# JOURNAL OF AGRICULTURAL PRODUCTION

Year: 2024 | Volume: 5 | Issue: 4 | e-ISSN 2757-6620 | agriprojournal.com

PRENSIP

# JOURNAL OF AGRICULTURAL PRODUCTION

## 2024 • VOLUME 5 • ISSUE 4





agriprojournal.com agriprojournal@gmail.com
prensip.gen.tr info@prensip.gen.tr

***Journal of Agricultural Production*** is a peer-reviewed scientific journal published by *Gökhan ARSLAN* twice a year.
Use of any material hereunder requires a citation to ***Journal of Agricultural Production***.
(Publisher support: ***Prensip Publishing***)

# JOURNAL OF AGRICULTURAL PRODUCTION

## 2024 • VOLUME 5 • ISSUE 4


## TABLE OF CONTENTS

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSIP

https://prensip.gen.tr

RESEARCH ARTICLE

# First Report of Bacterial Wilt Caused by *Clavibacter michiganensis* subsp. *michiganensis* Affecting Tomato in Iğdır

Mesude Figen Dönmez✉

Iğdır University, Faculty of Agriculture, Department of Plant Protection, Iğdır/Türkiye

ARTICLE INFO

**Article History**
Received: 24.05.2024
Accepted: 01.08.2024
First Published: 31.12.2024

**Keywords**
Biolog
*Clavibacter michiganensis* subsp. *michiganensis*
FAME
Identification
*Solanum lycopersicum*

ABSTRACT

Tomato wilt disease caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is one of the most destructive tomato diseases and causes significant crop loss in both greenhouse and field tomato production areas worldwide. In this study, the presence of the causal agent of bacterial wilt disease in tomato plants was investigated in Aras Valley. Isolation was made from diseased plant samples and it was determined whether the strains were pathogenic by cellulase activity and HR test. The virulence, morphological and biochemical characteristics of the strains were determined. Strains that fatty acid methyl ester extraction, isolation and purification were performed were identified at species and subspecies level with % similarity index using gas chromatography system. The diagnosis was confirmed with the Biolog Gen III System and all strains were identified at the subspecies level with a % similarity index. As a result of this study, 57 strains were obtained in the isolation, and 39 of the strains were determined not to be pathogenic. Strain 18 was determined as the pathogen causing the most damage to tomato plants with 100 % disease severity. Strains were identified as Cmm at subspecies level with a similarity index of 71-87 % using gas chromatography system and 54-75 % similarity index with Biolog Gen III System. According to the heat map created, it was determined that the strains consisted of two main clusters. The presence of pathogen in Aras Valley was proven for the first time by this study.

**Please cite this paper as follows:**

Dönmez, M. F. (2024). First report of bacterial wilt caused by *Clavibacter michiganensis* subsp. *michiganensis* affecting tomato in Iğdır. *Journal of Agricultural Production*, *5*(4), 217-227. https://doi.org/10.56430/japro.1488826

## 1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable species grown worldwide, with high production and consumption and commercial value. Bacterial diseases are of great importance among the factors limiting the cultivation of tomato plants, and the production of quality tomatoes is made possible by the diagnosis and control of these disease-causing organisms. Among these diseases, tomato wilt disease caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is one of the most destructive tomato diseases and causes significant crop loss in both greenhouse and field tomato production areas worldwide (Gautam et al., 2020). The disease still remains a serious source of concern for the tomato industry worldwide with 50-80 % yield and quality losses (Takishita et al., 2018).

The aerobic, gram-positive pathogen penetrates the plant through natural openings and wounds, then passes into the xylem and creates characteristic symptoms such as light brown colouring of the vascular bundles, wilting of one-sided leaves, necrotic lesions on the stem and petioles (Eichenlaub & Gartemann, 2011). When the pathogen infects the plant during the seed and seedling period, systemic infection occurs and leads to the death of the plant. When the infection occurs in the later stages of plant development, bird's eye symptom occurs

✉ Correspondence
E-mail address: sudefigen@hotmail.com

on the fruit. The pathogenicity is manifested by transcriptional regulators and virulence factors encoded by chromosomes and two plasmids. Virulence factors consist of serine proteases and cell wall degrading enzymes (cellulases, xylanases, pectinases). In studies, it was detected that the virulence genes viz. Cel A encoding cellulase enzyme and pat1, chp C and ppa A encoding serine protease were stimulated after the entry of the pathogen into the host (Nandi et al., 2018).

The successful use of fatty acid methyl ester (FAME) analysis and Biolog Gene III System in the diagnosis of bacteria was reported by different researchers (Anak et al., 2021; Belgüzar et al., 2016; Çakmakçı et al., 2010; Sunyar et al., 2021). Vauterin et al. (1995) used the Biolog System as one of the classification criteria for pathovars belonging to the genus *Xanthomonas*. Mirik et al. (2011) successfully identified *Pseudomonas cichorii* causing disease in tomato by FAME analysis with a similarity index of 84-97%. Aysan and Uygur (2005) detected mainly 12 fatty acids as a result of FAME analysis and identified the pathogen as *Pseudomonas viridiflava* with 81-96% similarity index. This study was carried out with the aim of investigating the presence of Cmm, which causes the disease in tomato plant that has economic value in Aras Valley, and determining the morphological, biochemical and pathological characteristics of the strains obtained and detecting their diagnosis by fatty acid methyl ester analysis and Biolog Gen III system.

## 2. Materials and Methods

### *2.1.* Collection and Isolation of Diseased Plant Samples

In 2020, tomato fields were visited in Iğdır, Aralık, Tuzluca and Karakoyunlu districts and Kasımcan, Oba and Melekli villages of Iğdır city center, and diseased plant samples were taken according to the simple random sampling method by selecting 3 fields from the district and each village. The surveys were carried out 2 times during seedling and fruiting periods of the plants. Fruits showing bird's eye symptoms and stems with browning of vascular bundles were taken as isolation material. Bacteria were isolated from the samples and yellow coloured colonies were purified from the bacteria growing on YDC (Yeast Dextrose Carbonate Agar) medium. Stock cultures of the strains were prepared in 500 ml Luria Bertani Broth and 500 ml 30% glycerol and stored at -80°C.

### *2.2.* Identification of Non-Pathogenic Strains

Cellulase activity of the strains was tested to identify non-pathogenic bacteria. For this test, a medium consisting of four different solutions recommended by Yin et al. (2010) was used. The prepared medium was inoculated with bacterial cultures cultivated on Nutrient Agar (NA) medium for 24-48 hours and incubated at 26°C for 4 days. After incubation, the petri surface was covered with 10 ml of 0.1% congo red solution and waited for 20 minutes. After this duration, the solution in the petri dish was removed from the medium. The medium was then covered with 10 ml of 1M NaCl and waited for 5 minutes. The yellow coloured open area formed around the colonies in the red coloured medium was evaluated as a positive result. The pathogenicity of strains with positive cellulase activity was confirmed by the hypersensitive reaction test in tobacco (*Nicotiana tabacum* L. Samsun) specified by Lelliot and Stead (1987).

### *2.3.* Evaluation of Virulence of Bacterial Strains

The pathogenicity test was carried out using super 5656 tomato variety. Plants were grown for five weeks in pots containing sterile sand + soil mixture prepared at a ratio of 1:4. Cells of the strains grown on NA medium were transferred to Nutrient Broth (NB) medium with a sterile core and incubated overnight at 150 rpm/min on a shaker set at 26°C. At the end of the incubation period, the inoculum density was set as $10^8$ CFU $ml^{-1}$ by turbidimeter. Tomato seedlings were inoculated with 100 µl of bacterial suspension using stem inoculation assay. The plants in the negative control group were treated with $sdH_2O$. After inoculation, polythene bags were placed over the plants and incubated at 26°C for 48 hours. At the end of the period, the bags were removed and the occurrence of disease symptom was monitored daily for 14-21 days. The study was carried out according to random plots experimental design with 3 repetitions. Disease appearance was evaluated according to a 0-5 scale (0: no disease symptoms; 1: wilting on 1-10% of the leaves; 2: wilting on 11-25% of the leaves; 3: wilting on 26-49% of the leaves; 4: wilting on 50-74% of the leaves; 5: all leaves of the plant wilted) (Soylu et al., 2003). The % disease severity was determined using the formula of Townsend and Heuberger (1943) (Formula 1).

$$\text{\% Disease Severity}=\frac{\Sigma\ (\text{scale value}\times\text{number of plants evaluated on the scale})}{\text{highest scale value}\times\text{total number of plants}}\ \text{x}\ 100 \qquad (1)$$

#### *2.3.1. Determination of colony and mobility characteristics of bacterial strains*

Colony characteristics of bacterial strains were determined in YDC medium. For the mobility test of bacterial strains, 10 g tryptone, 5 g NaCl and 5 g agar were added to one litre of $sdH_2O$. The pH of the mixture was adjusted to 7.2 and 5 ml of this mixture was placed in tubes and sterilised in autoclave at 121°C for 15 minutes. After inoculation of bacterial strains into the prepared media, bacterial growth was checked at 8th, 24th and 48th hours. Colony growth from the inoculation point to the surrounding area was recorded as a positive result (Schaad et al., 2001).

### *2.4.* Biochemical Characterization of Bacteria Strains

The gram reaction test was performed with 3 % potassium hydroxide (KOH) solution (Hyder et al., 2020), the catalase test with 7% $H_2O_2$, the oxidase test with discs containing 1% tetra methyl-p-phenylendiamine dihydrochloride (Narayanasamy, 2001). The arginine dehydrolase test was determined by the pinkish red colour of Thornley 2A medium, the levan test by the presence of convex, mucoid colonies on Nutrient Sucrose Agar medium, starch hydrolysis by the detection of a transparent zone around the colonies on Nutrient Starch Agar medium, and the pectolytic activity of bacterial strains by the formation of wells or watery appearance on Crystal Violet Pectate Agar (CVP) medium (Hélias et al., 2012).

### *2.5.* Identification of Microorganisms by Fatty Acid Methyl Ester Analysis

Bacterial strains maintained as pure cultures at -80°C were cultivated on Tryptic Soy Agar medium for 24-48 hours for fatty acid methyl ester extraction, isolation and purification. The colonies of the bacterial strains were collected with a sterile core and placed in glass test tubes with Teflon caps and each tube was treated with cell lysis solution [150 ml methyl alcohol (HPLC Grade, 45 g sodium hydroxide (ACS Grade), 150 ml sd$H_2O$]. 1 ml was added to free fatty acids. Then 2 ml of methylation solution [325 ml hydrochloric acid (6N), 275 ml methyl alcohol (HPLC Grade)] was added to the test tubes and methyl was added to the free fatty acids with ester bonds and fatty acid methyl esters were obtained and fatty acids were given high temperature volatility. After this process, the tubes were cooled rapidly and 2.5 ml of the purification solution [200ml methyl-tert-butyl-ether (HPLC Grade) 200 ml hexane (HPLC Grade)] was added. Meanwhile, the organic phase formed in the tube was retained and the acidic phase remaining at the bottom was discarded with a pasteur pipette. In the last step, 3 ml of basic washing solution [10.8 g solid sodium hydroxide (ACS Grade) 900 ml sd$H_2O$] was added to the test tubes and free fatty acid methyl esters were obtained in the pure form. At this stage, the phase containing fatty acid methyl esters collected at the top of the tube was collected with a pasteur pipette and transferred to 2 ml gas chromatography tubes, then the caps were firmly sealed and placed in the sample storage tray on the device. The cultured strains were identified at the species and subspecies level using the Microbial Identification System (Agilent 7890A GC System, MIDI, Inc., Newark, DE, Sherlock Software Version 6.1), which is a computer-controlled gas chromatography system. Fame profiles were compared with the RTSBA 6 library (Sasser, 1990). Analysis conditions of gas chromatography were presented in Table 1.

**Table 1.** Gas chromatography analysis conditions.

| | |
|---|---|
| **System** | Agilent 7890A GC System |
| **Column** | HP-Ultra 2 (25 m x 199 µm x 0.33 µm) |
| **$H_2$ Flow** | 30 ml/min |
| **Air Flow** | 350 ml/min |
| **$N_2$ Flow** | 28.771 ml/min |
| **Total Flow** | 53.37ml/min |
| **Septum Purge Flow** | 3 ml/min |
| **Pressure** | 20 psi |
| **Oven Temperature** | 120-210ºC |
| **Equilibration Time** | 0.25 min |
| **Injection Temperature** | 250ºC |
| **Column Temperature** | 60ºC for 2 min, 10ºC/min to 200ºC, 5ºC/min to 240ºC, hold 240ºC for 7 min |
| **Split Ratio** | 1/40 |
| **Split Flow** | 100 ml/min |
| **Flame Ionization Detector** | 250ºC |

### *2.6.* Diagnosis of Bacterial Strains with Biolog Gene III System

The obtained bacterial strains were grown on BUG (Biolog Universal Growth Agar) medium to determine their metabolic profiles. The bacterial cultures were suspended in IF-A buffer solution and the bacterial concentration in the tubes was adjusted by turbidimeter with a transmittance value of 92-95%. 100 µl of the adjusted bacterial suspensions were added to each well on the microplates and the plates were and the plates were incubated.

After incubation, the microplates were read on a Biolog reader. The metabolic profiles obtained for the test microorganisms were compared with the metabolic profiles of the microorganisms in the package programme of the system (MicroLogTM3 MicroStationTM Software, Version 5.2.2) (Saygılı et al., 2006).

### *2.7.* Statistical Analysis

The data obtained as a result of the pathogenicity test were subjected to analysis of variance in SPSS statistical programme (16.0). Significant values were grouped by using the Duncan multiple comparison test at $p\leq 0.01$ significance level.

Depending on the differences in the fatty acid profiles and metabolic profiles of the strains, a heat map was created by using the "heat map.2" command in the "glots" library in the R package programme.

## 3. Results and Discussion

As a result of isolation from the stems and fruits of diseased tomato plants, 57 bacterial strains were obtained. By using the cellulase activity test, 39 non-pathogenic strains were identified from the bacteria. The pathogenicity test was carried out with the remaining 18 strains and the results are given in Table 2 and Figure 1. The differences between the strains in terms of scale values were determined to be statistically significant ($p \le 0.01$). When the disease severity values were analysed, it was determined that 12 Cmm strains had high virulence.

**Table 2.** The pathogenicity test results and virulence of bacterial strains.

| Strain | Scala Value | Virulence Level | Strain | Scala Value | Virulence Level |
|---|---|---|---|---|---|
| **Cmm 1** | $1.7^{BC}$ | Low | **Cmm 10** | $4.7^{A}$ | High |
| **Cmm 2** | $3.0^{AB}$ | Moderate | **Cmm 11** | $4.3^{A}$ | High |
| **Cmm 3** | $1.7^{BC}$ | Low | **Cmm 12** | $3.0^{AB}$ | Moderate |
| **Cmm 4** | $3.0^{AB}$ | Moderate | **Cmm 13** | $3.0^{AB}$ | Moderate |
| **Cmm 5** | $4.0^{A}$ | High | **Cmm 14** | $4.3^{A}$ | High |
| **Cmm 6** | $4.0^{A}$ | High | **Cmm 15** | $4.3^{A}$ | High |
| **Cmm 7** | $4.0^{A}$ | High | **Cmm 16** | $4.3^{A}$ | High |
| **Cmm 8** | $4.3^{A}$ | High | **Cmm 17** | $3.0^{AB}$ | Moderate |
| **Cmm 9** | $4.3^{A}$ | High | **Cmm 18** | $5.0^{A}$ | High |
| | | | **Control** | $0^{C}$ | |

$^{A,B,C}$The values indicate the average of 3 replications, according to the Duncan multiple comparison test, the difference between the averages shown with different letters in the same column is significant with respect to $p \le 0.01$.

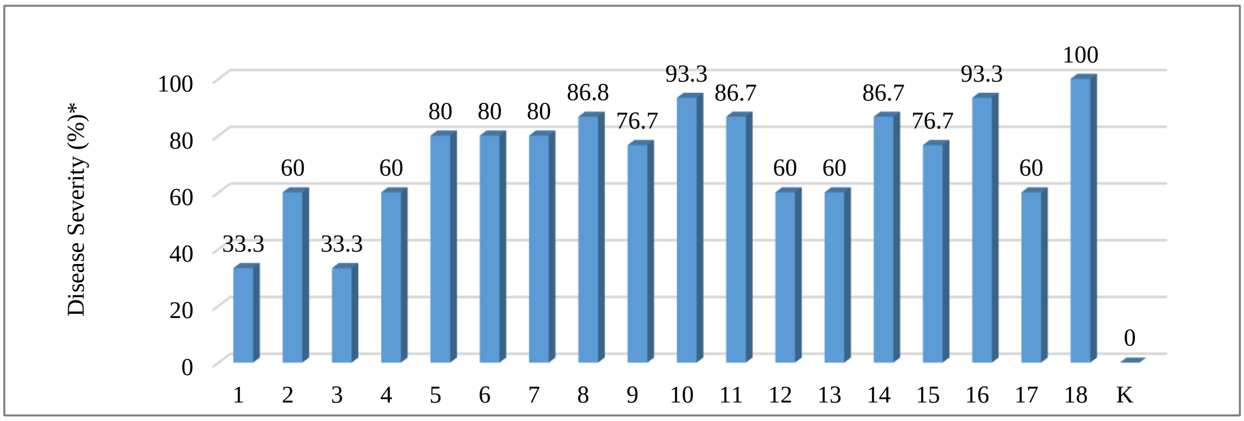


**Figure 1.** The disease severity index of Cmm strains.

The morphological and biochemical characteristics of the bacterial strains are given in Table 3. It was observed that all of the strains formed mucoid, yellow coloured colonies on YDC medium. The bacterial strains were identified as rod-shaped, gram positive bacteria by using the Biolog System. However, the strains were detected to be gram-negative in the KOH test. The results of the catalase and starch hydrolysis tests were positive, while the levan colony formation and pectolytic activities were negative.

**Table 3.** Morphological and biochemical characteristics of bacterial strains.

| SN | Colony Description | M | GR | BG | CT | OX | AD | LC | SH | PA |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **2** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **3** | Yellow, mucoid, rod | - | - | + | W+ | - | - | - | + | - |
| **4** | Yellow, mucoid, rod | - | - | + | + | - | - | - | W+ | - |
| **5** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **6** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **7** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **8** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **9** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **10** | Yellow, mucoid, rod | - | - | + | + | W+ | - | - | + | - |
| **11** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **12** | Yellow, mucoid, rod | - | - | + | + | - | - | - | W+ | - |
| **13** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **14** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **15** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **16** | Yellow, mucoid, rod | - | - | + | + | - | - | - | + | - |
| **17** | Yellow, mucoid, rod | - | - | + | W+ | - | - | - | + | - |
| **18** | Yellow, mucoid, rod | - | - | + | + | W+ | - | - | + | - |

SN: Strain No; M: Motility; GR: Gram reaction; BG: Biolog System gram reaction result; CT: Catalase; OX: Oxidase; AD: Arginine Dihydrolase; LC: Levan Colony; SH: Starch Hydrolysis; PA: Pectolytic Activity; +: Positive result; -: Negative result; W+: Weak positive.

