

*Sesbania herbacea* can easily become an invasive weed. It is an important weed that is a problem in soybean, cotton, sweet potato, rice, and summer crops in the world (Evan & Rotar, 1987; Smith, 1968; Woon, 1987, Norsworthy & Oliver, 2002). It can grow rapidly and increase its population in cash crops such as soybeans. Since it supports nitrogen-fixing symbiotic bacteria, it relatively does not need soil nitrogen for growth. It produces root exudates that inhibit soybean nodulation, so nitrogen fertilization sometimes allows soybeans to compete with this weed (King & Purcell, 1997). Since it tends to colonize the edges of waterways and spread seeds by water, it can easily spread over large areas by running water (Meert & Hester, 2009).

The economic threshold of *S. herbacea* is approximately 0.5 plants/m<sup>2</sup> in soybean fields

(McWhorter & Anderson, 1979). In its management, sowing soybeans early helps the soybean to grow more competitively against *S. herbacea* (King & Purcell, 1997). Tillage with a tine weeder and rotary hoe is partially effective in the control. This species does not usually emerge until late July or early August, but most competition occurs 4-10 weeks after soybean emergence (McWhorter & Anderson, 1979). However, it should be mowed just after flowering has begun to reduce seed production (Norsworthy & Oliver, 2002). On the other hand, In the control of this species, no pre or post-emergence herbicide could provide enough control throughout the season (Bryson, 1990). This shows us that this species can be harmful weed species in soybean and other irrigable cultures.

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### Author's Contributions

The contribution of the authors is equal.

### Statement of Conflict of Interest

The authors have declared no conflict of interest.

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## The Effect of Liquid Seaweed of Organic Origin on Seed Germination and Seedling Development of Some Winter Cereal Species

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### ABSTRACT

In this study, the effects of liquid seaweed on seed germination and seedling growth in some winter cereal species (triticale, barley, and wheat) were investigated. In the study, 6 different doses (D0: tap water D1:1000 ppm L<sup>-1</sup>, D2:2000 ppm L<sup>-1</sup>, D3:4000 ppm L<sup>-1</sup>, D4:8000 ppm L<sup>-1</sup>, D5:16000 ppm L<sup>-1</sup>) of seaweed were used. The experiment was conducted according to the split-plot trial design in random plots with 3 replications. Germination rate (%), radicle plumule and seedling length (cm), seedling fresh weight (g), seedling dry weight (g), and seedling vigor index were measured during the 14-day development period of cereal species at all seaweed doses. According to the study findings, except for germination rate in the cereal species; significant differences were found between the mean values of all the properties examined in the cereal types, fertilizer doses, and species x dose interactions. Wheat among cereals had the highest values regarding radicle length, seedling vigor index, seedling dry weight, plumule length, and seedling length. D2 dose from the doses of seaweed fertilizer form; germination rate, seedling vigor index, seedling dry weight, seedling fresh weight, plumule length, and seedling length were found to have the highest values, while D5 dose was the lowest. In terms of species x dose interaction, Germination rate, radicle length, seedling vigor index, and seedling length were found to be high in TxD1 interaction. Germination rate was found to be high in HxD0 interaction. Germination rate, radicle length, seedling vigor index, and seedling dry weight were found to be high in WxD2 interaction. As a result, in the germination study with liquid seaweed, D2 doses for wheat, D1 and D2 doses for triticale, D0, D1, and D2 doses for barley were found as encouraging.

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## Organik Kökenli Sıvı Deniz Yosununun Bazı Serin İklim Tahıl Türlerinde Tohum Çimlenmesi ve Fide Gelişimi Üzerine Etkisi

### ÖZET

Bu çalışmada sıvı deniz yosununun bazı kışlık tahıl türlerinde tohum çimlenmesi ve fide büyümesi üzerine etkileri araştırılmıştır. Araştırmada 6 farklı dozda (D0: saf su, D1:1000 ppm L<sup>-1</sup>, D2:2000 ppm L<sup>-1</sup>, D3:4000 ppm L<sup>-1</sup>, D4:8000 ppm L<sup>-1</sup>, D5:16000 ppm L<sup>-1</sup>) deniz yosunu kullanıldı. Deneme tesadüf parsellerinde bölünmüş parseller deneme desenine göre 3 tekerrürlü olarak yürütülmüştür. Tahıl türlerinin 14 günlük gelişim periyodu boyunca tüm deniz yosunu dozlarında çimlenme oranı (%), radikula, plumulu ve fide uzunluğu (cm), fide yaş ağırlığı (g), fide kuru ağırlığı (g) ve vigor indeksi ölçüldü. Araştırma bulgularına göre tahıl türlerinde çimlenme oranı dışında tahıl türleri, gübre dozları ve tür x doz etkileşimlerinde incelenen tüm özelliklerin ortalama değerleri arasında önemli farklılıklar bulunmuştur. Tahıllar arasında buğday, radikula uzunluğu, vigor indeksi, fide kuru ağırlığı, plumula uzunluğu ve fide uzunluğu bakımından en yüksek değerleri elde etmiştir. Deniz yosunu

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gübresinin dozlarından D2 dozu; çimlenme oranı, vigor indeksi, fide kuru ağırlığı, fide yaş ağırlığı, plumula uzunluğu ve fide uzunluğu en yüksek değerlere sahip olurken, D5 dozu en düşük değerlere sahip olmuştur. Türxdoz etkileşimi açısından bakıldığında TxD1 etkileşiminde çimlenme oranı, radikula uzunluğu, vigor indeksi ve fide uzunluğu yüksek bulunmuştur. HxD0 etkileşiminde çimlenme oranı yüksek bulunmuştur. WxD2 etkileşiminde çimlenme oranı, radikula uzunluğu ve vigor indeksi ile fide kuru ağırlığının yüksek olduğu tespit edilmiştir. Sonuç olarak sıvı deniz yosunu ile yapılan çimlendirme çalışmasında buğday için D2 dozları, tritikale için D1 ve D2 dozları, arpa için D0, D1 ve D2 dozları teşvik edici bulunmuştur.

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## INTRODUCTION

Winter and spring cereals have an important place in terms of cultivation area and production in the world and Türkiye. Especially in terms of winter cereals, Türkiye has a suitable ecology. Because Türkiye is a part of the Fertile Crescent and the most important gene center of winter cereals. Therefore, the cultivation of barley (*Hordeum* sp.), oat (*Avena* sp.), rye (*Secale* sp.), and triticale (*Triticale*) species, especially bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.), is carried out intensively in Türkiye.

According to TURKSTAT (2022), cereal sufficiency in Türkiye in 2020 was 97.4%. However, sufficiency in wheat was the highest with 102.3%. Among the winter cereals in the same year, the cultivation area of wheat was 5664180 ha for grain and 17866 ha for green grass. Wheat production was 16500000 tons for grain and 348838 tons for green grass. In wheat yield, grain was 2920 kg ha<sup>-1</sup> and green grass was 19530 kg ha<sup>-1</sup>. The cultivation area of barley was 2904637 ha in grain and 31319 ha in green grass. In addition, while the production of barley was 7700000 tons in grain and 537066 tons in green grass, the yield was 26500 kg ha<sup>-1</sup> for grain and 17150 kg ha<sup>-1</sup> for green grass. The cultivation area of triticale was recorded as 81115 ha for grain and 35009 ha for green grass. Triticale production was 276212 tons for grain and 558643 tons for green grass. In the yield, grain was 3410 kg ha<sup>-1</sup> and green grass was 15960 kg ha<sup>-1</sup>.

The issue that can be an alternative to chemical fertilizers or reduce their negative effects is the use of organic fertilizers in agricultural production. Seaweed fertilizer is one of the organic soil conditioners containing humic acid, which positively affects the development of the plant. Organic fertilizers or fertilizer forms provide root development by increasing the oxygen content of the soil and its water-holding capacity and protecting the structure of the soil by

reducing salinity in the soil (Kaya & Erdönmez, 2020). This seaweed, a form of organic fertilizer used versatilely in modern agriculture to improve plant growth and increase productivity is a valuable marine resource (Nanda et al., 2021) Seaweed is a form of organic fertilizer that increases germination, helps root development, protects plants against diseases, and pests and prolongs the life of the plant (Hong et al., 1995). For this reason, this study was carried out to determine the effects of seaweed applied in different doses on seed germination and seedling growth in wheat, barley, and triticale, which are winter cereals that have an important place in terms of production and consumption in the world and Türkiye.

## MATERIAL and METHOD

The seaweed fertilizer used in the study was produced by Kristal AG Kimya Tarım Sanayi ve Ticaret Ltd. Information about the fertilizer content is given below (Table 1).

Table 1. Seaweed fertilizer's content and amount  
*Çizelge 1. Deniz yosunu gübresinin içeriği ve miktarı*

| Seaweed Fertilizer Content                           | Amount    |
|--|-----------|
| Organic Matter (%)                                   | 15.00     |
| Organic Carbon (%)                                   | 10.00     |
| Total Nitrogen (%)                                   | 1.00      |
| Water-soluble Potassium Oxide (K <sub>2</sub> O) (%) | 2.00      |
| Alginate Acid (%)                                    | 1.50      |
| Gibberellic Acid (ppm)                               | 0.40      |
| pH   | 4.00-6.00 |

This research was carried out in the climate cabinet of Kahramanmaraş Sütçü İmam University Faculty of Agriculture Department of Field Crops Laboratory in April 2021. In the study, triticale (T: Ayşehanım variety), 2-row barley (B: Novosadski variety 565), and

bread wheat (W: Balkoni variety). which are winter cereal species. were used as seeds.

In the research, liquid seaweed fertilizer form was applied in 6 different ways as control (D0: tap water) and 5 doses (D1:1000 ppm L<sup>-1</sup>. D2:2000 ppm L<sup>-1</sup>. D3:4000 ppm L<sup>-1</sup>. D4:8000 ppm L<sup>-1</sup>. D5:16000 ppm L<sup>-1</sup>) (Kaya & Erdönmez. 2020). Tap water was used in the preparation of the doses. The experiment was carried out in 3 replications according to the split-plot trial design in random plots. After covering the bottom of 120 mm petri dishes with 2 layers of blotting paper. blotting papers were wetted with 12 ml of prepared solutions. The surface sterilization process was applied to 25 healthy seeds from each cereal type in a 5% NaOCl (sodium hypochlorite) solution for 5 minutes.

Afterward, the seeds were washed in tap water and sown in petri dishes to which the solution was added. Petri dishes were covered with parafilm (PM-992) in order to prevent the evaporation of irrigation water at different doses applied and left to germinate for 14 days in an incubator at 20±2 °C. Measurements for germination and seedling development were made on the 15th day.

In the experiment in cereal species, properties such as germination rate (GR) (%), radicle length (RL) (cm), plumule length (PL) (cm), seedling length (PL) (cm), seedling fresh weight (SFW) (g), seedling dry weight (SDW) (g) and seedling vigor index (SVI) were investigated. Images of the research are given below (Figure 1-8).



Figure 1. 6th dose 1st recurrence of triticale  
Şekil 1. 6. doz 1. tritikale nüksü



Figure 2. In triticale 1st dose 1st Recurrence  
Şekil 1. 6. doz 1. tritikale nüksü



Figure 3. In barley 2th dose 1st recurrence  
Şekil 3. Arpada 2. doz 1. nüks



Figure 4. In wheat 2th Dose 1st Recurrence  
Şekil 4. Buğdayda 2. doz 1. nüks



Figure 5. In triticale 1st dose 3th Recurrence  
Şekil 5. Tritikalede 1. Doz 3. nüks

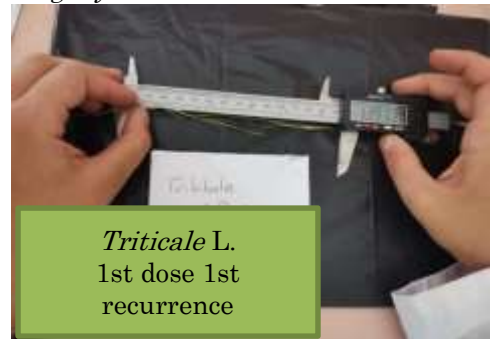


Figure 6. In triticale 1st dose 1st Recurrence  
Şekil 6. Tritikalede 1. Doz 1. nüks



Figure 7. In triticale 1st dose 3th Recurrence  
Şekil 7. Tritikalede 1. Doz 3. nüks



Figure 8. In the laboratory. on the 15th day after placing, while seeds are measured for germination and seedling development

Şekil 8. Laboratuvarıda. yerleştirmeden sonraki 15. günde; tohumlar çimlenme ve fide gelişimi için ölçülürken

The germination rate of the examined traits was found by counting the germinated seeds and dividing by the total number of seeds. and multiplying by 100. Radicle and plumule lengths were measured separately with the help of a caliper and then the seedling length was calculated by summing the radicle and plumule lengths. The fresh weights of the radicle and plumule length were pondered and the fresh weight of the collected seedlings was found. Then, the dry weight of the seedlings was calculated when they reached a constant temperature in the oven at 70°C. The seedling vigor index was obtained by multiplying the germination rate by the seedling length.

#### Statistical Analysis of Data

All data obtained from the research were processed by SAS (V. 9.0. 2002) statistical package. All the data were analyzed using analysis of variance (ANOVA) according to the split-plot trial design in random plots. Averages were compared by the Least Significant Difference multiple comparison test (Steel & Torrie, 1980).

## RESULTS and DISCUSSION

The average values of the effects of seaweed doses on the seedling growth and germination rate of wheat,

barley, and triticale seeds are given in Tables 2,3, 4, and 5.

#### Germination Rate (%)

According to the variance analysis results obtained, the difference between the doses was statistically very significant ( $p < 0.001$ ) in terms of germination rate, the difference between species x dose and species was insignificant (Table 2, Table 3, Table 4).

The germination rate of cereal species varied between 96.00-98.44 % (Table 2). The germination rate of the doses ranged from 92.00% to 100.00%. While the highest germination rate was observed in D0, D1, D2, and D3 (100.00, 100.00, 99.11, and 97.78 %, respectively) doses, the lowest germination rate was found in D4 and D5 doses (Table 3). Within the doses, D4 and D5 seem to have limits on the germination rate. The germination rate of the species x dose interaction varied between 86.67%-100.00%. In the species x dose interaction, the highest germination rate was found in TD0, TD1, TD2, TD4, BD0, BD1, WD0, WD1, and WD2, while the lowest germination rate was found in WD4 (Table 4).

Table 2. Mean values of GR, RL, PL, SL, SWF, SDW, and SVI across species

Çizelge 2. Türler arasında GR, RL, PL, SL, SWF, SDW ve SVI ortalama değerleri.

| Features | Species (S) |           |           |           |              |
|----------|-------------|-----------|-----------|-----------|--------------|
|          | T           | B         | W         | S average | LSD (0.05) S |
| GR (%)   | 98.44       | 96.00     | 96.00     | 96.81     | -            |
| RL (cm)  | 10.95 b     | 4.92 c    | 11.95 a   | 9.27      | 0.55**       |
| PL (cm)  | 13.17 b     | 13.81 a   | 13.84 a   | 13.61     | 0.58*        |
| SL (cm)  | 24.12 b     | 18.73 c   | 25.79 a   | 22.88     | 0.89**       |
| SFW (g)  | 3.58        | 3.31      | 3.34      | 3.41      | -            |
| SDW (g)  | 0.18 b      | 0.19 b    | 0.24 a    | 0.20      | 0.02**       |
| SVI      | 2390.17 a   | 1797.99 b | 2492.68 a | 2226.95   | 113.72**     |

GR: germination rate RL: radicle length PL: plumule length SL: seedling length SFW: seedling fresh weight SDW: seedling dry weight SVI: seedling vigor index. T: triticale B: barley W: wheat \*:  $P < 0.05$  \*\*:  $P < 0.01$ .

Table 3. Mean values of GR, RL, PL, SL, SFW, SDW, and SVI in seaweed doses.

Çizelge 3. Deniz yosunu dozlarında Deniz yosunu dozlarında GR, RL, PL, SL, SFW, SDW ve SVI ortalama değerleri.

| Features | Doses (ppm L <sup>-1</sup> ) |           |           |           |           |           | Dose average | LSD (0.05) |
|----------|------------------------------|-----------|-----------|-----------|-----------|-----------|--------------|------------|
|          | D0                           | D1        | D2        | D3        | D4        | D5        |              |            |
| GR (%)   | 100.00 a                     | 100.00 a  | 99.11 a   | 97.78 a   | 92.00 b   | 92.00 b   | 96.82        | 3.39 **    |
| RL (cm)  | 13.02 a                      | 11.62 b   | 12.37 ab  | 8.51 c    | 6.66 d    | 3.46 e    | 9.27         | 0.79 **    |
| PL (cm)  | 13.14 b                      | 12.61 bc  | 14.98 a   | 13.24 b   | 15.39 a   | 12.27 c   | 13.60        | 0.82 **    |
| SL (cm)  | 26.16 a                      | 24.23 b   | 27.35 a   | 21.76 c   | 22.05 c   | 15.73 d   | 22.88        | 1.26 **    |
| SFW (g)  | 3.64 a                       | 3.54 ab   | 3.70 a    | 3.40 b    | 3.27 b    | 2.88 c    | 3.40         | 0.35 **    |
| SDW (g)  | 0.21 b                       | 0.18 cd   | 0.31 a    | 0.15 d    | 0.19 bc   | 0.16 d    | 0.20         | 0.02 **    |
| SVI      | 2616.23 a                    | 2423.22 b | 2716.07 a | 2134.54 c | 2019.95 c | 1451.66 d | 2226.95      | 160.82 **  |

GR: germination rate RL: radicle length PL: plumule length SL: seedling length SFW: seedling fresh weight SDW: seedling dry weight SVI: seedling vigor index. Fertilizer Doses D0: Kontrol D1: 1000 ppm L<sup>-1</sup> D2: 2000 ppm L<sup>-1</sup> D3: 4000 ppm L<sup>-1</sup> D4: 8000 ppm L<sup>-1</sup> D5: 16000 ppm L<sup>-1</sup>. The same letters in each line are in the same group. In multiple comparison test; \*\*:P<0.01.

Table 4. Mean GR, RL, PL, SL, SFW, SDW and SVI values in seaweed doses of species.

Çizelge 4. Ortalama GR, RL, PL, SL, SFW, Türlerin deniz yosunu dozlarında SDW ve SVI değerleri.

| Species (S)      | Doses (ppm L <sup>-1</sup> ) | GR (%)        | RL (cm)         | PL (cm)          | SL (cm)          |
|------------------|------------------------------|---------------|-----------------|------------------|------------------|
| T                | D0                           | 100.00 ± 0.00 | 14.36 ± 0.65 bc | 13.63 ± 0.10 c-f | 27.99 ± 0.67 bcd |
|                  | D1                           | 100.00 ± 0.00 | 17.11 ± 0.80 a  | 15.23 ± 0.63 a-e | 32.34 ± 1.41 a   |
|                  | D2                           | 100.00 ± 0.00 | 15.18 ± 0.47 ab | 14.42 ± 0.28 b-e | 29.60 ± 0.66 abc |
|                  | D3                           | 98.67 ± 1.33  | 9.09 ± 0.46 d   | 12.90 ± 0.29 e-h | 21.99 ± 0.44 e   |
|                  | D4                           | 100.00 ± 0.00 | 6.48 ± 0.21 ef  | 13.31 ± 0.62 d-g | 19.79 ± 0.61 e   |
|                  | D5                           | 92.00 ± 2.31  | 3.48 ± 0.24 gh  | 9.53 ± 0.51 j    | 13.01 ± 0.60 f   |
| B                | D0                           | 100.00 ± 0.00 | 8.29 ± 0.09 de  | 13.88 ± 0.53 cde | 22.17 ± 0.61 e   |
|                  | D1                           | 100.00 ± 0.00 | 3.94 ± 0.15 g   | 11.10 ± 0.63 hij | 15.04 ± 0.75 f   |
|                  | D2                           | 97.33 ± 1.33  | 4.95 ± 0.33 fg  | 16.35 ± 0.49 ab  | 21.31 ± 0.37 e   |
|                  | D3                           | 96.00 ± 2.31  | 3.83 ± 0.26 gh  | 10.39 ± 0.48 ij  | 14.22 ± 0.58 f   |
|                  | D4                           | 89.33 ± 5.81  | 3.90 ± 0.15 g   | 10.70 ± 0.46 abc | 19.60 ± 0.61 e   |
|                  | D5                           | 93.33 ± 3.53  | 4.60 ± 0.32 fg  | 15.43 ± 0.82 a-d | 20.04 ± 1.03 e   |
| W                | D0                           | 100.00 ± 0.00 | 16.40 ± 0.47 a  | 11.92 ± 0.60 f-i | 28.33 ± 1.06 bc  |
|                  | D1                           | 100.00 ± 0.00 | 13.82 ± 0.88 bc | 11.49 ± 0.43 g-j | 25.31 ± 0.07 d   |
|                  | D2                           | 100.00 ± 0.00 | 16.97 ± 0.85 a  | 14.17 ± 0.49 cde | 31.15 ± 1.12 ab  |
|                  | D3                           | 98.67 ± 1.33  | 12.62 ± 0.80 c  | 16.44 ± 0.51 ab  | 29.06 ± 1.25 bc  |
|                  | D4                           | 86.67 ± 2.67  | 9.61 ± 0.54 d   | 17.15 ± 0.70 a   | 26.77 ± 1.16 cd  |
|                  | D5                           | 90.67 ± 1.33  | 2.29 ± 0.1 h    | 11.85 ± 0.43 f-i | 14.14 ± 0.41 f   |
| Overall Average  |                              | 96.81         | 9.27            | 13.33            | 22.88            |
| LSD (0.05) S x D |                              | -             | 2.41**          | 2.49**           | 3.96**           |
| CV (%)           |                              | 3.63          | 8.83            | 6.30             | 5.74             |

T: triticale. B: barley. W: wheat. GR: germination rate RL: radicle length PL: plumule length SL: seedling length CV: coefficient of variation T: species. D: dose. Fertilizer Doses; D0: control. D1: 1000 ppm L<sup>-1</sup> D2: 2000 ppm L<sup>-1</sup> D3: 4000 ppm L<sup>-1</sup> D4: 8000 ppm L<sup>-1</sup> D5: 16000 ppm L<sup>-1</sup>. There is no statistical difference between the same capital letters in the same column. There is no statistical difference between the same lowercase letters in the same column. In multiple comparison test; \*: P<0.05 \*\*:P<0.01.

In their previous studies on seaweed, Kaya & Erdönmez (2020) in their research to determine the effect of 6 different doses (D0: control, D1:1000 ppm L<sup>-1</sup>, D2:2000 ppm L<sup>-1</sup>, D3:4000 ppm L<sup>-1</sup>, D4:8000 ppm L<sup>-1</sup>, D5:16000 ppm L<sup>-1</sup>) of seaweed on soybean germination and seedling development; they found the highest germination rate at 84.00% to D2 dose and the lowest germination rate at 20.00% to D5 fertilizer dose. In the study, it was detected that the germination rate

decreased with the increase of the seaweed fertilizer dose, except for the control application, and this is consistent with our findings. Demirkaya (2016), in her study, examined the effect of germination rate in pepper seeds on the osmotic conditioning of seaweed extract applied at different times and doses with Methyl Jasmonate at different times. As a result of the data obtained, it was observed that the germination rate of pepper seeds would increase with seaweed extract at both 20 °C and 15 °C, the application period



of which was 1 day. Möller and Smith (1999) investigated the water sensitivity of seaweed suspensions from *Ascophyllum nodosum* (Linnaeus) Le Jolis (ANS) and *Laminaria hyperborea* (Gunn.) Foslie (LHS) in barley seeds in their study. As a result of the research, they reported that both seaweeds did not reduce the viability of barley seed but increased germination. Zodape (2001) reported in her study that the application of seaweed material before planting increased the germination rate of many vegetable seeds. As can be understood from the studies, it is seen that the germination rate varies depending on the type of plant used, variety, and seed size. Hong et al. (2007) found in their study that 20% of brown seaweed increased the seed germination percentage and produced a lower germination rate of 100%. Altuner et al. (2019) found in their study that the highest germination rate in the application of salt stress (0, 50, 100, and 200 mM) and gibberellic acid (0,100,200 and 300 ppm) to triticale seeds was 70.2% in the application of 300 ppm gibberellic acid in 0 mM salt stress.

### Radicle Length (cm)

According to the results of the analysis of variance, it was determined that the statistical difference between the cereal types, doses, and species x dose interaction ( $p < 0.001$ ) in terms of radicle length was very important (Table 2, Table 3, Table 4). The radicle length of the cereal species was found to be between 4.92 cm and 11.95 cm. While the highest radicle length was observed in W among cereal species, the shortest radicle length was found in B (Table 2). The radicle length of the doses was observed between 3.46 cm and 13.02 cm. While the longest radicle length was observed in the D0 dose, the shortest radicle length was found in the D5 dose. Among the doses, the D5 dose seems to have a limiting effect on the radicle length (Table 3). The radicle length of the species x dose interaction ranged from 2.29 cm to 17.11 cm. Within the species x dose interaction, the highest radicle length was found in TD1, WD2, and WD0 (17.11, 16.97, and 16.40 cm, respectively). and the shortest radicle length was found in WD5 (Table 4). In their previous studies on seaweed. Kaya & Erdönmez (2020) found the highest radicle length at 10.01 cm at a dose of D2, and the shortest radicle length at a fertilizer dose of 3.58 cm at D5. In the study, it was observed that the radicle length decreased with the increase of the seaweed fertilizer dose, except for the control application. and their findings support our findings. Bat et al. (2019) investigated the effects of seaweed doses (0 (control), 2, 4, and 6 cc L<sup>-1</sup>) applied in viols on echinacea plants under drought stress. As a result of the study, the highest radicle length was obtained in the seaweed extract application at 16.17 cm, and the shortest radicle length in the 6 cc L<sup>-1</sup>

application was obtained in the control application at 13.28cm. Mrogan & Tarjan. (1980) reported that seaweed material applied to tomato plants increased root growth in their study. Finnie & Staden (1985). in their study. found that the extract obtained from the seaweed material of *Ecklonia maxima* (sea bamboo) increased rooting in tomato plants. Kara et al. (2019) investigated the effect of seaweed applications (control (0), 2, 4, and 6 cc L<sup>-1</sup>) on salt stress (control, NaCl, KCl, and CaCl<sub>2</sub>) in the echinacea plant. As a result of the research, they reported that the highest average radicle length was obtained with 14.8 cm at the dose of 6 cc L<sup>-1</sup> seaweed, and the lowest average radicle length was obtained at 13.4 cm in the control application. As can be understood from the studies, the difference in seed size in different plant species and cultivars affects the root length. Güngör et al. (2017), in their study investigating the effect of different doses of salt concentration (0, 50, 100 mM) on the radicle length of the oat plant, observed that the highest radicle length was 6.87 cm at 0 mM salt concentration.

### Plumule Length (cm)

In the analysis of variance, the difference between doses and species x dose interaction in terms of plumule length was found to be statistically significant ( $p < 0.001$ ) while the difference between cereal species was significant ( $p = 0.042$ ) (Table 2, Table 3, Table 4). The plumule length of the cereal species was stated to be between 13.17-13.84 cm. While the highest plumule length was observed in W and B (13.84 and 13.81 cm, respectively) among cereal species, the shortest plumule length was detected in the T type (Table 2). The plumule length of the doses was observed between 12.27-15.39 cm. Among the doses, the longest plumule length was observed in D4 and D2 (15.39 and 14.98 cm, respectively) and the shortest plumule length was observed in D5 (12.27 cm) (Table 3). The plumule length of the species x dose interaction was determined between 9.53-17.15 cm. In the species x dose interaction, the highest plumule length was observed in WD4, and the shortest plumule length was observed in TD5. In their previous studies on seaweed (Table 4). Kaya & Erdönmez (2020) found the highest plumule length at 10.01 cm at a dose of D2, and the lowest plumule length at 3.58 cm at a fertilizer dose of D5. In the study, it was observed that the plumule length decreased with the increase of the seaweed fertilizer dose, except for the control application, and their findings support our findings. Bat et al. (2019) in their research; they determined that the highest plumule length was obtained in the application of 6 cc L<sup>-1</sup> seaweed extract at 21.42 cm, and the shortest plumule length was obtained at 18.21 cm in the control application. Kara et al. (2019) observed that the highest average plumule length was obtained with 18.00 cm at the dose of 2 cc L<sup>-1</sup> seaweed, and the lowest

average plumule length was obtained at 15.40 cm in the control application.

### Seedling Length (cm)

According to the results of the analysis, the statistical difference between cereal types, doses, and species x dose interaction in terms of seedling length was found to be very significant ( $p < 0.001$ ) (Tables 2, 3, and 4). The seedling length of the cereal species was found to be between 18.73-25.79 cm. Among the cereal species, the longest seedling length was determined in W type, and the shortest seedling length was determined in B type (Table 2). The seedling length of the doses was found to be between 15.73-27.35 cm. While the longest seedling length was observed at D2 and D0 (27.35 and 26.16 cm, respectively) doses, the shortest seedling length was observed at D5 dose. D2 seems to be more stimulating in terms of seedling length (Table 3). It has been determined that the species x dose interaction varies between 13.01-32.34 cm in seedling length. Within the species x dose interaction, the highest seedling length was observed in TD1, and the shortest seedling length was observed in TD5, BD3, WD5, and BD1 (13.01, 14.22, 14.14 and 15.04 cm, respectively) (Table 4). Allwright (1992) observed in a study that seaweed material increased plant height in wheat.

### Seedling Fresh Weight (g)

In the analysis of variance, the difference between the doses and the species x dose interaction in terms of seedling fresh weight was found to be statistically very significant ( $p < 0.001$ ), but it was found to be unimportant in terms of species (Table 2, Table 3 and Table 5). The seedling fresh weight of the cereal species varied between 3.31-3.58 g (Table 2). The seedling fresh weight of the doses varied between 2.88-3.70 g. The highest seedling fresh weight was obtained at D2 and D0 (3.70 and 3.64 g, respectively) doses, and the lowest seedling fresh weight was obtained at D5 doses (Table 3). The seedling fresh weight of the species x dose interaction ranged from 2.28 g to 4.06 g. Within the species x dose interaction, the highest seedling fresh weight was observed in TD2, and the lowest seedling fresh weight was observed in WD5 (Table 5). Kaya & Erdönmez (2020) found the highest seedling fresh weight at 1041.00 mg and D1 dose, and the lowest seedling fresh weight at 621.666 mg and D5 fertilizer dose. Their findings agree with our findings. Kaya & Coşkun (2020) in their research determined the effect of 6 different doses (D0: control (tap water). D1: 1000 ppm L<sup>-1</sup>. D2: 2000 ppm L<sup>-1</sup>. D3: 4000 ppm L<sup>-1</sup>. D4: 8000 ppm L<sup>-1</sup> and D5:16000 ppm L<sup>-1</sup>) of seaweed on rapeseed germination and seedling development; they determined that the highest seedling fresh weight was 79.23 mg with D2, the lowest seedling fresh weight was 46.60 mg with D1, and germination did not occur at D5.

### Seedling Dry Weight (g)

In the analysis of variance, the difference between cereal types, doses, and species x dose interaction in terms of seedling dry weight was found to be statistically very significant ( $p < 0.001$ ) (Table 2, Table 3, and Table 5). The seedling dry weight of the cereal species varied between 0.18-0.24 g. Among the species, the highest seedling dry weight was detected in the W type, and the lowest seedling dry weight was detected in the T and B types (0.18 and 0.19 g respectively) (Table 2). The seedling dry weight of the doses varied between 0.15-0.31 g. Among the doses, the highest seedling dry weight was obtained at the D2 dose, and the lowest seedling dry weight was obtained at the D3 and D4 doses (0.15 and 0.16 g, respectively) (Table 3). The seedling dry weight of the species x dose interaction ranged from 0.12 g to 0.48 g. Within the species x dose interaction, the highest seedling dry weight was observed in WD2, and the lowest seedling dry weight was observed in TD3, TD5, and BD3 (Table 5). Allwright (1992) stated that seaweed material increased plant height and plant dry weight in wheat. Kaya & Erdönmez (2020) in their research; they detected the highest seedling dry weight was detected at the dose of 155.667 mg and D2, and the lowest seedling dry weight was detected at the fertilizer dose of 96.833 mg and D5 doses. Their findings agree with our findings. Kaya & Coşkun (2020) found that the highest seedling dry weight was 8.716 mg with D2, the lowest seedling dry weight was 5.126 mg at D1, and germination did not occur at D5.