The fatty acid types and their % amounts in 18 Cmm strains were determined by the gas chromatography system. The identification results, the similarity index and the number of fatty acids contained in the strains are given in Table 4. The strains obtained were identified as Cmm at subspecies level with 71-87% similarity index. It was determined that the number of fatty acids detected varied in the strains. The highest number of fatty acids (18) was detected in Cmm 2 and Cmm 12.

**Table 4.** Diagnostic results of bacterial strains according to FAME profiles.

| SN | FAME Identification Result | SI (%) | NFA |
|---|---|---|---|
| **1** | *Clavibacter michiganensis* subsp. *michiganensis* | 72 | 11 |
| **2** | *Clavibacter michiganensis* subsp. *michiganensis* | 72 | 18 |
| **3** | *Clavibacter michiganensis* subsp. *michiganensis* | 76 | 11 |
| **4** | *Clavibacter michiganensis* subsp. *michiganensis* | 78 | 15 |
| **5** | *Clavibacter michiganensis* subsp. *michiganensis* | 71 | 12 |
| **6** | *Clavibacter michiganensis* subsp. *michiganensis* | 82 | 15 |
| **7** | *Clavibacter michiganensis* subsp. *michiganensis* | 87 | 14 |
| **8** | *Clavibacter michiganensis* subsp. *michiganensis* | 72 | 13 |
| **9** | *Clavibacter michiganensis* subsp. *michiganensis* | 83 | 13 |
| **10** | *Clavibacter michiganensis* subsp. *michiganensis* | 74 | 11 |
| **11** | *Clavibacter michiganensis* subsp. *michiganensis* | 85 | 14 |
| **12** | *Clavibacter michiganensis* subsp. *michiganensis* | 72 | 18 |
| **13** | *Clavibacter michiganensis* subsp. *michiganensis* | 72 | 13 |
| **14** | *Clavibacter michiganensis* subsp. *michiganensis* | 83 | 13 |
| **15** | *Clavibacter michiganensis* subsp. *michiganensis* | 71 | 12 |
| **16** | *Clavibacter michiganensis* subsp. *michiganensis* | 82 | 15 |
| **17** | *Clavibacter michiganensis* subsp. *michiganensis* | 78 | 15 |
| **18** | *Clavibacter michiganensis* subsp. *michiganensis* | 74 | 11 |

SN: Strain Number; FAME: Fatty Acid Methyl Ester; SI: Similarity Index; NFA: Number of Faty Acid.

When the FAME profiles of the strains were analysed, it was observed that 10 fatty acids (14:0, 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0, 16:0 iso, 17:0, 17:0 iso, 17:0 anteiso, 18:1 w9c) were common in all strains. Fatty acids 13:0, 14:0 anteiso and 17:1w8c were present in Cmm strain 2 and 12, while 18:0 3OH and 20:0 iso were present only in Cmm strain 7. It was determined that 15:0 anteiso (42.4-52.4%) and 17:0 antesiso (26.45-34.60%) fatty acids were present in all strains. The presence of 15:1 anteiso A fatty acid, having a diagnostic value for Cmm, was determined at a rate of 2.3-4.9%. A heat map was created based on the fatty acid types and % amounts determined in the strains with the R package programme (Figure 2). In this map, the relationship between the data of fatty acids that enable the grouping of bacterial strains is represented by colours. In the heat map, X axis represents fatty acids and Y axis represents bacterial strains. When the heat map is analysed, it is seen that 18 Cmm strains consist of 2 main clusters, A and B. The first cluster consists of two subgroups, A1 (Cmm strain 2-12) and A2 (Cmm strain 4-17). The second cluster consists of B1, which is represented only by Cmm 7, and B2 (Cmm strain 8-13, 14-9, 11, 5-15, 16-6, 18-10, 3-1) subgroups that contain a wide variety.

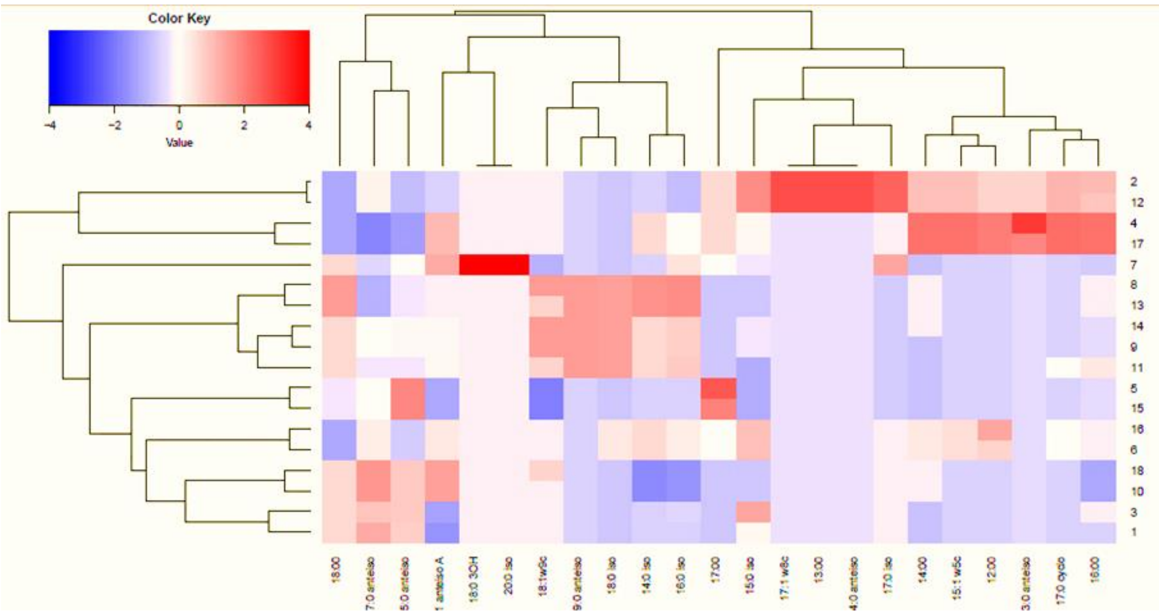


**Figure 2.** Heat map indicating the grouping of bacterial strains based on fatty acids.

The properties of the bacterial strains obtained in the study to utilise 71 different carbon sources and sensitivity tests for 23 chemicals were evaluated in the Biolog Gen III MicroPlate system and profiles were obtained accordingly. MicroPlates were evaluated visually, optically and spectrophotometrically. Visual reading revealed that redox reaction occurred in some of the wells, indicating that Cmm was metabolically active and as a result the wells were coloured purple. Optical reading was performed on the Biolog reader provided with a video camera that captured images at specific times. Spectrophotometric reading was carried out with a MicroStationTM reader at absorbance values of 590 nm and 750 nm at two intensities. Columns 1-9 were used for the carbon source utilisation tests. All reactions in these columns were compared to the reaction in the well A1, the negative control well. Therefore, reactions in wells in the columns 1-9 that gave a similar colour to the well A1 (absence of any colour) indicated that the respective carbon sources were not used and were considered negative. The purple coloured wells indicated that the strains metabolised the respective substrates using the carbon sources and were considered positive. The columns 10-12 were used for the chemical sensitivity tests. The reactions in these wells were compared with the reaction in the well A10, which was the positive control reaction. Since the colour of the positive control was purple, the reactions in the purple coloured wells in the columns 10-12 were considered positive and indicated that the strains were resistant to the respective chemical. The colourless well were considered negative as they demonstrated no growth and indicated that the strains exhibited a significant sensitivity to the chemical inhibitor. All of the strains were identified as *Clavibacter michiganensis* subsp. *michiganensis* at subspecies level. When the diagnosis report of the strains was analysed, the lowest similarity index was 54% and the highest was 75%. The diagnostic results of the strains are presented in Table 5.

**Table 5.** Identification of Cmm by Biolog Gen III System.

| Identification | Strain No | Prob*(%) | Sim (%) | Dist |
|---|---|---|---|---|
| Cmm | 1 | 100 | 97 | 0.3 |
| | 2 | 100 | 80 | 2.4 |
| | 3 | 99 | 80 | 2,7 |
| | 4 | 99 | 82 | 2.3 |
| | 5 | 99 | 88 | 1.6 |
| | 6 | 98 | 78 | 1.5 |
| | 7 | 98 | 76 | 1.4 |
| | 8 | 98 | 74 | 3.0 |
| | 9 | 98 | 54 | 1.2 |
| | 10 | 96 | 80 | 1.1 |
| | 11 | 94 | 71 | 1.6 |
| | 12 | 91 | 68 | 1.9 |
| | 13 | 87 | 75 | 0.2 |
| | 14 | 85 | 76 | 1.5 |
| | 15 | 75 | 72 | 1.2 |
| | 16 | 75 | 64 | 1.4 |
| | 17 | 75 | 75 | 1.7 |
| | 18 | 70 | 56 | 3.0 |

*Prob: Probability of correct identification; Sim: Similarity index value indicating the quality of each match; Dist: Distance rating indicating the number of mismatches.

When the profiles of Cmm strains were evaluated, it was determined that all of them used 3 carbon sources, namely Sucrose, D-Fructose and D-Mannitol. It was detected that only strains 9 and 14 grew in Tween 40 and the other strains did not grow in the presence of Tween 40. While all strains were observed to grow in 1% NaCl in the well B10, none of them were observed to grow in 8% NaCl in the well B12. In 4% NaCl in well B11, only 6 and 16 of the strains were observed to grow. When the tolerance of the strains to pH was analysed, it was detected that all of the strains grew at pH 6 and none of them grew at pH 8. When the reactions of Cmm strains against antibiotics were evaluated, it was observed that all of the strains did not grow in the wells containing troleandomycin, rifamycin, minocycline, lyncomycin and vanomycin, so they were sensitive to these antibiotics. In the wells containing Aztreonam antibiotic, all strains grew and therefore were resistant to this antibiotic. In the well containing nalidixic acid antibiotic, it was determined that all of the strains except 9 and 14 grew. A heat map was created based on the metabolic profiles determined with the R package programme (Figure 3). In the heat map, the relationship between the data belonging to the metabolic profiles that enable the grouping of Cmm strains is expressed in colours. In the heat map, the horizontal axis represents the bacterial strains and the vertical axis represents the wells in the Biolog Gen III MicroPlate. When the heat map is evaluated, it is seen that 18 Cmm strains consist of 2 main clusters, namely A and B. The first cluster consists of two subgroups, which are A1 (Cmm strain 9 and 14) and A2 (Cmm strain 6 and 16). The second main cluster (B) consists of two subgroups, namely B1 and B2. It is observed that the diversity in B1 is considerably high.

It is observed that the FAME and the Biolog Gen III System grouped Cmm strains differently based on their own characteristics. The results obtained demonstrate the value of both systems in the diagnosis of bacterial strains.

In this study, 57 bacterial strains were isolated from the stems and fruits of the diseased tomato plants. Jahr et al. (2000) reported that the endoglucanase gene celA of Cmm was an important virulence factor required for wilt induction on tomato. Therefore, cellulase activity test was used to determine the non-pathogenic *Clavibacter* strains. The results showed that 39 of the strains lacked cellulase activity, which was the proof of the non-pathogenicity. In the pathogenicity test, the disease-causing properties of 18 strains were investigated and it was determined that bacterial strains with cellulase activity caused wilt symptom on tomato plants. The results of the cellulase activity test were confirmed by determining that 12 of the bacterial strains had high, 4 of them had moderate and 2 of them had low levels of virulence. Załuga (2013) also investigated the presence of cellulase activity in the non-pathogenic Clavibacter strains and found that 15 non-pathogenic *Clavibacter* strains showed no cellulase activity, while the LMG 5616 and PD 5707 strains had high cellulase activity. It was determined that the strains formed yellow, mucoid colonies on the YDC medium. This result was found to be homogeneous with the results obtained in the study conducted by Li et al. (2018). In parallel with the findings of Cristina et al. (2018), it was determined in this study that the bacterial cells were rod-shaped and immobile. As a result of biochemical tests, catalase, amylase, oxidase and levan colony formation of the strains were detected positive and negative. These test results were consistent with the findings of Kolomiets et al. (2017). In this study, while the strains were determined as gram positive bacteria by the Biolog System, they were determined as gram negative as a result of the 3% KOH test. In the study conducted by Tripathi et al. (2022), Cmm strains were found to be positive as a result of gram staining, but negative in the gram reaction test conducted with KOH. As stated in EPPO (2016), it was determined that Cmm strains developed in 6% NaCl.

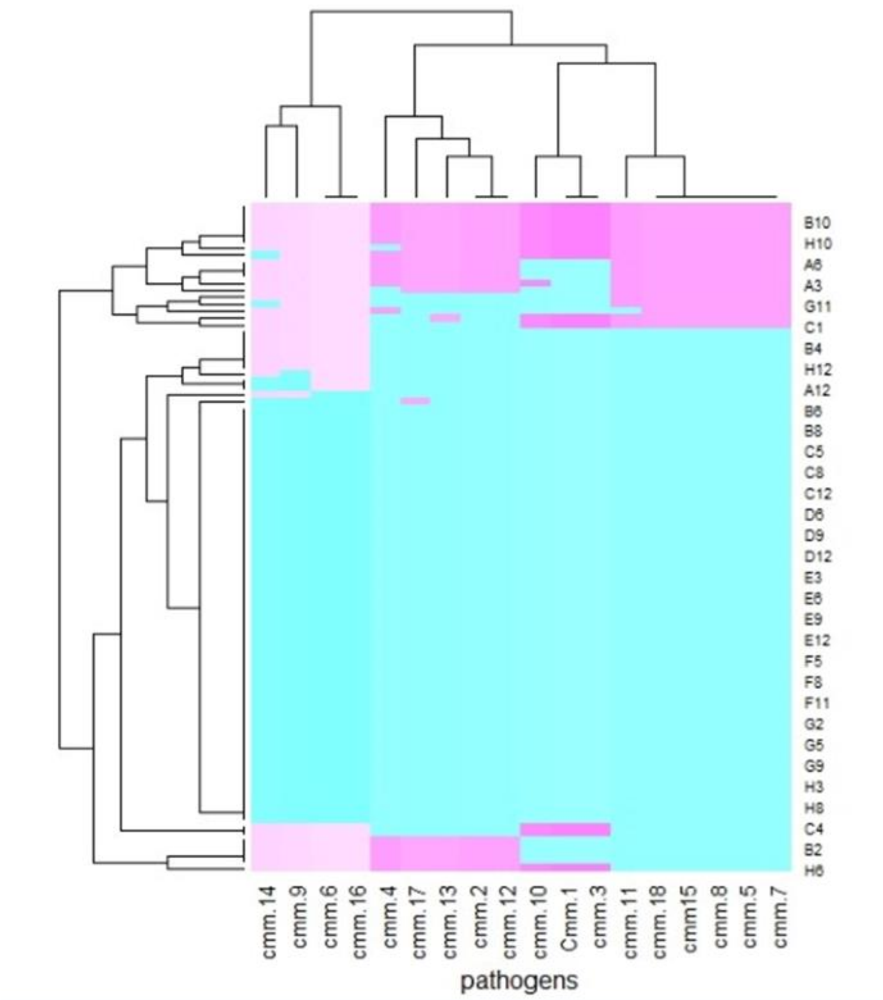


**Figure 3.** The heat map indicating the grouping of bacterial strains based on their metabolic profiles.

In numerous studies, the FAME analysis was used to classify microorganisms. A large library of fatty acid profiles including the Cmm fatty acid profile was established and the taxonomic significance of the FAME content in Coryneform bacteria, including *Clavibacter*, was reported. The anteisopenladecenoic acid, an unsaturated branched chain fatty acid with a carbon number of 15 and a double bond, was identified as an important criterion for the diagnosis of Cmm (Gitaitis & Beaver, 1990). In this study, the presence of 15:1 anteiso A fatty acid, which had a diagnostic value for Cmm, was identified in all strains at a rate of 2.33-4.95 %. In all strains, 15:0 anteiso (42.4-52.4%) and 17:0 antesiso (26.4-34.6%) fatty acids were detected at high rates and were identified as Cmm with a similarity index of 72-87%. These values were similar to other studies. For example, in the study conducted by Şahin et al. (2002) in the Eastern Anatolia Region of Türkiye, 16 strains were identified as Cmm with a similarity index of 47-89% as a result of the FAME analysis in the study in which the pathogen causing heavy yield losses up to 100% in tomato production areas was investigated. In the study conducted by Çetinkaya Yıldız (2007), 6 strains obtained from Mersin and Adana provinces were identified as Cmm with a similarity index of 41-81% and the 13 strains obtained from tomato production areas in Tokat province by Belgüzar et al. (2016) were identified as Cmm with a similarity index of 58-82% by using the Microbial Identification System. When the rates of fatty acids were analysed, it was observed that the strains were largely similar and 14:0 iso, 14:0, 15:1 anteiso A, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0, 17:0 iso, 17:0 anteiso and 17:0 fatty acids were detected in all strains. Especially 15:0 anteiso and 17:0 anteiso fatty acids were detected at high rates in the strains. It was determined that 15:1 anteiso A, which is characteristic for Cmm, was found in strains at an average rate of 2.2%. Basım and Basım (2018) isolated a total of 118 strains from diseased tomato samples in the Western Mediterranean region of Türkiye. As a result of the FAME analysis, it was determined that the anteisoheptadeconic acid (a15:0, 41.8-55.8%), palmitic acid (i16:0, 7.1-15.4%) and anteisoheptadeconic acid (a17:0, 24.8-30.9%) were the major components in all strains and were detected in high amounts. These results were consistent with the characteristics of the *Clavibacter* genus members. It was stated that the number, diversity and % amount of bacterial fatty acids remained unchanged as long as the environmental conditions were the same, therefore differences in fatty acid profiles were an indirect indicator of genetic kinships between strains (Yang et al., 1993). As a result, cellular fatty acid profiles were suggested to determine the differences between microorganisms. It was stated that quantitative and qualitative changes in cellular fatty acids could be used as an indicator of differentiation between species. However, in the comparison of fatty acid profiles, it was stated that standardisation of cultivation conditions was important since the composition of the medium, age of the culture, temperature and oxygen availability had a strong effect on fatty acids (Schumann et al., 2009).

The Biolog System is described as the gold standard for bacterial diagnosis (Morgan et al., 2009). However, most of the studies using this system did not focus on gram-positive corineform phytopathogens, i.e., bacteria belonging to the genus *Clavibacter*. In this study, *Clavibacter michiganensis michiganensis*, which causes wilt in tomato, was identified by using the Biolog Gen III system. When the profiles of bacterial strains obtained with the Biolog Gen III System were evaluated, 18 of them were identified as Cmm. It was determined that all of the strains used 3 carbon sources (Sucrose, D-fructose and D-mannitol). It was determined that they did not use 47 carbon sources in the MicroPlate and yielded negative results in the sensitivity test against 6 chemicals. Stancu and Mitrea (2020) conducted a study on the identification of Cmm strains obtained from different regions of Romania by using the Biolog Microbial Identification System. The bacterial strains were identified as *C. michiganensis* at the species level, but subspecies could not be identified. It was determined that all of the identified strains used α-D-glucose, dextrin, D-mannose, D-mannitol, D-fructose, D-galactose and sucrose carbon sources and 27 carbon sources were not used. It was determined that D-mannitol, D-fructose and sucrose were also used by the strains in this study, but the strains exhibited differences in the use of α-D-glucose, dextrin, D-mannose and D-galactose carbon sources. Regarding the chemical sensitivity tests, the growth of the strains in the presence of 1% NaCl, nalidixic acid, aztreonam, potassium tellurite and pH 6 was detected as positive, while their tolerance to 8% NaCl was detected as negative. In this study, the same results were obtained for all tests except growth in potassium tellurite in well G12. The strains were found to be sensitive to troleandomycin, lincomycin, vancomycin, fusidic acid, rimfamycin SV, guanidine HCl, D-serine, minocycline and niaproof 4. As reported in the study by Yasuhara-Bell and Alvarez (2015), it was determined in this study that Cmm strains did not hydrolyse gelatin. In the study conducted by Korus (2011) on the identification of subspecies of 32 *C. michiganensis* strains, only one strain was identified as Cmm by using the GEN III OmniLog ID System. The similarity index of the strain was relatively low (31%). In the study conducted by Yasuhara-Bell and Alvarez (2015) on the differentiation of *Clavibacter* subspecies from Cmm, Cmm strains were identified with the Biolog System with an average similarity index of 0.748 and 0.898 and a probability index of 0.564 and 0.686. Ialacci et al. (2016) used the Biolog System for the diagnosis of 63 strains obtained from samples taken from 17 farms during tomato bacterial cancer outbreaks in Sicily and identified 21 of the strains as Cmm with a similarity index of 0.50-0.70 and a probability index of 83-100%. In this study, 18 strains were identified as Cmm with a similarity index of 54-97% and a probability index of 70-100% and the result was confirmed by the pathogenicity test. Ialacci et al. (2016) and Stancu and Mitrea (2020) emphasised that the identification would be correct if the similarity index was greater than 0.5. In this study, it was determined that the similarity index of the strains was greater than 0.5. Harris-Baldwin and Gudmestad (1996) reported that the accuracy of the strain identification increased when the profiles of the known strains of a particular species were added to the Biolog System database. In this study, the strains were successfully identified with the Biolog Gen III System. However, in most of the studies, it was found that the differences between the strains studied with the Biolog System database did not alter their classification at the genus and species level, but were inadequate for their classification at the subspecies level (Morgan et al., 2009). The results indicated that *C. michiganensis* subspecies shared very similar dietary characteristics and therefore produced very similar profiles, which could pose a problem when trying to use profiles for subspecies differentiation. Yasuhara-Bell (2014) suggested that differences in metabolic profiles of strains could reflect loss or gain of genetic material and/or adaptation to a more specific niche.

## 4. Conclusion

In this study, all bacterial strains isolated from tomato plants were identified based on the FAME analyses and the metabolic profiles obtained using the Biolog Gene III System. The obtained morphological and biochemical test results provided valuable information for the diagnosis of Cmm. The results showed that morphological and biochemical properties could be used together to make a distinction between species, but it was necessary to use MIS and Biolog Gen III System to determine subspecies categories. It is thought that the profile data obtained in this study will be useful in the development of databases of systems for the diagnosis of Cmm.

## Conflict of Interest

The author has no conflict of interest to declare.

## References

Anak, H., Dönmez, M. F., & Çoruh, İ. (2021). Biological control of *Rhizoctonia solani* Kühn. with rhizobacteria isolated from different soil and *Calligonum polygonoides* L. subsp. *comosum* (L'hér.). *Journal of Agriculture*, *4*(2), 92-107. https://doi.org/10.46876/ja.986625

Aysan, Y., & Uygur, S. (2005). Epiphytic survival of *Pseudomonas viridiflava*, causal agent of pith necrosis of tomato, on weeds in Turkey. *Journal of Plant Pathology*, *87*(2), 135-139.

Basım, H., & Basım, E. (2018). Phenotypic and genotypic characterization of *Clavibacter michiganensis* subsp. *michiganensis* causing tomato bacterial canker and wilt disease in Turkey. *European Journal of Plant Pathology*, *151*, 355-369. https://doi.org/10.1007/s10658-017-1378-3

Belgüzar, S., Yanar, Y., & Aysan, Y. (2016). Tokat ilinde domates bakteriyel solgunluk hastalığının yaygınlığı ve etmenin (*Clavibacter michiganensis* subsp. *michiganensis*) tanılanması. *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi*, *33*(2), 34-40. https://doi.org/10.13002/jafag973 (In Turkish)

Çakmakçı, R., Dönmez, M. F., Ertürk, Y., Erat, M., Haznedar, A., & Sekban, S. (2010). Diversity and metabolic potential of culturable bacteria from the rhizosphere of Turkish tea grown in acidic soils. *Plant Soil*, *332*, 299-318. https://doi.org/10.1007/s11104-010-0295-4

Çetinkaya Yıldız, R. (2007). *Domates bakteriyel solgunluk hastalığı etmeni [Clavibacter michiganensis subsp. michiganensis (Smith) Davis et. al.]'nin tanılanması ve bitki büyüme düzenleyici rizobakteriler ile biyolojik mücadele olanaklarının araştırılması* (Doctoral dissertation, Çukurova University). (In Turkish)

Cristina, R. A., Roberto, S. O., Lourdes, C. D., Fidel, N. R., Hernán, C. M., & Edgar, R. P. (2018). Detection of *Clavibacter michiganensis* subsp. *michiganensis* in tomato and chili seeds and farming area of Sinaloa, Mexico. *Journal of Plant Science and Phytopathology*, *2*, 044-054. https://doi.org/10.29328/journal.jpsp.1001019

Eichenlaub, R., & Gartemann, K. H. (2011). The *Clavibacter michiganensis* subspecies: Molecular investigation of gram-positive bacterial plant pathogens. *Annual Review of Phytopathology*, *49*(1), 445-464. https://doi.org/10.1146/annurev-phyto-072910-095258

EPPO. (2016). PM 7/42 (3) *Clavibacter michiganensis* subsp. *michiganensis*. *EPPO Bulletin*, *46*(2), 202-225. https://doi.org/10.1111/epp.12302

Gautam, S., Sharma, R., Chauhan, A., Shirkot, C. K., & Kaushal, R. (2020). Biocontrol activities of rhizobacteria associated with apple, apricot and kiwi rhizosphere against bacterial canker caused by *Clavibacter michiganensis*. *Indian Phytopathology*, *73*, 45-56. https://doi.org/10.1007/s42360-019-00172-3

Gitaitis, R. D., & Beaver, R.W. (1990). Characterization of fatty acid methyl ester content of *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology*, *80*, 318-321. https://doi.org/10.1094/Phyto-80-318

Harris-Baldwin, A., & Gudmestad, N. C. (1996). Identification of phytopathogenic coryneform bacteria using the biolog automated microbial identification system. *Plant Disease*, *80*, 874-878. https://doi.org/10.1094/PD-80-0874

Hélias, V., Hamon, P., Huchet, E., Wolf, J. W. D., & Andrivon, D. (2012). Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, *61*(2), 339-345. https://doi.org/10.1111/j.1365-3059.2011.02508.x

Hyder, S., Gondal, A. S., Rizvi, Z. F., Ahmad, R., Alam, M. M., Hannan, A., Ahmed, W., Fatima, N., & Inam-ul-Haq, M. (2020). Characterization of native plant growth promoting rhizobacteria and their anti-oomycete potential against *Phytophthora capsici* affecting chilli pepper (*Capsicum annum* L.). *Scientific Reports*, *10*, 13859. https://doi.org/10.1038/s41598-020-69410-3

Ialacci, G. M., Bella, P., Licciardello, G., Strano, C. P., Eichenlaub, R., Gartemann, K. H., La Rose, R., & Catara, V. (2016). Clonal populations of *Clavibacter michiganensis* subsp. *michiganensis* are responsible for the outbreaks of bacterial canker in greenhouse tomatoes in Italy. *Plant Pathology*, *65*(3), 484-495. https://doi.org/10.1111/ppa.12424

Jahr, H., Dreier, J., Meletzus, D., Bahro, R., & Eichenlaub, R. (2000). The endo-β-1, 4-glucanase CelA of *Clavibacter michiganensis* subsp. *michiganensis* is a pathogenicity determinant required for induction of bacterial wilt of tomato. *Molecular Plant-Microbe Interactions*, *13*(7), 703-714. https://doi.org/10.1094/MPMI.2000.13.7.703

Kolomiets, J. V., Grygoryuk, I. P., & Butsenko, L. M. (2017). Bacterial diseases of tomato plants in terms of open and covered growing of Ukraine. *Annals of Agrarian Science*, *15*(2), 213-216. https://doi.org/10.1016/j.aasci.2017.05.010

Korus, K. A. (2011). *Evaluating commercially available diagnostic tests for the detection of Clavibacter michiganensis subsp. nebraskensis, cause of Goss's bacterial wilt and leaf blight in corn* (Doctoral dissertation, Nebraska University).

Lelliot, R. A., & Stead, D. E. (1987). *Methods for the diagnosis of bacterial diseases of plants*. Blackwell Scientific Publications, Oxford.

Li, X., Tambong, J., Yuan, K. X., Chen, W., Xu, H., Lévesque, C. A., & De Boer, S. H. (2018). Re-classification of *Clavibacter michiganensis* subspecies on the basis of whole-genome and multi-locus sequence analyses. *International Journal of Systematic and Evolutionary Microbiology*, *68*(1), 234-240. https://doi.org/10.1099/ijsem.0.002492

Mirik, M., Aysan, Y., & Sahin, F. (2011). Characterization of *Pseudomonas cichorii* isolated from different hosts in Turkey. *International Journal of Agriculture & Biology*, *13*(2), 203-209.

Morgan, M. C., Boyette, M., Goforth, C., Sperry, K. V., & Greene, S. R. (2009). Comparison of the Biolog OmniLog Identification System and 16S ribosomal RNA gene sequencing for accuracy in identification of atypical bacteria of clinical origin. *Journal of Microbiological Methods*, *79*(3), 336-343. https://doi.org/10.1016/j.mimet.2009.10.005

Nandi, M., Macdonald, J., Liu, P., Weselowski, B., & Yuan, Z. C. (2018). *Clavibacter michiganensis* ssp. *michiganensis*: Bacterial canker of tomato, molecular interactions and disease management. *Molecular Plant Pathology*, *19*(8), 2036-2050. https://doi.org/10.1111/mpp.12678

Narayanasamy, P. (2001). *Plant pathogen detection and disease diagnosis*. CRP Press.

Şahin, F., Uslu, H., Kotan, R., & Dönmez, M. F., (2002). Bacterial canker, caused by *Clavibacter michiganensis* ssp. *michiganensis*, on tomatoes in eastern Anatolia region of Turkey. *Plant Pathology*, *51*(3), 399. https://doi.org/10.1046/j.1365-3059.2002.00715.x

Sasser, M. (1990), Identification of bacteria by gas chromatography of cellular fatty acids. *MIDI Technical Note*, *101*, 1-6.

Saygılı, H., Şahin, F., & Aysan, Y. (2006). *Fitobakteriyoloji*. Meta Basım.

Schaad, N. W., Jones, J. B., & Chu, W. (2001). *Laboratory guide for identification of plant pathogenic bacteria*. American Phytopathological Society.

Schumann, P., Kämpfer, P., Busse, H. J., & Evtushenko, L. I. (2009). Proposed minimal standards for describing new genera and species of the suborder Micrococcineae. *International Journal of Systematic and Evolutionary Microbiology*, *59*(7), 1823-1849. https://doi.org/10.1099/ijs.0.012971-0

Soylu, S., Baysal, Ö., & Soylu, E. M. (2003). Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) in tomato seedlings. *Plant Science*, *165*(5), 1069-1075. https://doi.org/10.1016/S0168-9452(03)00302-9

Stancu, M. S., & Mitrea, R. (2020). Monitoring of the harmful organism *Clavibacter michiganensis* subsp. michiganensis *in* Romania, Between 2011-2018. *Current Trends in Natural Sciences*, *9*(17), 175-186. https://doi.org/10.47068/ctns.2020.v9i17.021

Sunyar, B., Dönmez, M. F., & Çoruh, I. (2021). Iğdır'da domates (*Solanum lycopersicon* L.)'te hastalığa neden olan bakterilerin izolasyonu ve tanısı. *Journal of Agriculture*, *4*(2), 108-129. https://doi.org/10.46876/ja.1015781 (In Turkish)

Takishita, Y., Charron, J. B., & Smith, D. L. (2018). Biocontrol rhizobacterium *Pseudomonas* sp. 23S induces systemic resistance in tomato (*Solanum Lycopersicum* L.) against bacterial canker *Clavibacter michiganensis* subsp. *michiganensis*. *Frontiers in Microbiology*, *9*, 2119. https://doi.org/10.3389/fmicb.2018.02119

Townsend, G. K., & Heuberger, J. W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter*, *27*(17), 340-343.

Tripathi, R., Vishunavat, K., & Tewari, R. (2022). Morphological and molecular characterization of *Clavibacter michiganensis* subsp. *michiganensis* causing bacterial canker in tomatoes. *Physiological and Molecular Plant Pathology*, *119*, 101833. https://doi.org/10.1016/j.pmpp.2022.101833

Vauterin, L., Hoste, B., Kersters, K., & Swings, J. (1995). Reclassification of *Xanthomonas*. *International Journal of Systematic and Evolutionary Microbiology*, *45*(3), 472-489. https://doi.org/10.1099/00207713-45-3-472

Yang, P., Vauterin, L., Vancanneyt, M., Swings, J., & Kersters, K. (1993). Application of fatty acid methyl esters for the taxonomic analysis of the genus Xanthomonas. *Systematic and Applied Microbiology*, *16*(1), 47-71. https://doi.org/10.1016/S0723-2020(11)80250-X

Yasuhara-Bell, J. (2014). *Establishment and validation of loop-mediated amplification for specific detection of tomato bacterial pathogen Clavibacter michiganensis subsp. michiganensis* (Doctoral dissertation, Hawai University).

Yasuhara-Bell, J., & Alvarez, A. M. (2015). Seed-associated subspecies of the genus *Clavibacter* are clearly distinguishable from *Clavibacter michiganensis* subsp. *michiganensis*. *International Journal of Systematic and Evolutionary Microbiology*, *65*(Pt_3), 811-826. https://doi.org/10.1099/ijs.0.000022

Yin, L. J., Huang, P. S., & Lin, H. H. (2010). Isolation of cellulase-producing bacteria and characterization of the cellulase from the isolated bacterium *Cellulomonas* sp. YJ5. *Journal of Agricultural and Food Chemistry*, *58*(17), 9833-9837. https://doi.org/10.1021/jf1019104

Załuga, J. (2013). *Diagnosis for quarantine plant pathogenic Clavibacter and relatives: molecular characterization, identification and epidemiological studies* (Doctoral dissertation, Ghent University).

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
https://prensip.gen.tr

RESEARCH ARTICLE

# Effect of Calcium Nitrate Applications on Plant Development and Some Physiological Characteristics of Sunflower Seedlings Grown Under Drought Conditions

Zeynep Gül

Atatürk University, Plant Production, Application and Research Center, Erzurum/Türkiye

ARTICLE INFO

**Article History**
Received: 27.07.2024
Accepted: 01.10.2024
First Published: 31.12.2024

**Keywords**
Calcium nitrate
Drought
Plant growth
Sunflower

ABSTRACT

Drought stress is one of the abiotic stresses that negatively affects plant growth, development and product yield. In recent years, nutrient solution applications to leaves have been frequently used to reduce the negative effects of drought stress. This study was conducted in 2024 at the Atatürk University, Plant Production Application and Research Center to determine the effect of calcium nitrate applications during the seedling period on the growth and some physiological characteristics of sunflower (*Helianthus annuus* L.) grown under drought stress. The study was based on two-factor completely randomized experimental design with three irrigation levels [full irrigation (100% ($I_0$), 70% ($I_1$) and 40% ($I_2$) of field capacity), two $Ca(NO_3)_2$ concentrations (15 mM and 30 mM)]. The study was carried out as a pot trial based on this experimental design. At the end of the trial period, plant growth parameters and some physiological measurements and analyzes were performed on sunflower plants and the differences between the treatments were evaluated. According to the research findings, significant differences were observed between the different treatments and irrigation levels. The application of $Ca(NO_3)_2$ significantly influenced plant growth parameters (such as plant height, stem diameter, fresh and dry weight) and physiological parameters [such as tissue relative water content (RWC)] in sunflower grown under varying irrigation levels. At the end of the study, it was determined that dry conditions negatively affected plant growth in sunflowers and reduced the RWC value. In conclusion; calcium nitrate applications reduced this negative effect of drought compared to the control application. It can be said that especially the results obtained from the application of 15 Mm CaN at 70% ($I_1$) irrigation level are relatively less affected by drought.

**Please cite this paper as follows:**
Gül, Z. (2024). Effect of calcium nitrate applications on plant development and some physiological characteristics of sunflower seedlings grown under drought conditions. *Journal of Agricultural Production*, *5*(4), 228-233. https://doi.org/10.56430/japro.1523145

## 1. Introduction

Sunflower (*Helianthus annuus* L.) is cultivated for snack purposes even in regions with short vegetation periods, such as the Eastern Anatolia Region and similar ecologies. This increases the importance of sunflowers in places where corn, the first plant that comes to mind when it comes to silage, cannot reach harvest maturity.