### Seedling Vigor Index

In the analysis of variance, the statistical difference between cereal types, doses, and species x dose interaction was found to be very significant ( $p < 0.001$ ) in terms of seedling vigor index weight (Table 2, Table 3, and Table 5). The seedling vigor index of cereal species varied between 1797.99 and 2492.68. The highest seedling vigor index among the cereal types was found in W and T (2492.68 and 2390.17, respectively) types, and the lowest seedling vigor index was found in the B type (Table 2). The seedling vigor index of the doses ranged from 1451.66 to 2716.07. Among the doses, the highest seedling vigor index was determined at the D2 and D0 doses (2716.07 and 2616.23, respectively), and the lowest seedling vigor index was found at the D5 dose (Table 3). The seedling vigor index of the species x dose interaction was observed to vary between 1198.98 and 3233.90. Among the species dose interaction, the highest seedling vigor index was in TD1, WD2, TD2, WD3, and WD0 (3233.90, 3114.83, 2959.99, 2870.63, and 2832.58, respectively). while the lowest seedling vigor index in TD5, WD5, and BD3 (1198.98, 1281.54, and 1363.11, respectively), was observed (Table 5). Kaya & Erdönmez (2020) found the highest seedling vigor

index in 1991.09 at 1000 ppm L<sup>-1</sup> dose. and the lowest seedling vigor index at 236.58 at D5 fertilizer dose. Kaya & Coşkun (2020) they found that the highest seedling vigor index was 14848.52 in the D0

application. the lowest seedling vigor index was 354.74 at the dose of D2. and the germination did not occur at the dose of D5.

Table 5. Mean SFW, SDW and SVI values in seaweed doses of species  
*Çizelge 5. Ortalama SFW, Türlerin deniz yosunu dozlarında SDW ve SVI değerleri*

| Species (S)             | Doses (ppm L <sup>-1</sup> ) | SFW (g)         | SDW (g)         | SVI                |
|-------------------------|------------------------------|-----------------|-----------------|--------------------|
| <b>T</b>                | <b>D0</b>                    | 3.36 ± 0.18 bcd | 0.20 ± 0.00 cde | 2798.99 ± 67.3 ab  |
|                         | <b>D1</b>                    | 4.01 ± 0.15 ab  | 0.20 ± 0.01 cde | 3233.90 ± 141 a    |
|                         | <b>D2</b>                    | 4.06 ± 0.07 a   | 0.23 ± 0.01 bc  | 2959.99 ± 66.4 a   |
|                         | <b>D3</b>                    | 3.85 ± 0.19 ab  | 0.12 ± 0.01 g   | 2169.89 ± 59.9 c-f |
|                         | <b>D4</b>                    | 3.50 ± 0.05 abc | 0.19 ± 0.01 def | 1979.25 ± 60.9 def |
|                         | <b>D5</b>                    | 2.67 ± 0.10 ef  | 0.12 ± 0.01 g   | 1198.98 ± 80.3 h   |
| <b>B</b>                | <b>D0</b>                    | 3.76 ± 0.21 ab  | 0.24 ± 0.01 b   | 2217.12 ± 61.2 cde |
|                         | <b>D1</b>                    | 3.21 ± 0.07 cde | 0.16 ± 0.01 ef  | 1504.52 ± 75.3 gh  |
|                         | <b>D2</b>                    | 3.68 ± 0.11 abc | 0.21 ± 0.01 cd  | 2073.40 ± 37.3 def |
|                         | <b>D3</b>                    | 2.85 ± 0.04 de  | 0.12 ± 0.00 g   | 1363.11 ± 36.7 h   |
|                         | <b>D4</b>                    | 3.38 ± 0.74 bc  | 0.17 ± 0.05 cd  | 1755.36 ± 157 fg   |
|                         | <b>D5</b>                    | 3.68 ± 0.15 abc | 0.21 ± 0.01 cd  | 1874.45 ± 152 ef   |
| <b>W</b>                | <b>D0</b>                    | 3.80 ± 0.23 ab  | 0.19 ± 0.01 de  | 2832.58 ± 106 a    |
|                         | <b>D1</b>                    | 3.41 ± 0.16 bc  | 0.18 ± 0.01 ef  | 2531.24 ± 107 bc   |
|                         | <b>D2</b>                    | 3.37 ± 0.12 bc  | 0.48 ± 0.01 a   | 3114.83 ± 112 a    |
|                         | <b>D3</b>                    | 3.51 ± 0.04 abc | 0.22 ± 0.01 bcd | 2870.63 ± 161 a    |
|                         | <b>D4</b>                    | 3.67 ± 0.19 abc | 0.21 ± 0.01 bcd | 2325.25 ± 166 cd   |
|                         | <b>D5</b>                    | 2.28 ± 0.07 f   | 0.15 ± 0.00 f   | 1281.54 ± 26.4 h   |
| <b>Overall Average</b>  |                              | 3.45            | 0.20            | 2226.95            |
| <b>LSD (0.05) S x D</b> |                              | 0.68**          | 0.04**          | 501.83**           |
| <b>CV (%)</b>           |                              | 10.66           | 12.99           | 7.50               |

**T:** triticale **B:** barley **W:** wheat **SFW:** seedling fresh weight **SDW:** seedling dry weight **SVI:** seedling vigor index **CV:** coefficient of variation **T:** species **D:** dose. **Fertilizer Doses; D0:** Control **D1:** 1000 ppm L<sup>-1</sup>. **D2:** 2000 ppm L<sup>-1</sup> **D3:** 4000 ppm L<sup>-1</sup> **D4:** 8000 ppm L<sup>-1</sup> **D5:** 16000 ppm L<sup>-1</sup>. There is no statistical difference between the same capital letters in the same column. There is no statistical difference between the same lowercase letters in the same column. In multiple comparison test; \*: P<0.05 \*\*:P<0.01.

## CONCLUSION

Many studies on seaweed organic material are mostly done on vegetable species, but not many studies have been done on cereal species. In addition, it is seen that there is not much research on seaweed material in germination studies. With this study, the effect of seaweed organic material on the germination of cereal species will be determined and will guide many future studies. In the study, it was observed that the data on the germination and seedling development of the cereal species of seaweed doses were statistically significant, D2 (2000 ppm L<sup>-1</sup>) dose from the 6 different seaweed doses used in the experiment; the highest values in terms of germination rate, seedling vigor index, seedling dry weight, seedling fresh weight, plumule length and seedling length, D5 (16000 ppm L<sup>-1</sup>) dose gave the lowest values. Among the cereal types, wheat; radicle length, seedling vigor index, seedling dry weight, plumule length, and seedling length

reached the highest values among other species. In species x dose interaction, T x D1 (Triticale x 1000 ppm L<sup>-1</sup>) in terms of germination rate, radicle length, seedling vigor index, and seedling length; In terms of BD0 (barley x control) germination rate; WD2 (wheat x 2000 ppm L<sup>-1</sup>) was at the forefront in terms of germination rate, radicle length, seedling vigor index, and seedling dry weight.

As a result, while D2 (2000 ppm L<sup>-1</sup>) dose of seaweed organic material is generally recommended for wheat from cereal species, D1 (1000 ppm L<sup>-1</sup>) and D2 (2000 ppm L<sup>-1</sup>) doses in triticale, D0 (control) among other species, D1 (1000 ppm L<sup>-1</sup>) and D2 (2000 ppm L<sup>-1</sup>) doses are recommended as incentives in barley.

## Contribution Rate Statement Summary of Researchers

The experiment was planned by A.R. Kaya. The

experiment was planned by A.R. Kaya. All authors also carried out the statistical analysis. wrote the first draft of the paper. and had it accepted.

### Conflict of Interest

The authors declare that he/she has no conflict of interest.

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## Sustaining Soil Biological Activity: The Role of Extended Reduced and No-Tillage Techniques

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### ABSTRACT

Soil management techniques can have varying effects on various soil properties. This study investigated the impact of various tillage techniques on soil properties for 14 years. The experiment was conducted at the Çukurova University Research Station, located in a region with a dominant Mediterranean climate. The research aimed to assess the changes in soil organic matter (SOM) content, soil respiration (SR), dehydrogenase enzyme activity (DHA), and soil temperature (ST) under seven different long-term tillage practices. The results revealed significant increases ( $p \leq 0.05$ ) in SOM (17-115%), SR (19-37%), and DHA (63-142%), under conservation tillage compared to conventional tillage practices. Additionally, conventional tillage with stubble burned consistently had the lowest values across all measured properties. Seasons variations also significantly ( $p \leq 0.05$ ) affected the observed values. These findings suggest that conventional tillage practices have a negative effect on the analyzed biological activities, with stubble burning further exacerbating this impact. Further research exploring the long-term effects of different tillage practices under varying crop rotations and soil conditions can contribute to the sustainable development of agricultural production in the region.

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## Toprak Biyolojik Aktivitesinin Sürdürülmesi: Genişletilmiş Azaltılmış ve Sıfır Toprak İşlemenin Rolü

### ÖZET

Toprak yönetimi tekniklerinin çeşitli toprak özellikleri üzerinde farklı etkileri olabilir. Bu çalışmada 14 yıl boyunca çeşitli toprak işleme tekniklerinin toprak özelliklerine etkisi araştırılmıştır. Deney, Akdeniz ikliminin hâkim olduğu bir bölgede yer alan Çukurova Üniversitesi Araştırma İstasyonu'nda gerçekleştirilmiştir. Araştırma, uzun süreli yedi farklı toprak işleme uygulaması altında toprağın organik madde (SOM) içeriği, toprak solunumu (SR), dehidrogenaz enzim aktivitesi (DHA) ve toprak sıcaklığındaki (ST) değişiklikleri değerlendirmeyi amaçlamıştır. Sonuçlar, geleneksel toprak işleme uygulamalarıyla karşılaştırıldığında koruyucu toprak işleme altında SOM (%17-115), SR (%19-37) ve DHA'da (%63-142) önemli artışlar ( $p \leq 0.05$ ) göstermiştir. Ek olarak, anızları yakılmış geleneksel toprak işleme, ölçülen tüm parametrelerde en düşük değerleri göstermiştir. Mevsim değişimleri de incelenen parametreleri önemli ölçüde ( $p \leq 0.05$ ) etkilemiştir. Bu bulgular, geleneksel toprak işleme uygulamalarının incelenen biyolojik aktiviteler üzerinde olumsuz bir etkiye sahip olduğunu ve anız yakmanın bu etkiyi daha da artırdığını göstermektedir. Farklı toprak işleme tekniklerinin uzun vadeli etkilerini çeşitli bitki rotasyonları ve toprak koşulları altında araştırmak, bölgedeki tarımsal üretimin sürdürülebilir gelişimine katkıda bulunabilir.

### Toprak Bilimi

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi : 31.01.2024

Kabul Tarihi : 14.06.2024

### Anahtar Kelimeler

Toprak işleme

Akdeniz iklim koşulları

Dehidrogenaz enzim aktivitesi

Organik madde

Toprak solunumu

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## INTRODUCTION

Soil organic matter (SOM) influences soil fertility by altering the physical, chemical, and biological aspects of the soil through different pathways. Elevated SOM content reduces soil bulk density (Zhang & Peng, 2021), boosts cation exchange capacity (Yost & Hartemink, 2019), and increases microbial diversity (Tian et al., 2018). Soil organic matter acts as a source of nutrients for microorganisms and enhances soil structure (Gök & Coskan, 2002). Therefore, maintaining high SOM content is crucial, not only for soil health but also because SOM is the largest global carbon pool (Wani et al., 2022). Each practice, particularly tillage, is linked to global warming and climate change (Bilen et al., 2010).

Tillage is defined as the process of loosening, crumbling, and mixing soil thoroughly with a tool at a specific depth for purposes such as seedbed preparation and weed management (Bilim & Korucu, 2016; Celik et al., 2017), and incorporating plant residues into the soil (Zhang & Peng, 2021). Tillage significantly impacts soil physical, chemical, and biological properties. Sustaining these attributes is essential for sustainable agriculture and soil management practices. Soil health refers to the ability of a particular soil type to function as a living system within an ecosystem, supporting plant and animal life, maintaining air and water quality, and contributing to human well-being (Doran & Parkin, 1997; Alkorta et al., 2003). Reduced and no-tillage practices, known as conservation tillage, are recognized as some of the most beneficial approaches for soil health. In conventional tillage systems, plows cultivate the top 30 cm of soil thoroughly. In contrast, reduced tillage approaches leave at least 30% of the soil surface covered with crop residue to reduce water and wind erosion. No-tillage, on the other hand, involves direct seeding into existing crop residue without any prior plowing (Köller, 2003). Conservation tillage is a well-established land management technique that continues to be refined and studied for optimal soil benefits (Tang et al., 2020).

Annually two crops are harvested in the Çukurova Region, one of Turkey's most fertile plains, and consecutive tillage is used for each crop. The microbial populations in the surface soil are negatively affected by frequent tillage applications, which also continuously degrade the soil structure (Mirzavand et al., 2022). Tillage damages aggregates generated in the surface soil over time, and also microorganism habitats are destroyed (Celik et al., 2011). Consequently, the tillage has an impact on several nutrient cycles in the soil.

Existing studies demonstrated that changes in microbial activity might be positive, negative, or

neutral as a result of soil tillage applications. The direction and severity of these effects are also closely influenced by the intensity and duration of the tillage applications. For instance, tillage may have a positive or negative impact on the soil system in the short term, but due to the long-term structural degradation of the soil, this impact may be neutral or negative (Das et al., 2014).

Soil organic matter in the soil directly influences the physical, chemical, and biological characteristics resulting in improvement of soil productivity (Li et al., 2021). While aggregates are maintained in no-tillage, they are broken down and exposed to microbial decomposition in standard tillage techniques, which stimulates the mineralization of SOM (Chen et al., 2009; Wang et al., 2020). Aside from being the primary SOM input, stubble supports SOM by promoting aggregate formation and soil stabilization (Pu et al., 2019). Furthermore, the stubble covers the soil surface and reduces temperature rise by reflecting sunlight (Salem et al., 2015). Soil temperature may rise (Bogužas et al., 2018) or fall (Hou & Li, 2019) due to tillage techniques, which that temperature fluctuations influence the biological activity of soil (Muñoz-Romero et al., 2015).

Biological properties are sensitive indicators reflecting the impact of frequently used agricultural practices. They can warn of structural changes in the soil (Futa et al., 2021; Mirzavand et al., 2022). Therefore, many researchers recommended determining soil biological properties due to their rapid response and high sensitivity (Mikanová et al., 2009). Among soil biological parameters, soil respiration, or CO<sub>2</sub> formation indicates the presence of active microorganisms (Mijangos et al., 2006). Dehydrogenase enzyme activity (DHA) is another indicator of overall microbial performance (Gajda & Przewloka, 2012). While some researchers (Akbolat et al., 2009; Moraru & Rusu, 2012) reported increased microbial activity with higher tillage intensity, long-term studies generally indicate the opposite trend (Li et al., 2021; Wang et al., 2022). For example, Moraru & Rusu (2012) reported the lowest soil respiration in no-tillage applications after three years of different tillage practices, whereas Celik et al. (2011) found the lowest value in conventional tillage. In contrast, studies by Cooper et al. (2020) and Nath et al. (2021) observed higher soil respiration in no-tillage treatments after 5 and 7 years of tillage practices, respectively.

Land management practices can significantly influence soil quality over time (Van Eerd et al., 2014). To investigate this effect, we conducted a long-term (14-year) field experiment in a Mediterranean climate, examining how different tillage practices affect soil biological properties. Soil samples were collected and

analyzed for soil organic carbon (SOC) content, soil respiration, and DHA, which serves as a marker of microbial activity. In this study, we hypothesized that: 1) Reduced tillage enhances SOM and biological activity: Less intensity tillage over time allows for greater accumulation of SOM, a key energy source of microbes, This, in turn, is expected to support higher soil respiration and DHA activity. 2) Stubble burning detracts soil biology: Burning crop residue removes potential organic matter input and disrupts microbial communities, leading to lower biological activity, 3) Reduced and conservation tillage moderate soil temperature: Leaving plant residues on the surface regulates soil temperature by reflecting sunlight, potentially influencing biological properties. By analyzing these factors, this study aims to quantify the long-term impacts of tillage practices on soil biology in a Mediterranean region. The findings will contribute to the development of sustainable soil management strategies that promote healthy biological activity.

## MATERIALS and METHOD

### Research Site, Experimental Design, and Tillage Practices

The long-term experiment was conducted at the Research Station of Çukurova University in Adana, Turkey in 2006, with different tillage practices implemented. The is located at 37°00'54.0" N 35°21'27.0" E, with an elevation of 32 meters above sea level (Figure 1). With a Mediterranean climate, the experimental area experiences monthly temperatures

of 9.4 (the lowest), 19.1 (average) and 28.6 °C (the highest) Precipitation follows a similar pattern, with July receiving the least (9.8 mm) and December the most (127.3 mm for a total annual precipitation of 671 mm (AMS, 2021). According to the World Reference Base, the soil in the research area is categorized as Haplic Vertisol. The soil developed on the former Seyhan River terraces and contains 49% clay, 33% silt, and 18% sand (clay texture) (Group, 2014). According to the analyses performed on the soil sample (0-30 cm) at the beginning of the experiment, pH, EC, CaCO<sub>3</sub> and SOM were 7.82, 0.15 dS m<sup>-1</sup> 24.4%, and 1.51%, respectively (Celik et al., 2011).

The experiment was arranged in a randomized complete block design with three replicates to evaluate the long-term effects of seven tillage practices. These practices included: two conventional tillage treatments (CT-1 and CT-2), three reduced tillage treatments (RT-1, RT-2, and RT-3), and two no-till treatments (NT and newly added ST) (Table 1). Initially, the experiment consisted of 18 plots, each measuring 40 x 12 m (480 m<sup>2</sup>). Six soil tillage practices were applied from 2006 to 2015 (9 years). In 2015, half of the NT plots (240 m<sup>2</sup>) were tilled once with a plow to a depth of 30-33 cm, creating a new strategic tillage (ST) treatment while maintaining no-till characteristics on the other half. This increased the total number of plots to 21. A disc harrow was used throughout this process to break up the large clods and level the soil surface after tillage events (Table 1). A 4-meter space was left between plots to minimize treatment interference.

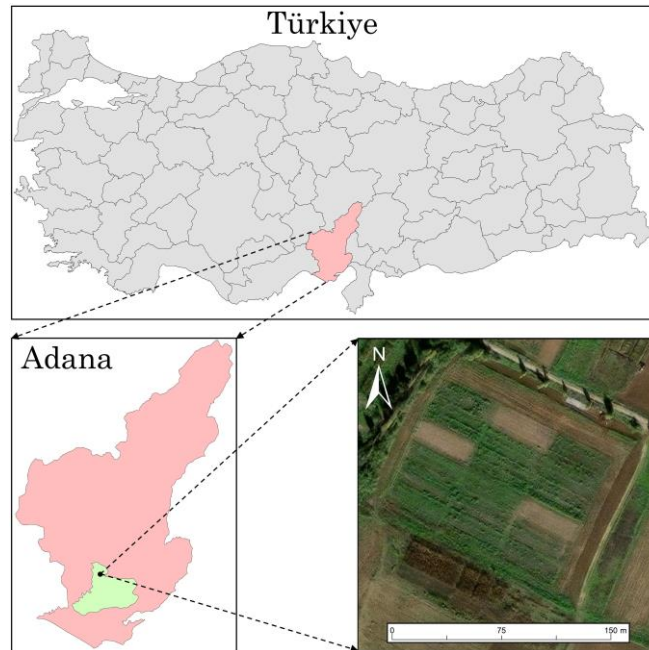


Figure 1. Location of the study area  
Şekil 1. Çalışma alanının konumu

Wheat (*Triticum aestivum* L.) - corn (*Zea mays* L.) or soybean (*Glycine max* L.) -alternatively- rotation was

followed throughout the 14-year long-term experiment. Plots with wheat (*Triticum aestivum* L.)

were examined for the objected parameters (Soil organic matter, soil respiration, dehydrogenase enzyme activity, soil temperature) in 2020. Glyphosate (500 g ha<sup>-1</sup>) was applied as a non-selective herbicide on RT-3, NT, and ST plots for weed control two weeks before sowing the wheat. Duration of the inspected year of the long-term experiment, the plots were

fertilized considering the results of soil analysis, with 260 kg ha<sup>-1</sup> di-ammonium phosphate (46.8 kg N ha<sup>-1</sup>; 119.6 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) before planting. At the tillering and bolting stages, 150 kg ha<sup>-1</sup> urea (69 kg N ha<sup>-1</sup>) and 180 kg ha<sup>-1</sup> calcium ammonium nitrate (46.8 kg N ha<sup>-1</sup>) were applied, respectively.

Table 1. Summary of tillage methods and equipment used in the long-term experiment

Çizelge 1. Denemede kullanılan toprak işleme yöntemleri ve ekipmanlar

| Tillage Methods   | Soil Tillage for Winter Wheat   | Soil Tillage for Second Crop Maize and Soybean   |
|---|---|--|
| Conventional tillage with stubbles (CT-1)               | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Moldboard plow</li> <li>▪ Disc harrow (2 passes)</li> <li>▪ Float (2 passes)</li> <li>▪ Drill</li> </ul> | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Heavy tandem disc harrow</li> <li>▪ Disc harrow (2 passes)</li> <li>▪ Float (2 passes)</li> <li>▪ Planter</li> </ul> |
| Conventional tillage with stubbles burned (CT-2)        | <ul style="list-style-type: none"> <li>▪ Stover burning of second crop</li> <li>▪ Moldboard plow</li> <li>▪ Disc harrow (2 passes)</li> <li>▪ Float (2 passes)</li> <li>▪ Drill</li> </ul>  | <ul style="list-style-type: none"> <li>▪ Stubble burning of wheat</li> <li>▪ Chisel plow</li> <li>▪ Disc harrow (2 passes)</li> <li>▪ Float (2 passes)</li> <li>▪ Planter</li> </ul>               |
| Heavy disc harrow reduced tillage (RT-1)                | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Heavy tandem disc harrow (2 passes)</li> <li>▪ Float (2 passes)</li> <li>▪ Drill</li> </ul>              | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Rotary tiller</li> <li>▪ Float (2 passes)</li> <li>▪ Planter</li> </ul>  |
| Reduced tillage with a rototiller (RT-2)                | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Rotary tiller</li> <li>▪ Float (2 passes)</li> <li>▪ Drill</li> </ul>                                    | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Rotary tiller</li> <li>▪ Float (2 passes)</li> <li>▪ Planter</li> </ul>  |
| Reduced tillage with a rototiller and no-tillage (RT-3) | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Heavy tandem disc harrow</li> <li>▪ Float (2 passes)</li> <li>▪ Drill</li> </ul>                         | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Herbicide treatment</li> <li>▪ No-till planter</li> </ul>  |
| No-till or zero tillage (NT)                            | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Herbicide treatment</li> <li>▪ No-till drill</li> </ul>  | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Herbicide treatment</li> <li>▪ No-till planter</li> </ul>  |
| Strategic tillage (ST)*                                 | <ul style="list-style-type: none"> <li>▪ Stover chopping of second crop</li> <li>▪ Herbicide treatment</li> <li>▪ No-till drill</li> </ul>  | <ul style="list-style-type: none"> <li>▪ Stubble chopping of wheat</li> <li>▪ Herbicide treatment</li> <li>▪ No-till planter</li> </ul>  |

\*This treatment continued as NT from 2006 until November 2015. Afterwards, it was tilled with moldboard plow only once in November 2015 and then, the same operations as in NT were implemented.

### Soil Sampling and Analysis

The basic physical and chemical properties of the soil were determined at a depth of 0 – 20 cm before the wheat sowing (November 02, 2020). Similarly, to monitor biological parameters throughout the wheat vegetation, samples were collected at depths from 0-20 cm. Soil samples were collected by using a soil auger at frequent intervals from the beginning of the experiment, the sampling interval was extended by time (11 to 35 d). The first sample was made just after wheat planting (November 19, 2020) and the last

sample was made just before wheat harvest (May 26, 2021). Due to the fertilizer application stimulating several features of the soil, after every single fertilization application, more frequent samplings were done. Each treatment including replicates was represented for one composite soil sample which was prepared by homogenized soil samples taken in 3 different representative sampling points of each plot. To prevent changes in the soil biological properties of the soil, samples were transferred to the laboratory within one hour. On each sampling day, CO<sub>2</sub> formation and DHA were determined. Carbon dioxide production



was determined according to Öhlinger (1996a) in which 100 g of fresh soil equivalent to dry soil was weighed and incubated for 24 h at 30 °C. Emitted CO<sub>2</sub> from the soil within 24 hours was collected by barium hydroxide, and soil respiration was determined by titrating the residual barium hydroxide with 0.05 M HCl. The DHA was evaluated at 10 g of dry soil equivalent to fresh soil according to Öhlinger (1996b) in which TTC (2,3,5-Triphenyltetrazolium chloride) was added to soil, incubated at 30 °C for 24 h. Emerged TPF (triphenyl formazan) was extracted by acetone and the TPF concentration of filtrate was measured by spectrophotometer at a wavelength of 546 nm. Soil temperatures were recorded on each sampling day by TP101 digital temperature sensor. The organic matter content of the soil was determined in the first sampling only (November 19, 2020) using chromate oxidation as described by (Kandeler, 1996).

### Statistical Analysis

Data gathered from 21 randomized plots was subjected to a one-way analysis of variance (ANOVA) using the IBM SPSS Statistics program. Duncan's test was also used to compare differences between the means.

Additionally, the Pearson correlation test was used to assess the correlations between the parameters. The Origin 2021 software was used to represent statistical findings.

## RESULTS and DISCUSSION

### The Effect of Long-Term Different Tillage Practices on Soil Organic Matter Content

The effects of various long-term tillage techniques on SOM content were statistically significant ( $p \leq 0.001$ ) after 14 years (Figure 2). The SOM content of the soils ranged from 1.57 to 3.38%, with NT application yielding the greatest value whereas the lowest value was obtained from CT-2 application. The lowest SOM content in conservation tillage practice was 17% higher than the highest value observed in conventional tillage treatment. Additionally, the highest SOM content was found in the conservation soil cultivation application (NT) as 115% more SOM than that of the CT-2 which is the conventional soil tillage application with the lowest SOM content. These findings show that 14 years of tillage methods boosted the soil's SOM contents by 17 to 115%.

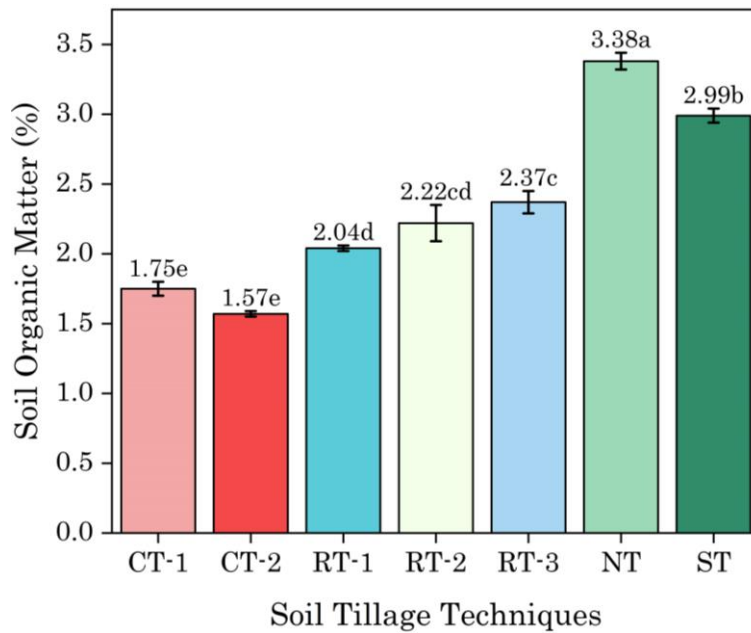


Figure 2. The effect of long-term different tillage practices on the soil organic matter content (%). Values are the average of samples from three plots. Means shown with the same letter are not statistically significant according to the Duncan test ( $p \leq 0.001$ ). CT-1: Conventional tillage with wheat stubble incorporated, CT-2: Conventional tillage with burned-off stubble, RT-1: Heavy disc harrow reduced tillage, RT-2: Reduced tillage with a rototiller, RT-3: Reduced tillage with a rototiller and no-tillage practice combination, NT: Direct seeding without tillage, ST: Strategic tillage.

Şekil 2. Uzun süreli farklı toprak işleme uygulamalarının toprağın organik madde içeriğine etkisi (%). Değerler üç parselden alınan örneklerin ortalamasıdır. Aynı harfle gösterilen ortalamalar Duncan testine göre istatistiksel olarak anlamlı değildir ( $p \leq 0.001$ ). CT-1: Anızlı geleneksel toprak işleme, CT-2: Anızı yakılmış geleneksel işleme, RT-1: Ağır diskli tırmıklı azaltılmış toprak işleme, RT-2: Rototillerli azaltılmış toprak işleme, RT-3: Ağır diskli tırmıklı azaltılmış ve sıfır toprak işleme kombinasyonu, NT: Doğrudan ekimli sıfır toprak işleme, ST: Stratejik sıfır toprak işleme.

Conventional tillage operations overturn the soil at depths ranging from 0 to 25 cm, producing soil

deterioration as well as stimulating organic carbon mineralization (Wang et al., 2020). While 170 to 1000

kg ha<sup>-1</sup> of organic carbon disappears every year in conventional tillage practices, no-tillage practices even result in SOM accumulation in the soil (Valkama et al., 2020). Similar to the results obtained in other studies, SOM increased as a result of reducing tillage intensity. While stubble management alone provided an 11% increase in SOM, without tillage practices increased this difference to 115%. Stubble contributes to the soil as a carbon source, and conservation tillage encourages their accumulation. Supporting this finding, Li et al. (2021) reported increased organic carbon contents in the soil because of conservative tillage and no-till practice, based on the existing 264 studies in the literature dealing with the effects of tillage practices on organic carbon. A distinction in conservative tillage techniques was also noted, and a 7.4% increase in organic carbon content was found in the no-till treatment compared to the reduced tillage application. This was attributed to less soil degradation and/or the addition of stubble resulting in the elevation of microbial biomass. According to Chen et al. (2009), no-tillage results in surface soil with 34% more SOM than conventional tillage. Several researchers reported that conventional soil cultivation is disturbing soil aggregates that are exposed to microbial decomposition leading to loss of SOM. Wang et al. (2020) reported that the organic carbon content in the surface soil increased significantly in both reduced and no-till applications compared to conventional tillage treatments. This difference resulted in more organic carbon accumulation with no-till application as well as the addition of plant residues. Similar results were also observed in the ST treatments, which were formed by dividing the NT plot and cultivated once, after 9 years after the beginning of the experiment in 2015 (Figure 2). Even single tillage on ST stimulated mineralization, therefore, a considerable decrease in the SOM of soil was observed compared to the NT. Similar to the existing literature, this study revealed that the management of stubble in the soil was found to alter the SOM content of the soil. The lowest SOM content was determined in CT-2, which is the only tillage method in the stubble is burned. Soil organic matter content was found to be 11% higher in CT-1 than in CT-2, which uses typical tillage techniques with the main variation being stubble management, although the difference was not statistically significant (Figure 2). The stubble, which is a potential source of organic materials, is removed from the soil by burning due to easy soil cultivation. However, Gök & Coskan (2002) stated that organic compounds with a high C/N ratio such as stubble can be considered as an important source of soil SOM content. Plant residues physically preserve SOM by providing aggregate formation and stabilization in the soil, in addition to being the primary source of SOM inputs (Pu et al., 2019). Furthermore, stubble promotes the establishment of a fluctuating carbon

pool (Chen et al., 2009). Similar findings have been frequently reported by researchers focusing on stubble residue incorporation and the organic carbon content of soil (Gök & Coskan, 2002; Wang et al., 2020). Pu et al. (2019) reported higher SOM accumulation in surface soil in case of stubble addition. Stubble is the primary source of SOM, hence organic carbon levels increase through convenient stubble management. On the other hand, soil cultivation seems to be more effective for SOM pools in which SOM content was increased in no-tillage application compared to conventional practices independent of stubble management. This phenomenon is possibly associated with less degradation of soil and suppression of mineralization. Increased mineralization is also reported due to the increased contact of SOM that is conservation in soil aggregates with microorganisms.

### **The Effect of Long-Term Different Tillage Practices on Soil Respiration**

The determined soil respiration values are presented in Table 2. The findings revealed that both different tillage techniques and sampling days had statistically significant ( $p=0.024$  and  $p\leq 0.001$ , respectively) effects on soil respiration. Considering the sampling day averages, the highest mean value was observed at ST as  $36.5 \mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$  whereas the lowest mean was obtained in CT-2 with  $26.8 \mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . Based on these findings, the conventional tillage application (CT-2), has a 36% lower soil respiration rate than the ST applications, representing lower soil biological activity as a function of conventional tillage systems. Sampling day averages showed great variability among the mean values, the highest CO<sub>2</sub> formation was achieved on May 26, 2021, as  $67.4 \mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$  whereas the lowest value was on November 19, 2020, with  $17.2 \mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . Based on these data, CO<sub>2</sub> formation on May 26, 2021, is %292 greater than that of the sampling day of November 19, 2020. The temporal fluctuation of CO<sub>2</sub> output across the wheat vegetation appears to be mostly influenced by soil temperature. Soil respiration increased consistently around 172% and 168% following the sample period ending on February 24, 2021, depending on the temperature rise. The relationship between soil respiration and soil temperature is similar considering the data recorded between the dates of February 24, 2021, to May 26, 2021 (Tables 2 and 4).

Soil respiration after fertilization increased during the tillering period, while the increase in respiration was not statistically significant ( $p=0.146$ ). The first sampling following fertilization during the bolting period showed an increase in soil respiration, but it is unclear whether this increase was due to fertilization or climate.

Table 2. The effect of long-term different tillage practices on soil respiration ( $\mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ )

Çizelge 2. Uzun süreli farklı toprak işleme uygulamalarının toprak solunumuna etkisi ( $\mu\text{g CO}_2\text{-C g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ )

| Soil Tillage Techniques <sup>1</sup> | Sampling Date   |                |                 |                 |                |                 |             |                |                |                 |                         |                |                 |  | Mean |
|--------------------------------------|---|----------------|-----------------|-----------------|----------------|-----------------|-------------|----------------|----------------|-----------------|-------------------------|----------------|-----------------|--|------|
|                                      | 19.11.2020 <sup>6</sup>   |                | 30.11.2020      |                 | 11.12.2020     |                 | 31.12.2020  |                | 13.01.2021     |                 | 26.01.2021 <sup>7</sup> |                | Mean            |  |      |
|                                      | p<0.001   |                | p<0.001         |                 | p<0.001        |                 | p<0.001     |                | p=0.029        |                 | p=0.007                 |                |                 |  |      |
| <b>CT-1</b>                          | 11.7 <sup>2</sup> ±0.8 <sup>3</sup> d <sup>4</sup> F <sup>5</sup> | 23.2 ±1.2 b DE | 17.1 ±0.3 c EF  | 30.7 ±0.0 c C   | 22.4 ±1.5 a DE | 17.8 ±1.5 c EF  | <b>CT-2</b> | 8.1 ±0.8 e F   | 23.8 ±0.9 b D  | 19.2 ±1.4 c E   | 24.8 ±0.6 d D           | 18.0 ±0.4 b E  | 24.6 ±1.1 ab D  |  |      |
| <b>RT-1</b>                          | 16.8 ±1.0 c F   | 30.7 ±0.5 a DE | 32.5 ±1.9 a CD  | 34.8 ±0.4 ab CD | 24.9 ±2.1 a E  | 23.8 ±2.3 b E   | <b>RT-2</b> | 16.9 ±0.4 c E  | 20.5 ±0.5 b E  | 23.6 ±0.8 b E   | 24.3 ±0.6 d E           | 24.4 ±1.6 a E  | 23.0 ±2.1 bc E  |  |      |
| <b>RT-3</b>                          | 24.5 ±0.2 a D   | 28.7 ±2.3 a CD | 24.0 ±0.7 b D   | 34.0 ±0.2 b C   | 25.5 ±1.3 a D  | 28.1 ±0.6 ab CD | <b>NT</b>   | 19.8 ±0.7 bc G | 31.8 ±1.0 a EF | 25.9 ±1.3 b E-G | 33.2 ±2.5 bc DE         | 24.1 ±1.5 a FG | 24.8 ±2.0 ab FG |  |      |
| <b>ST</b>                            | 22.4 ±1.8 ab F  | 28.3 ±2.5 a DE | 25.7 ±0.7 b D-F | 37.4 ±0.3 a C   | 24.1 ±0.5 a EF | 30.2 ±2.3 a D   | <b>Mean</b> | 17.2 F         | 26.7 E         | 24.0 E          | 31.3 D                  | 23.3 E         | 24.6 E          |  |      |

| Soil Tillage Techniques | Sampling Date   |                 |                |                |                         |                 |            |             |                 |                 |                | Mean           |                |                |          |
|-------------------------|-----------------|-----------------|----------------|----------------|-------------------------|-----------------|------------|-------------|-----------------|-----------------|----------------|----------------|----------------|----------------|----------|
|                         | 8.02.2021       |                 | 24.02.2021     |                | 31.03.2021 <sup>8</sup> |                 | 16.04.2021 |             | 03.05.2021      |                 | 26.05.2021     |                | Mean           |                |          |
|                         | p<0.001         |                 | p<0.001        |                | p=0.014                 |                 | p<0.001    |             | p=0.028         |                 | p<0.001        |                |                |                |          |
| <b>CT-1</b>             | 18.4 ±0.6 e E   | 22.9 ±2.2 c DE  | 41.6 ±3.9 ab B | 27.7 ±0.7 b CD | 47.2 ±1.4 bc AB         | 52.3 ±4.3 d A   | 27.7 bc    | <b>CT-2</b> | 27.9 ±1.9 bc CD | 17.9 ±0.8 d E   | 31.4 ±0.9 c C  | 27.5 ±1.3 b CD | 43.9 ±3.2 c B  | 54.9 ±1.1 cd A | 26.8 c   |
| <b>RT-1</b>             | 28.3 ±2.5 bc DE | 25.0 ±1.4 a-c E | 39.3 ±2.7 bc C | 28.1 ±2.2 b DE | 53.1 ±1.1 a-c B         | 67.4 ±5.1 b A   | 33.7 a-c   | <b>RT-2</b> | 21.4 ±0.7 de E  | 24.1 ±1.5 bc E  | 40.2 ±2.9 bc C | 32.5 ±1.2 b D  | 59.7 ±3.7 a B  | 86.3 ±5.2 a A  | 33.1 a-c |
| <b>RT-3</b>             | 34.4 ±0.8 a C   | 24.8 ±1.4 a-c D | 50.6 ±3.2 a B  | 30.1 ±1.8 b CD | 50.4 ±4.5 a-c B         | 82.1 ±1.7 a A   | 36.4 a     | <b>NT</b>   | 23.9 ±1.0 cd FG | 28.9 ±0.9 ab EF | 43.9 ±3.4 ab C | 39.7 ±2.4 a CD | 56.3 ±5.2 ab B | 65.4 ±4.0 bc A | 34.8 ab  |
| <b>ST</b>               | 29.2 ±1.7 b DE  | 29.6 ±1.6 a DE  | 43.1 ±2.1 ab B | 44.8 ±3.7 a B  | 59.6 ±1.4 a A           | 63.3 ±0.5 b-d A | 36.5 a     | <b>Mean</b> | 26.2 E          | 24.8 E          | 41.5 C         | 32.9 D         | 52.9 B         | 67.4 A         |          |

Means shown with the same letter are not statistically significant according to Duncan's test. The ANOVA value is  $p \leq 0.001$  for sampling dates and is shown in the table for comparisons between treatments.

<sup>1</sup>: CT-1: Conventional tillage with wheat stubble incorporated, CT-2: Conventional tillage with burned-off stubble, RT-1: Heavy disc harrow reduced tillage, RT-2: Reduced tillage with a rototiller, RT-3: Reduced tillage with a rototiller and no-tillage practice combination, NT: Direct seeding without tillage, ST: Strategic tillage. <sup>2</sup>: It is the average of the samples in three plots. <sup>3</sup>: It is the standard error of the means. <sup>4</sup>: Lowercase letters give statistical comparisons among treatments. <sup>5</sup>: Uppercase letters give statistical comparison among sampling dates. <sup>6</sup>: It is the sampling performed after pre-planting fertilization. <sup>7</sup>: It is the first sampling performed after fertilization during the tillering period. <sup>8</sup>: It is the first sampling performed after fertilization during the bolting period.

No-tillage and reduced tillage strategies improve the soil's organic matter content by undergoing less physical degradation of SOM than typical tillage techniques (Das et al., 2019) by encouraging biological activity. Previously, Çelik et al. (2021) have taken soil samples from the same long-term experiment, and they reported higher organic carbon content in the surface soil than in conventional tillage on the same soil layer. Similarly, Li et al. (2021) reported that the conservation tillage practices increase SOM content and lead promotion to the of microbial activity with this enhancement (Mirzavand et al., 2022). The findings of the SOM analysis conducted in this study area during the sampling date of November 19, 2020, revealed a pattern that was similar to the findings of the study with soil respiration indicated above. Figure 3 shows the correlation between soil respiration and SOM, which was found to be a positive correlation ( $r=0.182$ ;  $p<0.01$ ). According to the results acquired, during the sample dated November 19, 2020, both soil respiration and SOM content were determined as the lowest in CT-2 among tillage methods, followed by CT-1, RT-1, and RT-2. Although the highest CO<sub>2</sub> production was measured in RT-3 and ST-applied plots, the highest SOM content was obtained in ST applications. While RT-3 and ST applications produced the highest CO<sub>2</sub> formation, NT applications provided the highest SOM content. This could be attributed to tillage applied soon before the sampling date on November 19, 2020. In the RT-3 application, however, unlike the NT and ST applications, processing with both a heavy disc harrow and a tiller may have resulted in an increase in CO<sub>2</sub> emission from the soil. When soil respiration during the sampling time is considered, the highest CO<sub>2</sub> production was recorded in RT-3, and the lowest CO<sub>2</sub> production was observed in CT-2 application. When CO<sub>2</sub> generation was compared between these two tillage techniques, RT-3 produced nearly three times more soil respiration than CT-2. This is the date with the largest inter-application variation among all sampling dates. This can be explained by the fact that the aggregates that degrade after soil tillage applications (Celik et al., 2011) boost CO<sub>2</sub> emissions (Fiedler et al., 2016). Therefore, a lowering tendency on CO<sub>2</sub> production is expected after the tillage practice. Table 2 shows that the CO<sub>2</sub> output differs, which grew between soil tillage applications as the aggregates broke down, reduced throughout the sampling date of November 30, 2020, which was conducted 11 days after the initial sampling. Similar results were reported by Buragienė et al. (2019) and Zhang et al. (2021). All tillage methods were found to be significantly greater than other sampling intervals on May 26, 2021, when soil respiration was at its peak. The increased soil temperature is responsible for the highest values seen in tillage practices during this date (Table 4 and Figure

3). In addition, the increase in plant root growth from wheat planting to harvest, as well as the associated root exudate, may have enhanced CO<sub>2</sub> flow by promoting microbial activity. Consistent with these findings, a study by Bilen et al. (2010), the highest soil respiration was observed during the harvest period and stated that this was due to increases in root growth and microbial activity.

Furthermore, the dry climate during the sampling time of May 26, 2021, the lack of irrigation in the wheat growth, and the low seasonal soil moisture content may have reduced the rise in soil respiration during this date. Another study found that as temperatures decreased, SOM in the soil remained steady, resulting in little CO<sub>2</sub> emission, which was consistent with the findings in this study (Moreno et al., 2021). Under ideal temperature and humidity conditions, according to Gök et al. (1999), microbial activity increased in soil. The increase in soil temperature causes an increase in microbial activity and promotes the decomposition of SOM, which enhances soil heterotrophic respiration (Zhang et al., 2021). Furthermore, fertilizer had no obvious influence on soil respiration.

Soil respiration was strongly influenced ( $p=0.024$ ) by the management of stubble which is another soil management practice, studied. When the average of the sampling dates is taken into consideration, CT-2, which is the only application of the tillage methods in which the stubble is burned, has the lowest CO<sub>2</sub> output. The amount of soil respiration was decreased due to the microbial community in the surface soil being adversely impacted by rising temperature. Destruction of the stubble which is the main carbon source was also responsible for lower CO<sub>2</sub> formation. Regarding the issue, Shakoore et al. (2021) stated that stubble supports heterotrophic respiration by providing ready-to-use C and N substrates for the microbial population. By leaving the stubble in the soil and reducing tillage density, Mirzavand et al. (2022) found that SOM increased, enhancing the diversity of microbial biomass and promoting CO<sub>2</sub> formation. When the average of the sample dates of the other six tillage techniques, in which the stubble is incorporated into the soil or left on the surface, is examined, there were no significant differences in soil respiration (Table 2). The temporal fluctuation of CO<sub>2</sub> production across the wheat vegetation appears to be mostly influenced by soil temperature. Particularly, based on the sampling dates soil respiration followed a proportionate path with temperature after the samples were taken on February 24, 2021. The association between soil respiration and soil temperature was comparable when taking into account the average of tillage techniques in the sample dated February 24, 2021, and May 26, 2021, and these values rose by 172% and 169%, respectively. Similar findings are obtained when research on the issue is evaluated (Du et al.,



2021; Zhang et al., 2021). Additionally, a positive correlation ( $r=0.758$ ) between soil respiration and soil temperature was determined (Figure 3). Furthermore, fertilizer had no obvious influence on soil respiration.

Although no-tillage techniques decrease CO<sub>2</sub> output in the short term, long-term effects may vary. While short-term studies show low CO<sub>2</sub> formation owing to the preservation of SOM from tillage methods (Akbolat et al., 2009), long-term studies show an increase in the accumulated SOM pool and microbial population, which promotes increased soil respiration (Mirzavand et al., 2022).

### The Effect of Long-Term Different Tillage Practices on Dehydrogenase Enzyme Activity

Determined DHA values are presented in Table 3. Similar to CO<sub>2</sub> production results (Table 2), DHA was influenced by both soil tillage practice and sampling interval significantly ( $p \leq 0.001$  and  $p \leq 0.001$ , respectively). Based on the mean values, the highest value was determined in NT treatment as 84.8  $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . The CT-2 application yielded the lowest mean with 35.1  $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . According to these findings, DHA activity in NT was 142% higher than in CT-2 treatment. As mentioned earlier, sampling day was also significant on DHA activity in which the highest value was achieved on February 8, 2021, with 113.4  $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . The lowest mean value was observed on November 30, 2020, as 28.4  $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ . The observed DHA activity on February 8, 2021, was 299% higher than the value obtained on November 30, 2020. This difference was probably due to temperature changes.

The DHA levels significantly increased in samples collected after fertilization during tillering, followed by a significant decrease in samples from the bolting period. However, the conflicting results cast doubt on fertilization being the direct cause. The conventional tillage application CT-2 had the lowest average DHA value when the average of the sample intervals across the wheat plant vegetation was analyzed. This value was followed by CT-1, and the difference between them was found to be statistically insignificant. Both no-tillage and reduced tillage practices demonstrated higher DHA than conventional tillage practices, and there were no statistically significant differences between no-tillage and reduced tillage treatments according to the results of these various tillage practices ongoing for 14 years. In research carried out in the same long-term experiment where several tillage techniques were used over three years, similar results were attained by Celik et al. (2011). In this study, the lowest DHA was found in the conventional tillage methods CT-2 and CT-1, whereas the highest DHA was found in NT and RT-3, which are no-tillage and reduced tillage techniques. It was shown that

there was a statistically significant difference between conventional tillage techniques ( $p < 0.05$ ). Furthermore, Gajda & Przewłoka (2012) reported that direct sowing and reduced tillage treatments produce 18-28% and 13-17% more DHA activity, respectively, than conventional tillage due to lowering the usage of plows. On the other hand, Moreno et al. (2021) reported that biological activity increased by creating favorable temperature and humidity conditions in no-tillage applications compared to traditional tillage.

Soil organic matter content is enhanced by reduced tillage practices and stubble incorporation (Gök & Coskan, 2002; Wang et al., 2020); therefore, suitable habitat for microbial activity appears (Kumar et al., 2021) leading to higher DHA activity. Furthermore, because the majority of microorganisms in the soil are chemoorganotrophic, using organic carbon as an energy and substrate source promotes enzyme activity in the soil (Nugis et al., 2016). The correlation analysis performed in this study revealed a positive correlation ( $r=0.428$ ) between DHA and SOM (Figure 3). Existing literature points out an increase in DHA activity with a decrease in tillage density (Nath et al., 2021; Saurabh et al., 2021). According to Mikanová et al. (2009), the use of conservation tillage causes SOM to build on the soil surface, and the accumulated SOM serves as a source of energy and a substrate for the soil biota, increasing the activity of enzymes.

Among the soil cultivation techniques, CT-2, which involves burning the stubble, was found to have the lowest statistical group average throughout several sample dates, making it the soil cultivation technique with the lowest date average. The two conventional tillage techniques, CT-1 and CT-2, with their sole distinction being how stubble is managed, had the lowest results. Within CT-1 and CT-2, CT-1 has 15% more DHA than CT-2, which is still a significant difference. The primary reason for this is that stubble, which is a rich source of carbon and energy for soil microflora, has a favorable effect on soil microbial activity (Mrunalini et al., 2021). According to Das et al. (2019), incorporation of the stubble into the soil enhanced the physical characteristics of the soil increased the SOM content, and hence encouraged microbial activity.

DHA rose over the first six sample dates, peaked on February 8, 2021, and then fluctuated throughout the rest of the experiment. During this time, fertilization did not affect DHA levels. All tillage techniques appear to follow a similar trend (Table 3). The lowest DHA was measured on November 30, 2020. The low DHA at this date is probably related to both the low soil temperature (Table 4) and the damage to the microbiota due to the recent tillage. Wolinska & Stepniewska (2011) stated that DHA shows strong fluctuations depending on the seasons.

Table 3. The effect of long-term different tillage practices on dehydrogenase enzyme activity ( $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ )  
 Çizelge 3. Uzun süreli farklı toprak işleme uygulamalarının dehidrogenaz enzim aktivitesi üzerine etkisi ( $\mu\text{g TPF g}_{\text{dry soil}}^{-1} 24\text{h}^{-1}$ )

| Soil Tillage Techniques <sup>1</sup> | Sampling Date                       |                               |            |      |            |       |            |       |            |      |                         |       |         |  | Mean |
|--------------------------------------|-------------------------------------|-------------------------------|------------|------|------------|-------|------------|-------|------------|------|-------------------------|-------|---------|--|------|
|                                      | 19.11.2020 <sup>6</sup>             |                               | 30.11.2020 |      | 11.12.2020 |       | 31.12.2020 |       | 13.01.2021 |      | 26.01.2021 <sup>7</sup> |       | p≤0.001 |  |      |
|                                      | p≤0.001                             |                               | p≤0.001    |      | p≤0.001    |       | p≤0.001    |       | p≤0.001    |      | p≤0.001                 |       |         |  |      |
| <b>CT-1</b>                          | 21.0 <sup>2</sup> ±0.3 <sup>3</sup> | d <sup>4</sup> E <sup>5</sup> | 15.8 ±1.2  | c EF | 33.3 ±1.4  | c D   | 35.2 ±2.8  | d D   | 36.8 ±0.9  | d D  | 50.2 ±0.7               | d BC  |         |  |      |
| <b>CT-2</b>                          | 17.7 ±0.5                           | e G                           | 18.6 ±1.7  | bc G | 20.4 ±0.7  | d FG  | 23.8 ±0.2  | e E-G | 29.7 ±2.1  | e E  | 39.7 ±0.4               | d CD  |         |  |      |
| <b>RT-1</b>                          | 30.4 ±1.2                           | c F                           | 36.2 ±2.3  | a EF | 34.3 ±2.0  | c F   | 47.6 ±0.7  | c DE  | 54.7 ±1.7  | c D  | 105.1 ±3.4              | b B   |         |  |      |
| <b>RT-2</b>                          | 34.7 ±0.7                           | b FG                          | 22.7 ±1.0  | b G  | 33.4 ±1.8  | c FG  | 71.0 ±3.0  | ab DE | 61.2 ±1.6  | b E  | 133.3 ±7.0              | a A   |         |  |      |
| <b>RT-3</b>                          | 32.5 ±0.9                           | bc F                          | 36.4 ±0.3  | a F  | 39.2 ±2.5  | bc F  | 76.7 ±3.7  | a D   | 60.7 ±0.5  | b E  | 92.5 ±3.5               | bc BC |         |  |      |
| <b>NT</b>                            | 44.5 ±0.1                           | a GH                          | 37.6 ±3.0  | a H  | 47.5 ±1.4  | a F-H | 71.9 ±1.8  | ab E  | 63.4 ±0.8  | b EF | 106.8 ±11.9             | b CD  |         |  |      |
| <b>ST</b>                            | 43.5 ±1.9                           | a H                           | 31.9 ±2.6  | a I  | 41.9 ±3.1  | ab H  | 67.3 ±1.4  | b FG  | 74.5 ±1.0  | a EF | 86.3 ±0.6               | c CD  |         |  |      |
| <b>Mean</b>                          | 32.0                                | E                             | 28.4       | E    | 35.7       | E     | 56.2       | D     | 54.4       | D    | 87.7                    | B     |         |  |      |

| Soil Tillage Techniques | Sampling Date |       |            |       |                         |       |            |      |            |       |            | Mean  |         |  |
|-------------------------|---------------|-------|------------|-------|-------------------------|-------|------------|------|------------|-------|------------|-------|---------|--|
|                         | 8.02.2021     |       | 24.02.2021 |       | 31.03.2021 <sup>8</sup> |       | 16.04.2021 |      | 03.05.2021 |       | 26.05.2021 |       | p≤0.001 |  |
|                         | p≤0.001       |       | p≤0.001    |       | p≤0.001                 |       | p≤0.001    |      | p≤0.001    |       | p≤0.001    |       |         |  |
| <b>CT-1</b>             | 58.7 ±5.0     | d A   | 48.6 ±3.2  | d C   | 17.3 ±0.7               | d E   | 60.4 ±3.6  | c A  | 51.4 ±1.1  | c BC  | 56.5 ±2.1  | c AB  | 40.4 c  |  |
| <b>CT-2</b>             | 68.0 ±4.7     | d A   | 39.7 ±4.4  | d CD  | 27.9 ±1.2               | c EF  | 49.5 ±4.8  | c B  | 47.5 ±4.0  | c BC  | 38.4 ±1.2  | d D   | 35.1 c  |  |
| <b>RT-1</b>             | 122.9 ±8.0    | bc A  | 79.6 ±8.1  | c C   | 33.2 ±2.1               | c F   | 89.1 ±3.6  | b C  | 76.4 ±2.7  | b C   | 80.6 ±5.6  | ab C  | 65.8 b  |  |
| <b>RT-2</b>             | 142.3 ±9.1    | ab A  | 90.7 ±3.7  | bc BC | 42.4 ±1.1               | b F   | 104.0 ±7.8 | ab B | 79.7 ±4.0  | ab CD | 94.9 ±4.0  | a B   | 75.8 ab |  |
| <b>RT-3</b>             | 136.3 ±8.6    | a-c A | 80.7 ±1.8  | c CD  | 56.2 ±4.2               | a E   | 101.1 ±7.9 | ab B | 83.7 ±2.8  | ab CD | 88.6 ±8.5  | a B-D | 73.7 ab |  |
| <b>NT</b>               | 151.5 ±4.7    | a A   | 133.7 ±8.8 | a B   | 60.0 ±4.8               | a E-G | 118.7 ±8.7 | a BC | 90.0 ±4.1  | a D   | 92.1 ±4.2  | a D   | 84.8 a  |  |
| <b>ST</b>               | 114.2 ±6.9    | c A   | 101.8 ±1.7 | b B   | 58.4 ±3.7               | a G   | 91.8 ±2.7  | b C  | 80.0 ±3.4  | ab DE | 71.5 ±2.8  | b EF  | 71.9 ab |  |
| <b>Mean</b>             | 113.4         | A     | 82.1       | BC    | 42.2                    | DE    | 87.8       | B    | 72.7       | C     | 74.6       | BC    |         |  |

Means shown with the same letter are not statistically significant according to Duncan's test. The ANOVA value is p≤0.001 for sampling dates and is shown in the table for comparisons between treatments.

<sup>1</sup>: CT-1: Conventional tillage with wheat stubble incorporated, CT-2: Conventional tillage with burned-off stubble, RT-1: Heavy disc harrow reduced tillage, RT-2: Reduced tillage with a rototiller, RT-3: Reduced tillage with a rototiller and no-tillage practice combination, NT: Direct seeding without tillage, ST: Strategic tillage. <sup>2</sup>: It is the average of the samples in three plots. <sup>3</sup>: It is the standard error of the means. <sup>4</sup>: Lowercase letters give statistical comparisons among treatments. <sup>5</sup>: Uppercase letters give statistical comparison among sampling dates. <sup>6</sup>: It is the sampling performed after pre-planting fertilization. <sup>7</sup>: It is the first sampling performed after fertilization during the tillering period. <sup>8</sup>: It is the first sampling performed after fertilization during the bolting period.

In a study by Piotrowska & Długosz (2012), higher DHA was detected in April compared to August, and this situation increased with the growth of the wheat plant in April, polysaccharides, organic acids, etc. associated with the increase such as substrates. In addition, in the same study, it was stated that the highest DHA was observed in spring and the lowest in winter months in humid and slightly arid areas.

The maximum mean DHA throughout the wheat vegetation was obtained on February 8, 2021. When the DHA values among the applications were compared, the no-tillage application had the highest value, followed by the RT-2, RT-3, and ST applications. There was no statistically significant difference between these treatments. The lowest values were obtained from CT-1 and CT-2, which are conventional tillage methods.

### **The Effect of Long-Term Different Tillage Practices on Soil Temperature**

Recorded soil temperature data are presented in Table 4. The soil temperature ranged from 7.9 to 25.1 °C, and the sampling days had a statistically significant ( $p \leq 0.001$ ) effect on soil temperature. While the RT-3 application received the greatest value of 25.1 °C on the last sampling day before the harvest, the lowest value recorded from the CT-2 application was 7.9 °C on January 26, 2021. Based on the mean values, the temperature difference between the lowest and the highest value was 0.7 °C the lowest value was 13.5 °C in CT-2, and the highest value was 14.2 in RT-3. Although there was a 5% difference between the two practices those were not found to be statistically significant. Considering the averages of soil tillage practices on the sampling days, the highest average was 24.4 °C on the last sampling date on May 26, 2021; the lowest average was obtained on the sampling day on January 26, 2021, with 8.1 °C. These significant ( $p \leq 0.001$ ) differences showed that the soil temperature on May 26, 2021 was 201%, higher than the sampling day on January 26, 2021. An increase in soil temperature was associated with elevated air temperature due to seasonal changes.

Soil temperature appeared to fluctuate in response to air temperature changes across different sampling times (data not included). However, these fluctuations were independent of tillage techniques employed, as no statistical differences were observed. This finding aligns with some previous research. For instance, Hou & Li (2019) reported no statistically significant impact of tillage practices on soil temperature. Their study also noted a trend of higher soil temperatures in no-tillage treatments compared to conventional tillage, no statistical difference was determined by Bogužas et al. (2018) between tillage practices in two of the six different measurement dates. In one measurement date, higher soil temperature was obtained in

conventional tillage application compared to no-tillage. It has been stated that the reason for this situation is due to the faster drying and warming of the disturbed soil. According to Muñoz-Romero et al. (2015), both conventional and no-tillage treatments resulted in a comparable pattern of soil temperature. However, the findings revealed that at all measurement dates, conventional tillage operations had higher soil temperatures than no-tillage approaches, while the difference was not statistically significant. The soil absorbs more heat and has lesser thermal conductivity under traditional tillage than it does with no tillage, according to the study. Salem et al. (2015) in a related study, conventional tillage produced the greatest soil temperature. Researchers have shown that no-tillage methods result in increased soil moisture contents, and wet soils get warmer or cooler more slowly. Additionally, it was claimed that when no-tillage is used, more residues are left behind in the soil, and these residues reflect sunlight to prevent soil temperatures from rising.

### **Correlations Between Soil Properties**

Pearson correlation test revealed that there was a statistically significant ( $p \leq 0.01$ ) positive correlation between SOM content and soil respiration ( $r = 0.182$ ) as well as SOM content and DHA ( $r = 0.428$ ); however, no correlation was observed between SOM and soil temperature (Figure 3). A positive correlation was determined between soil respiration and both DHA ( $r = 0.240$ ) and soil temperature ( $p \leq 0.01$ ). There was no statistically significant correlation between DHA and soil temperature.

### **CONCLUSION**

Tillage techniques had impacts on the examined parameters in all sampling days, although the first sample date following the tillage practices showed the greatest variations. The lowest soil respiration and dehydrogenase enzyme activity were determined because of stubble burning. In the initial measurement dates, increasing tillage methods had a considerable impact on the biological characteristics of the soil, but this influence subsided in the last stages of wheat growth.

On the other hand, dehydrogenase enzyme activity and soil respiration decreased because of the intensifying soil tillage. Moreover, burning stubble had a detrimental effect on soil respiration and DHA activity as an essential biological indicator of the soil. Observed parameters revealed that the largest variability between sampling dates was due to air/soil temperature. Along with the rise in soil temperature, increases in microbial activity were observed.

Table 4. The effect of long-term different tillage practices on soil temperature (°C)  
 Çizelge 4. Uzun süreli farklı toprak işleme uygulamalarının toprak sıcaklığına etkisi (°C)

| Soil Tillage Techniques <sup>1</sup> | Sampling Date     |                   |                |                |            |      |   |   |            |      |   |     |            |      |   |   |            |      |   |    |            |      |   |   |
|--------------------------------------|-------------------|-------------------|----------------|----------------|------------|------|---|---|------------|------|---|-----|------------|------|---|---|------------|------|---|----|------------|------|---|---|
|                                      | 19.11.2020        |                   |                |                | 30.11.2020 |      |   |   | 11.12.2020 |      |   |     | 31.12.2020 |      |   |   | 13.01.2021 |      |   |    | 26.01.2021 |      |   |   |
|                                      | p=0.316           |                   |                |                | p=0.467    |      |   |   | p=0.763    |      |   |     | p=0.833    |      |   |   | p=0.843    |      |   |    | p=0.616    |      |   |   |
| <b>CT-1</b>                          | 15.0 <sup>2</sup> | ±0.2 <sup>3</sup> | a <sup>4</sup> | C <sup>5</sup> | 11.0       | ±0.4 | a | G | 12.6       | ±0.3 | a | F   | 9.1        | ±0.7 | a | H | 12.3       | ±0.2 | a | F  | 8.0        | ±0.1 | a | I |
| <b>CT-2</b>                          | 14.9              | ±0.2              | a              | C              | 10.9       | ±0.3 | a | F | 12.4       | ±0.2 | a | E   | 9.1        | ±0.4 | a | G | 12.4       | ±0.1 | a | E  | 7.9        | ±0.0 | a | H |
| <b>RT-1</b>                          | 14.5              | ±0.1              | a              | C              | 10.7       | ±0.4 | a | E | 12.6       | ±0.2 | a | D   | 9.0        | ±0.5 | a | F | 12.3       | ±0.1 | a | D  | 8.1        | ±0.1 | a | G |
| <b>RT-2</b>                          | 16.0              | ±0.8              | a              | C              | 11.3       | ±0.2 | a | G | 12.8       | ±0.2 | a | EF  | 9.7        | ±0.4 | a | H | 12.4       | ±0.1 | a | GF | 8.1        | ±0.0 | a | I |
| <b>RT-3</b>                          | 16.3              | ±0.7              | a              | C              | 11.4       | ±0.4 | a | G | 12.9       | ±0.1 | a | F   | 9.8        | ±0.4 | a | H | 12.4       | ±0.0 | a | F  | 8.2        | ±0.1 | a | I |
| <b>NT</b>                            | 15.9              | ±0.8              | a              | C              | 11.6       | ±0.4 | a | G | 12.8       | ±0.2 | a | EF  | 9.7        | ±0.4 | a | H | 12.4       | ±0.0 | a | FG | 8.2        | ±0.0 | a | I |
| <b>ST</b>                            | 15.9              | ±0.8              | a              | C              | 11.6       | ±0.4 | a | G | 12.7       | ±0.3 | a | E-G | 9.6        | ±0.7 | a | H | 12.3       | ±0.1 | a | FG | 8.1        | ±0.1 | a | I |
| <b>Mean</b>                          | 15.5 C            |                   |                |                | 11.2 H     |      |   |   | 12.7 FG    |      |   |     | 9.4 I      |      |   |   | 12.4 G     |      |   |    | 8.1 J      |      |   |   |

| Soil Tillage Techniques | Sampling Date |      |    |    |            |      |    |    |            |      |   |    |            |      | Mean |    |            |      |   |   |            |      |    |   |         |   |  |  |
|-------------------------|---------------|------|----|----|------------|------|----|----|------------|------|---|----|------------|------|------|----|------------|------|---|---|------------|------|----|---|---------|---|--|--|
|                         | 08.02.2021    |      |    |    | 24.02.2021 |      |    |    | 31.03.2021 |      |   |    | 16.04.2021 |      |      |    | 03.05.2021 |      |   |   | 26.05.2021 |      |    |   |         |   |  |  |
|                         | p=0.032       |      |    |    | p=0.144    |      |    |    | p=0.456    |      |   |    | p=0.532    |      |      |    | p=0.954    |      |   |   | p=0.126    |      |    |   | p=0.997 |   |  |  |
| <b>CT-1</b>             | 12.8          | ±0.1 | b  | EF | 9.0        | ±0.2 | ab | H  | 14.3       | ±0.5 | a | CD | 13.8       | ±0.2 | a    | DE | 21.8       | ±0.1 | a | B | 24.3       | ±0.3 | ab | A | 13.7    | a |  |  |
| <b>CT-2</b>             | 12.8          | ±0.1 | b  | E  | 8.7        | ±0.1 | b  | GH | 13.7       | ±0.4 | a | D  | 13.7       | ±0.3 | a    | D  | 22.0       | ±0.4 | a | B | 23.7       | ±0.0 | b  | A | 13.5    | a |  |  |
| <b>RT-1</b>             | 13.0          | ±0.2 | b  | D  | 9.0        | ±0.2 | ab | F  | 13.9       | ±0.2 | a | C  | 14.1       | ±0.3 | a    | C  | 22.1       | ±0.3 | a | B | 24.1       | ±0.3 | ab | A | 13.6    | a |  |  |
| <b>RT-2</b>             | 13.1          | ±0.1 | ab | EF | 9.2        | ±0.2 | a  | HI | 14.9       | ±0.9 | a | CD | 14.1       | ±0.4 | a    | DE | 22.0       | ±0.4 | a | B | 25.0       | ±0.7 | a  | A | 14.1    | a |  |  |
| <b>RT-3</b>             | 13.3          | ±0.0 | a  | EF | 9.3        | ±0.1 | a  | H  | 15.1       | ±0.5 | a | D  | 14.2       | ±0.1 | a    | DE | 22.3       | ±0.4 | a | B | 25.1       | ±0.3 | a  | A | 14.2    | a |  |  |
| <b>NT</b>               | 13.0          | ±0.1 | b  | EF | 9.2        | ±0.1 | a  | H  | 14.6       | ±0.5 | a | D  | 13.8       | ±0.1 | a    | DE | 21.9       | ±0.4 | a | B | 24.5       | ±0.1 | ab | A | 14.0    | a |  |  |
| <b>ST</b>               | 13.0          | ±0.1 | b  | EF | 9.1        | ±0.1 | ab | HI | 14.4       | ±0.3 | a | D  | 13.7       | ±0.1 | a    | DE | 22.1       | ±0.2 | a | B | 24.3       | ±0.3 | ab | A | 13.9    | a |  |  |
| <b>Mean</b>             | 13.0 F        |      |    |    | 9.1 I      |      |    |    | 14.4 D     |      |   |    | 13.9 E     |      |      |    | 22.0 B     |      |   |   | 24.4 A     |      |    |   |         |   |  |  |

Means shown with the same letter are not statistically significant according to Duncan's test. The ANOVA value is  $p \leq 0.001$  for sampling dates and shown in the table for comparisons between treatments.