Sunflower is easier to cultivate than corn. It is drought resistant and can be grown in arid areas without irrigation (Arıoğlu, 2000). With its deep root system, sunflower can use groundwater with a depth of approximately 2 m. For this reason, sunflower can be considered an alternative to corn as a silage forage plant in periods and places where rainfall or water is low (Bremner et al., 1986). It is resistant to lower temperatures than corn; It is much less damaged by the last

Correspondence
E-mail address: zdumlu@atauni.edu.tr

frosts of spring and the first frosts of autumn. The appropriate growth temperature in corn agriculture is 24-32°C, and the total temperature requirement during the development period varies between 2000-4000°C depending on the region and varieties (Kırtok, 1998). It also needs a relative humidity that does not fall below 60%. On the other hand, sunflower requires a total temperature of around 2600-2850°C during its growing period (Arıoğlu, 2000). In addition, the vegetation period of the sunflower plant until harvest maturity is quite short (90-120 days) (Ahmad et al., 2014). In addition to being a very valuable plant for silage, it is one of the four most important oilseeds in the world. In terms of vegetable oil production, sunflower ranks second after cotton and outperforms other non-traditional oilseed crops such as soybean and canola (Government of Pakistan, 2007).

Although Türkiye has suitable ecological conditions for sunflower cultivation, our sunflower cultivation areas cannot be increased (Meral, 2019). Although the sunflower plant is drought tolerant due to its taproot structure, the drought that occurs as a result of being a summer plant and not enough rainfall in this season reduces the yield per decare considerably (Kolsarıcı et al.,, 1995). It is known that drought has increased in recent years and is one of the main abiotic factors that negatively affects crop production worldwide (Reddy et al., 2004). Unlike other stress factors, drought stress does not appear suddenly, but develops gradually and increases in severity over time, causing damage (Larcher, 2003). Drought negatively affects plant growth and productivity by affecting water and nutrient supply to plants (Erdem et al., 2001; Jones & Qualset, 1984). Limited water in the plant prevents the accumulation of mineral elements in the tissues, negatively affecting normal root development, nutrient uptake and mobility in the soil (Luo et al., 2011). There are studies showing that the water potential, osmotic activity and gas exchange properties of sunflower are suppressed during drought (Tezara et al., 1999). Ashraf and O'Leary (1996) found that stomatal conductance and transpiration of sunflower plants grown under limited water stress were negatively affected. Studies aimed at reducing the negative effects of drought stress by spraying nutrient solutions on the leaves have been frequently used in recent years. Application of water-soluble fertilizers is foliar to increase nutrient availability (Al-Shammari et al., 2018, 2019).

Calcium nitrate fertilizer contains nitrate nitrogen and calcium, two essential nutrients for plants (Anonymous, 2024). It can be said that top fertilization is suitable for all types of soil and plants. Since the interaction of calcium and nitrate is favorable, there is no residue or salinity in the soil. It is generally used in foliar applications with drip and sprinkler irrigation systems on plants grown outdoors and in greenhouses. It has a white-colored granular structure and contains 15.5% N in the form of nitrate and 26.5% Ca, which is completely soluble in water. When given too little, it causes the plant to wilt, while when given too much, it causes crop maturation and harvest delays. Increasingly widespread agricultural drought limits irrigation opportunities in cultivation, and leading producers to seek alternative ways against the negative effects of drought stress. It has been determined that calcium has a reducing effect on drought stress in sugar beet, Arabidopsis thaliana, tea and corn plants (Hosseini et al., 2019; Huang et al., 2018; Malyukova et al., 2022; Naeem et al., 2018).

Numerous studies have shown that mineral nutrition plays an important role in increasing plant tolerance to abiotic stresses. However, despite the positive effect of calcium on plant growth and stress tolerance, studies on its application in sunflower are limited. The aim of this study is to investigate whether foliar application of calcium nitrate provides tolerance to drought stress in sunflower plants exposed to drought stress.

## 2. Materials and Methods

The study was conducted as a pot experiment in the greenhouses of the Atatürk University Plant Production Application and Research Center in 2024. The 'Bright Gold' variety of sunflower was used as the plant material in the research. The 2-liter pots were filled with the prepared soil mixture (garden soil (2): sand (1): peat mixture (1)) and 5 seeds were planted in each pot at a depth of 2-3 cm. After reaching the seedling stage, the plants were thinned to leave four uniform plants in each pot.

### *2.1.* Calcium Nitrate and Water Restriction Practices

The study included nine different treatments: 100% irrigation (control), 70% irrigation, 40% irrigation, 15 (mM) $Ca(NO_3)_2$ + 100% irrigation, 15 (mM) $Ca(NO_3)_2$ + 70% irrigation, 15 ( mM) $Ca(NO_3)_2$ + 40% irrigation, 30 (mM) $Ca(NO_3)_2$ + 100% irrigation, 30 (mM) $Ca(NO_3)_2$ + 70% irrigation, 30 (mM) $Ca(NO_3)_2$ + 40% irrigation. The experiment was conducted using a two-factor completely randomized experimental design, with three replications and four plants per replication. The 100% irrigation treatment ($I_0$) represents field capacity, while the 70% ($I_1$) and 40% ($I_2$) irrigation treatments represent water restriction levels relative to field capacity. Field capacity was calculated by weight percentage usingthe following formula (Güngör et al., 1996). 70% and 40% water restriction applications were calculated according to field capacity. Tap water was used in the study.

Water retention capacity = (Moisture level at field capacity (weight percentage) - Moisture level at wilting point (weight percentage))*Volume weight of the soil*Depth of the soil.

### *2.2.* Physical Methods

Calcium nitrate and water restriction treatments were initiated when the first true leaves formed at the seedling stage.

Fertilizer applications were made by spraying the leaves using a hand sprayer.

Within the scope of the study, seedling height (cm) and the number of leaves were measured. Additionally, fresh and dry weights of stems and roots (g) determined. Samples were dried at 68°C until a stable weight was achieved. Leaf area was measured using a LICOR model LI-3100 (Lincoln, NE, USA). Chlorophyll content was assessed with a SPAD502 chlorophyll meter (Konica Minolta Sensing Inc., Japan).

The stem diameter (mm) of the above-ground parts of the plants was measured at the harvest stage using a digital caliper (Gullap et al., 2022).

Leaf relative water content (LRWC) was determined according to Kaya et al. (2003).

The experiment was designed using a two-factor completely randomized experimental design. All data obtained at the end of the research were analysed using the variance analysis test with the SPSS 18 package program, and means were compared using the Duncan multiple comparison test (N. Yıldız & Bircan, 1991).

## 3. Results and Discussion

Differences in the average stem diameter (mm), plant fresh and dry weight (g), as well as root fresh and dry weight (g) under water restriction and calcium nitrate treatments are presented in Table 1. It is known that water stress negatively affects nutrient uptake in plants and reduces productivity (Ahmad et al., 2014). As a matter of fact, in Table 1, the decrease in irrigation level led to a decrease in all parameters. The highest and lowest stem diameter values were obtained from the control application (4.84) and the lowest irrigation level (3.31 mm), respectively. In both 15 mM and 30 mM $Ca(NO_3)_2$ applications, the stem diameter under 70% irrigation applications was higher than in the control applications and was included in the same statistical group. While a 59.42% weight loss was observed in fresh plant weight between $I_0$ and $I_2$ irrigation levels in control applications, this value was 48.65% in 15 mM $Ca(NO_3)_2$ applications. The weight loss in 30 mM $Ca(NO_3)_2$ applications was determined to be 44.52%. These values suggest that calcium nitrate applications can reduce the yield loss in plants due to drought. In plant dry weight, the weight loss in control applications was 68.57%, the weight loss in 15 mM $Ca(NO_3)_2$ applications was 61.66%, and the weight loss in 30 mM $Ca(NO_3)_2$ applications was 72.35%. The highest root fresh weight was obtained from $CaN_1$-$I_1$ treatment (2.25 g), While the lowest fresh root weight was from $CaN_2$-$I_2$ treatment (0.54g). The highest values for root dry weight were obtained from $CaN_1$-$I_0$ (1.01) and $CaN_1$-$I_1$ (1.15 g) treatments (Table 1). It is known that drought stress reduces photosynthesis, suppresses plant growth and food production, and causes decreases in total biomass and yield (Sarıyer et al., 2023). In addition, it has been determined that leaf water content and relative humidity in plants decrease significantly (Anjum et al., 2011). Clapco et al. (2018) stated that sunflower seedling fresh weight was negatively affected by increasing drought doses.

**Table 1.** Effect of applications on plant development in sunflower.

| Treatments | Stem diameter (mm) | Plant fresh weight (g) | Plant dry weight (g) | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|
| **$I_0$** | 4.84 a | 13.16 a | 5.25 a | 2.03 ab | 0.79 c |
| **$I_1$** | 4.32 c | 8.28 bc | 3.15bc | 1.95 ab | 0.82 bc |
| **$I_2$** | 3.31 d | 5.34 d | 1.65 d | 1.17 cd | 0.55 d |
| **$CaN_1$-$I_0$** | 4.74 ab | 12.60 a | 5.53 a | 2.08 ab | 1.01 a |
| **$CaN_1$-$I_1$** | 4.46 bc | 9.47 b | 3.99 b | 2.25 a | 1.15 a |
| **$CaN_1$-$I_2$** | 3.57 d | 6.47 cd | 2.12 cd | 1.80 abc | 0.49 d |
| **$CaN_2$-$I_0$** | 4.40 c | 9.14 b 9,14 | 5.57 a | 2.04 ab | 0.98 ab |
| **$CaN_2$-$I_1$** | 4.52 bc | 7.78 bc | 2.99 bc | 1.40 bc | 0.48 d |
| **$CaN_2$-$I_2$** | 3.48 d | 5.07 d | 1.54 d | 0.54 d | 0.10 e |

Means marked with different letters are statistically different.

The effects of calcium nitrate applications aimed at reducing the negative effects of different water restriction levels on seedling height, number of leaves, chlorophyll content, leaf area and tissue relative water content (RWC) in sunflower are presented in Table 2. The highest seedling height (52.00 cm and 52.67 cm) were obtained from control irrigation ($I_0$) and 15 mM $Ca(NO_3)_2$ with $I_0$ irrigation applications, both of which were in the same statistical group. The highest seedling height under $I_2$ irrigation application, which is the lowest irrigation level, was obtained from 15 mM $Ca(NO_3)_2$ application (42 cm). The number of leaves decreased with reduced irrigation levels in all treatments. The highest number of leaves (11 pieces per plant) was recorded with $I_0$ treatment, while the lowest number of leaves (6.67 pieces per plant) was observed with $CaN_2$-$I_2$ treatment. $CaN_1$-$I_1$ and $CaN_1$-$I_2$ treatments had higher leaf numbers compared to $I_1$ and $I_2$ irrigation levels of other treatments. Previous studies have determined that drought stress in sunflower, especially in the early development period

(4 to 8 leaves), causes a decrease in the number and area of leaves, a decrease in absorption during the maturity phase, and also a shorter life span of the plants (Göksoy et al., 2004). It is known that chlorophyll is the pigment that provides the green and healthy appearance of the plant. According to the results of our study, $I_0$ irrigation levels of all treatments and $CaN_1$-$I_1$ treatment had high chlorophyll content and were in the same statistical group. As expected, chlorophyll contents decreased with reduced irrigation levels. The stress caused by limited water in the plant develops due to the decrease in impulse growth, the expansion of cells in the root and shoot meristems, and the cessation of cell division. The suppression of cell expansion or division is parallel to the decrease in the rate of photosynthesis due to water deficiency (Anjum et al., 2011). Reducing irrigation levels decreased leaf area in all treatments. In the control application, there was a leaf area loss of 51.02 $cm^2$ between $I_0$ and $I_2$ irrigation levels, 32.05 $cm^2$ between 15 mM $Ca(NO_3)_2$ applications, and 37.62 $cm^2$ between 30 mM $Ca(NO_3)_2$ applications. Accordingly, it can be said that at least 15 mM $Ca(NO_3)_2$ applications of the leaf area are affected by water restriction applications. Researchers have stated that leaf area index is one of the most important growth indicators in sunflower. The reason for this is that sunflower reaches the highest leaf area and reaches maximum photosynthesis when it is not under any stress (Göksoy et al., 2004). Similarly, Pekcan et al. (2015) reported in their study that leaf area is the parameter most affected by drought stress in sunflower lines and that stress reduces leaf area up to 75%. In our study, it was determined that the highest RWC values were at $I_0$ levels, and as irrigation levels decreased, RWC values also decreased. Our results are parallel to other studies (Gür, 2019; S. Yıldız, 2017).

**Table 2.** Effect of applications on seedling height, number of leaves, chlorophyll value (SPAD), leaf area and tissue proportional water content in sunflower.

| Treatments | Seedling height (cm) | Number of leaves (pcs per plant) | Chlorophyll SPAD | Leaf area (cm² per plant) | RWC (%) |
|---|---|---|---|---|---|
| **$I_0$** | 52.00 a | 11.0 a | 40.00 a | 194.83 a | 67.00 bc |
| **$I_1$** | 45.33 bc | 8.67 cd | 36.66 b | 139.90 c | 63.66 c |
| **$I_2$** | 39.00 d | 7.67 de | 29.66 d | 95.42 d | 55.66 d |
| **$CaN_1$-$I_0$** | 52.67 a | 10.67 ab | 39.44 a | 187.82 ab | 71.96 a |
| **$CaN_1$-$I_1$** | 49.33 ab | 9.33 bc | 40.66 a | 151.89,bc | 56.48 d |
| **$CaN_1$-$I_2$** | 42.00 cd | 8.33 cd | 33.28 c | 127.62 cd | 42.79 f |
| **$CaN_2$-$I_0$** | 45.00 bc | 9.67 abc | 40.00 a | 153.80 bc | 70.33 ab |
| **$CaN_2$-$I_1$** | 44.00 c | 8.67 cd | 36.33 b | 135.11 cd | 56.33 d |
| **$CaN_2$-$I_2$** | 32.00 e | 6.67 e | 29.00 d | 95.93 d | 50.66 e |

Means marked with different letters are statistically different.

## 4. Conclusion

Sunflower is a valuable silage alternative forage plant in regions where the corn plant has a risk of freezing. Although it is a drought-resistant plant due to its taproot structure, drought caused by the fact that it is a summer plant and there is insufficient rainfall in this season significantly reduces the yield per decare. It is known that mineral nutrition plays an important role in increasing tolerance to drought stress, which negatively affects plant production. In fact, it was determined in our research that different irrigation levels and calcium nitrate applications significantly affected plant development and some physiological characteristics in sunflower. Increasing drought levels negatively affected all parameters. However, it was determined that calcium nitrate applications reduced this negative effect caused by drought compared to the control application. It can be said that the results obtained from the application of 15 mM CaN $Ca(NO_3)_2$ at 70% ($I_1$) irrigation level were relatively less affected by drought. Testing the results of this research, which was carried out controlled greenhouse conditions, with different plants and varying fertilizer doses under field conditions will be useful for further evaluation in terms of yield and quality.

## Acknowledgment

The greenhouses used in the study belong to the Atatürk University, Plant Production Application and Research Center. The authors thank Atatürk University for this.

## Conflict of Interest

The author has no conflict of interest to declare.

## References

Ahmad, R., Waraich, E. A., Ashraf, M. Y., Ahmad, S., & Aziz, T. (2014). Does nitrogen fertilization enhance drought tolerance in sunflower? A review. *Journal of Plant Nutrition*, *37*(6), 942-963. https://doi.org/10.1080/01904167.2013.868480

Al-Shammari, A. M. A., Abood, M. A., & Hamdi, G. J. (2018). Effect of foliar application of Tecamin flower on some vegetative growth characters of tomato plant under water stress conditions. *Syrian Journal of Agricultural Research*, *5*(2), 35-44.

Al-Shammari, A. M. A., Abood, M. A., & Hamdi, G. J. (2019). Foliar application of Tecamin max to alleviate water deficit on vegetative growth and yield of okra. *International Journal of Vegetable Science*, *25*(3), 278-284. https://doi.org/10.1080/19315260.2018.1509922

Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stres. *African Journal of Agricultural Research*, *6*(9), 2026-2032. https://doi.org/10.5897/AJAR10.027

Anonymous. (2024). *Kalsiyum gübresi nedir?* Retrieved July 07, 2024, from https://www.toros.com.tr/Kalsiyum%20Nitrat(1).pdf (In Turkish)

Arıoğlu, H. (2000). *Yağ bitkileri yetiştirme ve ıslahı*. Çukurova Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Ashraf, M., & O'Leary, J. W. (1996). Effect of drought stress on growth, water relations and gas exchange of two lines of sunflower differing in degree of salt tolerance. *International Journal of Plant Science*, *157*(6), 729-732. https://doi.org/10.1086/297395

Bremner, P. M., Preston, G. K., & Groth, C. F. (1986). A field comparison of sunflower (*Helianthus annuus* L.) and sorghum (*Sorghum bicolor*) in long srying cycle. I. Water extraction. *Australian Journal of Agricultural Research*, *37*(5), 483-493. https://doi.org/10.1071/AR9860483

Clapco, S., Tabara, O., Mutu, A., Gisca, I., Port, A., Joita-Pacureanu, M., & Duca, M. (2018). Screening of some sunflower hybrids for drought tolerance under laboratory conditions. *Lucrari Stiintifice*, *61*(1), 205-210.

Erdem, T., Delibas, L., & Orta, A. H. (2001). Water-use characteristics of sunflower (*Helianthus annuus* L.) under deficit irrigation. *Pakistan Journal of Biological Sciences*, *4*(7), 766-769. https://doi.org/10.3923/pjbs.2001.766.769

Göksoy, A. T., Demir, A. O., Turan, Z. M., & Dağüstü, N. (2004). Responses of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. *Field Crops Research*, *87*(2-3), 167-178. https://doi.org/10.1016/j.fcr.2003.11.004

Government of Pakistan. (2007). *Agriculture*. https://www.finance.gov.pk/survey/chapters/02-Agriculture08.pdf

Gullap, M. K., Severoglu, S., Karabacak, T., Yazici, A., Ekinci, M., Turan, M., & Yildirim, E. (2022). Biochar derived from hazelnut shells mitigates the impact of drought stress on soybean seedlings. *New Zealand Journal of Crop and Horticultural Science*, *52*(1), 19-37. https://doi.org/10.1080/01140671.2022.2079680

Güngör, Y., Erözel, A. Z., & Yıldırım, O. (1996). *Sulama ders kitabı*. Ankara Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Gür, A. (2019). *Kurak koşullarda yetiştirilen biber fidelerinde metil amin uygulamalarının bitki gelişimi ile bazı fizyolojik ve biyokimyasal özellikleri üzerine etkisi* (Master's thesis, Atatürk University). (In Turkish)

Hosseini, S. A., Réthoré, E., Pluchon, S., Ali, N., Billiot, B., & Yvin, J. C. (2019). Calcium application enhances drought stress tolerance in sugar beet and promotes plant biomass and beetroot sucrose concentration. *International Journal of Molecular Sciences*, *20*(15), 3777. https://doi.org/10.3390/ijms20153777

Huang, K., Peng, L., Liu, Y., Yao, R., Liu, Z., Li, X., Yang, Y., & Wang, J. (2018). Arabidopsis calcium-dependent protein kinase AtCPK1 plays a positive role in salt/drought-stress response. *Biochemical and Biophysical Research Communications*, *498*(1), 92-98. https://doi.org/10.1016/j.bbrc.2017.11.175

Jones, R. A., & Qualset, C. O. (1984). Breeding crops for environmental stress tolerance. In G. B. Collins & J. G. Petolino (Eds.), *Application of genetic engineering to crop improvement* (pp. 305-340). Advances in Agricultural Biotechnology. https://doi.org/10.1007/978-94-009-6207-1_10

Kaya, C., Higgs, D., Ince, F., Amador, B. M., Cakir, A., & Sakar, E. (2003). Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. *Journal of Plant Nutrition*, *26*(4), 807-820. https://doi.org/10.1081/PLN-120018566

Kırtok, Y. (1998). *Mısır üretimi ve kullanımı*. Kocaoluk Basın ve Yayınevi. (In Turkish)

Kolsarıcı, Ö., Bayraktar, N., İşler, N., Mert, M., & Arslan, B. (1995). *Yağlı tohumlu bitkilerin üretim projeksiyonları ve üretim hedefleri*. IV. Teknik Tarım Kongresi Bildiri Kitabı. (In Turkish)

Larcher, W. (2003). *Physiological plant ecology*. Springer-Verlag.