<sup>1</sup>: CT-1: Conventional tillage with wheat stubble incorporated, CT-2: Conventional tillage with burned-off stubble, RT-1: Heavy disc harrow reduced tillage, RT-2: Reduced tillage with a rototiller, RT-3: Reduced tillage with a rototiller and no-tillage practice combination, NT: Direct seeding without tillage, ST: Strategic tillage. <sup>2</sup>: It is the average of the samples in three plots. <sup>3</sup>: It is the standard error of the means. <sup>4</sup>: Lowercase letters give statistical comparison among treatments. <sup>5</sup>: Uppercase letters give statistical comparison among sampling dates.



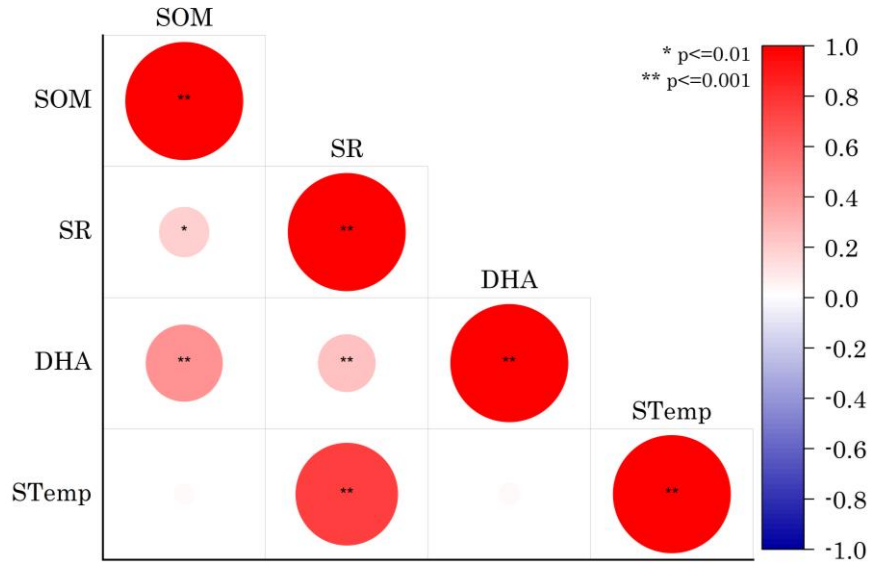


Figure 3. Pearson correlation test between soil organic matter (SOM), soil respiration (SR), dehydrogenase enzyme activity (DHA), and soil temperature (STemp)

Şekil 3. Toprak organik maddesi (SOM), toprak solunumu (SR), dehidrogenaz enzim aktivitesi (DHA) ve toprak sıcaklığı (STemp) arasındaki Pearson korelasyon testi

Consequently, conservation tillage practices are the most ideal tillage practices in terms of the biological qualities of the soil when all the above findings are assessed together. Therefore, further research is required in which physical, chemical, and biological properties are evaluated together to find out the impact of long-term different soil tillage practices in terms of sustainable and ecological agriculture. The sustainability of agricultural production in the area will also be improved by deep looking into the long-term consequences of various tillage systems under various plant rotations as well as in various climate and soil conditions.

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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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## Ege Bölgesi Zeytin Üretiminde Etkinliğin ve Belirleyicilerinin Tespiti: Bootstrap VZA Yaklaşımı

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### ÖZET

Bu çalışmanın amacı Ege Bölgesi'nde zeytin yetiştiren işletmecilerin etkinliklerinin ölçülmesi ve bu etkinliğe etki eden belirleyicilerin ortaya konmasıdır. Tabakalı Örneklem Yöntemi kullanılarak üç tabakaya ayrılan işletmelerden 154 tanesi ile yüz yüze anket yapılmış ve elde edilen verilerle Bootstrap VZA yöntemi kullanılarak, işletmelerin etkinlik skorları bulunmuştur. İkinci aşamada, Kırpılmış Regresyon yöntemiyle işletmelerin etkinlik skorlarını etkileyen faktörler belirlenmiştir. Bootstrap VZA yöntemiyle elde edilen düzeltilmiş etkinlik skorları ortalaması 0,528 olmuştur. Düzeltilmiş VRS teknik etkinlik değerlerine bakıldığında, birinci tabakadaki işletmelerin ortalaması 0,590; ikinci tabakadaki işletmelerin ortalaması 0,471 ve üçüncü tabakadaki işletmelerin ortalaması ise 0,472 olmuştur. Etkin olan işletmelerin etkin olmayanlara göre %31,99 daha az ağaç, %0,37 daha az işgücü, %13,03 daha az azot, %48,09 daha az fosfor, %14,49 daha az potasyum, %29,95 daha az ilaç, %18,81 daha az su kullandığı ve %13,02 daha fazla mazot kullandığı belirlenmiştir. İkinci aşama olan Kırpılmış Regresyon analiz sonuçlarında Muğla ili işletmeleri, tabaka 2 ve tabaka 3, işletmecinin ortaokul veya lise eğitim seviyesine sahip olması, işletmeci tecrübesi, aile birey sayısı ve ÇKS'ye kayıtlı olma etkinlik skorunu pozitif yönde etkileyen faktörler olarak bulunmuştur. Kooperatif üyeliği ve tarım dışı faaliyette bulunma ise etkinlik skorunu negatif etkileyen faktörler olarak ortaya çıkmıştır. Elde edilen bulgular, zeytin üreticilerinin VRS teknolojileri altında %47,2 daha az girdi kullanarak aynı üretimi gerçekleştirmelerinin mümkün olduğunu göstermektedir. Zeytin işletmelerinin kullandığı girdileri doğru tahsis etmelerinin etkinliklerini artırabileceği sonucuna ulaşılmıştır. Ayrıca çevre kirliliğini azaltmak ve gıda güvenliğini sağlamak için özellikle kimyasal girdilerin ve fosil yakıtların optimum şekilde kullanılmasının gerekli olduğu sonucuna varılmıştır.

### Tarım Ekonomisi

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

Etkinlik

Bootstrap VZA

Zeytin

Kırpılmış regresyon

## Determination of Efficiency and Its Determinants in Olive Production in the Aegean Region: Bootstrap DEA Approach

### ABSTRACT

The aim of this thesis study is to measure the efficiency of operators growing olives in the Aegean region and to reveal the determinants affecting this efficiency. The efficiency scores of the farms were determined by using the Bootstrap Data Envelopment Analyze (DEA) method with the data obtained by conducting a face-to-face survey with 154 of the farms divided into three groups using the Stratified Sampling Method. In the second stage, the significant impacts of the factors affecting the efficiency scores of the farms were revealed with the Truncated Regression method. The average of the bias-corrected efficiency scores obtained by the Bootstrap DEA method was 0,528. While the least efficient olive farms had a bias-corrected efficiency value of 0,119, the most efficient farms had 0,840. Considering the bias-corrected VRS technical efficiency values, the average of farms in the first group was 0,590; the average of the farms in the second group was 0,471 and

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the average of the farms in the third group was 0,472. Additionally, inefficient farms had an average efficiency of 54.69% less than the efficient ones. Efficient farms used 31,99% fewer trees, 0,37% less labor, 13,03% less nitrogen, 48,09% less phosphorus, 14,49% less potassium, 29,95% less pesticide, and 18,81% less and 13,02% more diesel compared to inefficient farms. In addition, experience, number of family members, FRS membership, cooperative membership, and non-agricultural activity variables were found to be significant in the Truncated Regression Analysis as well as Muğla province, secondary school, high school, Group 2 and Group 3 dummy variables. These results show that it is possible for olive producers to achieve the same production using 47.2% less input under VRS technologies. It has been concluded that the correct allocation of inputs used by olive enterprises can increase their efficiency. It has also been concluded that it is necessary to use chemical inputs and fossil fuels optimally in order to reduce environmental pollution and ensure food safety.

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## GİRİŞ

Zeytin ağacı her daim yeşil olan, uzun ömürlü ve 30-45 enlemleri arasında her iki yarım kürede de yetişebilen bir bitkidir (Russo vd. 2016). Ancak, Dünya genelinde zeytinin yaklaşık %97'lik kısmı Akdeniz Kuşağı'nda üretilmektedir. Türkiye ise Dünya zeytin üretiminin %7,81'ini gerçekleştirmektedir (Anonim, 2022a). İspanya, İtalya ve Yunanistan'dan sonra en çok zeytin üretimi Türkiye'de gerçekleşmektedir (Anonim, 2022a). Dolayısıyla Türkiye'nin zeytin üretiminde önemli bir potansiyeli vardır. Ayrıca, Türkiye'de zeytin arazilerinin toplam tarım arazilerindeki payı yaklaşık %4,4'tür (Anonim, 2019). Zeytin, Türkiye tarımının en önemli ürünlerinden birisidir ve yaklaşık 400.000 çiftçinin geçim kaynağıdır (Erdal & Vural, 2017). Zeytin üretim alanı ve üretim miktarlarında İspanya, İtalya ve Yunanistan gibi Avrupa ülkelerinin yanı sıra Türkiye'de de geçen yıllarda önemli artışlar kaydedilmiştir (Beltrán-Esteve, 2013; Çukur ve ark. 2013; Galluzzo, 2014; Niavis ve ark. 2018). Son 20 yıl incelendiğinde Türkiye'de yaklaşık olarak zeytin ağacı sayısında %89 ve zeytin üretiminde %119 artış olduğu belirlenmiştir. 2001 yılında 99.000.000 olan ağaç sayısı 2020 yılında 187.163.252'ye ve 2001 yılında 600.000 ton olan üretim miktarı 2020 yılında 1.316.626 tona çıkmıştır (Anonim, 2022b).

Zeytin Türkiye sınırlarında birçok bölgede yetişebilen bir bitkidir. Doğu Karadeniz Bölgesi'nden Güneydoğu'ya, Akdeniz, Ege ve Marmara Bölgelerinde ve hatta bazı iç kesimlerde bile üretilmektedir. Ancak, yoğun olarak Ege, Akdeniz ve Güney Marmara Bölgelerinde yetiştiriciliği yapılmaktadır. En yoğun zeytin üretiminin gerçekleştiği bölge ise Ege Bölgesi'dir. Ege Bölgesi'nde ise en fazla üretimi gerçekleştiren iller Aydın, İzmir, Manisa ve Muğla

illeri olmuştur.

Üretilen zeytinin verim miktarına bakıldığında Türkiye, ortalama zeytin veriminde Dünya ortalamasının yaklaşık %2,4 üzerindedir. Avrupa Birliği ülkelerinin verim ortalamaları incelendiğinde Türkiye ortalamasının Avrupa Birliği ortalamasından yaklaşık %28,7 daha az olduğu görülmektedir. Verim miktarında AB ortalamasının altında yer alan Türkiye'de, verimde artış sağlayacak tedbirlerin alınması gerekmektedir. Türkiye'de zeytin üretiminde verimin artırılarak AB ülkeleri düzeyine gelmesi hem üretim ve kârlılığı hem de rekabeti arttıracaktır. Zeytin üretiminde maliyetin düşük olması ülkeler arasındaki rekabette önemlidir (Semerci, 2018). Verim düşüklüğü maliyetin yükselmesine neden olmaktadır. Verimde azalmaya yol açan birçok neden bulunmaktadır. Bu nedenlerden en önemlileri girdi kullanım düzeyi ile işletme ve işletmecisi özelliklerinin üretim etkinliğine etkisidir. Zeytin üreten tarım işletmelerinde işletmecisi kararının sonucu olarak ortaya çıkan girdi seçimi ve kullanım miktarı yanında işletmecinin (yaş, cinsiyet, tecrübe, eğitim vb) ve işletmenin (zeytinlik alanı, arazi parçalılık durumu, sulanabilme durumu, arazinin eğimi, ailedeki birey sayısı, vb) özelliklerinin elde edilen verime dolayısıyla da işletmenin üretim etkinliğine katkısının belirlenmesi gerekmektedir.

Dünya nüfusu ve tüketimdeki artış, tarımsal üretimde girdi yoğun kullanılan üretim modelini zorunlu hale getirmiştir. Dolayısıyla, tarımsal üretimde yoğun girdi kullanılmaktadır ve böylece üretim miktarında önemli artışlar görülmektedir. Ancak, daha sürdürülebilir tarımsal faaliyetler ve çevre için kullanılan girdilerin en aza indirilmesi ya da optimum olarak üretime katılması büyük önem arz etmektedir. Çünkü tarımsal üretim için gerekli olan, özellikle akaryakıt, kimyasal

ilaç ve gübreler çevreye de zarar vermektedir. Bu petrol ürünleri ve kimyasalların bilinçsizce ve fazla miktarda kullanılması hava, su ve toprak kirliliğine neden olmaktadır. Neden olunan kirlilikler ise uzun vadede doğaya ve insan sağlığına zarar vermektedir. Bunun yanı sıra, tarımsal üretimde kalite ve verim de bundan negatif olarak etkilenmektedir.

Bu çalışma ile zeytin üretimi yapan üreticilerin girdi kullanım düzeyleri belirlenmiştir. Yüz yüze yapılan anket formları ile insan gücü, makine, ilaç, gübre, mazot, elektrik ve sulama suyu gibi girdilerin mevcut kullanılan ve optimum miktarları elde edilmiştir. Bununla birlikte işletmecinin yaşı, eğitimi ve tecrübesi, ailedeki birey sayısı, arazi miktarı, üretim tekniği, zeytincilikle ilgili bir tarımsal örgüte üye olma durumu, bilgi kaynağına başvuru durumu, zeytin üretimine yönelik teşviklerden faydalanma durumu vb. değişkenler ikinci aşamada modele dâhil edilerek işletmecilerin etkinliklerine olan etkileri ortaya konulmuştur. Sonuç olarak, zeytin üreticilerinin girdi odaklı etkinlik düzeylerinin belirlenmesi, yani aynı üretim düzeyi korunarak ne kadar girdi tasarrufunda bulunulabilecekleri ve yukarıda belirtilen demografik faktörlerin üretim etkinliğine nasıl bir katkı sağlayacağı araştırılmıştır.

## MATERYAL ve METOD

Çalışmanın ana materyalini zeytin üretiminin yoğun olduğu Aydın, İzmir ve Muğla illerinde zeytin yetiştiren işletmelerle yapılan anket verileri oluşturmaktadır. Çalışmada Tabakalı Örneklemeye Metodu kullanılmıştır.

Çizelge 1. Tabakaların Belirlenmesi

Table 1. Determination of Layers

| Tabaka No | Tabaka Alt ve Üst Sınırları (da) | Tabakadaki İşletme Sayısı (Nh) | Standart Sapma (Sh) | (Nh*Sh)   | Örnek Sayısı |
|-----------|----------------------------------|--------------------------------|---------------------|-----------|--------------|
| 1         | 1-10                             | 1343                           | 2.824               | 3792.632  | 73           |
| 2         | 11-40                            | 1111                           | 8.275               | 9193.525  | 38           |
| 3         | 41-200                           | 435                            | 30.985              | 13478.475 | 43           |
| Toplam    | 1-200                            | 2889                           | 42.084              | 26464.632 | 154          |

## Veri analiz yöntemi (Etkinlik Analizi)

İlk olarak Farrell tarafından 1957 yılında etkinlik ölçülmüştür. Etkinliği ölçmek için çeşitli parametrik ve parametrik olmayan yöntemler mevcuttur. Parametrik yöntemler en iyi performansı gösteren karar birimine göre etkinliği ölçmek yerine, ortalama performansa göre etkinliği ölçmektedir (Yeşilyurt, 2018). Parametrik olmayan yöntemlerde ise göreceli etkinlik ölçülmektedir. Yani popülasyonu temsil eden örnek içerisinde bulunan karar birimleri içerisinde verilen girdileri en etkin şekilde kullanarak maksimum ürünü elde eden karar birimleri etkin

## Örneklemeye yöntemi

Popülasyon ana kitlesinin büyük olduğu durumlarda, zamandan ve diğer maddi faktörlerden tasarruf etmek için örneklemeye yöntemleri kullanılmaktadır (Yamane, 2006). Dolayısıyla çalışma kapsamında belirlenen Aydın ilinin Çine, Kuyucak ve Söke ilçelerinden; İzmir ilinin Bayındır, Bergama ve Seferihisar ilçelerinden ve Muğla ilinin Dalaman, Fethiye ve Milas ilçelerinden her birinden üçer adet köy belirlenmiştir. Seçilen bu köylerin ilçeleri; seçilen ilçelerin de illeri en iyi şekilde temsil edeceği düşünülmüştür. Çalışmanın ana kitlesini belirlenen bu köylerde zeytin yetiştiren 2889 tarım işletmesi oluşturmaktadır. Bu çalışmada ana kitleyi en iyi şekilde temsil edecek örnek hacminin hesaplanmasında tabakalı örneklemeye yöntemi uygun görülmüştür. Popülasyon bireyleri arasında büyük farklılıklar olması durumunda popülasyonu tabakalara ayırmak çalışma sonuçlarını daha güvenilir hale getirecektir. Tabakalı örneklemeye yönteminin formülü aşağıda verilmiştir (Yamane, 2006).

$$n = \frac{Nz^2 \sigma^2}{d^2(N-1) + z^2 d^2}$$

Formülde,

$n$ : Örnek hacmi

$\sigma$ : Standart sapma

$N$ : Anakitle büyüklüğü

$d$ : Hata değeridir.

Yapılan araştırmada %90 güven aralığı ve %10 hata payı ile örnek hacmi 154 olarak bulunmuştur.

Yukarıda verilen formüle göre oluşturulan tabakalar ve herbir tabakada örneğe çıkan işletme sayıları Çizelge 1'de verilmiştir.

birimler olarak değerlendirilmekte ve diğer karar birimleri bu etkin karar birimlerine göre değerlendirilerek etkinlik skorları elde edilmektedir. Parametrik olmayan yöntemlerden en çok kullanılan Veri Zarflama Analizi (VZA) yöntemidir. VZA yöntemi kaynak etkinliğinin ölçülmesinde bankacılık, sigortacılık, sağlık sektörü ve tarım sektöründe yaygın olarak kullanılmaktadır (Temur & Bakırcı, 2008; Bedihoğlu & Özcan, 2009; Gündüz ve ark., 2011; Çukur ve ark., 2013). VZA yöntemi parametrik olmayan ve tarım sektöründe de oldukça yaygın biçimde kullanılan başarılı bir etkinlik ölçme yöntemidir (Toma ve ark., 2017). VZA yönteminde

girdi odaklı ve çıktı odaklı olmak üzere iki farklı yaklaşım vardır. Girdi odaklı VZA yaklaşımda mevcut üretim (çıkıtı) seviyesi minimum girdiyle sağlanmaya çalışılırken çıktı odaklı VZA'da mevcut girdiyle maksimum üretim (çıkıtı) sağlanmaktadır. Tarımsal üretimde, üreticilerin girdi üzerinde, çıktılara oranla daha fazla kontrol gücü olduğu için girdi odaklı VZA daha uygun bir yöntem olmaktadır. Bu çalışmada girdi odaklı VZA kullanılması uygun görülmüştür. Diğer bir ifadeyle, aynı üretim düzeyinde kullanılan girdilerde sağlanabilir tasarruf miktarı ortaya konacaktır. Çalışmada her zeytin üreticisinin  $j$  ( $j=1,2,3,...,n$ ) tek çıktısı zeytin üretim miktarı girdi olarak ise  $x_{ij}$  (ağaç, işgücü, mazot, azot, fosfor, potasyum, ilaç ve sulama suyu) kullanılmaktadır. Cooper ve ark. (2007) tarafından kullanılan CRS (Ölçeğe göre sabit getiri) varsayımı altında VZA formülü aşağıda verilmiştir:

$$\text{Minimum } \theta - \left( \sum_r s_r^+ + \sum_i s_i^- \right)$$

$$\sum_{j=1}^n x_{ij} \lambda_j + s_i^- = \theta_0 x_{i0}, \quad i = 1, 2, \dots, m$$

$$\sum_{j=1}^n Y_{rj} \lambda_j - s_r^+ = Y_{r0} \quad r = 1, 2, \dots, p$$

Bütün  $i, j$  ve  $r$  için;  $\lambda_j, s_i^-, s_r^+ \geq 0$  olmaktadır.

Burada  $x_{ij}$  ve  $y_{rj}$  sırasıyla önceden tanımlanan girdiler ve çıktılardır.  $\lambda_j$  vektör ağırlıkları.  $\theta$  işletmelerin 0 ile 100 arasında yer alan teknik etkinlik endeksidir. Yukarıdaki eşitlik CCR (Charnes ve ark., 1981) tarafından kullanılan CRS varsayımıdır. Bu varsayımda girdi miktarında belli bir değişim olduğunda aynı değişim çıktı miktarında da olacağı öngörülmektedir.

CCR modeli üretilen ürünün genel teknik etkinliğini vermektedir. Ancak, ölçek ekonomisinden dolayı tarımsal üretim Ölçeğe Göre Değişir Getiri (VRS) varsayımı altındadır. CCR modelinden elde edilen teknik etkinlik (TE) saf teknik etkinlik ve ölçek etkinliği olarak ikiye ayrılır. Genel Teknik Etkinlik (TE<sub>CCR</sub>) = Saf teknik etkinlik (TE<sub>BCC</sub>) \* Ölçek etkinliği (ÖE) ya da

$$TE_{CCR} = TE_{BCC} * \text{ÖE}$$

Burada TE<sub>BCC</sub> çiftçinin yönetim etkinliğini yansıtırken ÖE genel TE ve TE<sub>BCC</sub> arasındaki kalıntıdır ve işletmenin optimum ölçekte çalışıp çalışmadığını göstermektedir (Heidari ve ark., 2012). Banker ve ark. (1984) CRS varsayımına ilave bir kısıt daha ekleyerek  $\sum_j \lambda_j = 1$  Charnes ve ark. (1978)'in çalışmasını genişletmiştir. Çözüm aşağıdaki gibidir:

$$\text{Minimum } \theta - \left( \sum_r s_r^+ + \sum_i s_i^- \right)$$

$$\sum_{j=1}^n x_{ij} \lambda_j + s_i^- = \theta_0 x_{i0}, \quad i = 1, 2, \dots, m$$

$$\sum_{j=1}^n y_{rj} \lambda_j - s_r^+ = y_{r0}, \quad r = 1, 2, \dots, s$$

$$\sum_j \lambda_j = 1 \quad \text{bütün } i, j \text{ ve } r \text{ için } \lambda_j, s_i^-, s_r^+ \geq 0 \text{ eşitliği geçerli olacaktır.}$$

Yukarıdaki eşitlik TE<sub>BCC</sub> modelidir ve genel TE'yi saf teknik ve ölçek etkinliğine ayırır. Ölçek etkinlik skorunun 100 olması, işletmenin CRS'de ya da optimum ölçekte üretim yaptığını göstermektedir. Diğer tarafta, ölçek etkinlik skorunun 100'den küçük bir değere sahip olması ise işletmenin IRS (Ölçeğe göre artan getiri)'de ya da DRS (Ölçeğe göre azalan getiri)'de üretim yaptığını tanımlar. Ölçek etkisizliğinin mevcut olduğu durumda DRS varsayımı altında üretim yapan işletmelerin girdilerini azaltması, IRS varsayımı altında üretim yapan işletmelerin girdi kullanımını artırması tavsiye edilir ve böylece işletmelerin ortalama etkinliği artmış olur.

### Bootstrap VZA yöntemi

Daha önce yapılan birçok çalışmadan elde edilen sonuçlar, standart VZA'nin, rastsal hataların negatif etkilerini elemine edemediğini ve bu yüzden yanlış etkinlik skorlarını tahmin ettiğini göstermiştir (Simar & Wilson, 2000a). Çalışmalar aynı zamanda tahmin edilen etkinlik skorlarının, elde edilen sınırın örnek varyansına duyarlı olduğunu göstermiştir. Standart VZA'ni iyileştirmek için Simar ve Wilson (2000b) etkinlik skorları için belirsizliğin ölçümünü (yanlı tahminleri düzeltmek ve güven aralıklarının belirlenmesi gibi) sağlayabilen güçlü bir metod olan Bootstrap VZA yöntemini geliştirmişlerdir.

Bootstrap VZA yaklaşımı aşağıdaki gibi özetlenebilir (Simar & Wilson, 2000b).

1. Farklı  $s$  çıktılarını  $Y_{rj}$  ( $r = 1, 2, \dots, r$ ) yani  $j$  karar biriminin  $r$ 'inci çıktısını üretmek için farklı  $m$  girdisi  $x_{ij}$  ( $i = 1, 2, \dots, m$ ) kullanan her bir karar birimi ( $KB_j, j = 1, 2, \dots, n$ ) için VZA etkinlik skorları ( $\theta$ ) aşağıdaki doğrusal programlama modeli çözümlenerek hesaplanır. VZA, Banker ve ark. (1984) tarafından standart genel teknik etkinliği tahmin etmek ve onu saf teknik etkinlik ve ölçek etkinliğine ayırtmak için geliştirilmiştir.

$$\theta^* = \text{Min } \theta$$

$$\sum_{j=1}^n \lambda_j x_{ij} \leq \theta x_{i0} \quad i = 1, \dots, m;$$

$$\sum_{j=1}^n \lambda_j Y_{rj} \geq Y_{r0} \quad r = 1, \dots, s;$$



$$\sum_{j=1}^n \lambda_j = 1$$

$$\lambda_j \geq 0 \quad j = 1, \dots, n;$$

$\theta^*$  teknik etkinlik skorunu temsil etmektedir.  $\theta^* < 1$  olması değerlendirilen karar biriminin etkisiz olmasını ifade eder.  $\theta^* = 1$  karar biriminin tamamen etkin olduğu anlamına gelir.  $\sum_{j=1}^n \lambda_j = 1$  olması dışbükeylik sınırlandırmasıdır. Bu model CRS varsayımı altındaki VZA modelidir.

2.  $\{\hat{\theta}_1, \dots, \hat{\theta}_n\}$  den çekilen n adet tesadüfi örnek büyüklüğünü  $\{\theta_{1b}^*, \dots, \theta_{nb}^*\}$  türetme işlemi gerçekleştirilir. "Smooted Bootstrap" metodu. Silverman (1986) yansıtma metodu ve Kernel yoğunluk metodu kullanılarak gerçekleştirilir.

3. Bootstrap tekniğini yapılandırmak için  $x_{jb}^* = \frac{\hat{\theta}_j}{\hat{\theta}_{jb}} x_j$  formülü kullanılarak  $\{(x_{jb}^*, Y_j); j = 1, \dots, n\}$  yalancı veri seti oluşturulur.

4. Sunulan önceki doğrusal programlama modelinin bootstrap karşılığı çözümlenerek her bir karar birimi ( $j = 1, 2, \dots, n$ ) için  $\theta_{jb}$  etkinlik skorlarının  $\hat{\theta}_{jb}$  bootstrap tahmini hesaplanır.

5.  $j = 1, \dots, n$  için bir bootstrap tahminleri  $\{\hat{x}_{jb}^*; b = 1, \dots, B\}$  setini oluşturmak için B sayısı kadar 2-4 adımları tekrarlanır. Simar ve Wilson (2007)'a göre B makul bir güven aralığı tahminini yapabilmek için 2000'e eşit olmalıdır.

Bootstrap tahminlerini yaptıktan sonra her bir karar biriminin etkinlik skorları için yüzde olarak güven aralıklarını yapılandırabiliriz. Bunu yapmak için  $a_\alpha$  ve  $b_\alpha$  değerlerini bularak  $(\hat{\theta}_j - \theta_j)$  nın dağılımını bilmemiz gerekmektedir.

$$Prob(-b_\alpha \leq \hat{\theta}_j - \theta_j \leq -a_\alpha) = 1 - \alpha$$

$(\hat{\theta}_j - \theta_j)$ 'nin dağılımı bilinmediği için  $a_\alpha$  ve  $b_\alpha$  değerlerini bulmanın mümkün olmadığını Simar ve Wilson (2007) göstermiştir. Bu problemi çözmek için bootstrap tahminlerinin  $\{\hat{x}_{jb}^*; b = 1, \dots, B\}$  dağılımından  $\hat{a}_\alpha$  ve  $\hat{b}_\alpha$  değerlerini bulabiliriz.

$$Prob(-\hat{b}_\alpha \leq \hat{\theta}_j - \theta_j \leq -\hat{a}_\alpha) \approx 1 - \alpha$$

Eşitlik 3'teki  $\hat{a}_\alpha$  ve  $\hat{b}_\alpha$  değerleri. artan sırada  $b = 1, \dots, B$  için  $(\hat{\theta}_j - \theta_j)$  değerleri sıralanarak ve daha sonra sıralanan listenin sonundaki her iki uçta yer alan elemanların yüzdesi  $(\alpha/2 \times 100)$  silinerek hesaplanan  $a_\alpha$  ve  $b_\alpha$ 'nın yaklaşık değerleridir.  $\hat{a}_\alpha$  ve  $\hat{b}_\alpha$ .  $\hat{a}_\alpha \leq \hat{b}_\alpha$  sıralı dizilişinin son noktasına eşit olacak şekilde ayarlanarak her bir  $KB, j = 1, 2, \dots, n$

için etkinlik skoru aşağıdaki gibi tahmin edilebilir:

$$\hat{\theta}_j + \hat{a}_\alpha \leq \theta_j \leq \hat{\theta}_j + \hat{b}_\alpha$$

Bootstrap yaklaşımı aynı zamanda aşağıdaki gibi tahmin edilen yanlış etkinlik skorlarını  $\hat{\theta}_j, j = 1, \dots, n$  değerlendirmemize izin vermektedir.

$$\widehat{Bias}_j(\hat{\theta}_j) = B^{-1} \sum_{b=1}^B \hat{\theta}_{jb}^* - \hat{\theta}_j$$

Eşitlik 5'ten her bir etkinlik skorunun  $(\theta_j, j = 1, \dots, n)$  düzeltilmiş yanlış tahmini aşağıdaki gibi belirlenir:

$$\hat{\tilde{\theta}}_j = \hat{\theta}_j - \widehat{Bias}_j(\hat{\theta}_j)$$

Bunun yanında Bootstrap değerlerinin örnek varyansını temsil eden  $\hat{\sigma}^2$  aşağıdaki formülle bulunmaktadır:

$$\hat{\sigma}^2 < \frac{1}{3} [\widehat{Bias}_j(\hat{\theta}_j)]^2$$

VZA sonucunda. girdilerin optimum kullanım düzeyleri de hesaplanabilmektedir. Bu durum işletmelerin elde etmiş oldukları zeytin üretim miktarını girdilerde ne kadarlık bir tasarruf yapılarak elde edilebileceğini vermektedir. Böylece aynı üretim miktarını elde edebilmek için girdilerde yapılacak tasarruf miktarları hesaplanabilmektedir.

Double-Bootstrap yönteminin bir sonraki adımı olan kırılmış regresyon analizi. etkinlik skorlarının diğer bağımsız değişkenler tarafından ölçülmesidir. Bu ölçüm. teknik etkinlik değerlerinin tersinin alınmasını ve  $\delta_i = (1/\hat{\theta}_i)$  olarak tanımlanmasını içerir. Sonuç olarak. bağımsız değişkenler kümesine bağlı değişken çift sınır boyutundan tek sınır boyutuna dönüştürülür. Böyle bir durumda  $\delta_i, \delta_{i\epsilon} [1, \infty)$  aralığı ile sınırlıdır ve teknik etkinlik puanlarının karşılığı ile ilişkili faktörleri belirlemek için sol limit kesme regresyonu kullanılır.  $\hat{\delta}_i$  etkin bir işletmeyi gösterirken. daha büyük olan  $\delta_{i\epsilon}$  değeri etkin olmayan bir işletmeyi gösterir.

Bağımlı değişken ile bağımsız değişkenler arasındaki ilişki şu şekilde gösterilebilir:

$$\delta_i = z_i' \beta + \varepsilon_i \text{ eğer : } \delta_i \geq 1$$

Bu matematiksel modelde  $z^j$  bağımsız değişkenlerin bir  $(N \times K)$  matrisidir.  $\beta$  ilgili tahmin edilecek parametrelerin bir vektörüdür ve  $\varepsilon_i$  sürekli bir rasgele hata terimidir.  $\delta_i \geq 1 - z_i \beta$  eşitsizliği dikkate alındığında.  $\varepsilon_i$  soldan kırılmıştır  $(1 - z_i \beta)$  ve standart sapma  $\sigma_\varepsilon$  ile normal olarak dağılır. Bu varsayım altında. yukarıdaki denklemin parametreleri modelin parametreleri soldan kırılmış olabilirlik fonksiyonu kullanılarak şu şekilde elde edilir:

$$L = \prod_{i=1}^N \left( \frac{1}{\sigma\varepsilon} \right) \phi \left( \frac{\delta_i - z'_i \beta}{\sigma\varepsilon} \right) \Phi \left[ 1 - \Phi \left( \frac{1 - z'_i \beta}{\sigma\varepsilon} \right) \right]^{-1}$$

Burada  $\phi$  ve  $\Phi$  standart normal için sırasıyla tek değişkenli olasılık yoğunluğu ve kümülatif dağılım fonksiyonlarıdır. Veri oluşturma aşaması tanımlandığında. Double-Bootstrap yöntemi  $\sigma\varepsilon$  ve  $\beta$ 'nin örnekleme dağılımlarını ampirik olarak tahmin etmek ve doğru çıkarımlar yapmak için güven aralığı oluşturur (Monchuk ve ark. 2010).

### BULGULAR ve TARTIŞMA

Bu çalışmada zeytinde üretim etkinliği hesaplanırken gözlemlerin örneklendiği kümenin CRS veya VRS göstermesinin test edilmesi amaçlanmıştır. Bu hipotez aşağıda verilmiştir.

H<sub>0</sub>: CRS teknolojisi

H<sub>a</sub>: VRS teknolojisi

$$S = \frac{\sum_{k=1}^K E_{CRS}^k}{\sum_{k=1}^K E_{VRS}^k} = \frac{70.532}{102.078} = 0.691$$

Radyal VZA için S=0.691 olarak hesaplanmıştır. S ve 10000 yinelemeli Bootstrap VZA yaklaşımıyla hesaplanan Sb için p= 0.025 olarak bulunmuştur. Buna göre  $\alpha=0.05$  için  $p<\alpha$  olduğu için sıfır hipotezi reddedilir. O halde, zeytin üretiminde VRS söz konusudur. Bu nedenle etkinliği artırmak amacıyla yapılması gerekenleri ortaya koyabilmek için, girdiye dönük VRS düzeltilmiş etkinlik değerlerinden yararlanmak gerekmektedir.

Çizelge 2'de Bootstrap VZA'da kullanılan çıktı ve girdilere ait ortalama değerler, minimum ve maksimum değerleri ve standart sapmaları verilmiştir. Gösterilen bu değerlere ait birimler verilmiştir. Ayrıca bütün bu değerler ağaç başına değerlerdir.

Çizelge 2. Bootstrap VZA'da Kullanılan Çıktı ve Girdilere Ait Tanımlayıcı İstatistikler

Table 2. Descriptive Statistics of Outputs and Inputs Used in Bootstrap DEA

| Çıktı ve Girdiler       | Ortalama | Minimum | Maksimum | Standart Sapma |
|-------------------------|----------|---------|----------|----------------|
| Verim (Kg)              | 19.76    | 1.50    | 100.00   | 15.38          |
| Meyve Veren Ağaç (Adet) | 590.56   | 40.00   | 2200.00  | 574.47         |
| İşgücü (EİB)            | 0.28     | 0.02    | 1.77     | 0.19           |
| Mazot (Litre)           | 0.97     | 0.12    | 5.88     | 0.91           |
| Azot (Kg)               | 0.59     | 0.04    | 4.92     | 0.66           |
| Fosfor (Kg)             | 0.46     | 0.00    | 5.00     | 0.49           |
| Potasyum (Kg)           | 0.30     | 0.00    | 1.76     | 0.25           |
| İlaç (TL)               | 2.99     | 0.14    | 36.00    | 3.47           |
| Sulama (Ton)            | 4.67     | 0.05    | 38.40    | 5.64           |

İşletmelerin bootstrap VZA sonuçları Çizelge 3'te verilmiştir.

Çizelge 3. İşletmelerin Bootstrap VZA Sonuçları

Table 3. Bootstrap DEA Results of Farms

| Değişkenler       | Ortalama | Std. Sapma | Min.  | Maks. |
|-------------------|----------|------------|-------|-------|
| <b>CRS</b>        |          |            |       |       |
| Düzeltilmemiş TE  | 0.458    | 0.302      | 0.047 | 1.000 |
| TE=1 (%)          | 14.29    |            |       |       |
| TE $\geq$ 0.8 (%) | 18.18    |            |       |       |
| TE $\geq$ 0.5 (%) | 37.66    |            |       |       |
| Düzeltilmiş TE    | 0.344    | 0.211      | 0.036 | 0.816 |
| TE $\geq$ 0.8 (%) | 0.65     |            |       |       |
| TE $\geq$ 0.5 (%) | 25.97    |            |       |       |
| <b>VRS</b>        |          |            |       |       |
| Düzeltilmemiş TE  | 0.663    | 0.279      | 0.143 | 1.000 |
| TE=1 (%)          | 29.87    |            |       |       |
| TE $\geq$ 0.8 (%) | 37.66    |            |       |       |
| TE $\geq$ 0.5 (%) | 64.29    |            |       |       |
| Düzeltilmiş TE    | 0.528    | 0.206      | 0.119 | 0.840 |
| TE $\geq$ 0.8 (%) | 4.55     |            |       |       |
| TE $\geq$ 0.5 (%) | 50.00    |            |       |       |

Elde edilen sonuçlar, zeytin üreticilerinin CRS teknolojileri altında %65.6 oranında daha az girdi ile

aynı üretim miktarını elde edebileceklerini ortaya koymaktadır. CRS için orijinal VZA etkinlik

skorlarının 0.047 ile 1.00 arasında değiştiği tespit edilmiştir. En kötü performans gösteren işletmenin sınırını kaydırarak girdilerden %95.3 oranında tasarruf edebileceği görülmektedir. CRS teknolojileri altında tamamen etkin olan işletmelerin toplam işletmelerin %14.3'ünü; 0.5'in üzerinde etkinlik skoruna sahip olan işletmelerin ise toplam işletmelerin %37.66'sını oluşturduğu tespit edilmiştir. VRS teknolojileri altında ise zeytin yetiştiren işletmeler. %47.3 daha az girdi kullanarak aynı üretimi gerçekleştirmelerinin mümkün olduğu

belirlenmiştir. Orijinal VZA etkinlik skorları VRS için 0.143 ve 1.00 arasında değişmektedir. Bu durum en kötü performans sergileyen işletmenin sınırını kaydırarak girdilerinden %85.7 oranında tasarruf sağlayabileceğini göstermektedir. VRS teknolojileri için tamamen etkin olan işletmelerin oranının 29.87 olduğu, işletmelerin %64.29'unun etkinlik skorlarının 0.5'in üzerinde olduğu tespit edilmiştir. Galluzzo (2014) ise konvansiyonel zeytin işletmelerinde teknik etkinlik skorunu 0.388 olarak bulurken, organik zeytin işletmelerinde 0.481 olarak bulmuştur.

Çizelge 4. İşletmelerin Teknik Ekinlik Skorlarının Tanımlayıcı İstatistikleri  
Table 4. Descriptive Statistics of Technical Efficiency Scores of Farms

| VRS                  | Ortalama | 1     | 2     | 3     |
|----------------------|----------|-------|-------|-------|
| Etkinlik             | 0.663    | 0.741 | 0.587 | 0.597 |
| Düzeltilmiş Etkinlik | 0.528    | 0.590 | 0.471 | 0.472 |
| Fark                 | 0.135    | 0.151 | 0.116 | 0.124 |

Çizelge 4'te işletmelerin teknik etkinlik skorları, düzeltilmiş etkinlik skorları ve fark ortalamaları tabakalara göre verilmiştir. Örnekleme yer alan 154 işletmenin teknik etkinlik skorları ortalaması 0.663 olurken, birinci tabakadaki işletmelerin teknik etkinlik skoru ortalaması 0.741 değeriyle ilk sırada yer almaktadır. 0.597 teknik etkinlik skoru ortalamasıyla üçüncü tabaka, ikinci sırada yer alırken; 0.587 teknik etkinlik skoru ortalamasıyla ikinci tabaka son sırada yer almıştır. Düzeltilmiş teknik etkinlik skorlarının ortalamalarına bakıldığında ise teknik etkinlik skorlarına paralel bir durum görülmektedir.

İşletmeler genelinin ortalaması 0.528 olurken, birinci tabaka ortalaması 0.590 değeriyle en yüksek olmuş, üçüncü ve ikinci tabaka ise 0.472 ve 0.471 ortalama değerleriyle sırasıyla ikinci ve üçüncü olarak yer almıştır. Bu sonuçlar doğrultusunda, birinci tabakada yer alan işletmelerin diğer işletmelere göre daha etkin olduğu ortaya çıkmaktadır.

Kırpılmış Regresyon modelinde kullanılan değişkenler, bu değişkenlerin isimleri, tanımlamaları ve birimleri, ilgili değişkenlere ait maksimum ve minimum değerler Çizelge 5'te sunulmuştur.

Çizelge 5. Kırpılmış Regresyon Verilerine Ait Tanımlayıcı İstatistikler  
Table 5. Descriptive Statistics of Truncated Regression Data

| Değişken  | Tanım                                   | Ort.   | Std. Sapma | Min. | Maks. |
|-----------|---|--------|------------|------|-------|
| Mugla     | Muğla İlindeki Üreticiler=1. Diğer=0    | 0.331  | 0.472      | 0.0  | 1.00  |
| Izmir     | İzmir İlindeki Üreticiler=1. Diğer=0    | 0.325  | 0.470      | 0.0  | 1.00  |
| Ortokul   | Ortaokul Mezunları=1. Diğer=0           | 0.149  | 0.358      | 0.0  | 1.00  |
| Lise      | Lise Mezunları=1. Diğer=0               | 0.104  | 0.306      | 0.0  | 1.00  |
| Tecrube   | Tecrübe (Yıl)                           | 36.240 | 14.327     | 5.0  | 70.00 |
| Ailesay   | Ailedeki Birey Sayısı (Kişi)            | 3.305  | 1.325      | 1.0  | 11.00 |
| Cks       | ÇKS Kaydı 1=Evet. 0=Hayır               | 0.844  | 0.381      | 0.0  | 1.00  |
| Araziuz   | Arazi Uzaklığı (Km)                     | 2.188  | 2.785      | 0.0  | 15.00 |
| Araziyar  | Arazi Parça Sayısı (Adet)               | 2.864  | 2.744      | 1.0  | 15.00 |
| Birlik    | Birlik Üyeliği 1=Evet. 0=Hayır          | 0.058  | 0.235      | 0.0  | 1.00  |
| Koop      | Kooperatif Ortaklığı 1=Evet. 0=Hayır    | 0.500  | 0.502      | 0.0  | 1.00  |
| Hayvncik  | Hayvancılık Faaliyeti 1=Evet. 0=Hayır   | 0.292  | 0.456      | 0.0  | 1.00  |
| Tardis    | Tarım Dışı Faaliyet 1=Evet. 0=Hayır     | 0.195  | 0.397      | 0.0  | 1.00  |
| Araziyegm | Arazi Eğimi 0=Düz. 1=Az eğimli. 2=Yamaç | 0.396  | 0.631      | 0.0  | 2.00  |
| Destek    | Destekleme Alma 1=Evet. 0=Hayır         | 0.825  | 0.381      | 0.0  | 1.00  |
| Tabaka2   | 2. Tabaka İşletmeleri=1. Diğer =0       | 0.247  | 0.433      | 0.0  | 1.00  |
| Tabaka3   | 3. Tabaka İşletmeleri=1. Diğer =0       | 0.279  | 0.450      | 0.0  | 1.00  |

Çizelge 6'da kırpılmış regresyon analizi sonuçları yer almaktadır. Kırpılmış regresyon analiz sonuçlarına göre istatistiksel olarak anlamlı çıkan değişkenlere baktığımızda Muğla ilinde zeytin yetiştiren

işletmelerin Aydın ilindekilere göre daha etkin oldukları %1 önem seviyesinde anlamlı bulunmuştur. Muğla'da bulunan işletme sayısındaki 1 adetlik bir artış Aydın'da bulunan işletmelere göre etkinliği

yaklaşık %111 oranında artırmaktadır.

İşletmecisi ortaokul mezunu olan işletmelerin ilkökul ve lise üstü eğitime sahip olanlara göre daha etkin olduğu %5 önem seviyesinde önemli bulunurken yine lise mezunu işletmecilerin ilkökul ve lise üzeri eğitime sahip işletmecilerin bulunduğu işletmelere göre %1 önem seviyesinde anlamlı bulunmuştur. İşletmecinin ortaokul ve lise mezunu olması sırasıyla işletmenin etkinliğini ilkökul mezunu ve lise üstü eğitim

seviyesine sahip işletmecilere göre işletme etkinliğini yaklaşık olarak sırasıyla %208 ve %156 artırmaktadır. Bu durum ortaokul ve lise mezunu olan işletmecilerin lise üstü eğitime sahip işletmecilere göre geçimini tarımdan sağlayan ve başka geliri bulunmayan ilkökul mezunu olanlara göre ise daha yeni ve etkin üretim yöntemleri benimsemeleri bu yüzden de tarımsal gelirini artırmak için çabalayarak girdilerini etkin kullanma gayreti içerisinde bulunmaları nedeniyle böyle bir sonucun oluştuğu düşünülmektedir.

Çizelge 6. Kırpılmış Regresyon Modelinde Kullanılan Değişkenler ve Analiz Sonuçları

Table 6. Variables Used in Truncated Regression Model and Analysis Results

| Değişkenler | Katsayı  | %90 Güven Aralığı |           | %95 Güven Aralığı |           | %99 Güven Aralığı |           |
|-------------|----------|-------------------|-----------|-------------------|-----------|-------------------|-----------|
|             |          | Alt Sınır         | Üst Sınır | Alt Sınır         | Üst Sınır | Alt Sınır         | Üst Sınır |
| (Intercept) | -8.314** | -1.443            | -5.362    | -15.017           | -3.796    | -15.824           | 0.969     |
| Mugla       | 1.110*   | 6.995             | 2.660     | -0.315            | 2.834     | -1.279            | 3.362     |
| Izmir       | 0.440    | -9.057            | 1.960     | -1.238            | 2.270     | -2.235            | 2.956     |
| Ortokul     | 2.085**  | 7.318             | 4.107     | 0.401             | 4.462     | -0.707            | 4.965     |
| Lise        | 1.560*   | 5.113             | 3.799     | -0.470            | 4.114     | -1.418            | 5.270     |
| Tecrube     | 0.069**  | 3.021             | 0.126     | 0.015             | 0.133     | -0.020            | 0.150     |
| Ailesay     | 0.875*** | 5.342             | 1.415     | 0.434             | 1.489     | 0.100             | 1.608     |
| Cks         | 2.278*   | 2.434             | 4.975     | -0.336            | 5.470     | -1.745            | 6.471     |
| Araziuz     | -0.056   | -2.673            | 0.202     | -0.306            | 0.272     | -0.410            | 0.403     |
| Araziparça  | 0.121    | -6.996            | 0.377     | -0.115            | 0.435     | -0.235            | 0.547     |
| Birlik      | -1.006   | -3.436            | 2.260     | -3.825            | 3.529     | -4.739            | 6.201     |
| Koop        | -2.139** | -3.705            | -1.148    | -3.881            | -0.763    | -4.300            | 0.185     |
| Hayvncık    | -0.925   | -2.299            | 0.392     | -2.555            | 0.746     | -2.919            | 1.689     |
| Tardis      | -1.947*  | -3.715            | -0.457    | -3.941            | 0.013     | -4.477            | 1.515     |
| Araziygm    | -0.165   | -9.973            | 0.762     | -1.143            | 0.951     | -1.483            | 1.591     |
| Destek      | -1.929   | -4.709            | 0.014     | -5.193            | 0.498     | -6.442            | 1.445     |
| Tabaka2     | 3.053*** | 1.973             | 5.162     | 1.436             | 5.398     | 0.096             | 5.757     |
| Tabaka3     | 4.521*** | 3.443             | 6.967     | 2.685             | 7.227     | 0.734             | 7.610     |
| Sigma       | 2.264*** | 2.091             | 3.079     | 1.929             | 3.139     | 1.540             | 3.253     |

\*%10 önem düzeyi. \*\* %5 önem düzeyi.\*\*\* %1 önem düzeyi

İşletmecinin tecrübesi arttıkça işletmenin etkinliğinin %10 önem seviyesinde arttığı analiz sonucundan çıkarılmaktadır. İşletmecinin zeytin üreticiliğiyle uğraştığı yıl sayısı 1 yıl arttığında işletmenin etkinliğinin %7.00 oranında arttığı belirlenmiştir. Zira. zeytin üretimi ile uğraşan işletmeciler ne kadar çok tecrübeli olurlarsa üretim tekniği ve girdi kullanımı konusunda da o kadar bilgi birikimine sahip olurlar. Bu durum işletmecinin tecrübesi ile bilgi birikiminin ve işletmenin etkinliğinin artmasına neden olmaktadır.

Ailedeki birey sayısının yani potansiyel işgücü miktarının artması analiz sonuçlarına göre %1 önem seviyesinde yine etkinliği artırmaktadır. Bu sonuç beklenen bir sonuçtur. Analize göre ailedeki birey sayısının 1 birim artması işletme etkinliğini yaklaşık

olarak %88 oranında artırmaktadır.

İşletmelerin ÇKS kayıtlarının olması. etkinliği %10 önem düzeyinde ve pozitif yönde etkilemektedir. ÇKS kaydı olan işletmeler kaydı bulunmayan işletmelere göre %228 oranında yüksek etkinlik skoruna sahiptir. Bu durum işletme yönetiminin kanun ve yönetmeliklere uygun olarak gerçekleştirilmesinden kaynaklanmaktadır. Bu yönüyle skor artışı beklenen bir sonuç olarak karşımıza çıkmaktadır.

Yapılan analizler 2. ve 3. tabakada bulunan işletmelerin birinci tabakada yer alan işletmelere göre %1 önem seviyesinde daha etkin olduklarını göstermiştir. Bu da işletmelerin zeytin bahçesi genişliği ile etkinliğinin arttığı anlamına gelmektedir. Bu durum işletmelerin zeytin bahçesi genişliği ile



etkinliğinin arttığı. büyük işletmelerin daha profesyonel bir şekilde işletme faaliyetini yürüttüklerini ortaya koymaktadır.

Yürütülen araştırmada elde edilen analiz sonucuna göre işletmenin tarımsal kooperatif üyeliği ve tarım dışı gelirinin olması etkinliği negatif yönde etkilemektedir. Sırasıyla bu değişkenler istatistiksel olarak %5 ve %1 oranında önemli bulunmuştur. İşletmelerin kooperatif ortağı olmasının etkinliği %214 oranında. tarım dışı gelire sahip olmasının ise %195 oranında işletmenin etkinliğini azalttığı belirlenmiştir. Bu sonuçlara göre. tarımsal kooperatife ortak olan işletmelerin yeterince ortaklık olanaklarından yararlanmadığını diğer yandan tarım dışı gelire sahip olan işletmelerin ise zeytin üretiminden ziyade tarım dışından elde ettiği gelire güvendiği şeklinde yorumlanabilir.

### SONUÇ ve ÖNERİLER

Bu çalışmada. İzmir. Aydın ve Muğla illerinde 154 zeytin üreticisiyle yapılan anket çalışması sonucunda. Bootstrap VZA yöntemi kullanılarak teknik etkinlik skorları elde edilmiştir. Klasik VZA. rastsal hataların negatif etkilerini bertaraf edememesi nedeniyle yanlış etkinlik skorlarına sahiptir. Çalışmada bootstrap VZA analizi. düzeltilmiş etkinlik skorlarını vermesinden dolayı tercih edilmiştir.

Girdi odaklı VRS Bootstrap analiz sonuçlarına göre işletmelerin ortalama düzeltilmiş etkinlik skoru 0.528 olarak belirlenmiştir. Bunun anlamı düzeltilmiş etkinlik skoruna göre işletmeler aynı üretim düzeyini girdilerinde yaklaşık %47 oranında tasarruf yaparak ulaşabilecekleridir. Düzeltilmemiş etkinlik skorları incelendiğinde ortalama etkinlik skorunun 0.663 olduğu belirlenmiş olup. aynı üretim düzeyinin girdilerde yapılacak yaklaşık %34'lük bir tasarrufla ulaşılacağı anlamı taşımaktadır.

İkinci aşama olan Kırpılmış Regresyon analiz sonuçlarında anlamlı bulunan değişkenlerden Muğla ili. tabaka 2 ve tabaka 3 kukla değişkenlerinin yanı sıra ortaokul eğitim seviyesi. lise eğitim seviyesi. tecrübe. aile birey sayısı ve ÇKS kayıt durumu etkinlik skorunu pozitif etkileyen faktörler olarak bulunmuştur. Muğla ili işletmelerinin Aydın ve İzmir ili işletmelerine göre. ortaokul ve lise eğitim seviyesinin ise diğer eğitim seviyelerine göre etkinliği artırdığı sonucuna varılmıştır. Ayrıca. kooperatif üyeliği ve tarım dışı faaliyet değişkenleri de analiz sonuçlarına göre anlamlı bulunan ve etkinlik skorunu negatif etkileyen faktörler olarak ortaya çıkmıştır. Yani. kooperatif üyesi olmayan işletmelerin üye olan işletmelere göre. tarım dışı faaliyette bulunmayan işletmelerin ise tarım dışı faaliyette bulunan işletmelere göre daha etkin olduğu sonucu çıkarılabilir.

Artan dünya nüfusuyla birlikte girdi yoğun tarımsal

üretim her geçen gün yaygınlaşmaktadır ( Cengil ve Kuşvuran 2012; Gökırmaklı ve Bayram 2018; Şahin ve Külekçi 2022). Ancak. girdi maliyetlerindeki artış. son dönemlerde girdi kullanım düzeyinin önemini daha çok ön plana çıkarmaktadır. Kaynakların israfını önlemek. maliyet artışlarından daha az etkilenmek ve en önemlisi; kimyasal girdi kullanımını azaltarak çevre kirliliğini en aza indirmek zeytin üreticilerinin etkinliklerini artırarak daha az girdi kullanımıyla mevcut üretimi gerçekleştirmeleri. hem zeytin üretiminin sürdürülebilirliğini artıracaktır hem de mevcut çevre koşullarının korunmasına katkı sağlayacaktır.

Tarım ilaçları ve kimyasal gübrelerin kullanımı gıda güvenliğini riske atmaktadır. Dolayısıyla. tarımsal üretimde yoğun olarak kullanılan gübre ve ilaç gibi kimyasalların. fosil yakıt olan mazotun doğru miktarlarda kullanılarak. diğer bir ifadeyle aynı üretim miktarı daha az kimyasal girdilerle sağlanarak gıda güvenliğinin artırılacağı düşünülmektedir.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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## Çekirdeksiz Kuru Üzüm Üretiminde Teknik Etkinliğin Belirlenmesi

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**ÖZET** Bu çalışmada Türkiye'de çekirdeksiz kuru üzüm üretiminin tamamına yakınının gerçekleştirildiği Manisa, İzmir ve Denizli illerindeki üretici işletmelerin teknik etkinlik puanları belirlenmiştir. Etkinlik hesaplaması veri zarflama yöntemiyle yapılmış olup 150 işletmenin 2020 hasat yılına ait verileri kullanılmıştır. Çal ve Bekilli ilçelerindeki verimlilik farkının nedeni metafrontier analizi yaklaşımıyla açıklanmış ve bu bölgedeki 33 işletme Çal grubu, diğerleri Ova grubu olarak gruplandırılarak etkinlik analizleri yapılmıştır. Ova grubunun etkinlik puanı (crste) ortalamasının 0.726, Çal grubu etkinlik puanı ortalamasının (crste) 0.815 olduğu belirlenmiştir. Çal grubundaki verim seviyesi farklılığı üretim teknolojilerinden kaynaklanmaktadır. Ova grubu işletmelerin metafrontier (üst sınır) teknolojisini temsil ettikleri, Çal grubundaki işletmelerin üretimlerini sınırlayan bir üretim teknolojisine sahip oldukları ve üretebilecekleri maksimum çıktının üst sınıra ait üretim teknolojisini kullandıklarında elde edeceklerinin %56'sı kadar olduğu belirlenmiştir. Ova grubunda teknik etkinliğe (crste) ulaşan 16 işletme bir dekar üretim alanında 591.12 kg verim almış, 132.26 TL gübre, 408.84 TL pestisit, 6,89 erkek işgücü ve 25.5 litre mazot kullanmıştır. Çal grubunda teknik etkinliğe (crste) ulaşan 15 işletme bir dekar üretim alanında 127.88 kg verim almış, 73.51 TL gübre, 91.90 TL pestisit, 3.71 erkek işgücü ve 16.0 lt mazot kullanmıştır.

Tarım Ekonomisi

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## Determination of Technical Efficiency in Seedless Raisin Production

**ABSTRACT** In this study, technical efficiency scores of producing enterprises in Manisa, Izmir, and Denizli provinces, where almost all the seedless raisins are produced in Turkey, were determined. Efficiency calculation was made with the data envelopment method and data from 150 enterprises for the 2020 harvest year were used. The reason for the difference in productivity in the Çal and Bekilli districts was explained with the metafrontier analysis approach, and efficiency analyses were carried out by grouping 33 enterprises in this region as the Çal group and the others as Ova group. It was determined that the average efficiency score (crste) of the Ova group was 0.726, and the average efficiency score (crste) of the Çal group was 0.815. The reason for the difference in productivity in the Çal group is due to the difference in production technology. It has been determined that the enterprises in the Ova group represent the metafrontier (upper limit) technology. The enterprises in the Çal group have a production technology that limits their production, and the maximum output they can produce is 56% of what they would achieve when they use the upper-limit production technology. In the plain group, 16 enterprises that achieved technical efficiency (crste) yielded 591.12 kg in one decare of production area, used 132.26 Turkish Liras fertilizer, 408.84 Turkish Liras pesticide, 6.89 male labor force, and 25.5 liters of diesel oil. In the Çal group, 15 enterprises that reached technical efficiency (crste) yielded 127.88 kg in one decare of production area, used 73.51 Turkish Liras fertilizer, 91.90 Turkish Liras pesticide, 3.71 male labor force, and 16.0 liters of diesel oil.

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## GİRİŞ

Tarım ekonomisi çalışma ve araştırmaları verimliliği arttırmayı amaçlar. Bu amaca ulaşılması için, belirli bir ürün miktarının mümkün olan en az girdi ile elde edilmesi veya belirli bir miktarda girdi kullanılarak mümkün olan en fazla ürünün elde edilmesi yani teknik etkinliğe ulaşılması gerekmektedir (Doll & Orazem, 2005).

Etkinlik analizi ile ilgili çalışmalar II. Dünya Savaşı'ndan sonra doğrusal programlama yöntemi ve bilgisayar teknolojisi sayesinde gelişim göstermiş olup Farrell (1957) çalışmasıyla bu konuda öncü olmuştur. Günümüzde etkinlik analizi çalışmalarında yaygın olarak kullanılan veri zarflama ve stokastik sınır analizi yöntemleri 1970'li yılların sonlarında ortaya çıkmıştır (Aigner et al, 1977; Meeusen & Broeck, 1977; Charnes ve ark. , 1978). Türkiye'de tarım sektöründe pamuk, elma, tütün, mısır gibi pek çok ürün için etkinlik analizi çalışması yapılmıştır (Aktürk & Kıral, 2002; Gül, 2006; Ören & Alemdar, 2006; Kaçıra, 2007). Bu çalışmalarda yöntem olarak daha çok veri zarflama analizi (VZA) kullanılmıştır (Özden, 2017; Bayav & Karlı, 2020). Dünya genelinde VZA yönteminin kullanıldığı bilimsel makale sayısının 2004 yılından sonra üssel bir artış göstererek 10 bini geçtiği ve son yıllarda en çok kullanıldığı alanların başında tarım sektörünün geldiği bildirilmektedir (Emrouznejad & Yang, 2018). Bölgesel verimlilik farklılıklarının ise metafrontier analizi yaklaşımıyla incelendiği görülmektedir (Chen & Song, 2008; Villano ve ark. 2010, Özden & Di'os-Palomares, 2016; Bayav ve ark., 2022).

Türkiye üzümün anavatanı olarak köklü bir bağcılık geleneğine sahiptir (Güvenç, 2020). Türkiye'nin kuru üzüm ihracatının neredeyse tamamını Ege Bölgesinde üretilen ve dünyada "sultana" olarak bilinen çekirdeksiz kuru üzüm oluşturmaktadır. Dünyada beş yılın ortalamasına göre yıllık 1.77 milyar dolar değerinde kuru üzüm ihracatı yapılmakta olup ihracatçı ülkeler arasında Türkiye, %27.9'luk pay ile ilk sırada yer almaktadır (ITC, 2021).

Türkiye'de çekirdeksiz kuru üzüm ile ilgili yapılan iki etkinlik çalışmasında da (Bayramoğlu & Gündoğmuş, 2008; Atış ve ark., 2013) organik ve geleneksel üretim ayırımına gidilmiştir. Bu çalışmada, Türkiye'de çekirdeksiz kuru üzüm üretiminin tamamına yakınının gerçekleştirildiği bölgeler çalışma kapsamına alınmış ve bölgesel verimlilik farklılığının nedenleri metafrontier yaklaşımıyla incelenmiştir.

## MATERYAL ve METOD

Çalışmanın materyali, Türkiye'de çekirdeksiz kuru

üzümün üretildiği Manisa, Denizli ve İzmir illerindeki üreticilerle yüz yüze anketler yoluyla elde edilmiş olan, 2020 yılı üretim yılına ait verilerdir.

Ege Bölgesi, Ege Denizi'ne açılan geniş bir cepheye sahip olan Ege Bölümü ve iç kısımda kalan deniz etkisinin olmadığı İç Batı Anadolu Bölümü olarak iki alt bölüme ayrılmıştır (Koçman, 1993). Çalışmanın yürütüldüğü ilçelerden Çal ve Bekilli İç Batı Anadolu Bölümü'nde, diğer ilçeler ise Ege Bölümü'nde yer almaktadır. Ege Bölümü'ndeki ilçelerden Kemalpaşa ve Menemen İzmir ili, Buldan Denizli ili, Şehzadeler, Saruhanlı, Ahmetli, Turgutlu, Gölarmara, Salihli, Alaşehir ve Sarıgöl ilçeleri Manisa ili sınırları içerisinde bulunmaktadır. Çal ve Bekilli bölgesinde, yer bağlarında üretim yapılmakta olup verim seviyeleri diğer bölgelerden oldukça düşüktür.

## Örnekleme Yöntemi

Örneklem sayısının belirlenmesi için ilk olarak üç ildeki kayıtlı üretici sayıları İl Tarım Orman Müdürlüklerinden sorulmuş ve 12590 kişinin çekirdeksiz kuru üzüm üreticisi olarak kayıtlı olduğu öğrenilmiştir. Yapılacak anket sayısı Eşitlik 1'de yer alan oransal örnek hacmi formülü kullanılarak (Newbold, 1995) %95 güven aralığı ve %8 hata payı için 150 olarak belirlenmiştir.

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

Eşitlik 1'de yer alan n örneklem sayısını, N ana kütle büyüklüğünü, p tahmin oranını (en fazla örneklem sayısı için 0.5 alınmıştır),  $\sigma_{px}^2$  varyansı ifade etmektedir.

Anket sayıları illere payları oranında dağıtılmış olup Manisa'da 96, İzmir'de 16, Denizli'de 38 adet üretici anketi gerçekleştirilmiştir.

## Etkinlik Analizi

Etkinlik analizi literatüründe bölgeler arası verimlilik farklılıklarının incelenmesi için metafrontier (üst sınır) yaklaşımı geliştirilmiştir (Rao ve ark. , 2003). Metafrontier yaklaşımda etkinlik; biri girdi-çıkıtı noktasından grup sınırına olan mesafeyi ölçen bileşen (teknik etkinliğin yaygın ölçüsü), diğeri grup sınırı ile üst sınır arasındaki mesafeyi ölçen (üretim ortamının kısıtlayıcı yapısını temsil eden) bileşen olarak ikiye ayrılmaktadır. Metafrontier analizi etkinlik puanının grup analizi etkinlik puanına bölünmesiyle metateknoloji oranı hesaplanmaktadır. Metateknoloji oranı bir bölgedeki üretim ortamının kısıtlayıcı yapısını ortaya koymakta, verimliliğin arttırılması için sulama ve arazi islahı gibi altyapı yatırımlarına gerek



olup olmadığı konusunda politik karar vericilere yol gösterici olabilmektedir (O'Donnell ve ark. , 2008). Metafrontier yaklaşımı bağlamında Çal ve Bekilli ilçelerinde yer alan 33 üretici Çal grubu, diğer 117 üretici Ova grubu olarak gruplandırılmıştır. Bu gruplar için ayrı ayrı yapılan etkinlik analizleri ile grup analiz sonuçları elde edilmiştir. Bütün işletmelerin birlikte yer aldığı analiz ise metafrontier olarak adlandırılmıştır.