Luo, Y., Zhao, X., Zhou, R., Zuo, X., Zhang, J., & Li, Y. (2011). Physiological acclimation of two psammophytes to repeated soil drought and rewatering. *Acta Physiologiae Plantarum*, *33*, 79-91. https://doi.org/10.1007/s11738-010-0519-5

Malyukova, L. S., Koninskaya, N. G., Orlov, Y. L., & Samarina, L. S. (2022). Effects of exogenous calcium on the drought response of the tea plant (*Camellia sinensis* (L.) Kuntze). *PeerJ*, *10*, e13997. https://doi.org/10.7717/peerj.13997

Meral, Ü. B. (2019). Ayçiçeği (*Helianthus annuus* L.,) bitkisinin önemi ve üretimine genel bir bakış. *International Journal of Life Sciences and Biotechnology*, *2*(2), 58-71. https://doi.org/10.38001/ijlsb.535889 (In Turkish)

Naeem, M., Naeem, M. S., Ahmad, R., Ihsan, M. Z., Ashraf, M. Y., Hussain, Y., & Fahad, S. (2018). Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. *Archives of Agronomy and Soil Science*, *64*(1), 116-131. https://doi.org/10.1080/03650340.2017.1327713

Pekcan, V., Evci, G., Yilmaz, M. I., Nalcaiyi, A. B., Erdal, Ş. Ç., Cicek, N., & Kaya, Y. (2015). Evaluating foliar responses of sunflower genotypes under drought stress. *International Journal of Scientific Research in Science and Technology*, *1*(6), 54-63.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, *161*(11), 1189-1202. https://doi.org/10.1016/j.jplph.2004.01.013

Sarıyer, T., Özbay, N., & Karaca, A. (2023). Kuraklık stresinin biberin morfolojisi, fizyolojisi, büyümesi, biyokütle ve verim komponentleri üzerine etkileri. In Z. Gökbayrak, N. Keskin & B. İşçi (Eds.), *Türkiye'de agroekoloji: Bilim, uygulama ve sürdürülebilirlik*, (pp. 233-260). İKSAD Yayınevi. (In Turkish)

Tezara, W., Mitchell, V. J., Driscoll, S. P., & Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, *401*, 914-917. https://doi.org/10.1038/44842

Yıldız, N., & Bircan, H. (1991). *Araştırma ve deneme metotları*. Atatürk Üniversitesi Yayınları. (In Turkish)

Yıldız, S. (2017). *Kuraklık ve tuz stresinin lahanada fide gelişimi ve bazı fizyolojik özellikler üzerine etkisi* (Master's thesis, Atatürk University). (In Turkish)

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

# Mycoflora of Stored Wheat in Bafra District of Samsun Province

Ümit Eser[1]✉ • Bayram Kansu[2] • Berna Tunalı[3]

[1]Black Sea Agricultural Research Institute, Department of Plant Protection, Samsun/Türkiye
[2]Ondokuz Mayıs University, Samsun Vocational School, Department of Plant & Animal Production, Samsun/Türkiye
[3]Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun/Türkiye

ARTICLE INFO

**Article History**
Received: 14.05.2024
Accepted: 16.10.2024
First Published: 31.12.2024

**Keywords**
*Alternaria*
*Fusarium*
Samsun
Storage
Wheat

ABSTRACT

In this study, the fungal flora in wheat grain samples taken six months after harvest from warehouses in 13 villages of Bafra district of Samsun province was investigated. A total of 600 seeds were processed isolates of endophytic, saprophytic, or pathogenic fungi recovered were identified as 15 fungal genera. *Alternaria alternata, Alternaria* spp., *Chaetomium* spp., *Phoma* spp., *Epicoccum nigrum* were the fungi that showed the highest colonization frequency in analyzed grain. Fungi such as *Penicillium, Aspergillus, Fusarium graminearum, F. poae*, which are known to produce mycotoxins, were among the isolated fungi. *Fusarium graminearum, F. poae* and *Bipolaris sorokiniana* are among the important pathogens of wheat. The other microorganisms were present at intermediate or low values. On the other hand, fungi such as *Chaetomium, Epicoccum nigrum, Torula* species were isolated as antagonist organisms. *Stemphylum, Ulocladium, Cladosporium, Popularia, Nigrospora oryzae,* which are thought to be saprophytes or endophytes, were also isolated. Some are also known as weak pathogens. On average, 31.5% of the seeds examined had one or more fungal infections, while 68.5% had no fungal infections.

**Please cite this paper as follows:**

Eser, Ü., Kansu, B., & Tunalı, B. (2024). Mycoflora of stored wheat in Bafra District of Samsun Province. *Journal of Agricultural Production*, *5*(4), 234-240. https://doi.org/10.56430/japro.1484041

## 1. Introduction

Wheat is one of the most cultivated and consumed agricultural products by mankind since the day it was cultivated. It provides about 20% of the daily caloric requirement on average and about 21% of the daily protein intake in the human diet (Shiferaw et al., 2013). Wheat grains are also widely used in the flour and bakery sector as well as in the fermentation industry to produce beer, alcohol, vodka and biodiesel. Most of the products in the cereal group are stored in various ways for future use after harvest. The preferred general storage method for most of the other cereals, particularly wheat, is storage in silos or stacks in warehouses (Ertugay, 2010; Muir, 1980; Olgun, 2011). In these storage methods, the moisture content of the grain to be stored and the storage temperature are two important factors affecting the storage time of the grains (Muir, 1980). The moisture content of storage of 14% or less for wheat, ensures safe and long-lasting preservation (Wallace et al., 1983). Wolter (1986) reported that the positive effect of temperature on the flour quality extent of within 10˚C to 30˚C in stored wheat. Generally, storage temperature is kept at 15˚C and below to reduce metabolic activity and variability in stored grains (Timm et al., 2020; Wallace et al., 1983). Wheat grains can be infected by many microorganisms in the field condition or at post-harvest, which has a significant negative impact on food safety and product quality. Species belonging to fungal

✉ Correspondence
E-mail address: umiteser16@gmail.com

genera such as *Fusarium*, *Penicillium*, *Alternaria* and *Aspergillus* cause spoilage in stored wheat and the formation of mycotoxins that adversely affect human and animal health (Magan et al., 2011; Placinta et al., 1999; Solanki et al., 2021).

In this study, it was aimed to determine the fungal flora in stored wheat grain samples collected from different villages in Bafra district of Samsun province and to identify the fungal genera and species based on their morphological characteristics.

## 2. Materials and Methods

This study was carried out in 13 different villages of Bafra district (41°38′23″ North; 35°59′7″ East, Elevation = 20 m) of Samsun province, where wheat is cultivated and collected from 21 different wheat stores in 2017. The warehouses were selected as closed warehouses belonging to farmers or closed areas where bagged products were located. Sampling was done from the products stored within 6 months after harvest.

These villages in Bafra district of Samsun province include Yakıntaş, Tütüncüler, Evrenuşağı, Karıncak, Koşuköyü, Yeşilyazı, Kuşcular, Kaygusuz, Emenli, Harız, Azay, Çataltepe, Gökçeağaç villages. In the process of determining the villages where the study was carried out, it was important that the villages were in different locations and topographical features from each other. Totally, twenty samples were collected from stores in located these villages. The collected wheat samples were kept in sealed paper envelopes and brought to the laboratory.

Each collected sample batch was divided into two parts and the part not to be used for isolation was kept for further studies. From the remaining sample batch, thirty wheat grains were randomly selected. For surface disinfection, these grains were soaked in 1% NaOCl for 3 minutes, washed with distilled water and dried on blotting paper. After drying, the grains were placed on Potato Dextrose Agar (PDA) medium (39 g per liter distilled water with sterilized for 15 minutes at 121˚C and added Streptomycin sulfate in 0.1 g $L^{-1}$ and Oxytetracycline dehydrate 0.05 g $L^{-1}$) in 3 replicates with 10 seeds in each petri dish. The petri dishes were then sealed with parafilm and left to incubate for 5-8 days in cabinets with a temperature of 24±1˚C and Black Light with 12 hours of light and 12 hours of darkness (Booth, 1977; Burgess et al., 1994; Nelson et al., 1983).

At the end of approximately 1 week, cultures with mycelial growth were examined under a light microscope. The seeds in the petri dishes were evaluated one by one and the fungi were identified, and the number of affected seeds was recorded. Barnett and Hunter (1998) were used for identification some fungi that did not develop spores and resembling *Fusarium* sp. transferred on CLA (Carnation Leaf Agar) medium. All identified fungi and other identified fungi on carnation leaf agar (CLA) media were transferred to ½ PDA media and stored in the refrigerator at 4˚C for a certain period.

The SPSS v21 statistical packages (IMB, Statistic, OMU Licensed for online users) were used analysis of differences between the variances by One-Way ANOVA. The variance homogeneity was analysis Levene Test (Levene, 1960) and means were grouped by Duncan multiple range test (Duncan, 1955).

## 3. Results

In this study, 600 wheat seeds from 21 different storage samples were examined to investigate the fungal flora in wheat grains stored for 6 months after harvest. As a result, 68.5% (411) of the seeds examined did not show any fungal growth, while 38.5% (189) were found to be infected with at least one fungal genus or species (Figure 1). A total of 192 isolates belonging fifteen fungal genera were obtained from 189 wheat seeds in which fungal growth was observed.

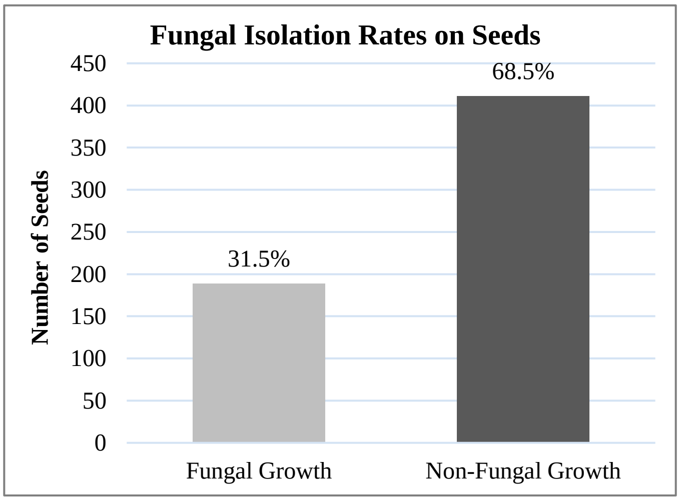


**Figure 1.** The percentage and number of fungal growth/non-growth on stored wheat seeds.

Fungal isolates obtained from the seeds on PDA were varied to base on their morphological characteristics and they were identified under the light microcopy. *Alternaria alternata* was the most dominant species with 41.5% among the isolated fungi from the seeds and together with other *Alternaria* isolates was the most common fungal genera with 60.4% in this study (Table 1). The morphology of *Alternaria* is typical and its conidia are dark colored, typically elliptical or spherical in shape, with both transverse and longitudinal compartments (Figure 2A and B). Following these, *Chaetomium* with 14.5% and *Phoma* with 6.7% were observed second and third most common fungal genera, respectively. The genus *Chaetomium* is generally characterized by rounded, ovoid, or obovate ostiolate ascomata covered with characteristic hairs (Figure 2C). *Epicoccum*, *Cladosporum* and *Penicillium* were among the other common fungi with 3.2%, 3% and 2.6%, respectively (Figure 2D and E). *Fusarium* was less common at 2.1%, but three different species had been identified as *F. graminearum*, *F. tabacinum* and *F. poae* (Figure 2F, G, H). *Bipolaris*, *Ulocladium* and *Septonema* were represented by two isolates for each genus, while *Aspergillus*, *Stemphylium*, *Nigrospora*, *Torula*, *Papularia* were rarely identified as only one isolate for each (Figure 2).

**Table 1.** The fungal genera/species and isolation rate of stored wheat seeds.

| Fungal Genera & Species | No. Isolates | Isolation Rate* (%) | Std. Deviation | Groups** |
|---|---|---|---|---|
| *Alternaria* spp. | | | | |
| *Alternaria alternata* | *79* | 41.46 | ±3.40 | a |
| *Alternaria* others | *37* | 18.96 | ±5.32 | ab |
| *Fusarium* spp. | | | | |
| *Fusarium graminearum* | *2* | 0.96 | ±0.86 | e |
| *Fusarium tabacinum* | *1* | 0.50 | ±0.86 | e |
| *Fusarium poae* | *1* | 0.63 | ±1.09 | e |
| *Bipolaris* spp. | | | | |
| *Bipolaris spicifera* | *1* | 0.63 | ±1.09 | e |
| *Bipolaris sorokiniana* | *1* | 0.63 | ±1.09 | e |
| *Phoma* spp. | *13* | 6.66 | ±1.42 | d |
| *Aspergillus* sp. | *1* | 0.50 | ±0.86 | e |
| *Penicillium* spp. | *5* | 2.63 | ±3.06 | de |
| *Epicoccum nigrum* | *6* | 3.23 | ±1.62 | de |
| *Chaetomium* spp. | *29* | 14.50 | ±6.06 | c |
| *Cladosporum* spp. | *6* | 3.03 | ±1.15 | de |
| *Septonema* spp. | *2* | 1.13 | ±1.00 | e |
| *Stemphylium* sp. | *1* | 0.63 | ±1.09 | e |
| *Ulocladium* spp. | *3* | 1.73 | ±1.92 | e |
| *Torula* sp. | *1* | 0.63 | ±1.09 | e |
| *Nigrospora oryzae* | *1* | 0.63 | ±1.09 | e |
| *Papularia* sp. | *1* | 0.50 | ±0.86 | e |
| Sterile fungus | *1* | 0.50 | ±0.86 | e |

* There is a significant difference among the variances (F=54.74, df=19, $p<0.01$).
**Duncan multiple range test.

## 4. Discussion

When the seeds were examined randomly by eye although a few seeds were chalky, embryos were blackened and spindly, 95% of the seeds appeared healthy. It is thought that most of the fungi obtained from these seeds may be endophytes. Researchers have shown that endophytes have an effect on plant growth and that phytohormones, such as indole-3-acetic acid, cytokinin, and other plant growth regulators play a role in increasing plant growth (Tan & Zou, 2001). Some researchers have also reported that endophytes contribute to the uptake of nutrients such as nitrogen and phosphorus by the host (Malinowski & Belesky, 1999; Reis et al., 2000). Gibberellins also play an important role in plant development. However, 12 fungal species have been found to produce gibberellins so far (Kawaide, 2006; MacMillan et al., 2005; Vandenbussche et al., 2007). In one study, the gibberellin production capacity of 19 endophytic fungal isolates was determined in Waito-C paddy cultivar and their effect on shoot growth was investigated, and more plant height growth was recorded in cucumber plants compared to the control (Hamayun et al., 2010).

On the other hand, it is known that isolates of *Torula*, *Ulocladium, N. oryzae, E. nigrum, Penicillium* and *Aspergillus* species are saprophytic (Barnet & Hunter, 1998). However, it should be taken into consideration that *Aspergillus* and *Penicillium* species, for example, produce mycotoxins (Prasher et al., 2024). It is known that about 250 of the fungal species whose existence has been revealed to date produce mycotoxins and about 20 of them cause poisoning in humans and animals (Erdem & Özen, 1990).

In a study on mycotoxin formation in cereals, it was stated that there is a very suitable environment for mycotoxin formation in cereals. The probability of formation of aflatoxin B1 and other aflatoxins in stored and carbohydrate-rich foodstuffs such as wheat and flour are very high (Evren, 1999). Aflatoxins are known to have toxigenic, mutagenic, teratogenic and carcinogenic effects for humans and animals (Ünlütürk & Turantaş, 1998). Aflatoxin formed by *Aspergillus* species and ochratoxin A formed by *Penicillium* species are the leading mycotoxins that cause significant health problems. The mycotoxins mainly grow on the food before or after harvesting and during storage. Most mycotoxins are chemically stable, and they survive food processing.