Üreticilerin etkinlik ölçümü veri zarflama analizi yöntemiyle, DEAP 2.1 (Coelli, 1996) bilgisayar paket programı kullanılarak yapılmıştır. Bu program ile analizde yer alan işletmelere etkin birimlerin oluşturduğu üretim sınırına olan uzaklıklarına göre puanlar verilmektedir. Etkin sınırdaki işletmeler "1" puanını alırken diğer işletmeler "0" ile "1" arasında puanlar almaktadır. Analizde üretimin çıktısı olarak çekirdeksiz kuru üzüm miktarı (kg) kullanılmıştır. Kullanılan arazi miktarı (da), kullanılan gübre değeri (TL), kullanılan Pestisit değeri (TL), kullanılan akaryakıt (mazot) miktarı (litre) ve kullanılan işgücü miktarı (erkek işgücü birimi) girdi setini oluşturmuştur. Gübre ve pestisitler dışındaki girdiler teknik birimler ile analizde yer almış, çok farklı içerik ve formda kullanıldığı görülen gübre ve pestisitler parasal karşılıkları alınarak toplulaştırılmıştır. İşgücü kullanımı günlük sekiz saatlik çalışmayı ifade eden erkek işgünü (yevmiye) birimi ile kullanılmış olup kadın iş gücü 0.75 katsayısı ile çarpılarak (Açıl & Demirci, 1984) erkek iş gücü birimine (EİB) çevrilmiştir.

Çalışmanın yürütülmesi Ege Üniversitesi Fen ve Mühendislik Bilimleri Bilimsel Araştırma ve Yayın Etiği Kurulu'nun 01.02.2021 tarihli ve 778 protokol numaralı kararıyla etik açıdan uygun bulunmuştur.

## BULGULAR ve TARTIŞMA

Çekirdeksiz kuru üzüm üreten işletmelerin grup ve metafrontier analizde elde ettikleri etkinlik puanlarının ortalamaları Çizelge 1'de verilmiştir. Üreticilerin ölçeğe sabit getiri (constant return scale)

varsayımı altında grup analizi teknik etkinlik puanlarının (crste) ortalaması 0.746 iken aynı değer metafrontier analizde 0.666 olarak hesaplanmıştır. Oluşan bu farkın neredeyse tamamının nedeni Çal grubunda yer alan işletmelerdir. Grup analizinde Çal grubu işletmelerin etkinlik puanı ortalamasının 0.815 olduğu ve 15 işletmenin etkin olduğu görülmektedir. Metafrontier analizde ise Çal grubunun etkinlik puanı ortalaması 0.459 puandır ve sadece bir işletme tam olarak etkindir. Bu durum Çal grubunda üretim sınırının diğerlerinden farklı olduğunu, bu bölgede üretimi sınırlayan farklı bir teknoloji kullanıldığını göstermektedir.

Üreticilerin metafrontier analizden aldıkları etkinlik puanlarının grup analizinden aldıkları puanlara bölünmesiyle (O'Donnell ve ark. , 2008) metateknoloji oranları belirlenmiş olup ortalama değerler Çizelge 1'de verilmiştir. Ova grubu işletmelerin metafrontier teknolojisini temsil ettikleri, buna karşılık Çal grubundaki işletmelerin sahip oldukları mevcut teknoloji ile alacakları maksimum çıktı miktarının, metafrontier üretim teknolojisine sahip olsalardı elde edeceklerinin %56'sı kadar olduğu belirlenmiştir. Çal yöresindeki teknolojik açığın kapatılması için anahtar kelime sulamadır. Bu yörede sulama ve fertigasyon (sulama ile gübreleme) sayesinde daha uzun verim çubuklarının kullanıldığı, birim alanda daha çok asmanın olduğu yüksek sistem bağ teknolojisine geçiş yapılabilecektir.

Çekirdeksiz kuru üzüm üretimi için yapılan önceki iki çalışmada da organik ve geleneksel üretim ayrımı yapılmıştır. Bayramoğlu & Gündoğmuş (2008) ortalama teknik etkinlik puanlarını (crste), organik üretim için 0.862 ve geleneksel üretim için 0.903 olarak belirlemiştir. Atış ve ark., (2013) tarafından yapılan diğer çalışmada bu değerlerin sırasıyla 0.410 ve 0.593 olduğu hesaplanmıştır. Bu çalışmada ise organik ve geleneksel üretim ayrımına gidilmemiş olup elde edilen Ova grubuna ait ortalama etkinlik puanı (0.726), önceki iki çalışmadan elde edilenlerin arasında bir değerdir.

Çizelge 1. Etkinlik analizi puanları (crste) ve metateknoloji oranları

Table 1 Efficiency analysis scores (crste) and metatechnology rates

| Metafrontier analiz | Ortalama | En az | En çok | Std. Sapma |
|---------------------|----------|-------|--------|------------|
| Ova grubu           | 0.725    | 0.263 | 1.000  | 0.202      |
| Çal grubu           | 0.459    | 0.201 | 1.000  | 0.172      |
| Genel               | 0.666    | 0.201 | 1.000  | 0.224      |
| Grup analizleri     |          |       |        |            |
| Ova grubu           | 0.726    | 0.282 | 1.000  | 0.201      |
| Çal grubu           | 0.815    | 0.414 | 1.000  | 0.216      |
| Genel               | 0.746    | 0.282 | 1.000  | 0.207      |
| Metateknoloji oranı |          |       |        |            |
| Ova grubu           | 0.997    | 0.923 | 1.000  | 0.013      |
| Çal grubu           | 0.559    | 0.369 | 1.000  | 0.122      |
| Genel               | 0.901    | 0.369 | 1.000  | 0.191      |

Ova grubunda tam etkinliğe ulaşan (crste=1) ve verim gruplandırması yapılan işletmelerin girdi kullanım değerleri Çizelge 2'de verilmiştir. Belirlenen gübre ve pestisit değerleri enflasyon nedeniyle kısa sürede anlamını yitirmiş olup bu değerlerin temsil kabiliyeti olan belli ürünler cinsinden ifade edilmesi yerinde olacaktır. Ova grubu etkin işletmeler geneli için hesaplanan gübre kullanımı (132.26 TL da<sup>-1</sup>) 2020 yılı fiyatlarıyla 66 kg da<sup>-1</sup> 15.15.15 gübresine veya 103 kg da<sup>-1</sup> Amonyum sülfat gübresine veya 50 kg da<sup>-1</sup> DAP gübresine denk gelmektedir. Etkin işletmelerin belirlenen ortalama gübre kullanım değeri, Ülgen ve Yurtsever (1995) tarafından Ege Bölgesi sulanan

bağları geneli için önerilen 11-13 kg da<sup>-1</sup> Azot (N), 8-10 kg da<sup>-1</sup> Fosfor (P) ve Potasyum (K) değerlerine uygundur. Etkin işletmeler geneli için hesaplanan dekara ortalama gübre kullanımı, 60 kg 15.15.15 gübresine ile birlikte 9,5 kg Amonyum sülfat gübresine karşılık gelmekte olup bu gübreler kullanıldığında 11 kg N ve 9'ar kg P ve K verilmiş olmaktadır. Pestisit değerinin (408.84 TL da<sup>-1</sup>) karşılığının ise 22 kg da<sup>-1</sup> bordo bulamacı (%20 metalik bakır eşdeğerli) veya 46 kg da<sup>-1</sup> Kükürt (%80, WP) olduğu belirlenmiştir. Genel olarak, verim grupları fark etmeksizin etkin işletmelerin 400 TL da<sup>-1</sup> dolayında pestisit kullandıkları, gübre giderinin ise yüksek verim gruplarında daha fazla olduğu görülmektedir.

Çizelge 2. Ova grubu etkin işletmelerin verim ve girdi kullanım değerleri (ağırlıklı ortalama)  
Table 2. Ova group efficiency enterprises' yield and input usage values (weighted averages)

| Verim Grubu (kg da <sup>-1</sup> ) | İşletme sayısı | Verim (kg da <sup>-1</sup> ) | Gübre (TL da <sup>-1</sup> ) | Pestisit (TL da <sup>-1</sup> ) | Mazot (litre da <sup>-1</sup> ) | İşçilik (EİB da <sup>-1</sup> ) |
|------------------------------------|----------------|------------------------------|------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 700-900                            | 5              | 745.21                       | 217.15                       | 396.04                          | 30.91                           | 7.73                            |
| 500-699                            | 7              | 618.25                       | 130.75                       | 416.33                          | 22.19                           | 7.19                            |
| 300-499                            | 4              | 341.71                       | 20.63                        | 414.29                          | 23.43                           | 5.29                            |
| Genel                              | 16             | 591.12                       | 132.26                       | 408.84                          | 25.50                           | 6.89                            |

Çal grubunda yer alan 33 işletme arasından 15 üreticinin etkin olduğu belirlenmiştir. Bu üreticilerin ortalama verim değeri 127.88 kg da<sup>-1</sup>, gübre girdi tutarı 73.51 TL da<sup>-1</sup>, pestisit girdi tutarı 91.90 TL da<sup>-1</sup>, mazot kullanımı 16.0 litre da<sup>-1</sup> ve kullanılan işçilik miktarının 3.71 EİB da<sup>-1</sup> olduğu belirlenmiştir.

DEAP 2.1 (Coelli, 1996) bilgisayar programının verdiği sonuçlar arasında her bir üreticinin etkin olabilmesi

için azaltması gereken girdi miktarları da bulunmakta olup bu veriler kullanılarak girdi israf oranları hazırlanmıştır (Çizelge 3). Genel olarak, Ova grubunda Çal grubuna göre girdi israfının daha çok olduğu, girdiler arasında en fazla oransal israfın pestisit ve gübre girdilerinde yapıldığı görülmektedir. Ayrıca aynı çıktı miktarının %23.91 daha az arazi kullanılarak elde edilebileceği ortaya çıkmıştır.

Çizelge 3. İsrar edilen girdi oranları (%)  
Table 3. Rates of wasted inputs (%)

| Gruplar   | Alan % | Gübre % | Pestisit % | Mazot % | İşçilik % |
|-----------|--------|---------|------------|---------|-----------|
| Ova grubu | 23.83  | 42.91   | 43.12      | 30.07   | 23.13     |
| Çal grubu | 25.25  | 27.56   | 29.69      | 26.57   | 15.88     |
| Genel     | 23.91  | 42.63   | 42.97      | 29.95   | 22.95     |

DEAP 2.1 (Coelli,1996) bilgisayar programı ile çıktı hedefleri de elde edilmektedir. Çıktı hedefleri potansiyel kabul edilerek gerçek verim değerlerinin hedeflenen değerlere oranlanmasıyla potansiyel kullanım oranları belirlenmiştir (Çizelge 4). İşletmeler genelinde çıktı potansiyelinin %79.37'si

gerçekleştirilmiştir. Etkinlik analizinde kullanılan veriler 2020 hasat yılına ait olup bu yılın rekoltesinin 270 bin ton olduğu açıklanmıştır. Bu bilgilerden hareketle, üretim alanlarının tamamında uygun şartlar gerçekleşir ve çıktı hedeflerine ulaşırsa, 340 bin ton düzeyinde yıllık ürün elde edilebilecektir.

Çizelge 4. Verim hedefleri ve potansiyelin kullanım oranı  
Table 3. Yield targets and utilization ratio of potential

| Gruplar   | Gerçek verim (kg da <sup>-1</sup> ) | Hedef verim (kg da <sup>-1</sup> ) | Potansiyel kullanım (%) |
|-----------|-------------------------------------|------------------------------------|-------------------------|
| Ova grubu | 506.15                              | 638.23                             | 79.31                   |
| Çal grubu | 101.09                              | 118.84                             | 85.06                   |
| Genel     | 483.39                              | 609.04                             | 79.37                   |

## SONUÇ ve ÖNERİLER

Çekirdeksiz kuru üzüm üreticileri için gerçekleştirilen etkinlik analizi sonucunda yüksek sistem bağ teknolojisine sahip olan Ova grubu üreticilerinin etkinlik puanları ortalaması 0,726 iken yer bağlarında ve sulama yapılmadan üreticilik yapılmakta olan Çal grubundaki üreticiler için etkinlik puanı ortalaması 0.815 olarak belirlenmiştir. İşletmeler geneli için grup etkinlik puanları ortalamasının 0.746 olduğu görülmektedir. Bir başka deyişle çekirdeksiz kuru üzüm üretimindeki girdi kullanım etkinliği %74.6 düzeyinde gerçekleşmiştir. Ova grubunda Çal grubuna göre girdi israfının daha fazla olduğu ve israfın oransal olarak daha çok pestisit ve gübrede yapıldığı görülmektedir. Çal yöresinde sulama imkanlarının geliştirilmesi verimliliği arttıracak olup halihazırdaki düşük kimyasal girdi kullanımını ise yörenin organik üretim bölgesi olarak değerlendirilebileceğini düşündürmektedir.

Üretim alanı dışındaki girdilerin etkin kullanılmasıyla işletmelerin toplam brüt karlarında %10 kadar artış olacağı hesaplanmıştır. Girdi israfının azaltılması, gübreleme, ilaçlama ve toprak işlemenin gerektiği kadar yapılmasıyla mümkün olacaktır. En uygun gübreleme programının toprak analizi ile belirleneceği bir gerçek olmakla birlikte çoğu üreticinin önerilen gübreleme programını uygulayabilecek temel düzeyde (gübrelerin besin içerikleri, dozaj hesaplama vb.) yeterli bilgi ve farkındalığa sahip olmadığı da görülmüştür. Ayrıca toprakların genel olarak organik madde bakımından fakir ve pH değerlerinin yüksek olması gübrelemenin etkinliğini azaltmaktadır. Benzer durum zirai ilaçlama uygulamaları için de geçerli olup üretim bölgeleri için bağ hastalıkları tahmin ve erken uyarı sistemlerinin yaygınlaşması, zarar eşiği ve ilaç etki sürelerine dikkat edilmesi etkinliğin sağlanması yönünde fayda sağlayacaktır. Bu bağlamda etkili ve etkin gübreleme ve ilaçlama uygulamaları konusunda üreticilere yapılacak eğitim ve yayım faaliyetleri de önem arz etmektedir. Verim düşüklüğünden kaynaklanan üretim alanı israfının ortadan kaldırılması ise yaşanan ve/veya çeşitli asma odun dokusu hastalıkları ile verimden düşen bağların nitelikli asma fidanlarıyla yenilenmesi ile mümkün olacaktır. Bu doğrultuda asma fidanı üreticilerinin üretim altyapılarını güçlendirecek desteklemelerle birlikte bu üreticilere etkili bir denetimin yapılması fayda sağlayacaktır.

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çalışmasından elde edilmiştir.

## 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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## Assessment of Key Factors Affecting Farmers' Migration Intentions Due to the Syrian Conflict

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### ABSTRACT

This study aims to identify the migration patterns of Syrian farmers in northern Aleppo and to investigate the main factors affecting their migration patterns. In this context, the study data were obtained through face-to-face surveys with a total of 210 farmers from 17 villages in three administrative districts of Aleppo Governorate using a proportional sampling method. The data were analyzed using descriptive statistics and a logistic regression model. According to the results of the study, education level, land size, and household income were found to decrease the tendency to migrate. On the other hand, the factors that increase the tendency of farmers to migrate include being temporarily settled in the region and having family members abroad as refugees. As the war-induced environment has reduced participation in non-agricultural work, more farmers consider agriculture as their main source of livelihood.

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## Suriye Çatışması Sebebiyle Çiftçilerin Göç Niyetlerini Etkileyen Temel Faktörlerin Değerlendirilmesi

### ÖZET

Bu çalışma, Halep'in kuzeyindeki Suriyeli çiftçilerin göç şekillerini belirlemeyi ve göç etme niyetlerini etkileyen temel faktörleri araştırmayı amaçlamaktadır. Bu bağlamda çalışma verileri Halep Valiliği'nin üç ilçesindeki 17 köyde toplam 210 çiftçi ile oransal örnekleme yöntemi kullanılarak yüz yüze anket yoluyla elde edilmiştir. Veriler tanımlayıcı istatistikler ve lojistik regresyon modeli kullanılarak analiz edilmiştir. Araştırma sonuçlarına göre eğitim düzeyi, arazi büyüklüğü ve hane gelirinin göç eğilimini azalttığı tespit edilmiştir. Diğer taraftan, çiftçilerin göç eğilimini artıran faktörler arasında bölgeye geçici olarak yerleşmek ve aile bireylerinin yurt dışında mülteci olarak bulunması yer almaktadır. Savaşın neden olduğu ortam tarım dışı işlere katılımı azalttığından, giderek daha fazla çiftçi tarımı ana geçim kaynağı olarak görmektedir.

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### INTRODUCTION

The Syrian conflict, which started in 2011, is considered very complex because of the diversity of ethical and social factors that have affected the entire situation in Syria, in addition to the multiplicity of parties to the conflict at the local, regional, and international levels. This complexity has made this crisis very difficult (Hove & Mutanda, 2015; Haran, 2016; Phillips & Valbjørn, 2018). Thus, the agriculture sector has also been influenced by the circumstances of war, leading to a decrease in the cultivated area and a subsequent reduction in the total amount of

agricultural production (Mohammed et al., 2020).

Before the war, Syria was considered one of the few nations that had achieved self-sufficiency in a wide range of agricultural products, particularly major crops such as wheat, barley, and cotton (Carnegie Middle East Center, 2015). Significant improvements were also made in the quality of crops and land management through five-year plans, which formed the basis of the directed agricultural economy. In 2010, Syria had amassed a strategic reserve of wheat amounting to 3.5 million tons, distributed across 140 silos. Moreover, agriculture served as the primary

source of income for over 20% of the population before the war, and it employed more than 17% of the workforce (Harmoon Center, 2017). The irrigated lands had been expanded to 1.4 million hectares by 2010 as a result of an increase in the number of dams and irrigation canals, in addition to efforts to control the excessive use of groundwater, especially during drought periods, as witnessed in 2008 (SASG, 2011). In this context, some studies illustrated that the negative impact of climate change can be a reason for the Syrian crisis, where many farmers from the eastern parts of Syria have moved to other cities because of the drought (Mohammed et al., 2020); besides the Syrian government's tendency toward what is called a social market economy following price liberalization policy (Kelley et al., 2015).

Before the outbreak of the war, the government supported an ambitious project aimed at modernizing irrigation. This was achieved by providing farmers with easy loans and encouraging their participation through extension programs sponsored by government institutions. However, as the conflict continued for a decade, affecting the entire Syrian territory, particularly rural areas, the agricultural sector suffered gradual and catastrophic destruction on multiple fronts. This destruction included critical infrastructure, such as irrigation canals, roads, silos, agricultural research centers, agricultural extension services, and seed stores. Furthermore, the systems of marketing and geographical communication, which facilitated the movement of agricultural products between production areas and major markets, were severely damaged. Additionally, there was a gradual and significant shortage of essential agricultural inputs and production requirements, including machinery, irrigation tools, seeds, and fertilizers. The extent of damage inflicted on the agricultural sector was estimated to be approximately 60 percent by the end of 2016 (FAO, 2018). However, 16 billion dollars was estimated as a loss of the Syrian agriculture sector between 2011 and 2016, with 7.2 billion resulting from crop production losses (FAO, 2017). Therefore, a significant loss of Syrian GDP happened due to the damage witnessed in the agricultural sector, especially in the Syrian strategic crops (Mohammed et al., 2020). Similarly, Jaubert et al. (2014) illustrated that 70% of the agricultural production value was lost in the Qusair region, because of the military operations that took place there.

The mass forced displacement of citizens from conflict zones to safer areas poses one of the most significant challenges facing the agricultural sector, leading to a reduction in labour in productive areas. This migration can be classified into two distinct categories: international migration, which involves movement beyond the borders of the country and leads to the permanent loss of labour from the nation, and internal

migration characterized by a transition from a temporary to a semi-permanent status as a consequence of the ongoing war (David et al., 2019; Ahmadzai & Akbay).

Conflicts lead to direct losses in the labour force due to death, disability, displacement from places of conflict, or engagement in hostilities and away from agricultural work (Azam et al., 2022). Therefore, it is essential to identify these losses and specify the current source of labour and its associated costs.

Wars lead to decreased crop yields. For example, wheat production in Syria decreased by as much as 40% in the first two years of the conflict, while Iraq saw a reduction of over 10% in processed food production. During Sierra Leone's civil war in the 1990s, livestock losses reached 70%, while palm oil and rice production declined by over 25% (FAO, 2018). Comparing crop productivity before the war with the time of this study will provide insight into the war's impact on the productivity of essential crops in the study area.

Climate change and drought have exacerbated the conflict's impact on the local population, with water resources often becoming a focal point of the conflict (OCHA, 2021). Conflicts lead to increased production costs due to input scarcity and difficulty in procurement. Additionally, inadequate funding and low returns result in reduced agricultural investment (Arias et al., 2014).

Migration from the Middle East is divided into three types; regular labour migration, forced migration, and mixed migration (IOM, 2015). In the case of Syria, migration can be considered to follow the second and third modes. At the beginning of the war years, it was characterized by the second pattern – forced migration resulting from the violence of military operations. This is supported by an equal distribution of Syrian migrants across gender and age groups. However, even with a relative decrease in conflict intensity, emigration continued due to the devastating economic impact of the war and the absence of hope for economic recovery. Consequently, it shifted to the third type of mixed migration (IOM, 2019). Understanding the determinants of forced migration is a fundamental prerequisite for formulating appropriate policies for prevention, assistance, and resettlement (Engel & Ibanez, 2007). As long as the war continues, it can be predicted that people of Syrian origin will continue to migrate to other countries.

Statistical data obtained from the 2020 Report of the United Nations High Commissioner for Refugees (UNHCR) reveal that roughly 6.7 million individuals undertook internal migration within the country, with approximately 20% of this demographic located in the Euphrates Shield (UNHCR, 2021). Moreover, Syria has been the main country of origin for refugees since 2014. At the end of 2019, there were 6.6 million Syrian

refugees hosted by 126 countries worldwide. The majority of those who migrated (83%) stayed in neighboring countries or the region. In this context, Türkiye continues to host the largest number of Syrian refugees (3.6 million), followed by Lebanon, Jordan, Iraq and Egypt (UNHCR, 2024). Many studies have been conducted internationally on the willingness to migrate (Ibáñez & Velez, 2008; Bohra-Mishra & Massey, 2011; Verwimp & Maystadt, 2015; Bertoli & Ruysen, 2018; Sav & Sayın, 2018; Balcilar & Nugent, 2019; Aslany et al., 2021; Alrababah et al., 2023; Ruhe et al., 2023; Walk et al., 2023). However, there are limited studies on the willingness to migrate of farmers living in rural areas and negatively affected by the war (Czaika & Kis-Katos, 2009). Therefore, this research aims to investigate the key determinants shaping the migration intentions of farmers in northern Aleppo, who are contemplating emigration from Syria. The study offers precise insights into the impact of the Syrian conflict on agricultural practices in northern Aleppo while elucidating the factors that drive farmers' decisions to migrate. Doing so aims to contribute to a deeper understanding of the complex interplay between conflict, agriculture, and migration dynamics in the region.

## MATERIAL and METHOD

### The Study Areas

The northern Aleppo region was selected due to its current security stability and ease of movement for data collection. This part of the Aleppo Governorate includes three regions: Jarabulus, Azaz, and Al-Bab (Table 1). It is considered one of the crucial agricultural areas in Syria, as a portion of it lies along the banks of the Euphrates River, boasting several major irrigation projects. Before the war, it was considered one of the most important areas for the production of Syria's two strategic crops: wheat and cotton (SASG, 2011). A significant portion of the population was engaged in rural occupations. Currently, this area continues agricultural production, albeit with changes in the types of crops, sources of agricultural inputs, production quantities, and working conditions.

This region is connected to Türkiye through efficient commercial border crossings, with Türkiye serving as a vital lifeline for this area. The study area shares similarities with most Syrian agricultural regions regarding its exposure to the direct and indirect effects of the conflict. Therefore, the research results can be considered relatively representative of the situation in other regions. The geographical location of the region, serving as a gateway for goods, commodities, and production elements coming from Türkiye to Syria, also acts as a route for Syrian travelers to access international destinations via Turkish airports.

### Study Design and Data

The study data were collected through the proportional sampling method. In this method, the studied community was divided according to the administrative regions in the province, and the data was gathered from three specific administrative regions within the province of Aleppo: Al-Bab, Azaz, and Jarabulus. These areas currently enjoy security stability and have experienced a gradual improvement in services, reminiscent of their pre-war conditions. They still serve as significant agricultural production areas profoundly affected by the war. Additionally, several international organizations and independent entities operate within these regions (Maharramov, 2022). By using the proportional sampling method (with a 5.5% margin of error and 90% confidence interval), a total of 210 questionnaire forms were collected from 13 villages across the three administrative regions of Aleppo province, as detailed in Table 1. Data collection via the questionnaire took place between February 1, 2021, and March 3, 2021.

Simultaneously, they attract refugees and serve as corridors for refugees crossing into Türkiye to settle there or continue to other destinations. These administrative regions differ from one another in terms of cultivated areas and available water sources. The data were collected through personal interviews with farmers using a questionnaire prepared according to the research objectives. The questionnaire was divided into several sections, each containing questions relevant to the respective section's focus.

### Data analysis

The descriptive analysis approach was followed in the study. Regardless of the level of education, closed-ended or multiple-choice questions were asked to the subjects for ease of data collection and to shorten the time to complete the data required for the research without grumbling. To analyze whether surveyed individuals would like to immigrate to another country, we used the following question: '1' represents the desire to migrate outside the country, and zero otherwise. In data analysis, the binary logistic regression model was employed due to the nature of the data and the binary type of the dependent factor. This research is grounded in the following hypothesis: being a temporarily settled person significantly impacts one's desire to emigrate from the country. The logistic regression equation takes the following form (Draper & Smith, 1981; Ağır and Akbay, 2018):

$$Prob(y = 1) = \frac{e^{x\beta}}{1 + e^{x\beta}} = f(x\beta)$$
$$odds (Exp B) = \frac{P}{1 - P}$$

$$\text{Log e} \left( \frac{p}{q} \right) = \beta_0 + \sum_{i=1}^k \beta_i X_i$$
$$i = 1, 2, \dots, \dots, k$$

In the equation,  $p$  is a desire to emigrate,  $q=(1-p)$  is an unwillingness to emigrate,  $f(x\beta)$  is the standard logistics distribution function,  $\text{Log} e(\frac{p}{q})$  or  $\text{Ln}(\frac{p}{q})$  is the Logit transformation,  $X_i$  is the explanatory variables such as age, gender, education, income, farm size, conflict, labour status, and  $\beta_i$  are coefficients of corresponding variables as explained in Table 4. The odds ratio ( $\text{Exp } \beta$ ) shows how many times (relatively) a

unit increase in this variable increases the probability of migration of a business for a single explanatory variable if all other variables are kept constant. According to migration research, there are a variety of socioeconomic and demographic individual and farm-level variables that influence migration such as age, gender, education, income, farm size, conflict, labour status, etc (Migali & Scipioni, 2019; Aslany et al., 2021; Ruhe et al., 2023).

Table 1. Distribution of the sample according to regions  
*Çizelge 1. İşletmelerin bölgelere göre dağılımı*

| District | Village         | Frequency | Per cent |
|----------|-----------------|-----------|----------|
| Jarablus | Gandora         | 16        | 7.6      |
|          | Jamel           | 16        | 7.6      |
|          | Al jamel        | 15        | 7.1      |
|          | Jarablus markez | 15        | 7.1      |
| Al-Bab   | Al-Bab          | 17        | 8.1      |
|          | Bzaha           | 15        | 7.1      |
|          | Al rahi         | 15        | 7.1      |
|          | Kabaseen        | 17        | 8.1      |
|          | Tadef           | 15        | 7.1      |
| Azaz     | Azaz            | 18        | 8.6      |
|          | Ahtareen        | 17        | 8.1      |
|          | Mareh           | 17        | 8.1      |
|          | Dabek           | 17        | 8.1      |
| Total    |                 | 210       | 100.0    |

## RESULTS and DISCUSSION

According to the survey results, all farmers in the region were male and married, with an average age of 46.8 years. Among these farmers, 44.8% had completed primary or middle school, 36.7% had finished high school, and 19.1% held university degrees. The average household size was 6.5. All analyzed respondents were individuals engaged in farming. They were asked about their birthplaces and whether they originated from the regions where they currently reside. The results indicated that 83.8% of farmers were originally from the region (natives).

Before the war, this area was among the regions where

the government implemented an agricultural plan, specifically targeting wheat and cotton crops. The plan aimed to support farmers by providing improved seeds, affordable fertilizers, guidance from agricultural extension services, and, importantly, purchasing these crops from farmers at an annually fixed price.

During the study period, there was a significant diversification in the cultivated crops. Apart from continuing to cultivate the crops they had grown before the war, farmers' reasons for selecting cultivated crops had changed from their previous motivations. Their responses were ranked in order of importance, as shown in Table 2.

Table 2. Reasons for choosing cultivated crops due to their importance  
*Çizelge 2. Seçilen ürünlerin önem düzeylerine göre seçilme nedenleri*

| Reasons for choosing the type of crop                             | Frequency | Percentage |
|---|-----------|------------|
| According to market requirements and expected return              | 103       | 49,05      |
| Because of my accumulated experience in these crops               | 44        | 20,95      |
| Because of the scarcity of water, its low requirements for water  | 32        | 15,24      |
| Because of the comparatively low cost of seeds and service        | 23        | 10,95      |
| I grow the same crops according to the previous government's plan | 8         | 3,81       |
| Total   | 210       | 100,00     |

The reasons for farmers' choosing crop types include market demand and expected financial returns, capitalizing on accumulated expertise in growing particular crops, prioritizing crops with low water requirements due to water scarcity, preference for

crops with relatively cheap seeds and services, and adhering to the crop selection outlined in the agricultural plan that guided the previous government agricultural policies.

Table 3 presents results indicating a significant



reduction in the number of individuals engaged in alternative employment outside of farming. Before the war, the non-agricultural labour force participation rate stood at a robust 62%. However, in the aftermath of the war, this figure took a substantial hit, plummeting to 38%. Consequently, there is a rise in the number of individuals relying on agriculture as their sole source of income compared to the pre-war period. This suggests that the war has likely disrupted other economic activities, leading more people to rely on agriculture for their livelihoods. The interruption of irrigation canals and the control of dams by hostile forces have resulted in a significant decline in farmers' ability to access irrigation water. This limitation has severely impacted crop yields and agricultural productivity, exacerbating food insecurity in affected regions. Additionally, the results reveal that there has been a serious decrease in the number of people satisfied with the sales prices of agricultural products compared to the pre-war period. This illustrates the economic strain and market disruptions faced by farmers, possibly due to decreased demand or other market dynamics affected by the war. Table 4 provides summary statistics and a complete description of the variables used in the logistic regression model. The means are presented with standard deviations. According to the table, 61.4% of farmers desire to migrate outside the country.

Table 5 displays the results of binary logistic regression. The Chi-square value of 72.989 indicates that the model is a significant fit for the data at a p-value of 0.00. Finally, the results suggest that there are no issues of multicollinearity among the predictor variables in the model. Therefore, the absence of

multicollinearity ensures the reliability of the model. The logistic regression model's accuracy of 0.781 indicates that the model correctly predicts the outcome for approximately 78.1% of the observations in the dataset. Notably, the regression coefficients for ten variables were found to be statistically significant, as presented in Table 5.

The results show that individuals originally from the region are less likely to migrate out of the country than farmers localized to the study area from other regions for different reasons and at different times. Natives have approximately 83 percent lower odds of wishing to migrate than later settlers to the region. Consequently, they believe that migrating abroad might offer a more economically viable and expeditious path to rebuilding their lives. This observation aligns with the findings of the IOM (2015), which identified a higher percentage of individuals wishing to emigrate abroad among Palestinians residing in refugee camps compared to those living outside the camps.

An intriguing observation is the inverse relationship between educational level and the intensity of the desire to emigrate. This result appears to be at odds with the findings of David et al. (2019), who reported that in Egypt, the inclination to emigrate increased as educational levels rose. This increase was primarily attributed to the high unemployment rate among university and institute graduates in Egypt. However, the Syrian context presents a different scenario, where mass displacements from various regions have led to an increase in unemployment rates among individuals with low levels of education, especially those working in the agricultural sector.