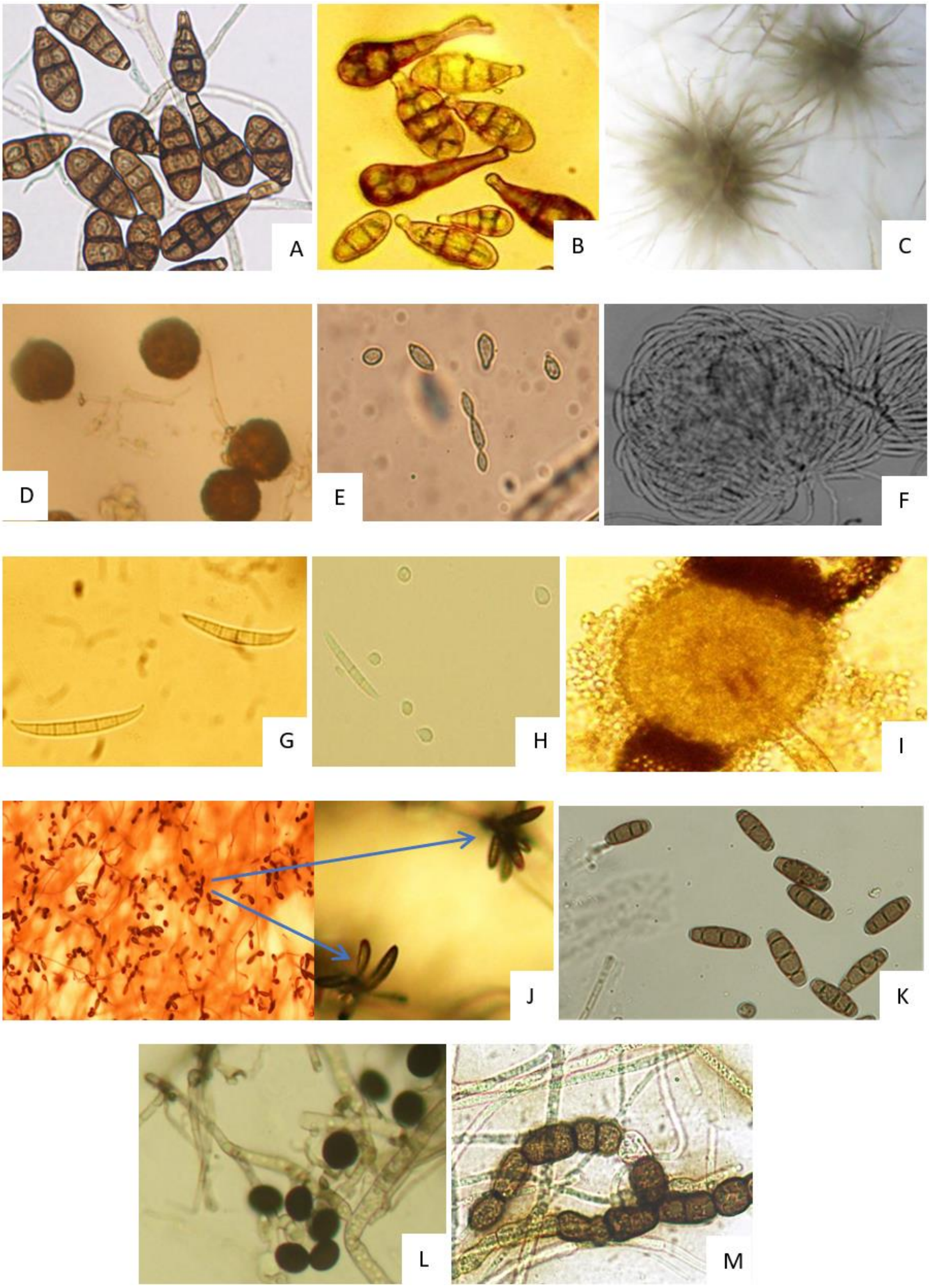


**Figure 2.** The microscopic images of fungal genus/species: A=*Alternaria alternata*, B= *Alternaria* sp., C= *Chaetomium* spp., D= *Epicoccum nigrum*, E= *Cladosporum* sp., F= *Fusarium graminearum* sporodochium, G= *F. graminearum* macroconidium, H= *Fusarium poae*, I= *Aspergillus* sp., J= *Bipolaris sorokiniana* conidia, K= *Bipolaris spicifera* conidia, L= *Nigropora oryzae*, M= *Torula* sp.

When the results are analyzed, it is seen that *Alternaria* spp. is the most common genus in wheat seeds and *A. alternata* is the most common species. In some other studies, it was determined that *A. alternata* was the most common species in the leaves, stems, ears and grains of wheat plants (Larran et al., 2002, 2007). The most frequently detected Alternaria toxins, which have significant toxicity, are alternariol (AOH), alternariol monomethyl ether (AME), altertoxins (ATXs; I, II and III), altenuene (ALT), tenuazonic acid (TEA), tentoxin (TEN) and *A. alternata* f. sp. *lycopersici* toxins (AALs) (EFSA, 2011). *Alternaria alternata* (Fr.) Keissl. is the most important and widespread *Alternaria* species, both in terms of its wide biological activity (pathogen, saprophyte, etc.) and host distribution, and mycotoxin production and diversity (Barkai-Golan, 2008; Bottalico & Logrieco, 1998; Logrieco et al., 2009; Pinto & Patriarca, 2017; Tunalı et al., 2023). *Alternaria* spp. can produce many different secondary metabolites at different stages of pathogenicity and these are defined as host-specific toxins (HSTs) and non-HSTs (Berestetskly, 2008; Friesen et al., 2008). Both groups of toxins are considered to be a "virulence factor" of *Alternaria* species, especially in terms of plant pathogenicity (Andrew et al., 2009). *Fusarium* species produce three most important classes of mycotoxins namely: trichothecenes, zearalenone (ZEN), and fumonisins (FBs). Among the fungi isolated from wheat grain, *F. graminearum* and *F. po*ae are also important pathogens. These fungi are among the leading agents causing head blight disease in small grains. Although several species have now been described within the clade, *F. graminearum* sensu stricto remains the most economically important toxigenic species in the genus, as it is the most frequent cause of Fusarium head blight of small grains and Gibberella ear rot of maize throughout most of the world. Several mycotoxins with different chemical structures have been reported to be associated with health problems in humans and animals (Munkvold et al., 2021). *Fusarium graminearum* can produce multiple mycotoxins, but production of the DON during the development of Fusarium head blight of cereals is most significant. *F. graminearum* is the species from which DON was first characterized (Vesonder et al., 1973, Yoshizawa & Morooka, 1973).

In this study, fungal flora was examined nine months after harvest. Isolates belonging to *Penicillium* and *Aspergillus* genera were also identified as a result of the examination, while in a study, the flora six months later was compared with the flora immediately after harvest. Analyses of the mycoflora revealed that in the tested varieties of grains at harvest, field fungi were overwhelmingly predominant constituting more than 90 % of the total number of species. *Alternaria alternata* was most predominant followed by other field fungi, *Curvularia pallescens, Cladosporium herbarum, B. sorokiniana* and species of *Fusarium* spp. and sterile fungi. The number of field fungi was found to decrease significantly with prolonged storage in all cases. The percentage of *Aspergillus* and *Penicillium*, on the other hand, which were present only occasionally at harvest, showed a continuous increase during the storage period (Ghosh et al., 1981).

## 5. Conclusion

As a result of this study, it was determined that a large number of fungal species were present in the fungal flora of wheat seeds nine months after harvest. With this study, we think that it would be useful to examine what kind of differences in the fungal flora in producer warehouses immediately after harvest and after certain periods of time after harvest. As a matter of fact, there are studies on this subject in the world. In addition, both endophytic fungi that can be used in biological control and toxigenic and saprophytic fungi obtained as a result of the research should be emphasized.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Andrew, M., Peever, T. L., & Pryor, B. M. (2009). An expanded multilocus phylogeny does not resolve morphological species within the small-spored *Alternaria* species complex. *Mycologia*, *101*(1), 95-109. https://doi.org/10.3852/08-135

Barkai-Golan, R. (2008). *Alternaria* mycotoxins. In R. Barkai-Golan & N. Paster (Eds.), *Mycotoxins in fruits and vegetables* (pp. 185-203). Elsevier. https://doi.org/10.1016/B978-0-12-374126-4.00008-5

Barnett, H. L. & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi*. APS Press.

Berestetskly, A. O. (2008). A review of fungal phytotoxins: From basic studies to practical use. *Applied Biochemical and Microbiology*, *44*, 453-465. https://doi.org/10.1134/S0003683808050013

Booth, C. (1977). *Fusarium. Laboratory guide to the identification of the major species*. Commenwealth Mycology Institute.

Bottalico, A., & Logrieco, A. (1998). Alternaria species of economic importance. In K. K. Sinha & D. Bhatnagar (Eds.), *Mycotoxins in agriculture and food safety* (pp. 65-108). Marcel Dekker.

Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P., & Backhouse, D. (1994). *Laboratory manual for Fusarium research*. Fusarium Research Laboratory Department of Crop Sciences University of Sydney and Royal Botanic Gardens.

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42. https://doi.org/10.2307/3001478

EFSA. (2011). Scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal*, *9*(10) 2407. https://doi.org/10.2903/j.efsa.2011.2407

Erdem, H., & Özen, N. (1990). Aflatoksinlerin insan ve hayvan sağlığı açısından önemi. *Ondokuz Mayıs Üniversitesi Ziraat fakültesi Dergisi*, *5*(1-2). (In Turkish)

Ertugay, Z. (2010). Buğday, un ve ekmek arasındaki kalite ilişkileri. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *13*(1-2), 165-176. (In Turkish)

Evren, M. (1999). Aflatoksinlerin etki şekilleri, gıdalarda bulunma durumları ve önleme çareleri. *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi*, *14*(2), 159-172. (In Turkish)

Friesen, T. L., Faris, J. D., Solomon, P. S., & Oliver, R. P. (2008). Host-specific toxins: Effectors of necrotrophic pathogenicity. *Cell Microbiology*, *10*(7), 1421-1428. https://doi.org/10.1111/j.1462-5822.2008.01153.x

Ghosh, J., Nandi, B., & Fries, N. (1981). Deterioration of stored wheat caused by fungal infections under different conditions of temperature and relative humidity. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, *88*, 9-17.

Hamayun, M., Khan, S. A., Khan, A. L., Rehman, G., Kim, Y. H., Iqbal, I., Hussain, J. & Lee, I. J. (2010). Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). *Mycologia*, *102*(5), 989-995. https://doi.org/10.3852/09-261

Kawaide, H. (2006). Biochemical and molecular analyses of gibberellin biosynthesis in fungi. *Bioscience, Biotechnology, and Biochemistry*, *70*(3), 583-590. https://doi.org/10.1271/bbb.70.583

Larran, S., Perello, A., Simon M. R., & Moreno, V. (2002). Isolation and analysis of endophytic microorganisms in wheat (*Triticum aestivum* L.) leaves. *World Journal of Microbiology & Biotechnology*, *18*, 683-686, 2002. https://doi.org/10.1023/A:1016857917950

Larran, S., Perello, A., Simon M. R., & Moreno, V. (2007). The endophytic fungi from wheat (*Triticum aestivum* L.). *World Journal of Microbiology & Biotechnology*, *23*, 565-572. https://doi.org/10.1007/s11274-006-9266-6

Levene, H. (1960). Robust tests for equality of variances. In I. Olkin (Ed.), *Contributions to probability and statistics* (pp. 278-292). Stanford University Press.

Logrieco, A., Moretti, A., & Solfrizzo, M. (2009). *Alternaria* toxins and plant diseases: An overview of origin, occurence and risks. *World Mycotoxin Journal*, *2*(2), 129-140. https://doi.org/10.3920/WMJ2009.1145

MacMillan, C. P., Blundell, C. A., & King, R. W. (2005). Flowering of the grass *Lolium perenne*. Effects of vernalization and long days on gibberellin biosynthesis and signaling. *Plant Physiology*, *138*(3), 1794-1806. https://doi.org/10.1104/pp.105.062190

Magan, N., Medina, A., & Aldred, D. (2011). Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. *Plant Pathology*, *60*(1), 150-163. https://doi.org/10.1111/j.1365-3059.2010.02412.x

Malinowski, D. P., & Belesky, D. P. (1999). *Neotyphodium coenophialum*-endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. *Journal of Plant Nutrition*, *22*(4-5), 835-853. https://doi.org/10.1080/01904169909365675

Muir, W. E. (1980). Grain drying and storage in Canada. *The Society of Agricultural Structures, Japan*, *10*(2), 91-105.

Munkvold, G. P., Proctor, R. H., & Moretti, A. (2021). Mycotoxin production in Fusarium according to contemporary species concepts. *Annual Review of Phytopathology*, *59*, 373-402. https://doi.org/10.1146/annurev-phyto-020620-102825

Nelson, P. E., Toussoun, T. S., & Marasas, W. F. O. (1983). *Fusarium species: An illustrated manual for identification*. Pennsylvania State University Press.

Olgun, M. (2011). *Tarımsal yapılar*. Ankara Üniversitesi Ziraat Fakültesi Yayınları. (In Turkish)

Pinto, V. E. F., & Patriarca, A. (2017). *Alternaria* species and their associated mycotoxins. In A. Moretti & A. Susca (Eds.), *Mycotoxigenic fungi: Methods and protocols* (pp. 13-32). Springer. https://doi.org/10.1007/978-1-4939-6707-0_2

Placinta, C. M., D'Mello, C. P. F., & MacDonald, A. M. C. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology*, *78*(1-2), 21-37. https://doi.org/10.1016/s0377-8401(98)00278-8

Prasher, P., Sharma, M., & Kumar, T. (2024). Chapter 6: Biocontrol of *Aspergillus* and *Penicillium* mycotoxins: Benefits and limitations. In K. A. Abd-Elsalam & H. I. Mohamed (Eds.), *Fungal secondary metabolites: Synthesis and applications in agroecosystem; a volume in nanobiotechnology for plant protection* (pp. 117-129). Elsevier. https://doi.org/10.1016/B978-0-323-95241-5.00021-6

Reis, V. M., Dos Reis, J., Fabio, B., Salles, J. F., & Schloter, M. (2000). Characterisation of different polyclonal antisera to quantify *Herbaspirillum* spp. in elephant grass (*Pennisetum purpureum* Schun.). *Symbiosis*, *29*(2), 139-150.

Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, *5*, 291-317. https://doi.org/10.1007/s12571-013-0263-y

Solanki, M. K., Droby, S., & Sionov, E. (2021). The wheat microbiome in relation to Mycotoxin occurrence in stored grain: An overview. In D. Spadaro, S. Droby & M. L. Gullino (Eds.), *Postharvest pathology: Next generation solutions to reducing losses and enhancing safety* (pp. 129-139). Springer. https://doi.org/10.1007/978-3-030-56530-5_8

Tan, R. X., & Zou, W. X. (2001). Endophytes: A rich source of functional metabolites. *Natural Product Reports*, *18*(4), 448-459. https://doi.org/10.1039/b100918o

Timm, N. S., Lang, G. H., Ramos, A. H., Pohndorf, R. S., Ferreira, C. D., & Oliveira, M. (2020). Effects of drying methods and temperatures on protein, pasting, and thermal properties of white floury corn. *Journal of Food Processing and Preservation*, *44*(10), e14767. https://doi.org/10.1111/jfpp.14767

Tunalı, B., Küçüktopçu, Y., Tunalı, N., Erken Meral, S., Eker, S., & Kansu, B. (2023). Alternaria mikotoksinleri ve önemi. *Bursa Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, *37*(1), 195-219. https://doi.org/10.20479/bursauludagziraat.1111062 (In Turkish)

Ünlütürk, A., & Turantaş, F. (1998). *Gıda mikrobiyolojisi*. Ege Üniversitesi Yayınları. (In Turkish)

Vandenbussche, F., Fierro, A. C., Wiedemann, G., Reski, R., & Van Der Straeten, D. (2007). Evolutionary conservation of plant gibberellin signalling pathway components. *BMC Plant Biology*, *7*, 1-17. https://doi.org/10.1186/1471-2229-7-65

Vesonder, R. F., Ciegler, A., & Jensen, A. H. (1973). Isolation of emetic principle from Fusarium-infected corn. *Applied Microbiology*, *26*(6), 1008-1010. https://doi.org/10.1128/am.26.6.1008-1010.1973

Wallace, H. A. H., Sholberg, P. L., Sinha, R. N., & Muir, W. E. (1983). Biological, physical and chemical changes in stored wheat. *Mycopathologia*, *82*, 65-76. https://doi.org/10.1007/BF00437333

Wolter, K. (1986). Flour quality flour storage flour temperature. *Allgemeine Baecker Zeitung*, *41*(3-49, 6.

Yoshizawa, T., & Morooka, N. (1973). Deoxynivalenol and its monoacetate: New mycotoxins from *Fusarium roseum* and moldy barley. *Agricultural and Biological Chemistry*, *37*(12), 2933-2934. https://doi.org/10.1080/00021369.1973.10861103

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSİP
https://prensip.gen.tr

RESEARCH ARTICLE

# Evaluating the Efficacy of Fungicides for Controlling Late Blight in Tomatoes Induced by *Phytophthora infestans*

Iqra Kanwal[1] • Fatih Ölmez[2] • Amjad Ali[2] • Muhammed Tatar[2]✉ • Fatih Dadaşoğlu[3]

[1]Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun/Türkiye
[2]Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technologies, Department of Plant Protection, Sivas/Türkiye
[3]Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum/Türkiye

ARTICLE INFO

**Article History**
Received: 14.08.2024
Accepted: 16.10.2024
First Published: 31.12.2024

**Keywords**
Chemical management
Disease incidence
Foliar spray
*Phytophthora infestans*
Tomato late blight

ABSTRACT

Tomato (*Solanum lycopersicum* L.), is an important crop in tropical and subtropical regions, but it is highly susceptible to biotic stresses, particularly late blight caused by *Phytophthora infestans.* This fungus disease can lead to sudden outbreaks, resulting in severe crop losses. Chemical control remains a vital strategy for managing such outbreak. This study evaluated the effectiveness of 20 different fungicides, sprayed at recommended doses, for controlling late blight tomato and improving tomato production. A susceptible tomato variety, Nagina, was grown under randomized complete block design (RCBD) *in vivo.* Based on the percentage of disease infections produced on tomato plants and statistical analysis results, the results found that Chlorostrobin (13.62%), Cabrio Top (14.91%), Curzate M (15.38%), Ridomil Gold (16.77%), Jalva (17.13%), Nanok (19.2%), and Antracol (19.34%) were the most effective fungicides against *P. İnfestans.* Other fungicides such as Co-pride (21.1%), Flumax (21.54%), Alliette (23.81%), Score (24.35%), Success 40 WSP (25.13%), and Melody Due (28.82%) also exhibited effective results. However, fungicides like Rally (32.23%), Cytrol (34.28%), Thrill (37.46%), Evito (37.52%), Shincar (43.63%), Topas (45.83%), and Tilt (48.59%) were less effective in controlling the disease. These findings highlight the importance of using Chlorostrobin, Cabrio Top, Curzate M, Ridomil Gold, Jalva, Nanok, and Antracol are highly effective fungicides to combat late blight. This targeted approach ensures that fungicides are applied when they are most effective at preventing disease outbreaks, reducing overall fungicides use and costs.

**Please cite this paper as follows:**

Kanwal, I., Ölmez, F., Ali, A., Tatar, M., & Dadaşoğlu, F. (2024). Evaluating the efficacy of fungicides for controlling late blight in tomatoes induced by *Phytophthora infestans*. *Journal of Agricultural Production*, *5*(4), 241-247. https://doi.org/10.56430/japro.1533073

## 1. Introduction

The increasing frequency and intensity of plant disease outbreaks present significant and escalating threats to primary productivity, global food security, and biodiversity, particularly in vulnerable regions (Mubeen et al., 2024; Nauman et al., 2023). These outbreaks result in considerable losses, with pathogens and pests alone causing as estimated annual crop loss of US$220 billion globally. This directly affects food security, economic stability, and a range of interconnected socio-economic factors significantly affecting both regional economies and global markets (Ali et al., 2020; Anwaar et al., 2022; Azeem et al., 2020; Iftikhar et al., 2024; Rehman et al., 2023).

✉ Correspondence
E-mail address: mtatar@sivas.edu.tr

Pakistan is recognized for its favourable agro-climatic conditions, which support the robust cultivation of various of various fruits and vegetables. The country's total vegetable production has reached approximately 7.07 million tonnes, contributing to the overall agriculture output of 13.764 million tonnes (Abbas et al., 2023; Ahmad et al., 2021; Naqvi et al., 2024). The primary vegetables cultivated in the country are as tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), onion (*Allium cepa* L.), chilies (*Capsicum* spp.), turnip (*Brassica rapa* L.), okra (*Abelmoschus esculentus* L.), carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* var. *botrytis*), peas (*Pisum sativum* L.), tinda (*Praecitrullus fistulosus*) gourd, collectively occupying 78% of the total vegetable acreage and contributing 81% of the overall production. Its adaptability to both tropical and subtropical climates, coupled with its status as a key organic vegetable, underscores its role in sustainable agricultural practices in the region Tomatoes are a nutrient-rich source of essential vitamins, minerals, and dietary fibre, contributing to a well-balanced diet. They are particularly valued for their high concentration of lycopene content, a carotenoid recognized for its potent antioxidant activity, which has been extensively studies for its role in reducing the risk of various chronic diseases including cardiovascular disease and certain cancers. Interestingly, the bioavailability of the lycopene is significantly increased through thermal processing, such as during production of tomato juice or puree, enhancing its absorption and efficacy in the human body. This increased bioavailability likely explains why more than 80% of global tomato consumption occur in processed forms. In addition to lycopene, tomatoes are rich in other antioxidants, including vitamin C, beta-carotene, and phenolic compounds, which may act synergistically to provide a broad spectrum of health benefits by reducing oxidative stress and inflammations (Helyes & Lugasi, 2006).

Late blight, caused by the oomycete *Phytophthora infestans*, is a highly destructive pathogen affecting both tomatoes and potatoes. Its origins are traced to the Andes Mountains, with its global spread facilitated by cool, humid environments. The co-evolutionary relationship between the host plants and the pathogen, hypothesized in 19th century following the Irish potato famine of 1845-46 (De Bary, 1876; Ghorbani et al., 2004) has been confirmed by modern genetic analyses, including isozyme profiling, DNA sequencing, and pathogenicity comparisons. These studies have revealed striking genetic similarities among *P. infestans* isolates from Peruvian, U.S., and Europe (Foolad et al., 2008; Nowicki et al., 2012; Vleeshouwers et al., 2011) supporting the hypothesis of a common evolutionary origin. On tomatoes, the disease was first reported in France by Payen in 1847, and it is now known to infect all parts of the tomato plant, including leaves, stems, fruits, and seeds, leading to substantial crop losses (Reeves et al., 2023; Vincent et al., 2023). Symptoms typically begin as water-soaked lesions on the leaves, which can rapidly progress to blight and death of the entire plant within days. In the presence of moist weather, a whitish growth forms on the margins of the lesions on the underside of the leaf.

Once an unprotected crop (field, greenhouse, and/or plastic-cover cultures) is infected by *P. infestans*, the whole crop can be devastated within seven to ten days (Foolad et al., 2008). Economic losses may be in the form of reduced yield, lower quality of the fruit (such as low specific gravity), diminished storability, and increased cost associated with fungicide applications (Nowicki et al., 2012).

Different management strategies are adopted by the various researchers and farmers towards late blight of tomato and the most economical and environment friendly is the use of resistant varieties (Kanwal et al., 2024). Late blight of tomato disease can also be managed through the application of biocontrol agents and crop rotation. However, farmers need quick action in case of severe infection or the emergence of epidemics, and quick action can be acquired through the use of fungicides. Integrated Pest Management (IPM) suggests the application of fungicides after the appearance of typical disease symptoms (Mazumdar et al., 2021). Spraying fungicides at seven to ten days interval leads high crop quality and best average yield potential (Zhi et al., 2021).

This study aimed to evaluate the effectiveness of twenty fungicides available in Pakistan against *P. infestans* in field conditions to identify the most potent antifungal agent for managing late blight in tomatoes. The widespread application of fungicides in Pakistan necessitated a meticulous evaluation to identify the most efficacious fungicide for disease control, thereby optimizing resource allocation and minimizing financial burdens on farmers. The fungicides tested included Cholorostobin, Score, Alliette, Success, Antracol, Melody Due, Cytrol, Ridomil Gold, Curzate M, Rally, Cabrio Top, Co-Pride, Thrill, Evito, Shincar, Topas, Score, Flumax, Jalva, and Nanok, each applied as foliar sprays at recommended doses.

## 2. Materials and Methods

### *2.1.* Study Design and Agronomic Practices

The study was conducted at the Vegetable Research Institute, AARI Faisalabad, Pakistan, during the 2017 growing season. The highly susceptible variety Nagina was used to evaluate the efficacy of twenty different fungicides (Table 1). The nursery was sown in October, and transplanting was completed in November. The seedlings were arranged in a randomized complete block design (RCBD) with three replications. Each treatment plot measured 1×10 m, with plant-to-plant spacing of 50 cm and row-to-row spacing of 60 cm. Conventional agronomic practices were followed throughout to maintain optimal crop conditions.

**Table 1.** List of used fungicides and their recommended dozes.

| **Fungicides** | **Formulations** | **Active Ingredients** | **Manufacturers** | **Recommended doses (per Liter)** |
|---|---|---|---|---|
| Chlorostrobin | 56 EC | Azoxystrobin Chlorothalonil | Sitara | 5 mL |
| Cabrio Top | 60 WDG | Pyraclostrobin Metiram | FMC | 5 g |
| Curzate M | 72 WP | Cymoxanil Mencozeb | Arysta | 6 g |
| Ridomil Gold | 68 WG | Metalaxyl-m, Mencozeb | Syngenta | 3 g |
| Jalva | 325 SC | Azoxystrobin Difenoconazole | Orange Protection | 3 mL |
| Nanok | 25 SC | Azoxystrobim Flutrifol | Swat Agro Chemicals | 3 g |
| Antracol | 70 WP | Propineb | Bayer | 5 g |
| Co-pride | 50 WP | Copper Oxychloride | FMC | 10 g |
| Flumax | 60 EC | Fluazinam Metalaxal-M | Evyol Group | 2 mL |
| Alliette | 80 WP | Fosetyl Aluminium | Bayer | 3 g |
| Score | 250 EC | Difenoconazole | Syngenta | 2 mL |
| Success 40 WSP | 72 WP | Chlorothalonil Metalaxyl | Arysta | 3 g |
| Melody due | 66.8 WP | Propineb, Iprovalicarb | Bayer | 3 g |
| Rally | 40 WSP | Myclobutanil | Dow AgroSciences | 1 g |
| Cytrol | 75 WP | Thiophanate Methyl Chlorothaloni | Sitara | 5 g |
| Thrill | 20 WP | Bismerthiazole | FMC | 3 g |
| Evito | 480 SC | Fluoxastrobin | Arysta | 1 mL |
| Shincar | 50 EC | Carbendazim | FMC | 3 mL |
| Topas | 100 EC | Penconazole | Syngenta | 1 mL |
| Tilt | 250 EC | Propiconazol | Syngenta | 2 mL |
| Control | - | Distilled water | - | - |

### *2.2.* Disease Inoculation and Fungicide Application

Late blight disease was artificially induced using a 20 µl zoospore suspension of *Phytophthora infestans* cultured from infected leaves, following the method described by Pliakhnevich and Ivaniuk (2008). High humidity levels were maintained by regular water spraying to promote disease development. Fungicides were applied as foliar sprays (Sharma et al., 2011), after the onset of disease symptoms, with two applications administered at a seven-day interval using a battery-operated knapsack sprayer. The fungicides used included Chlorostobin, Score, Alliette, Success, Antracol, Melody Due, and others, at recommended doses.

### *2.3.* Data Collection and Statistical Analysis

Disease incidence was recorded weekly for three intervals, beginning seven days after the fungicide treatments, using the following formula:

Disease incidence (%) = (Number of infected plants)/(Total number of plants) × 100 (1)

Statistical analysis was performed using Statistix 8.1 (Mitani et al., 2001), with graphical representation generated through Minitab 18. Pairwise comparisons were made using the Least Significant Difference (LSD) test at a 0.05 significance level to determine the effectiveness of the fungicides.

## 3. Results and Discussion

Pathogenicity tests confirmed symptoms consistent with late blight in tomatoes, as reported in the literature (Figure 1). The effectiveness of the tested fungicides varied significantly in controlling the late blight disease. Chlorostrobin was the most effective, reducing disease incidence to 13.62%, followed closely by Cabrio top (14.91%), Curzate M (15.38%), Ridomil Gold (16.77%), Jalva (17.13%), Nanok (19.2%), and Antracol (19.34%). These treatments demonstrated substantial reductions in disease prevalence compared to untreated plants.

Other fungicides such as Co-pride (21.1%), Flumax (21.54%), Alliette (23.81%), Score (24.35%), Success 40 WSP (25.13%), and Melody due (28.82%) showed moderate effectiveness but did not match the performance of the top-tier treatments. Despite reducing the incidence of late blight, their efficacy was comparatively lower.

In contrast, fungicides including Rally (32.23%), Cytrol (34.28%), Thrill (37.46%), Evito (37.52%), Shincar (43.63%), Topas (45.83%), and Tilt (48.59%), were far less effective. These treatments failed to sufficiently control the spread of the disease, with exhibited a significantly higher disease incidence at 68.31% (Figure 2, Table 2). The limited effectiveness of these fungicides suggests their reduced capacity to mitigate fungal infestation, further underscoring the superior performance of the top-performing fungicides.


**Figure 1.** Healthy tomato plants (A), late blight diseased tomato plants in the field (B).

The fungicides Chlorostrobin, Cabrio Top, and Curzate M showed the highest efficacy with the lowest disease incidences, making them ideal candidates for controlling late blight (Table 2). In contrast, fungicides like Rally, Cytrol, and Tilt exhibited lower efficacy, with significantly higher disease incidences. This highlights the critical importance of selecting the most effective fungicides, particularly those with a disease incidence below 20%, to ensure optimal late blight management in tomatoes.

**Table 2.** Correlation based on disease incidence and the efficacy of each fungicide.

| Fungicide | Disease Incidence (%) | Rank |
|---|---|---|
| Chlorostrobin | 13.62 | 1 |
| Cabrio Top | 14.91 | 2 |
| Curzate M | 15.38 | 3 |
| Ridomil Gold | 16.77 | 4 |
| Jalva | 17.13 | 5 |
| Nanok | 19.20 | 6 |
| Antracol | 19.34 | 7 |
| Co-pride | 21.10 | 8 |
| Flumax | 21.54 | 9 |
| Alliette | 23.81 | 10 |
| Score | 24.35 | 11 |
| Success 40 WSP | 25.13 | 12 |
| Melody Due | 28.82 | 13 |
| Rally | 32.23 | 14 |
| Cytrol | 34.28 | 15 |
| Thrill | 37.46 | 16 |
| Evito | 37.52 | 17 |
| Shincar | 43.63 | 18 |
| Topas | 45.83 | 19 |
| Tilt | 48.59 | 20 |

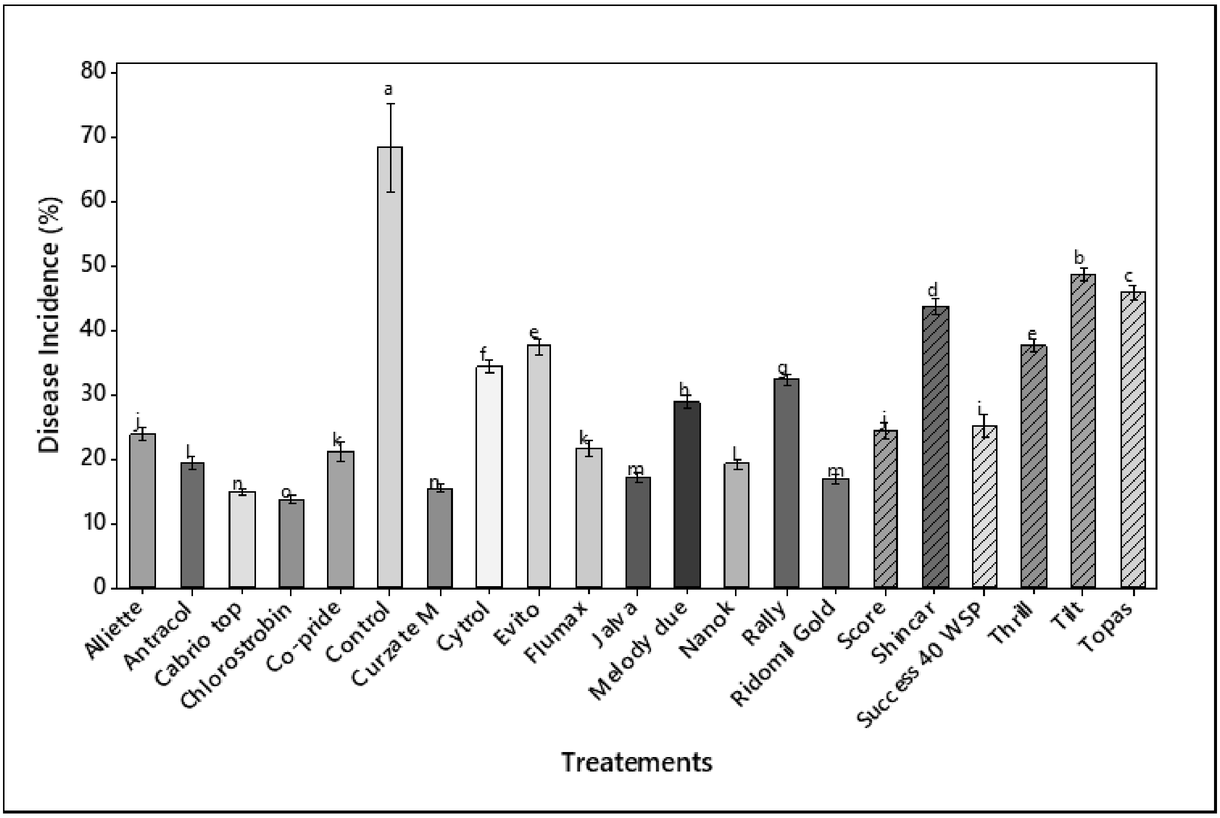


**Figure 2.** Fungicides' potential in mitigating the incidence of late blight of tomato.

Tomato a widely cultivated vegetable crop in tropical and subtropical regions, faces various biotic and abiotic challenges, particularly from fungal, bacterial, and viral pathogens, as well as several insect pests. Major fungal diseases of tomatoes include early blight, late blight, botrytis blight, alonf with widespread viral diseases include tomato leaf curl virus pathogen. All of these diseases reduce the tomato fruit size and leave it unmarketable. The best approach to control these diseases by use of effective fungicides.

Previous research by Peerzada et al. (2020) aligns with our findings, demonstrating the positive impact of fungicide application on controlling late blight. Their observations support the notion that fungicides effectively inhibit spore germination, sporulation, and intercellular mycelial growth of the late blight pathogen. This study further highlights a significant correlation: untreated control plots displayed higher AUDPC values, indicating a more rapid spread of the disease. Conversely, fungicide application significantly reduced AUDPC values, primarily by hindering spore germination and sporulation, which are crucial factors in the disease's progression. The severity of late blight directly translates to yield losses. These losses were quantified by measuring the yield difference between fungicide-treated and untreated plots. Notably, plants treated with systemic fungicides, particularly those receiving foliar sprays of Curzate, produced the highest tuber yield. In contrast, untreated control plots exhibited the lowest tuber yield (11.40 t/ha) alongside higher levels of defoliation at season's end and elevated AUDPC values. This observed variation in tuber yield across different treatments can be primarily attributed to the differing severities of late blight experienced in each treatment group.

Corroborating our findings, Neupane et al. (2018) investigated the efficacy of various active ingredients against late blight in tomato plants. Their study encompassed a broad spectrum of fungicides, including dimethomorph, cymoxanil, azoxystrobin, famoxadone, chlorothalonil, fenamidone, fosetyl-AL, cupric oxychloride, and cupric hydroxide, as well as several commercially available products. Notably, all tested fungicides with the exception of azoxystrobin (Quadris) demonstrated suppressive effects on late blight development. This aligns with previous research suggesting the effectiveness of contact fungicide applications applied towards the end of August in providing robust protection for both leaves and fruit throughout the growing season until harvest (Alexandrov, 2011; Mugao et al., 2020).

Our study aligns with the observations of Töfoli et al. (2014), who reported enhanced efficacy of fungicide combinations against potato blight under specific precipitation regimes. Their findings demonstrate that mefenoxam + mancozeb, mefenoxam + chlorothalonil, dimethomorph + ametoctradin, dimethomorph + chlorothalonil, propamocarb, fenamidone + propamocarb, benthiavalicarb + fuazinam, mandipropamid, mandipropamid + chlorothalonil, ametoctradin + metiram, cyazofamid, cymoxanil + zoxamide, and cymoxanil + famoxadone exhibited superior blight control when the initial rainfall event occurred 30 minutes after application, followed by progressively increasing simulated rain. Conversely, the activity of dimethomorph + mancozeb, fuopicolide + propamocarb, pyraclostrobin + metiram, fuazinam, chlorothalonil, and mancozeb NT was demonstrably diminished up to the 1-hour post-application interval (HAA). Notably, blight control by fenamidone treatments displayed a significant decline that extending up to the 2-hour HAA interval.

A growing body of research has investigated the effectiveness of various fungicides in combating late blight disease, a destructive illness caused by the pathogen *P. infestans* and plaguing crops like potato and tomato. Vincent et al. (2023) identified Bonsoin as the most potent fungicide against tomato late blight in their study, advocating for its use to control the disease and bolster tomato production within Cameroon. Furthermore, Ben Naim and Cohen (2023) observed that two applications of Zorvec Endavia yielded significantly superior late blight control compared to six applications of Ranman or four applications of Revus. These findings suggest these fungicides as potential alternatives to Mancozeb, offering the possibility of effective late blight management while reducing the risk of *P. infestans* developing resistance to mandipropamid and oxathiapiprolin.

Although my current research involves the use of fungicides to control plant infections, it is recommended that future strategies focus on prioritizing the utilization of biocontrol agents and the development of crop types that are more resistant to pathogens. Sometimes, the beneficial microorganisms and natural predators, known as biocontrol agents, provide a sustainable and ecologically sound substitute for synthetic fungicides (Ali et al., 2024a, 2024b). In addition, the process of breeding and genetically modifying crops to have improved resistance against infections might greatly decrease the need for chemical treatments (Ali et al., 2024c; Baloch et al., 2023). By implementing these strategies, the adverse environmental effects of fungicides can be reduced, biodiversity can be promoted, and long-term agricultural sustainability and food security can be ensured. It will be essential to prioritize the implementation of integrated pest management solutions for effective disease control in the future.