Table 3. Comparison of some inputs with the pre-war period  
*Çizelge 3. Bazı girdilerin savaş öncesi dönemle karşılaştırılması*

|                                    | Before the war |     | At the time of the study |     | P-value |
|------------------------------------|----------------|-----|--------------------------|-----|---------|
|                                    | No             | Yes | No                       | Yes |         |
| Having a job (other than farming)* | 76             | 134 | 130                      | 80  | 0,042   |
| Obtaining agricultural loans       | 56             | 154 | 90                       | 120 | 0,371   |
| Modern irrigation finance          | 44             | 166 | 207                      | 3   | 0,492   |
| Easy access to irrigation water*   | 82             | 128 | 144                      | 66  | 0,038   |
| Difficulty in getting inputs       | 182            | 28  | 77                       | 133 | 0,298   |
| the cost of the inputs (expensive) | 127            | 83  | 35                       | 175 | 0,547   |
| Satisfaction with the sale price*  | 144            | 66  | 188                      | 22  | 0,028   |
| Availability of labor              | 167            | 43  | 155                      | 55  | 0,351   |

\*: statistically important at  $\alpha=0.05$  level.

The research results reveal a noteworthy trend: the greater the number of first-degree immigrant relatives a person has, the stronger their desire to immigrate. This observation can be attributed to immigrants' expectations of receiving support and guidance from their relatives when embarking on their new lives in a foreign society. Additionally, these individuals may develop a more informed and positive perception of the new society through their immigrant relatives. This

finding corroborates the observations made by Khater (2001), who noticed a higher percentage of individuals aspiring to travel in societies with a substantial number of overseas immigrants, as evidenced in Lebanon.

The size of the farm emerged as a statistically significant factor, albeit in a negative manner. It was observed that an increase in the size of the farm correlated with a decrease in the desire to emigrate outside the country. This trend could be attributed to

an increase in income associated with larger farms and the subsequent increase in agricultural activities. This result is similar to the study of Aslany et al. (2021) showing that farms with large land areas have a low willingness to migrate. The income factor further substantiates this relationship, which also

demonstrated statistical significance in a negative direction. Specifically, as income levels rise, the desire to emigrate diminishes. This observation suggests that individuals with higher incomes may be less inclined to seek opportunities abroad.

Table 4. Description of the variables included in the model  
*Çizelge 4. Modeldeki değişkenlerle ilgili tanımlayıcı istatistikler*

| Variables                             | Variable description   | Mean  | Standard deviation |
|---------------------------------------|--|-------|--------------------|
| Desire to migrate outside the country | Wants to immigrate =1; otherwise = 0   | 0.614 | 0.487              |
| Later settlers                        | Originally from the region (Natives) =1; Being originally displaced from another area (Later settlers to the region = 0  | 0.823 | 0.381              |
| Age1                                  | Younger than 40 years = 1; Otherwise = 0   | 0.323 | 0.469              |
| Age2                                  | Between 40 and 55 years = 1; Otherwise = 0   | 0.481 | 0.500              |
| Age3                                  | Older than 55 years = 1; Otherwise = 0   | 0.195 | 0.397              |
| Edu1                                  | Elementary or Middle School graduate =1; Otherwise = 0   | 0.447 | 0.498              |
| Edu2                                  | Secondary or high school graduate =1; Otherwise = 0  | 0.357 | 0.480              |
| Edu3                                  | University and above=1; Otherwise = 0  | 0.195 | 0.397              |
| Ware injury                           | Injury to self or family member in war =1; Otherwise = 0   | 0.176 | 0.381              |
| Difficulty working                    | Difficulty continuing the same work or economic activity he was practising before the war =1; Otherwise = 0              | 0.571 | 0.496              |
| Immigrants                            | The presence of one or some family members outside the country and the desire to be reunited with them =1; Otherwise = 0 | 0.557 | 0.497              |
| Obtaining assistance                  | Obtaining regular assistance from organizations operating in the area =1; Otherwise = 0                                  | 0.390 | 0.489              |
| Farm size 1                           | Small farms (<26 decare) = 1; 0 otherwise  | 0.304 | 0.461              |
| Farm size 2                           | Middle farms (26 -50 decare) = 1; 0 otherwise  | 0.371 | 0.483              |
| Farm size 3                           | Big farms (51-100 decare) = 1; 0 otherwise   | 0.252 | 0.435              |
| Farm size 4                           | Very big farms (>100 decare) = 1; 0 otherwise  | 0.071 | 0.258              |
| Income 1                              | Low income (≤\$100) = 1; 0 otherwise   | 0.681 | 0.467              |
| Income 2                              | Medium income (\$101-\$200) = 1; 0 otherwise   | 0.238 | 0.426              |
| Income 3                              | High income (>\$200) = 1; 0 otherwise  | 0.081 | 0.273              |

Table 5. Coefficient estimates of the logit model for analysis of the Factors affecting farmers' desire to migrate outside the country

*Çizelge 5. Çiftçilerin ülke dışına göç etme istekliliğini etkileyen faktörlerin analizi için logit modeli katsayı tahminleri*

| Variables                                   | Coefficient | Std. Error | P-value | Exp(B) |
|---|-------------|------------|---------|--------|
| Constant***                                 | 1,911       | 0,560      | 0,001   | 6,759  |
| Later settlers***                           | -1,746      | 0,625      | 0,005   | 0,175  |
| Age2  | 0,182       | 0,402      | 0,650   | 1,200  |
| Age3  | -0,215      | 0,543      | 0,693   | 0,807  |
| Edu2**                                      | -0,799      | 0,403      | 0,047   | 0,450  |
| Edu3***                                     | -1,526      | 0,511      | 0,003   | 0,217  |
| War injury*                                 | 0,982       | 0,549      | 0,074   | 2,670  |
| Difficulty working                          | 0,145       | 0,355      | 0,683   | 1,156  |
| The presence of immigrants in the family*** | 1,047       | 0,370      | 0,005   | 2,850  |
| Obtaining assistance                        | -0,432      | 0,374      | 0,249   | 0,649  |
| FarmSize2*                                  | -0,774      | 0,469      | 0,099   | 0,461  |
| FarmSize3***                                | -1,379      | 0,512      | 0,007   | 0,252  |
| FarmSize4***                                | -2,556      | 0,736      | 0,001   | 0,078  |
| Income2***                                  | -1,835      | 0,417      | 0,000   | 0,160  |
| Income3**                                   | -1,572      | 0,617      | 0,011   | 0,208  |

Overall Percentage: 0,781; Nagelkerker R<sup>2</sup> : 0,399; The Chi-square value: 72,989; P-value: 0,000

\*, \*\*, \*\*\* indicate that the coefficient is statistically significant at the 10%, 5% and 1% level.

Higher-income levels often provide individuals with greater financial stability, access to better education, healthcare, and overall quality of life. Additionally, by providing advancement opportunities within their home country, it reduces the perceived need to relocate and potentially strengthens one's ties to their place of residence. This finding aligns with the outcomes of numerous studies, such as those by Justino (2011), Bertoli & Ruyssen (2018), Migali & Scipionii (2019), Aslany et al. (2021), and OCHA (2021), which consistently linked a heightened willingness to immigrate with lower income levels.

Conflicts in the region can often directly or indirectly affect the entire population living in the conflict-affected region. Therefore, wars and conflicts often threaten all age groups. However, model results show that age does not affect willingness to migrate.

## RESULTS and RECOMMENDATIONS

Studying the effects of war, such as the protracted Syrian conflict, which endured for many years and continues to this day with relative calm in most regions, demands an extensive body of research and numerous studies to comprehensively monitor these effects. The paramount importance of this study lies in its specific focus on a particular geographic area, the results of which can be extrapolated to other regions due to their analogous circumstances. With the current stability in northern Aleppo, the agricultural sector has initiated its recovery. However, it is of utmost importance to delve into the repercussions of the prolonged years of warfare on specific agricultural activities and practices. This study will enable the development of sound policies that can actively support the revival of this sector.

Furthermore, this study has investigated the root causes that push farmers to migrate from their home countries. Additionally, it examines the long-term consequences on the nation itself and its neighboring countries, particularly those striving to create secure conditions for the safe return of Syrian refugees to their homeland. The research findings unveil a substantial increase in the prices of agricultural inputs, coupled with the difficulties in sourcing them. As a result, local authorities must prioritize the task of securing these essential supplies at reasonable prices and maintaining the desired quality standards. This is particularly significant in light of the possibility of importing supplies through the open Turkish borders. The study identifies a discernible decline in the market prices of agricultural products, which do not align with the elevated production costs. This discordance is a result of deficiencies in the marketing process and the exploitation of certain traders. Therefore, it becomes imperative for the responsible authorities to regulate local markets and procure essential crops, such as wheat and cotton, from farmers at prices that are on

par with those in the neighboring Turkish market. The study also underscores the decrease in farmers' access to irrigation water, a challenge that extends beyond mere drought-related issues. Consequently, authorities must actively seek and implement solutions to address the problems associated with suspended irrigation canals. They should also facilitate the establishment of new wells and secure affordable fuel prices, given the increased reliance on pump-based irrigation systems. Moreover, it is crucial to support farmers by guiding them towards adopting state-of-the-art irrigation technologies. This can be achieved by ensuring that the necessary components are readily available at competitive prices, offering accessible loans, and fostering their adoption through comprehensive agricultural extension campaigns. Regarding the desire to migrate from the country once the security situation stabilizes, the study discerns that improving income levels, enhancing the work environment, providing consistent support for farmers, raising the educational levels of both farmers and their families, and assisting originally displaced individuals in the region all contribute significantly to reducing their inclination to seek migration opportunities abroad.

## Researchers' Contribution Rate Statement

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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## Ankara İlinde Memur Tüketicilerin Arı Ürünleri Tüketimi ve Tüketim Tercihleri

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### ÖZET

Bu çalışmanın amacı, Ankara ili kent merkezindeki memur tüketicilerin arı ürünleri tüketiminde bilinç düzeyini ortaya koymak, satın alma ve tüketme sıklıklarını ve arı ürünleri satın almada önem verdikleri konuları belirlemektir. Araştırmada örnek hacmi “Ana kitle Oranlarına Dayalı Basit Tesadüfi Olasılık Örneklemesi” yöntemi ile belirlenmiş ve 300 tüketici ile yüz yüze görüşülmüştür. Tüketicilerin satın alma sıklığı en yüksek balda bulunmuştur (4.15). Tüketicilerin arı sütü satın alma sıklığı bal mumunda ortalama 1.13 ve arı zehrinde ise 1.05'tir. Bal dışındaki diğer arı ürünlerinde satın alma ve tüketim sıklığının düşük olduğu belirlenmiştir. Tüketicilerin büyük çoğunluğu arı ürünlerinin sağlıklı ve besleyici ürünler olduğunu düşünmektedir. Tüketiciler arı ürünlerini satın alırken marka ve ambalaj konusuna önem vermektedir. Markalı ürünler tüketiciler açısından güvenli ürünler olarak bilinmektedir. Balı satın alırken %97.6 oranında cam kavanozda almayı, %63.3 oranında ise markalı almayı tercih etmektedirler. Tüketicilerin gelir düzeyi arttıkça satın aldıkları yerler farklılaşmaktadır. Çalışma sonucunda tüketicilerin arı ürünleri hakkında bilgi sahibi oldukları ancak bal dışındaki diğer arı ürünlerinde satın alma ve tüketim sıklığının artması için ürünlerin sağlıklı ve faydalı ürünler olduğu konusunda tanıtımların artması gerektiği, fiyatların daha makul düzeyde oluşması ile tüketim miktarlarının artacağı sonucuna varılmıştır.

### Makale Konusu

### Araştırma Makalesi

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### Anahtar Kelimeler

Ankara ili  
Tüketici  
Arı ürünleri  
Marka  
Ambalaj

## Consumption of Bee Products and Consumption Preferences of Civil Servant Consumers in Ankara Province

### ABSTRACT

This study aims to reveal the awareness level of civil servant consumers in the city center of Ankara regarding the consumption of honeybee products, to determine their purchasing and consumption frequency, and the issues they attach importance to in purchasing honeybee products. In the research, the sample volume was determined by the "Simple Random Probability Sampling Based on Main Population Proportions" method and 300 consumers were interviewed face-to-face. The highest purchasing frequency of consumers was found in honey (4.15). The average frequency of consumers purchasing royal jelly is 1.13 for beeswax and 1.05 for honeybee venom. It has been determined that the frequency of purchase and consumption of other honeybee products other than honey is low. The majority of consumers think that bee products are healthy and nutritious. Consumers attach importance to brand and packaging when purchasing honeybee products. Branded products are known to be safe for consumers. When purchasing honey, they prefer to buy it in glass jars 97.6% of the time and branded products 63.3% of the time. As the income levels of consumers increase, the purchasing places differ. As a result of the study, it was concluded that consumers are knowledgeable about honeybee products, but in order to increase the frequency of purchase and consumption of honeybee products other than honey, promotions of the products as healthy and beneficial products should be increased, and consumption amounts will increase as prices

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## GİRİŞ

Arıcılık; az bir sermaye ile toprağa bağlı olmadan yapılabilen, kısa sürede gelir getiren, farklı arı ürünleri sağlayan ve dünyada önemi giderek artan bir tarımsal uğraşı alanıdır. Arı ürünleri ekolojik denge ve beslenme açısından önemlidir (Gürel & Göstert, 2004; Tunca ve ark., 2015; Niyaz & Demirbaş, 2017). Arıların bitki tozlaşmasını sağlayan görevleri sayesinde, evrende birçok tür sürdürülebilirliğini sağlamaktadır (Korkmaz, 2003; Engindeniz ve ark., 2014; Niyaz & Demirbaş, 2017). Arıcılık faaliyeti ile bal, polen, propolis, arı sütü, arı zehri, bal mumu gibi arı ürünleri de elde edilmektedir (Kumova & Korkmaz, 2001; Bölüktepe & Yılmaz, 2008; Niyaz & Demirbaş, 2017). Arı ürünleri insan sağlığı açısından oldukça önemli ürünlerdir. Arı ürünlerinden biri olan bal özellikle kahvaltılarımızda sıkça tükettiğimiz bir üründür. İçerisinde karbonhidrat, mineral, vitamin, organik asit, enzim ve fenolik bileşikler bulunmaktadır (Mutlu ve ark., 2017; Saral & Yılmaz Yavuz, 2020). Bal yara iyileşmesi ve ülser tedavisinde kullanılmaktadır (Saral & Yılmaz Yavuz, 2020). İçeriğinde polen, protein, karbohidrat, lipid, vitamin ve mineral bulunmaktadır (Campos ve ark., 2003; Saral & Yılmaz Yavuz, 2020). Propolisin içeriğinde reçine, bal mumu, uçucu yağ bulunmaktadır. Kansersiz hücrelerin gelişimine engel olduğu ve karaciğer hasarını engellediği belirtilmiştir (Inoue ve ark., 2008; Saral ve ark., 2016; Saral ve Yılmaz Yavuz, 2020). Arı sütünün içeriğinde protein, şeker, lipid vitamin, serbest amino asitler ve biyoaktif bileşenler bulunmaktadır (Zong & Wu, 2014; Saral & Yılmaz Yavuz, 2020). Arı zehiri apamin, mellitin, fosfolipaz A2 gibi bileşenlerden oluşmaktadır. Romatoid artirit, MS (Multiple Sclerosis), ve kanser gibi hastalıkların tedavisinde kullanılmaktadır (Son ve ark., 2007; Saral & Yılmaz Yavuz, 2020). Bal mumu yüksek oranda alkali esterler, serbest yağ asitleri ve hidrokarbonlar içermektedir (Doğaroğlu, 2008; Saral & Yılmaz Yavuz, 2020). Yaraları, iltihabı ve yanıkları iyileştirmede etkili olduğu bildirilmiştir (Fratini ve ark., 2016; Saral & Yılmaz Yavuz, 2020).

Dünya'da kovan sayısı 2021 yılında bir önceki yıla göre %2,2 oranında artarak 102 milyon olmuştur. 2021 yılı verilerine göre, dünya toplam kovan sayısında Hindistan %12,6'lık pay ile ilk sırada yer alırken, Çin %9,1 pay ile ikinci sırada, Türkiye %8.6 pay ile üçüncü sırada yer almaktadır. Toplam kovan sayıları 2021 yılında bir önceki yıla oranla Hindistan'da %2,1, Çin'de %0,3 ve Türkiye'de ise %6,8 oranında artmıştır (TEPGE, 2023). TÜİK, 2024 yılı verilerine göre 2023

yılında arıcılık yapan işletme sayısı 100.399, yeni kovan sayısı 8.969.387, üretilen bal miktarı 114.886 ton olarak belirlenmiştir. 2019 yılı itibariyle kişi başına bal tüketimi 1.25 kg ve kişi başına bal üretimi ise 1.31 kg olarak bulunmuştur. Üretimin tüketimi karşılama oranı %94.96'dır (Şengül, 2020; Onuç & Saner 2022).

Bir çok dünya ülkesinde toplum bilinci hem üretici hemde tüketici açısından oluşmuştur. Türkiye'de yüksek bir üretim potansiyeli mevcuttur ancak yeterli bilinç olmaması ekonomik kayba sebep olmaktadır (Kumova & Korkmaz, 1998; Samancı & Sunay, 2011; Denizli Akdemir & Dağdemir, 2021). Arı ürünleri tüketiminde birçok faktör etkili olmaktadır. Tutum, algılama, seçim yapabilme, inanç ve değer yargıları tüketicilerin arı ürünleri tercihlerini etkilemektedir (Kavas, 1987; Denizli Akdemir & Dağdemir, 2021). Ancak arı arı ürünleri tüketim tercihlerine yönelik yapılan çalışmalarda özellikle bal üzerinde yoğunlaşmıştır (Merdan & Durmuş, 2018; Denizli Akdemir & Dağdemir, 2021).

Türkiye Dünya'da arıcılık ve bal üretimi bakımından ilk sıralarda olmasına rağmen; polen, propolis ve arı sütü üretim ve tüketim sıralaması bakımından alt sıralarda yer almaktadır (Saral, 2013; Denizli Akdemir & Dağdemir, 2021). Arı ürünleri tüketimi ile ilgili çalışmalar literatürde yer almaktadır (Murphy ve ark., 2000; Gambaro ve ark., 2007; Niyaz & Demirbaş, 2017; Marangoz & Tayçu Dolu, 2019; Aytıp ve ark., 2019; Karahan & Özmen Özbakır., 2020; Saral & Yılmaz Yavuz, 2020; Şahinler ve ark., 2021; Denizli Akdemir & Dağdemir 2021; Onuç & Saner, 2022; Gündal & Agayeva, 2022; Yüzbaşıoğlu, 2022). Ancak literatürde Ankara ilinde arı ürünleri tüketimi ile ilgili çalışmaya rastlanmamıştır. Bu çalışma ile Ankara ilinde memurlara yönelik olarak arı ürünleri tüketiminde bilinç düzeyini ortaya koymak, tüketicilerin arı ürünleri satın alma ve tüketme sıklıklarını belirlemek, arı ürünleri satın almada önem verdikleri konular ve bazı demografik ve ekonomik özellikler arasındaki ilişkileri ve arı ürünleri satın aldıkları yer tercihini belirlemek, ambalaj ve markalı ürün kullanımında taleplerini ortaya koymak amaçlanmıştır.

## MATERYAL

### Materyal

Araştırmanın ana materyalini Ankara ili kentsel alanda yaşayan ve memur olan tüketicilerden anket yöntemi ile elde edilen birincil veriler oluşturmuştur. Çalışmada kullanılan anket formları arı ürünleri

tüketimine yönelik olarak daha önce yapılmış çalışmalar incelenerek (Niyaz & Demirbaş, 2017; Marangoz & Tayçu Dolu, 2019; Karahan & Özmen Özbakır.,2020; Saral & Yılmaz Yavuz, 2020 ; Şahinler ve ark.,2021; Denizli Akdemir & Dağdemir 2021; Onuç & Saner, 2022; Gündal & Agayeva, 2022; Yüzbaşıoğlu, 2022) ve araştırmacılarca geliştirilerek hazırlanmıştır. Ayrıca araştırmada ikincil verilerden de yararlanılmıştır.

### Metotlar

Araştırma verileri 2022 yılında elde edilmiştir. Örnek hacmi "Anakitle Oranlarına Dayalı Basit Tesadüfi Olasılık Örneklemesi" yöntemi ile belirlenmiştir (Malhotra, 2004).

$$n = z^2 (p.q)/d^2 \quad (1)$$

n: Örnek büyüklüğü

z: 1,64 (%90 güven düzeyine karşılık gelen standart z değeri),

p: incelenen konuyla ilgili ön bilgi ya da tahmine dayalı olarak belirli bir özelliğe sahip ana kitle oranı (0.5 olarak belirlenmiştir).

q: (1-p) İlgili özelliğe sahip olmayan ana kitle oranı

d: Kabul edilen hata tolerans düzeyi ±%5 olarak kabul edilmiştir.

Çalışmada %90 güven aralığı ve %5 hata payına göre örnek hacmi 278 olarak hesaplanmıştır. Anket sayısı 300'e tamamlanmıştır. Verilerin analizinde SPSS 22 paket programı kullanılmıştır. Likert ölçeği kullanılarak arı ürünleri tüketimi ilgili sorulara cevaplar aranmıştır. Verilerin değerlendirilmesinde oransal dağılım ve frekans tabloları, çapraz tablolar kullanılmış, Mann Whitney U, Kruskal-Wallis H testleri yapılmıştır.

Bu çalışma için etik onay ve izin HMKÜ Sosyal ve Beşeri Bilimler Araştırmaları Etik Kurulu'ndan alınmıştır. Toplantı tarihi 07.04.2023, toplantı sayısı 02, karar no: 31,sayfa no: 5/6

### BULGULAR ve TARTIŞMA

Araştırma kapsamında olan tüketicilerin bazı demografik ve ekonomik özellikleri Çizelge 1'de verilmiştir. Tüketicilerin %50'sinin kadın %50'sinin ise erkeklerden oluştuğu belirlenmiştir. Denizli Akdemir & Dağdemir (2021), Erzurum ilinde yaptıkları çalışmada tüketicilerin %44.25'inin erkek, %55.75'inin kadın olduğunu belirlemişlerdir. Onuç & Saner (2022), İzmir ilinde yaptıkları çalışmada öğrencilerin %52.90'ının kız, %47.10'unun erkek olduğunu, Sayılı (2012), Tokat ilinde yaptığı çalışmada tüketicilerin %78,31'inin erkek ve %21,69'unun kadınlardan oluştuğunu, Yüzbaşıoğlu (2022) çalışmasında tüketicilerin %49,63'ünün erkek, %50.37'sinin kadın olduğunu belirlemiştir.

Araştırmada tüketiciler yaşlarına göre 3 gruba ayrılmıştır. Birinci grup tüketiciler 0-30 yaş, ikinci

grup tüketiciler 31-50 yaş ve üçüncü grup tüketiciler ise 50 yaş ve üzerindedir. 0-30 yaş grubu %19.3, 31-50 yaş grubu %59.3, 50 ve üzeri yaş grubu ise %21.4 oransal paya sahiptir. İncelenen tüketicilerin aileleri %59.7 oranında 1-3 kişiden oluşurken, %40.3 oranında ise 4-6 kişiden oluşmaktadır. Tüketicilerin %3.3'ü ilköğretim, %22.7'si lise %18.3'ü yüksek okul ve %55.7'si ise üniversite mezunlarından oluşmaktadır. Marangoz & Tayçu Dolu (2019) Türkiye'de yaptıkları çalışmada incelenen tüketicilerin, ilköğretim mezunu 4 kişi, ilköğretim mezunu 11, lise mezunu 32, ön lisans mezunu 45, lisans mezunu 103 kişi ve lisansüstü mezunu olarak da 113 kişiden oluştuğunu, Sayılı (2013), yılında Tokat ilinde yaptığı araştırmada tüketicilerin %8.09'unun doktora, %7.72'sinin yüksek lisans, %34.92'sinin üniversite, %9.56'sının ilköğretim ve %6.62'sinin ortaokul, %33.09'unun lise mezunu olduğunu, Niyaz & Demirbaş (2017) Çanakkale ilinde yaptıkları çalışmada tüketicilerin %2.3'ünün okur-yazar %24'ünün ilköğretim, % 35.4'ünün lise, %36.6'sinin üniversite, %1.7'sinin de yüksek lisans mezunu olduğunu, Şahinler ve ark. (2021), Uşak ilinde yaptıkları çalışmada tüketicilerin %50'sinin üniversite mezunu, %16'sının lise, %8'inin ortaokul, %10'unun ilköğretim mezunu olduğunu belirtmişlerdir.

İncelenen tüketicilere Ankara'da ikamet ettikleri süre sorulmuştur. Tüketicilerin %15.3'ü 0-5 yıldır, %27.3'ü 6-15 yıldır, %24.3'ü 16-30 yıldır, %33'ü ise 30 ve daha fazla yıldır ikamet ettiklerini söylemiştir. Tüketiciler gelirlerine göre üç gruba ayrılmıştır. Birinci grup 10 000 TL'ye kadar gelirleri olanlar olup, %46.3 oransal paya sahiptir. İkinci grup 11 000 TL ile 20 000 TL arasında geliri olanlardır. Bu gruptaki tüketicilerin oranı %46.7'dir. Üçüncü gelir grubu 21 000 TL ve üzeri olup bu grup tüketicilerin oranı %7'dir. Tüketiciler aylık gıda harcamalarına göre 3 gruba ayrılmış olup 0-5000 TL gıda harcaması yapanların oranı %77.3, 5 001-10 000 TL gıda harcaması yapanların oranı %21.4, 10 001 TL'den fazla yapanların oranı ise %1.3'tür. Tüketicilerin arı ürünlerine yaptıkları yıllık harcamalar gruplara ayrılmıştır. 1 000 TL'ye kadar harcama yapanların oranı %71.7, 1 001-5 000 TL harcama yapanların oranı %26.3, 5000 TL ve üzeri harcama yapanların oranı ise %2 olarak belirlenmiştir (Çizelge 1).

Araştırma kapsamında olan tüketicilerin bazı demografik ve ekonomik özellikleri Çizelge 1'de verilmiştir. Tüketicilerin %50'sinin kadın %50'sinin ise erkeklerden oluştuğu belirlenmiştir. Denizli Akdemir & Dağdemir (2021), Erzurum ilinde yaptıkları çalışmada tüketicilerin %44.25'inin erkek, %55.75'inin kadın olduğunu belirlemişlerdir. Onuç & Saner (2022), İzmir ilinde yaptıkları çalışmada öğrencilerin %52.90'ının kız, %47.10'unun erkek olduğunu, Sayılı (2012), Tokat ilinde yaptığı çalışmada tüketicilerin %78,31'inin erkek ve %21,69'unun kadınlardan oluştuğunu, Yüzbaşıoğlu (2022)



çalışmasında tüketicilerin %49,63'ünün erkek, %50,37'sinin kadın olduğunu belirlemiştir.

Araştırmada tüketiciler yaşlarına göre 3 gruba ayrılmıştır. Birinci grup tüketiciler 0-30 yaş, ikinci grup tüketiciler 31-50 yaş ve üçüncü grup tüketiciler ise 50 yaş ve üzerindedir. 0-30 yaş grubu %19,3, 31-50 yaş grubu %59,3, 50 ve üzeri yaş grubu ise %21,4 oransal paya sahiptir. İncelenen tüketicilerin aileleri %59,7 oranında 1-3 kişiden oluşurken, %40,3 oranında ise 4-6 kişiden oluşmaktadır. Tüketicilerin %3,3'ü ilköğretim, %22,7'si lise %18,3'ü yüksekokul ve %55,7'si ise üniversite mezunlarından oluşmaktadır. Marangoz & Tayça Dolu (2019) Türkiye'de yaptıkları çalışmada incelenen tüketicilerin, ilkökul mezunu 4 kişi, ilköğretim mezunu 11, lise mezunu 32, ön lisans mezunu 45, lisans mezunu 103 kişi ve lisansüstü mezunu olarak da 113 kişiden oluştuğunu, Sayılı (2013), yılında Tokat ilinde yaptığı araştırmada tüketicilerin %8,09'unun doktora, %7,72'sinin yüksek lisans, %34,92'sinin üniversite, %9,56'sının ilkökul ve %6,62'sinin ortaokul, %33,09'unun lise mezunu olduğunu, Niyaz & Demirbaş (2017) Çanakkale ilinde yaptıkları çalışmada tüketicilerin %2,3'ünün okur-yazar %24'ünün ilköğretim, % 35,4'ünün lise, %36,6'sının üniversite, %1,7'sinin de yüksek lisans

mezunu olduğunu, Şahinler ve ark. (2021), Uşak ilinde yaptıkları çalışmada tüketicilerin %50'sinin üniversite mezunu, %16'sının lise, %8'inin ortaokul, %10'unun ilkökul mezunu olduğunu belirtmişlerdir.

İncelenen tüketicilere Ankara'da ikamet ettikleri süre sorulmuştur. Tüketicilerin %15,3'ü 0-5 yıldır, %27,3'ü 6-15 yıldır, %24,3'ü 16-30 yıldır, %33'ü ise 30 ve daha fazla yıldır ikamet ettiklerini söylemiştir. Tüketiciler gelirlerine göre üç gruba ayrılmıştır. Birinci grup 10 000 TL'ye kadar gelirleri olanlar olup, %46,3 oransal paya sahiptir. İkinci grup 11 000 TL ile 20 000 TL arasında geliri olanlardır. Bu gruptaki tüketicilerin oranı %46,7'dir. Üçüncü gelir grubu 21 000 TL ve üzeri olup bu grup tüketicilerin oranı %7'dir. Tüketiciler aylık gıda harcamalarına göre 3 gruba ayrılmış olup 0-5000 TL gıda harcaması yapanların oranı %77,3, 5 001-10 000 TL gıda harcaması yapanların oranı %21,4, 10 001 TL'den fazla yapanların oranı ise %1,3'tür. Tüketicilerin arı ürünlerine yaptıkları yıllık harcamalar gruplara ayrılmıştır. 1 000 TL'ye kadar harcama yapanların oranı %71,7, 1 001-5 000 TL harcama yapanların oranı %26,3, 5000 TL ve üzeri harcama yapanların oranı ise %2 olarak belirlenmiştir (Çizelge 1).

Çizelge 1. Tüketicilerin demografik ve ekonomik özellikleri

Table 1. Demographic and economic characteristics of consumers

| Özellikler                  | Sayı         |       | %     |       | Özellikler                  | Sayı             |       | %     |       |
|-----------------------------|--------------|-------|-------|-------|-----------------------------|------------------|-------|-------|-------|
|                             | Erkek        | Kadın | Erkek | Kadın |                             | Erkek            | Kadın | Erkek | Kadın |
| Cinsiyet                    | Erkek        | 150   | 50    |       | Eğitim Düzeyi               | İlk. Öğr.        | 10    | 3,3   |       |
|                             | Kadın        | 150   | 50    |       |                             | Lise             | 68    | 22,7  |       |
| Yaş Grupları                | 30≤          | 58    | 19,3  |       |                             | Yüksekokul       | 55    | 18,3  |       |
|                             | 31-50        | 178   | 59,3  |       |                             | Üniversite       | 167   | 55,7  |       |
|                             | 50+          | 64    | 21,4  |       | Ankara'da ikamet edilen yıl | 0-5              | 46    | 15,3  |       |
| Aile Büyüklüğü              | 1-3          | 179   | 59,7  |       |                             | 6-15             | 82    | 27,3  |       |
|                             | 4-6          | 121   | 40,3  |       |                             | 16-30            | 73    | 24,3  |       |
|                             | 7+           |       |       |       |                             | 30+              | 99    | 33,0  |       |
| Aylık Gıda harcaması        | 0-5 000      | 232   | 77,3  |       | Aylık gelir grupları        | 0-10 000TL       | 139   | 46,3  |       |
|                             | 5 001-10 000 | 64    | 21,4  |       |                             | 10 001-20 000 TL | 140   | 46,7  |       |
|                             | 10 001+      | 4     | 1,3   |       |                             | 20 001+          | 21    | 7,0   |       |
| Yıllık arı ürün harcamaları | 0-1 000      | 215   | 71,7  |       | Yıllık arı ürün harcamaları | 0-1 000          | 215   | 71,7  |       |
|                             | 1 001-5 000  | 79    | 26,3  |       |                             | 1 001-5 000      | 79    | 26,3  |       |
|                             | 5 001+       | 6     | 2,0   |       |                             | 5 001+           | 6     | 2,0   |       |

### Tüketicilerin Arı Ürünlerinden Haberdar Olma Durumları

Tüketicilerin arı ürünlerinden haberdar olup olmadıklarını belirlemek amacıyla likert ölçeği kullanılarak sorular yöneltilmiştir. Çizelge 2'ye göre tüketicilerin bazı arı ürünlerinden bilgi sahibi olma oranı daha yüksek iken bazılarında bilgi sahibi olma oranı daha düşüktür. Arı ürünlerinden bilgi sahibi

olma durumu bal için ortalama 4,73 olarak bulunmuştur. Propolisin tüketicilerce bilinirliği ortalama 4,22, polenin 4,25, arı sütünün 3,78, balmumunun 3,50, arı zehrinin ise 2,19'dur (Çizelge 2). Buradan çıkaracağımız sonuç tüketicilerin Ankara'da arı ürünleri konusunda bilinç düzeylerinin düşük olmadığını, ancak daha çok artabileceğini göstermektedir. Marangoz & Tayçu Dolu (2019),

tüketicilerin arı ürünlerinden haberdar olma durumlarını petek bal için 4,34, süzme bal için 4,25, polen için 3,88, arı zehri için 2,52, balmumu için 3,36, arı sütü için 3,42, propolis için 3,04 olarak

belirlemişlerdir. Yapılan çalışma ve araştırmamız sonuçları kıyaslandığında ürünlerden haberdar olma ortalamaları yakın bulunmuştur.

Çizelge 2. Tüketicilerin arı ürünlerinden haberdar olma durumları  
Table 2. Consumers' awareness of bee products

| Arı Ürünleri | Kesinlikle bilmiyor (1) |      | Bilmiyor (2) |      | Kararsız (3) |      | Biliyor (4) |      | Kesinlikle biliyor (5) |      | Ort. | St. hata |
|--------------|-------------------------|------|--------------|------|--------------|------|-------------|------|------------------------|------|------|----------|
|              | Sayı                    | Oran | Sayı         | Oran | Sayı         | Oran | Sayı        | Oran | Sayı                   | Oran |      |          |
| Bal          | -                       | -    | -            | -    | -            | -    | 77          | 25.6 | 223                    | 74.4 | 4.73 | 0.028    |
| Propolis     | 21                      | 7.0  | 14           | 4.7  | 15           | 5.0  | 79          | 26.3 | 171                    | 57.0 | 4.22 | 0.068    |
| Polen        | 17                      | 5.7  | 11           | 3.7  | 18           | 6.0  | 87          | 29.0 | 167                    | 55.6 | 4.25 | 0.064    |
| Arı Sütü     | 36                      | 12.0 | 33           | 11.0 | 28           | 9.3  | 67          | 22.3 | 136                    | 45.4 | 3.78 | 0.082    |
| Balmumu      | 52                      | 17.3 | 49           | 16.3 | 19           | 6.3  | 58          | 19.3 | 122                    | 40.7 | 3.50 | 0.090    |
| Arı Zehri    | 97                      | 32.3 | 100          | 33.3 | 20           | 6.7  | 24          | 8.0  | 59                     | 19.7 | 2.19 | 0.086    |

Not:1: Kesinlikle bilmiyor 2: Bilmiyor 3: Kararsız 4: Biliyor 5: Kesinlikle biliyor

### Tüketicilerin Arı Ürünleri Satın Alma Sıklıkları

İncelenen tüketicilere arı ürünleri satın alma sıklıkları likert ölçeği kullanılarak sorulmuştur. Arı ürünlerinden en bilineni ve hepimizin vazgeçilmez besin kaynağı olan bal tüketiciler arasında en çok satın alınan arı ürünü olarak göze çarpmaktadır. Tüketicilerin 13 tanesi (%4.3) balı hiç almadığını ifade etmiştir. Tüketiciler arasında üç ayda bir kullanıma durumu en yüksek oranda bulunmuştur (%27.7), Tüketiciler balı %26.3 oranında ayda bir, %15.3 oranında ise 6 ayda bir kullanmaktadır. Yılda bir bal satın alıyorum diyen tüketicilerin oranı ise %23 olarak

hesaplanmıştır. Çizelge 3'e göre tüketicilerin en fazla satın aldığı arı ürünü bal olurken (4.15), en az alınan arı ürünü ise arı zehri (1.05) ve balmumu (1.13) olmuştur. Bal 13 (%4.3) tüketici tarafından hiç alınmazken, propolis 162 (%54), polen 192 (%64), arı sütü 228 (%76), balmumu 285 (%95), arı zehri ise 298 (99.3) tüketici tarafından hiç alınmamaktadır (Çizelge 3). Tüketicilerin arı ürünlerinden haberdar olma durumlarının yüksek olduğu belirlenmiş ancak, satın alma sıklığının daha düşük oranlarda olduğu görülmüştür. Bu durumun en önemli sebebinin ürün fiyatlarının yüksek olmasından kaynaklandığı yapılan görüşmeler sırasında belirlenmiştir.

Çizelge 3. Tüketicilerin arı ürünleri satın alma sıklığı

Table 3. Frequency of purchase of bee products by consumers

| Arı Ürünleri | Hiç almam (1) |      | 15 günde bir (2) |      | Ayda bir (3) |      | 3ayda bir (4) |      | 6 ayda bir (5) |      | Yılda Bir (6) |      | Ort. | St. hata |
|--------------|---------------|------|------------------|------|--------------|------|---------------|------|----------------|------|---------------|------|------|----------|
|              | Sayı          | Oran | Sayı             | Oran | Sayı         | Oran | Sayı          | Oran | Sayı           | Oran | Sayı          | Oran |      |          |
| Bal          | 13            | 4.3  | 10               | 3.3  | 79           | 26.3 | 83            | 27.7 | 46             | 15.3 | 69            | 23.0 | 4.15 | 0.078    |
| Propolis     | 162           | 54.0 | 0                | 0.0  | 0            | 0.0  | 0             | 0.0  | 0              | 0.0  | 138           | 46.0 | 2.54 | 0.124    |
| Polen        | 192           | 64.0 | 0                | 0.0  | 0            | 0.0  | 0             | 0.0  | 29             | 9.7  | 79            | 26.3 | 2.34 | 0.114    |
| Arı Sütü     | 228           | 76.0 | 0                | 0.0  | 3            | 1.0  | 6             | 2.0  | 14             | 4.7  | 49            | 16.3 | 1.44 | 0.132    |
| Balmumu      | 285           | 95.0 | 0                | 0.0  | 0            | 0.0  | 0             | 0.0  | 5              | 1.7  | 10            | 3.3  | 1.13 | 0.028    |
| Arı Zehri    | 298           | 99.3 | 0                | 0.0  | 0            | 0.0  | 0             | 0.0  | 0              | 0.0  | 2             | 0.7  | 1.05 | 0.059    |

1: Hiç almam 2: yılda bir 3: 6 ayda bir 4: Üç ayda bir 5: Ayda bir 6: 15 günde bir

### Tüketicilerin Arı Ürünü Tüketme Sıklıkları

Araştırma kapsamındaki tüketiciler arı ürünlerini tüketme sıklıkları bakımından incelenmiştir. Tüketicilerin %4.3'ü hiçbir zaman bal tüketmediklerini belirtmiştir. 124 (%41.3) tüketici ara sıra bal tükettiklerini, 89 (%29.7) tüketici sık sık tükettiklerini, 74 tüketici (%24.7) her zaman

tükettiklerini belirtmiştir. Propolis tüketimi 138 (%46) tüketici tarafından ara sıra yapılmaktadır (%46). Sık sık tüketirim ve her zaman tüketirim diyen tüketici bulunmamaktadır. Arı sütü 72 tüketici tarafından (%24) ara sıra tüketilmektedir. Balmumu 15 (%5) tüketici tarafından ara sıra, arı zehri ise 2 (%0.7) tüketici tarafından ara sıra tüketilmektedir. Arı

ürünü tüketme sıklığı ortalaması en yüksek balda görülürken (3.35), en az tüketim sıklığı ise arı zehri (1.01) ve balmumunda görülmektedir (1.01). Satın alma sıklığında olduğu gibi tüketim sıklığı da bal dışındaki diğer ürünlerde oldukça düşük düzeylerde bulunmuştur (Çizelge 4). Onuç & Saner (2022) yılında Ege üniversitesi öğrencilerine yönelik yaptıkları çalışmada çiçek balını her gün tüketenlerin oranını

%5.53, hiç tüketmeyenlerin oranını %40.00, petek balı her gün tüketenlerin oranını %1.84, hiç tüketmeyenlerin oranını %46.32, organik balı her gün tüketenlerin oranını %3.42, hiç tüketmeyenlerin oranını %63.95, propolisi her gün tüketenlerin oranını %1.05, arı sütünü her gün tüketenlerin oranını %0.79 olarak belirlemişlerdir.

#### Çizelge 4. Tüketicilerin arı ürünü tüketim sıklığı

Table 4. Consumers' frequency of bee product consumption

| Arı Ürünleri | Hiçbir zaman (1) |      | Arasıra (2) |      | Sık sık (3) |      | Her zaman (4) |      | Ort. | St. hata |
|--------------|------------------|------|-------------|------|-------------|------|---------------|------|------|----------|
|              | Sayı             | Oran | Sayı        | Oran | Sayı        | Oran | Sayı          | Oran |      |          |
| Bal          | 13               | 4.3  | 124         | 41.3 | 89          | 29.7 | 74            | 24.7 | 3.35 | 0.050    |
| Propolis     | 162              | 54.0 | 138         | 46.0 | 0           | 0.0  | 0             | 0.0  | 1.62 | 0.044    |
| Polen        | 192              | 64.0 | 108         | 36.0 | 0           | 0.0  | 0             | 0.0  | 1.52 | 0.033    |
| Arı Sütü     | 228              | 76.0 | 72          | 24.0 | 0           | 0.0  | 0             | 0.0  | 1.28 | 0.043    |
| Balmumu      | 285              | 95.0 | 15          | 5.0  | 0           | 0.0  | 0             | 0.0  | 1.05 | 0.005    |
| Arı Zehri    | 298              | 99.3 | 2           | 0.7  | 0           | 0.0  | 0             | 0.0  | 1.01 | 0.015    |

1:Hiçbir zaman

2:Arasıra

3:Sık sık

4:Her zaman

#### Tüketicilerin Arı Ürünü Satın Alırken Dikkat Ettikleri Konular

Tüketicilerin arı ürünleri satın alırken dikkat ettikleri hususların neler olduğu incelenmiştir. Buna göre tüketiciler için ürünün son kullanma tarihinin (5), üreten firmanın (5), koruyucu madde içermemesinin (5), tat ve aromasının (5) çok önemli olduğu, fiyat (4), marka (4), ambalajlı olmasının (4), çok talep edilen bir ürün olmasının (4), gramajının (4) önemli olduğu, reklamların ise hiç önemli olmadığı sonucu çıkmaktadır (1). Gürer & Akyol (2018), Niğde ilinde yaptıkları çalışmada tüketicilerin bal satın alırken kalite (4.69), tazelik (4.65), renk (4.55), koyuluk (4.59), içerdiği katkı maddesi (4.59), güvenilirlik (4.73) konularına dikkat ettiklerini ifade etmişlerdir. Denizli Akdemir & Dağdemir (2021) Erzurum ilinde yaptıkları çalışmada, Tüketicilerin arı ürünleri satın alırken ürünün kalitesine (4.75), tadına (4.69), sağlık açısından güvenilirliğine (4.66), katkı maddesi içerip içermediğine (4.62) ve üretim yapan firma adı ve markasına (3.60) önem verdiklerini belirtmişlerdir. Bal ve arı ürünleri satın alırken en az reklamların etkili olduğunu tespit etmişlerdir (2.68).

Tüketicilerin bazı demografik ve ekonomik özellikleri ile arı ürünü satın alırken dikkat ettikleri konular arasında ilişki olup olmadığını belirlemek için yapılan istatistiksel analizlerde, cinsiyet ile son kullanma tarihi ( $p=0.019$ ) ve üreten firma ( $p=0.020$ ), koruyucu madde içermeme durumu ( $p=0.039$ ) arasında anlamlı bir ilişki olduğu belirlenmiş olup, kadın tüketicilerin ürün tercihinde son kullanma tarihi, üreten firma ve koruyucu madde içermeme konularına daha çok hassasiyet gösterdikleri belirlenmiştir. Yaş grubu ile satın alırken dikkat edilen konular arasında ise fiyat ( $p=0.022$ ) ve üreten firma arasında ( $p=0.030$ ) istatistiksel açıdan anlamlı bir ilişki bulunmuştur.

Tüketicilerin yaşları ile doğru orantılı olarak, ürün fiyatı ve üretici firmaya daha çok önem verdikleri görülmektedir. Tüketicilerin aylık gelirleri ile ürün satın alırken dikkat edilen konular arasında fiyat ( $p=0.035$ ), son kullanma tarihi ( $p=0.045$ ), çok talep görmesi ( $p=0.001$ ), gramajı ( $p=0.048$ ) arasındaki ilişki anlamlı bulunmuştur. Geliri artan tüketiciler fiyatı daha az dikkate alırken, çok talep gören bir ürün olması ve ürün gramajı konusunda gelir yüksekliği daha etkili olmaktadır. Tüketicilerin aylık gıda harcamaları ile ürün satın alırken dikkat ettikleri konular arasındaki istatistiksel ilişkiye bakıldığında ise çok talep görmesi ( $p=0.009$ ), gramajı ( $p=0.063$ ) arasında anlamlı bir ilişki olduğu belirlenmiş ve aylık gıda harcaması arttıkça çok talep gören ürün tercihi ve ürünün gramajı etkili olmuştur (Çizelge 5).

#### Tüketicilerin Arı Ürünleri Hakkındaki Düşünceleri

Tüketicilerin arı ürünleri konusundaki düşünceleri 5'li likert ölçeği kullanılarak belirlenmiştir. Arı ürünlerinin sağlık için faydalı olduğuna tüketicilerin %67.7'si kesinlikle katılıyorum cevabını vermişlerdir (4.63), Kesinlikle katılmıyorum cevabı ise sadece 2 tüketici tarafından verilmiştir (%0.7). Tüketicilerin büyük bir bölümünde arı ürünlerinin sağlıklı ürünler olduğu düşüncesinin etkili olduğunu vurgulayabiliriz. Arı ürünlerinin güvenli ürünler olduğuna tüketicilerin %45.3'ü kesinlikle katılmaktadır (4.14). Arı ürünlerinin kolay erişilebilir ürünler olduğuna tüketicilerin %40.7'si katılmakta ve %22.7'si kesinlikle katılmaktadır. Tüm cevapların ortalamasına göre katılıyorum düşüncesi hakimdir (3.50). Tüketiciler arı ürünlerinin ucuz ürünler olduklarına %46.3 oranında katılmamaktadır. Tüketicilerin arı ürünlerinin ucuz ürünler olduğuna katılma ortalaması düşük bulunmuştur (1.90) (Çizelge 6).

Çizelge 5. Tüketicilerin arı ürünleri satın alırken dikkat ettikleri konular

Table 5. Issues that consumers pay attention to when purchasing bee products

| Unsurlar                  | mod | Cinsiyet *<br>(K/E)<br>Sig. | Yaş grubu**<br>(30≤<br>50+)<br>Sig. | Aylık gelir grubu**<br>Sig.<br>(0-10.000<br>10.001-20.000<br>20.001-+) | Aylık Gıda<br>Har ** Sig.<br>(0-5.000<br>5.001-10.000<br>10.001-+) |
|---------------------------|-----|-----------------------------|-------------------------------------|--|--|
| Fiyat                     | 4   | 0.087                       | <b>0.022</b>                        | <b>0.035</b>   | 0.687  |
| Markası                   | 4   | 0.835                       | 0.241                               | 0.317  | 0.727  |
| Ambalajlı olması          | 4   | 0.284                       | 0.794                               | 0.692  | 0.708  |
| Son kullanma tarihi       | 5   | <b>0.019</b>                | 0.602                               | <b>0.045</b>   | 0.804  |
| Üreten firma              | 5   | <b>0.020</b>                | <b>0.030</b>                        | 0.492  | 0.687  |
| Çok talep görmesi         | 4   | 0.950                       | 0.426                               | <b>0.001</b>   | <b>0.009</b>   |
| Organik olması            | 5   | 0.062                       | 0.527                               | 0.146  | 0.795  |
| Koruyucu madde içermemesi | 5   | <b>0.039</b>                | 0.210                               | 0.452  | 0.270  |
| Tat ve aroması            | 5   | 0.726                       | 0.754                               | 0.540  | 0.896  |
| Reklamlar                 | 1   | 0.694                       | 0.352                               | 0.297  | 0.186  |
| Gramajı                   | 4   | 0.506                       | 0.358                               | <b>0.048</b>   | <b>0.063</b>   |

1:Hiçönemli değil 2:Önemli değil 3:Kararsız 4:Önemli 5:Çok Önemli, \*Mann Whitney U \*\*Kruskal Wallis H

Çizelge 6. Tüketicilerin arı ürünleri konusundaki düşünceleri

Table 6. Consumers' opinions on bee products

| Unsurlar  | Kesinlikle katılmıyorum |      | Katılmıyorum |      | Fikrim yok |      | Katılıyorum |      | Kesinlikle katılıyorum |      | Ort  | St. Error |
|---|-------------------------|------|--------------|------|------------|------|-------------|------|------------------------|------|------|-----------|
|   | Sayı                    | Oran | Sayı         | Oran | Sayı       | Oran | Sayı        | Oran | Sayı                   | Oran |      |           |
| Arı ürünleri sağlık için faydalıdır             | 2                       | 0.7  | 3            | 1.0  | 2          | 0.7  | 90          | 30.0 | 203                    | 67.7 | 4.63 | 0.036     |
| Arı ürünleri güvenilir ürünlerdir               | 5                       | 1.7  | 25           | 8.3  | 30         | 10.0 | 104         | 34.7 | 136                    | 45.3 | 4.14 | 0.058     |
| Arı ürünlerine kolay erişilir                   | 24                      | 8.0  | 60           | 20.0 | 26         | 8.7  | 122         | 40.7 | 68                     | 22.7 | 3.50 | 0.073     |
| Arı ürünleri ucuzdur                            | 108                     | 36.0 | 139          | 46.3 | 31         | 10.3 | 18          | 6.0  | 4                      | 1.3  | 1.90 | 0.052     |
| Arı ürünleri her zaman tüketilebilen ürünlerdir | 12                      | 4.0  | 54           | 18.0 | 30         | 10.0 | 123         | 41.0 | 81                     | 27.0 | 3.65 | 0.068     |
| Fazla tüketimi sağlığa zararlı olabilir         | 15                      | 5.0  | 18           | 6.0  | 58         | 19.3 | 130         | 43.3 | 79                     | 26.3 | 3.80 | 0.061     |

1:Kesinlikle katılmıyorum 2:Katılmıyorum 3:Fikrim yok 4: Katılıyorum 5: Kesinlikle katılıyorum

### Tüketicilerin Arı Ürünü Tüketme Sebepleri

İncelenen tüketicilere arı ürünlerini tüketme sebebi birden fazla seçenek hakkı tanınarak sorulmuştur. Verilen cevapların %38'i bağışıklığa faydalı olduğu için, %29.3'ü sevdiğim için, %20.6'sı besleyici olduğu için, %12.1'i hastalıklara karşı koruduğu içindir. Verilen cevaplar dikkatli incelendiğinde tüketici

zihninde arı ürünlerinin lezzet ve aromasının yanında insan sağlığı için faydalı ürünler olduğu, birçok hastalığa iyi geldiği ve aynı zamanda besleyici olduğu yerleşmiştir. Bu bilincin daha geniş kitlelerde oluşması ve yaygınlaşması ürünlerin daha iyi tanıtımı (özellikle bal dışındaki diğer arı ürünlerinin) ile mümkün olacaktır (Çizelge 7).

Çizelge 7. Tüketicilerin arı ürünleri tüketme sebebi

Table 7. Reason for consumers to consume bee products

|                                  | Sayı | %    |
|----------------------------------|------|------|
| Bağışıklığa faydalı olduğu için  | 292  | 97.3 |
| Sevdiğim için                    | 225  | 75.0 |
| Besleyici olduğu için            | 158  | 52.7 |
| Hastalıklara karşı koruduğu için | 93   | 31.0 |
| Toplam                           | 768  |      |

Not: Birden fazla seçenek hakkı verilmiştir.

### Tüketicilerin Ambalaj Tercihleri

İncelenen tüketicilere arı ürünlerini satın alırken

tercih ettikleri ambalaj sorulmuştur. Tüketicilerin büyük bir bölümü arı ürünlerini cam kavanozla satın



almayı tercih etmektedir. Balı cam kavanozda satın almayı tercih edenlerin oranı %97.6'dır. Ambalaj tercihiinde cam kullanılması tüketici bilinç düzeyinin arttığını göstermektedir. Ambalaj olarak camın kullanılabilceği ürünlerde tüketici tercihi genellikle cam ambalajdan yana olmaktadır. Ürünlerin daha uzun süre korunması, buzdolabında saklanması, kışık olarak bekletilmesi gibi durumlarda çok yaygın olarak tercih edilmektedir. Tüketiciler cam ambalaj kullanımını daha sağlıklı bulmaktadır. Tüketiciler bal alırken %1.3 oranında teneke kutuları, %0.7 oranında plastik kapları ve %0.3 oranında ise poşeti tercih etmektedir. Tüketiciler propolis satın alırken cam kavonozu %42.3 oranında, polen satın alırken %29 oranında, arı sütü satın alırken %24 oranında,

balmumu satın alırken %5 oranında, arı zehri satın alırken %0.7 oranında tercih etmektedir. Propolis satın alırken plastik kap %3.3, polen alırken %6.3, oranında tercih edilirken arı sütü, balmumu ve arı zehri alımında tercih edilmemektedir (Çizelge 8). Aydurmuş ve ark. (2022) Bal tüketimi ve bilinç düzeyi isimli çalışmalarında bal satın alırken cam ambalaj tercih edenlerin oranını %87, teneke ambalaj tercih edenlerin oranı %8, plastik ambalaj tercih edenlerin oranını %1, diğer ambalaj türlerini tercih edenlerin oranını ise %4 olarak bulmuşlardır. Gürer & Akyol (2018), Niğde ilinde yaptıkları çalışmada, tüketicilerin %64,3'ünün cam kavanoz, %21'inin teneke, %12,2'sinin çıta ve %2,4'ünün ise plastik ambalajda bal satın almayı tercih ettiklerini ortaya koymuşlardır.

Çizelge 8. Tüketicilerin arı ürünü satın alırken ambalaj tercihleri

Table 8. Consumers' packaging preferences when purchasing bee products

| Arı Ürünleri | Cam Kavonoz |      | Plastik kap |      | Teneke kutu |      | Poşet |      | Tüketim yok |      |
|--------------|-------------|------|-------------|------|-------------|------|-------|------|-------------|------|
|              | Sayı        | Oran | Sayı        | Oran | Sayı        | Oran | Sayı  | Oran | Sayı        | Oran |
| Bal          | 293         | 97.6 | 2           | 0.7  | 4           | 1.3  | 1     | 0.3  | 13          | 4.3  |
| Propolis     | 127         | 42.3 | 10          | 3.3  | 1           | 0.3  | 0     | 0.0  | 162         | 54.0 |
| Polen        | 87          | 29.0 | 19          | 6.3  | 2           | 0.7  | 0     | 0.0  | 192         | 64.0 |
| Arı Sütü     | 72          | 24.0 | 0           | 0.0  | 0           | 0.0  | 0     | 0.0  | 228         | 76.0 |
| Balmumu      | 15          | 5.0  | 0           | 0.0  | 0           | 0.0  | 0     | 0.0  | 285         | 95.0 |
| Arı Zehri    | 2           | 0.7  | 0           | 0.0  | 0           | 0.0  | 0     | 0.0  | 298         | 99.3 |

### Tüketicilerin Marka Tercihleri

Marka tercihi günümüzde geçmiş yıllara kıyasla önemli oranda artmıştır. Günümüzde tüketiciler kaliteli, tanınmış, güvenilir markalar tercih ederken, markadan dolayı oluşan fiyat farklılığını göz ardı edebilmektedir. Teknolojik gelişmeler, tutundurma faaliyetlerinin modern pazarlama anlayışı ile giderek yaygınlaşması ve tüketicilerin bilinç düzeyindeki artış, sağlıklı yaşam arzusu gibi birçok nedenden dolayı markalı ürünlere talep artmıştır. Ürettiğimi satarımdan ziyade satış çabalarının ve müşteri memnuniyetinin ne kadar önemli olduğunu fark eden

işletmeler üretimde kalite, müşteri arzu ve istekleri doğrultusunda hareket etmektedir. Markalı ürünlere olan talebin artışı, üreticileri daha dikkatli bir üretim planlamasına itmektedir. Çizelge 9'da tüketicilerin arı ürünleri tercihiinde markalı ürünleri tercih etme durumları verilmiştir. Tüketicilerin %63.3'ü balı markalı tercih ettiklerini belirtmiştir. %27.7'si fark etmez derken, %9'u ise markasız tercih ettiklerini belirtmiştir. Marka tercihinin diğer arı ürünleri satın alırken daha düşük olduğu göze çarpmaktadır. Propoliste marka tercihi %33, polende %24.3, arı sütünde %20, balmumunda %5, arı zehrinde ise %0.7'dir.

Çizelge 9. Tüketicilerin arı ürünü satın alırken marka tercihi

Table 9. Consumers' brand preference when purchasing bee products

| Arı Ürünleri | Markalı |      | Markasız |      | Farketmez |      | Tüketim yok |      |
|--------------|---------|------|----------|------|-----------|------|-------------|------|
|              | Sayı    | Oran | Sayı     | Oran | Sayı      | Oran | Sayı        | Oran |
| Bal          | 190     | 63.3 | 27       | 9.00 | 83        | 27.7 | 13          | 4.3  |
| Propolis     | 99      | 33.0 | 10       | 3.33 | 29        | 9.6  | 162         | 54.0 |
| Polen        | 73      | 24.3 | 0        | 0.0  | 35        | 11.7 | 192         | 64.0 |
| Arı Sütü     | 60      | 20.0 | 0        | 0.0  | 12        | 4.0  | 228         | 76.0 |
| Balmumu      | 15      | 5.0  | 0        | 0.0  | 0         | 0.0  | 285         | 95.0 |
| Arı Zehri    | 2       | 0.7  | 0        | 0.0  | 0         | 0.0  | 298         | 99.3 |

Tüketicilerin gelir gruplarına göre ayrımı Çizelge 1'de verilmiştir. 2022 yılında asgari ücret net olarak 5.500 TL'dir. Araştırma kapsamındaki tüketicilerin büyük

bir bölümü asgari ücretten yüksek gelir düzeyine sahiptir. Buna göre birinci gelir grubunda 139, ikinci gelir grubunda 140, üçüncü gelir grubunda ise 21 kişi bulunmaktadır. Birinci gelir grubunda %43.2 oranında

marketler, %5 oranında pazar, %7.2 oranında alışveriş merkezleri, %5.8 oranında internet, %38.8 oranında ise arıcılar tercih edilmektedir. İkinci gelir grubunda dikkati çeken nokta %50 oranında arıcıların tercih edilmesidir. İkinci gelir grubundaki tüketiciler ürünleri yerinden ve doğrudan üretim noktasından almayı tercih etmektedir. İkinci gelir grubunda arıcılara olan güvenin diğer satış merkezlerinden daha fazla olduğu görülmüştür. Üçüncü ve en yüksek gelir grubunda ise birinci ve ikinci gelir grubundan daha farklı bir sonuç ortaya çıkmış ve ürün tercihinde %38.1 oranında alışveriş merkezleri tercih edilmiştir. Bunun sebebi olarak gelir yüksekliği ve markalı ürünlere ulaşımın daha kolay olması gösterilebilir (Çizelge 10).

Gürer & Akyol (2018), Niğde ilinde yaptıkları çalışmada Tüketicilerin bal satın alırken ilk sırada üreticileri, (%36,01), ikinci sırada tanıdıkları (%33,33) ve üçüncü sırada şarküterileri (%50,41) tercih ettiklerini belirlemişler, marketlerin genellikle yüksek gelir gruplarınca tercih edildiğini ifade etmişlerdir. Şahinler ve ark.(2020). Uşak ilinde yaptıkları çalışmada tüketicilerin arı ürünlerini %63 oranında marketlerden, %31 oranında arıcılardan aldığını belirlemişlerdir. Tunca ve ark. (2015) yaptıkları çalışmada %41 oranında tüketicilerin ürünlerini market ve pazardan aldıklarını, sanal ortamdan alışverişin %7.8 oranında yapıldığını ifade etmişlerdir.

Çizelge 10. Tüketicilerin gelirleri ile bal satın alma yerleri arasındaki ilişki

Table 10. Relationship between consumers' income and place of honey purchase

| Bal satın alınan yerler | Tüketici Gelir grupları |       |      |       |      |       | Toplam |
|-------------------------|-------------------------|-------|------|-------|------|-------|--------|
|                         | I                       |       | II   |       | III  |       |        |
|                         | Sayı                    | Oran  | Sayı | Oran  | Sayı | Oran  |        |
| Market                  | 60                      | 43.2  | 43   | 30.7  | 5    | 23.8  | 108    |
| Pazar                   | 7                       | 5.0   | 10   | 7.1   | 0    | 0.0   | 17     |
| Alışveriş merkezi       | 10                      | 7.2   | 9    | 6.4   | 8    | 38.1  | 27     |
| İnternet                | 8                       | 5.8   | 8    | 5.7   | 5    | 23.8  | 21     |
| Arıcılar                | 54                      | 38.8  | 70   | 50.0  | 3    | 14.3  | 127    |
| Toplam                  | 139                     | 100.0 | 140  | 100.0 | 21   | 100.0 | 300    |

İncelenen tüketicilerde birinci yaş grubunda olanların daha çok marketleri tercih ettiği görülmektedir (%36.7). İkinci olarak alışveriş merkezlerini (%23.3) tercih etmektedirler. İnternet tercihi %20, arıcının tercihi ise %20 oranındadır. İkinci yaş grubunda ise gelir grupları ayırımında olduğu gibi arıcılar daha çok

tercih edilmektedir (%50.0), ikinci olarak marketler (%38.0), üçüncü olarak alışveriş merkezleri ve internet (%4.5) tercih edilmiştir. %3 oranında ise pazarlar tercih edilmiştir. Üçüncü yaş grubunda marketler %45.8 oranında tercih edilirken, arıcılar %43.1 oranında tercih edilmektedir (Çizelge 11).

Çizelge 11. Tüketicilerin yaşları ile bal satın alma yerleri arasındaki ilişki

Table 11. The relationship between age of consumers and place of honey purchase

| Bal satın alınan yerler | Tüketici Yaşları |       |      |       |      |       | Toplam |
|-------------------------|------------------|-------|------|-------|------|-------|--------|
|                         | I                |       | II   |       | III  |       |        |
|                         | Sayı             | Oran  | Sayı | Oran  | Sayı | Oran  |        |
| Market                  | 11               | 36.7  | 75   | 38.0  | 33   | 45.8  | 119    |
| Pazar                   | 0                | 0.0   | 6    | 3.0   | 2    | 2.8   | 8      |
| Alışveriş merkezi       | 7                | 23.3  | 9    | 4.5   | 5    | 6.9   | 21     |
| İnternet                | 6                | 20.0  | 9    | 4.5   | 1    | 1.4   | 16     |
| Arıcı                   | 6                | 20.0  | 99   | 50.0  | 31   | 43.1  | 136    |
| Toplam                  | 30               | 100.0 | 198  | 100.0 | 72   | 100.0 | 300    |

## SONUÇ ve ÖNERİLER

Ankara ilinde memurlara yönelik olarak yapılan çalışmada arı ürünlerinin tüketiciler tarafından bilinirlik düzeyleri incelenmiş ve bal dışındaki diğer ürünlerde bilinç düzeyi ve farkındalığın düşük olduğu belirlenmiştir. Bu nedenle tüketici bilincinin tüm arı ürünlerinde yükselmesi için çeşitli tanıtım ve bilgilendirme programlarına öncelik verilmesi ve tüketicilerin bilinç düzeyini arttıracak faaliyetlerde

bulunulması gerekmektedir. Arıcılık faaliyeti kolay olmayan ve meşakkatli bir işlemdir. Elde edilen ürünler ise oldukça değerlidir. Bal gibi diğer arı ürünlerinin de insan sağlığına faydalı olduğu görülmektedir. Özellikle diğer arı ürünlerinin faydaları da tüketicilere anlatılmalıdır. Aynı bilinç düzeyinde olduğu gibi satın alma oranları da diğer arı ürünlerinde düşüktür. Tüketicilerin ürün satın alma miktarının artması bilinç düzeyinin artışı ve aynı zamanda tüketici gelirleri ile de alakalıdır. Arı

ürünleri bildiğimiz gibi fiyatı pahalı olan ürünlerdir. Fiyat düzeyinin yüksekliği tüketici taleplerini olumsuz etkilemektedir. Fiyatların makul düzeyde olması ancak üretim miktarındaki artışla ve maliyetlerdeki düşüşle sağlanabilecektir. Bu nedenle arı ürünleri üretici sayılarının artması gerekmektedir. Arıcılık yapan üretici sayısının artışı için devlet tarafından gerekli destek ve teşviklerin yeterli düzeyde olması gerekmektedir. Tüketiciler arı ürünleri satın alırken birtakım faktörlere dikkat etmektedir. Bunlar ürün fiyatı yanında ürün kalitesi, markası, ambalajı, üründeki katkı maddesi gibi faktörlerdir. Tüketicilerin önem verdiği bu konular dikkate alınmalıdır. Tüketimde sürdürülebilirliğin sağlanması için tüketici tercihlerine önem verilmelidir. Tüketiciler arı ürünlerinin güvenli ürünler olduğu konusunda bilinçlenmeli ve bu konuda tanıtıcı ve güven artırıcı faaliyetlerle bilgilendirilmelidir. Bu işlevler ilgili kurum ve kuruluşlar aracılığı ile yapılmalıdır. Tüketicilerin arı ürünlerinin ucuz ürünler olmadığını farkında olduğu açıktır. Arı ürünlerinin özellikle kalite ve güvenilirlik düzeyi arttıkça fiyatlarının çok yükseldiği görülmüştür. Kaliteli ve güvenilir ürün talebinin artışı ve tüm gelir gruplarında talebin yeterince oluşması makul fiyatlarla sağlanacaktır.

#### 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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