## 4. Conclusion

The results revealed Chlorostrobin, Cabrio top, Curzate M, Ridomil Gold, Jalva, Nanok, and Antracol as the most effective fungicides in field situations against *P. infestans*. Co-pride, Flumax, Alliette, Score, Success 40 WSP, and Melody Due were also found effective. On the other hand, Rally, Cytrol, Thrill, Evito, Shincar, Topas and Tilt were found less effective.

The on-time pervasive application of the most effective fungicide optimizes resource utilization and mitigates financial strains on farmers by minimizing the need for multiple fungicide applications throughout the growing season. Based on the current research, a fungicide program utilizing Chlorostrobin, Cabrio top, followed by sequential applications of Curzate M, Ridomil Gold, Jalva, Nanok, and Antracol is recommended for combating late blight in tomatoes and a range of other fungal diseases.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Abbas, A., Ali, A., Hussain, A., Ali, A., Alrefaei, A. F., Naqvi, S. A. H., & Baloch, F. S. (2023). Assessment of genetic variability and evolutionary relationships of *Rhizoctonia solani* inherent in legume crops. *Plants*, *12*(13), 2515. https://doi.org/10.3390/plants12132515

Ahmad, K., Afridi, M., Khan, N. A., & Sarwar, A. (2021). Quality deterioration of postharvest fruits and vegetables in developing country Pakistan: A mini overview. *Asian Journal of Agriculture and Food Sciences*, *9*(2), 83-90. https://doi.org/10.24203/ajafs.v9i2.6615

Alexandrov, V. (2011). Efficacy of some fungicides against late blight of tomato. *Bulgarian Journal of Agricultural Sciences*, *17*(4), 465-469.

Ali, A., Zeshan, M. A., Iftikhar, Y., Abid, M., Ehsan, S. F., Ghani, M. U., & Khan, A. A. (2020). Role of plant extracts and salicylic acid for the management of chili veinal mottle virus disease. *Pakistan Journal of Phytopathology*, *32*(2), 147-157. https://doi.org/10.33866/phytopathol.030.02.0583

Ali, A., Ölmez, F., Zeshan, M. A., Mubeen, M., Iftikhar, Y., Sajid, A., & Solanki, M. K. (2024a). Yeast-based solutions in controlling plant pathogens. *Biocatalysis and Agricultural Biotechnology*, *58*, 103199. https://doi.org/10.1016/j.bcab.2024.103199

Ali, A., Aasim, M., Çelik, K., Nadeem, M. A., & Baloch, F. S. (2024b). Frontiers in bacterial-based green synthesized nanoparticles (NPs): A sustainable strategy for combating infectious plant pathogens. *Biocatalysis and Agricultural Biotechnology*, *60*, 103293. https://doi.org/10.1016/j.bcab.2024.103293

Ali, A., Shahbaz, M., Ölmez, F., Fatima, N., Umar, U. U. D., Ali, M. A., Akram, M., Seelan, J. S. S., & Baloch, F. S. (2024c). RNA interference: A promising biotechnological approach to combat plant pathogens, mechanism and future prospects. *World Journal of Microbiology and Biotechnology*, *40*, 339. https://doi.org/10.1007/s11274-024-04143-3

Anwaar, H. A., Perveen, R., Chohan, S., Saeed, A., Cheema, M. T., Qadeer, A., & Ali, A. (2022). First report of *Alternaria alternata* causing Alternaria leaf spot of fig in Pakistan. *Plant Disease*, *106*(2), 759. https://doi.org/10.1094/PDIS-05-21-0963-PDN

Azeem, H., Ali, A., Zeshan, M. A., Ashraf, W., Ghani, M. U., Sajid, A., & Sajid, M. (2020). Biological control of plant pathogens by using antagonistic bacteria: A review. *Pakistan Journal of Phytopathology*, *32*(2), 273-290. https://doi.org/10.33866/phytopathol.030.02.0590

Baloch, F. S., Ali, A., Tajibayev, D., Nadeem, M. A., Ölmez, F., Aktaş, H., & Yang, S. H. (2023). Stripe rust resistance gene *Yr15* in Turkish and Kazakhstan wheat germplasms and the potential of Turkish wild emmer for stripe rust breeding. *Genetic Resources and Crop Evolution*, *71*, 2699-2719. https://doi.org/10.1007/s10722-023-01804-4

Ben Naim, Y., & Cohen, Y. (2023). Replacing Mancozeb with alternative fungicides for the control of late blight in potato. *Journal of Fungi*, *9*(11), 1046. https://doi.org/10.3390/jof9111046

De Bary, H. A. (1876). Researches into the nature of the potato fungus, *Phytophthora infestans*. *Journal of the Royal Agricultural Society of England*, *12*, 239-269.

Foolad, M. R., Merk, H. L., & Ashrafi, H. (2008). Genetics, genomics and breeding of late blight and early blight resistance in tomato. *Critical Reviews in Plant Sciences*, *27*(2), 75-107. https://doi.org/10.1080/07352680802147353

Ghorbani, R., Wilcockson, S. J., Giotis, C., & Leifert, C. (2004). Potato late blight management in organic agriculture. *Outlooks on Pest Management*, *15*(4), 176-180. https://doi.org/10.1564/15aug12

Helyes, L., & Lugasi, A. (2006). Formation of certain compounds having technological and nutritional importance in tomato fruits during maturation. *Acta Alimentaria*, *35*(2), 183-193. https://doi.org/10.1556/aalim.35.2006.2.5

Iftikhar, Y., Mubeen, M., Shakeel, Q., Lalarukh, I., Ali, A., Aasim, M., & Al-Shuraym, L. A. (2024). Spatial distribution and molecular characterization of Huanglongbing and its vector in various citrus cultivars. *Pakistan Journal of Agricultural Sciences*, *61*(2), 685-694. https://doi.org/10.21162/PAKJAS/24.155

Kanwal, I., Iffat, A., Shaukat, M. B., Shafique, T., Majeed, Y., Zafar, M. I., Awan, H. M., Tabbasum, I., Iqbal, A., Tatar, M., Mortazavi, P., Ali, A., Bejaoui, R., Aslam, H., & Seemab, F. (2024). Insights into Fusarium wilt of tomato (*Fusarium oxysporum* f. sp. *lycopersici*) and its management strategies. *Journal of Agriculture and Biology*, *2*(1), 31-42. https://doi.org/10.55627/agribiol.002.01.0753

Mazumdar, P., Singh, P., Kethiravan, D., Ramathani, I., & Ramakrishnan, N. (2021). Late blight in tomato:

Insights into the pathogenesis of the aggressive pathogen *Phytophthora infestans* and future research priorities. *Planta*, *253*, 119. https://doi.org/10.1007/s00425-021-03636-x

Mitani, S., Araki, S., Yamaguchi, T., Takii, Y., Ohshima, T., & Matsuo, N. (2001). Antifungal activity of the novel fungicide cyazofamid against *Phytophthora infestans* and other plant pathogenic fungi in vitro. *Pesticide Biochemistry and Physiology*, *70*(2), 92-99. https://doi.org/10.1006/pest.2001.2541

Mubeen, M., Ali, A., Iftikhar, Y., Shahbaz, M., Ullah, M. I., Ali, M. A., Fatima, N., Seelan, J. S. S., Tan, Y. S., & Algopishi, U. B. (2024). Innovative strategies for characterizing and managing huanglongbing in citrus. *World Journal of Microbiology and Biotechnology*, *40*, 342. https://doi.org/10.1007/s11274-024-04135-3

Mugao, L. G., Muturi, P. W., Gichimu, B. M., & Njoroge, E. K. (2020). In vitro control of *Phytophthora infestans* and *Alternaria solani* using crude extracts and essential oils from selected plants. *International Journal of Agronomy*, *2020*(1), 8845692. https://doi.org/10.1155/2020/8845692

Naqvi, S. A. H., Abbas, A., Farhan, M., Kiran, R., Hassan, Z., Mehmood, Y., Ali, A., Ahmed, N., Hassan, M. Z., Alrefaei, A. F., Ölmez, F., Yang, S. H., & Baloch, F. S. (2024). Unveiling the genetic tapestry: Exploring *Rhizoctonia solani* AG-3 anastomosis groups in potato crops across borders. *Plants*, *13*(5), 715. https://doi.org/10.3390/plants13050715

Nauman, M., Mushtaq, S., Khan, M. F., Ali, A., Naqvi, S. A. H., Haq, Z., & Umar, U. U. D. (2023). Morphological, b,ochemical, and molecular characterization of *Xanthomonas citri* subsp. citri, cause of citrus canker disease in Pakistan. *Pakistan Journal of Botany*, *55*(6), 2409-2421. http://doi.org/10.30848/PJB2023-6(14)

Neupane, P., Shrestha, R. K., & Shrestha, J. (2018). Efficacy of fungicides against late blight of potato. *Agricultura*, *107*(3-4), 39-43.

Nowicki, M., Lichocka, M., Nowakowska, M., Kłosińska, U., & Kozik, E. U. (2012). A simple dual stain for detailed investigations of plant-fungal pathogen interactions. *Journal of Fruit and Ornamental Plant Research*, *77*(1), 61-74. https://doi.org/10.2478/v10032-012-0016-z

Peerzada, S., Viswanath, H., & Bhat, K. (2020). In-vitro studies on effect of fungicides against mycelial growth and sporangial germination of *Phytophthora infestans* (Mont) de Bary) causing late blight of potato. *International Journal of Chemical Studies*, *8*(1), 2069-2075. https://doi.org/10.22271/chemi.2020.v8.i1ae.8569

Pliakhnevich, M., & Ivaniuk, V. (2008). Aggressiveness and metalaxyl sensitivity of *Phytophthora infestans* strains in Belarus. *Zemdirbyste*, *95*(3), 379-387.

Reeves, E. R., Strayer-Scherer, A., Panthee, D. R., Gardner, R., & Meadows, I. M. (2023). Variable yield responses among grafted and nongrafted late blight–resistant tomato (*Solanum lycopersicum* L.) hybrids in North Carolina. *HortScience*, *58*(8), 943-948. https://doi.org/10.21273/HORTSCI17145-23

Rehman, A. U., Rauf, A., Ali, A., Taimoor Shakeel, M., Hasan Naqvi, S. A., Shahid, M., & Umar, U. U. D. (2023). First report of *Fusarium equiseti* causing leaf spots of Bitter gourd (*Momordica charantia*) in Pakistan. *Plant Disease*, *107*(2), 584. https://doi.org/10.1094/PDIS-04-22-0786-PDN

Sharma, B. P., Manandhar, H. K., Forbes, G. A., Shrestha, S. M., & Thapa, R. B. (2011). Efficacy of fungicides against *Phytophthora infestans* in potato under laboratory and field conditions. *Nepal Agriculture Research Journal*, *11*, 28-39.

Töfoli, J. G., Domingues, R. J., de Melo, P. C. T., & Ferrari, J. T. (2014). Effect of simulated rain on the efficiency of fungicides in potato late blight and early blight control. *Semina: Ciências Agrárias*, *35*(6), 2977-2989. https://doi.org/10.5433/1679-0359.2014v35n6p2977

Vincent, D. P., Elie, K. K., & Gaston, T. N. (2023). Impact of introduced tomato cultivars and chemical fungicides on the occurrence and intensity of late blight in Western Cameroon. *Journal of Experimental Agriculture International*, *45*(9), 71-84. https://doi.org/10.9734/jeai/2023/v45i92177

Vleeshouwers, V. G., Raffaele, S., Vossen, J. H., Champouret, N., Oliva, R., Segretin, M. E., Rietman, H., Cano, L. M., Lokossou, A., Kessel, G., Pel, M. A., & Kamoun, S. (2011). Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology*, *49*(1), 507-531. https://doi.org/10.1146/annurev-phyto-072910-095326

Zhi, X., Shu, J., Zheng, Z., Li, T., Sun, X., Bai, J., Cui, Y., Wang, X., Huang, Z., Guo, Y., Du, Y., Yang, Y., Liu, L., & Li, J. (2021). Fine mapping of the *Ph-2* gene conferring resistance to late blight (*Phytophthora infestans*) in tomato. *Plant Disease*, *105*(4), 851-858. https://doi.org/10.1094/PDIS-03-19-0679-RE

*Journal of Agricultural Production* (2024), *5*(4), 248-254 https://doi.org/10.56430/japro.1515703

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
PRENSIP
https://prensip.gen.tr

RESEARCH ARTICLE

# A Study on Occupational Health and Safety in the Agricultural Sector in Türkiye: Analysis of Work Accidents, Occupational Diseases, and Lost Workdays

Mustafa Özdemir✉

Bayburt University, Faculty of Applied Sciences, Department of Emergency Aid and Disaster Management, Bayburt/Türkiye

ARTICLE INFO

**Article History**
Received: 22.07.2024
Accepted: 29.11.2024
First Published: 31.12.2024

**Keywords**
Agricultural worker
Occupational disease
Occupational health and safety
Temporary incapacity
Work accident

ABSTRACT

The agricultural sector holds strategic economic importance due to its roles in food production, employment, and providing raw materials to industry. Türkiye is a significant agricultural country with its biological diversity, favourable climate conditions, and agriculture-based sector. According to the labor force statistics of the Turkish Statistical Institute (TÜİK), as of 2023, approximately 4.7 million people are employed in the agricultural sector in Türkiye, with the majority being seasonal workers. The farm sector includes field farming, livestock, fisheries, and forestry. Occupational Health and Safety (OHS) is vital for the sustainability of the agricultural sector. The Occupational Health and Safety Law No. 6331, which came into force in 2012, has mandated OHS provisions for all enterprises and employees in the agricultural sector. This study examines the statistics on work accidents, occupational diseases, and lost workdays in Türkiye's plant and animal production sector between 2018 and 2022 and evaluates the current state of OHS practices. Based on data from the Social Security Institution (SGK), the analysis reveals that 13,993 work accidents and 96 fatal work accidents occurred in the plant and animal production sector between 2018 and 2022, with the highest frequency of work accidents recorded in 2022. Additionally, 13 cases of occupational diseases were reported during the same period. An examination of temporary incapacity durations shows 133,008 days of incapacity reports, with the highest number occurring in 2021, influenced by the COVID-19 pandemic. The study's findings indicate that OHS practices in the agricultural sector are inadequate and need improvement. In light of these findings, various policy recommendations have been developed, such as the widespread implementation of OHS training, increased health screenings, and stricter OHS inspections.

**Please cite this paper as follows:**

Özdemir, M. (2024). A study on occupational health and safety in the agricultural sector in Türkiye: Analysis of work accidents, occupational diseases, and lost workdays. *Journal of Agricultural Production*, *5*(4), 248-254. https://doi.org/10.56430/japro.1515703

## 1. Introduction

The agricultural sector holds strategic importance in national economies because it meets food needs, contributes to national income and employment, and provides raw materials for agriculture-based industries (Demir, 2015). With its biological diversity, rich climate and geographical conditions, presence of agriculture-based industries, and ranking in the top ten globally for producing fifty-five products, Türkiye is among the world's significant agricultural countries (Solmaz, 2023). According to 2023 employment data from the Turkish Statistical Institute (TÜİK), approximately 4.7 million people aged 15 and overwork in the agricultural sector, with 2.7 million being male workers and 2 million female workers, the majority of whom are seasonal workers (TÜİK, 2023).

✉ Correspondence
E-mail address: mozdemir@bayburt.edu.tr

When referring to the agricultural sector, it is essential to remember that it includes not only field farming with branches such as grain cultivation, vegetable growing, greenhouse farming, fruit growing, and seedling cultivation but also livestock breeding, including types such as large and small animal husbandry, dairy farming, and poultry farming; fishing, including marine and lake fishing, fish farming in ponds and farms; and forestry-logging (Suvla, 1949).

The sustainability of the agricultural sector is possible by ensuring the health and safety of agricultural workers. Regarding economy and employment, occupational health and safety in the agricultural sector are of indispensable importance. Until June 30, 2012, the occupational health and safety provisions of Labor Law No. 4857 were only applied to agricultural enterprises employing more than 50 workers, exempting those with fewer than 50 workers. However, with the publication of the Occupational Health and Safety Law No. 6331 on this date, the provisions of this law were applied to all enterprises and workers (Engin, 2014). In addition, agricultural activities were generally located in "Hazardous" occupational zones according to the Workplace Hazard Classes Communiqué published in the Official Gazette dated 27.2.2017 and numbered 29992.

Occupational health and safety in the agricultural sector are vital to meet workers' basic needs, ensure suitable working and living conditions, protect their health and welfare, and increase labour productivity. Agricultural workers face a series of potential occupational health and safety risks, including physical, chemical, biological, ergonomic, and psychosocial risks. These risks can lead to work accidents and occupational diseases, endangering the health and safety of workers. The health and safety problems faced by agricultural workers largely resemble those of industrial workers (Bakırcı, 2011; Engin, 2014; Seyhan & Seyhan, 2015; Yalçın et al., 2016).

Common or potentially developing diseases, injuries, and accidents among agricultural workers include severe mechanical risks such as death or limb loss related to the use of vehicles, tools, tractors, and agricultural machinery; cancers; respiratory diseases; skin disorders; animal-related accidents and diseases; exposure to pesticides and other chemicals; dust exposure; contact with hazardous plants and biological substances; infectious and parasitic diseases; noise exposure and hearing loss; vibration exposure and its effects; ergonomic problems such as heavy lifting and repetitive movements, leading to musculoskeletal disorders; working in adverse climatic conditions like extreme heat or cold, leading to thermal risks; and psychosocial factors and stress. Causes of fatal work accidents in the agricultural sector include transportation (being run over by tractors or agricultural machinery, or vehicle overturning), falls from height, being hit by moving or falling objects, drowning, livestock-related accidents, machinery-related accidents, and electrical accidents (Bakırcı, 2011; Engin, 2014; Seyhan & Seyhan, 2015; Yalçın et al., 2016).

Previous research on occupational health and safety (OHS) in the agricultural sector reveals that workers in this field face significant physical, chemical, biological, ergonomic, and psychosocial risks (Bakırcı, 2011; Engin, 2014). In Türkiye, these risks are further intensified due to the high rates of seasonal and informal employment in the agricultural sector. For instance, Demir (2015) notes that seasonal agricultural workers in Türkiye experience significant challenges in accessing social security rights. Yalçın et al. (2016) identified machinery-related accidents, pesticide exposure, and extreme weather conditions as primary causes of occupational accidents in agriculture. Although the Occupational Health and Safety Law No. 6331, enacted in 2012, aimed to extend OHS protection across all sectors, the unique structural issues of the sector, such as seasonal labor and informal employment, have posed various challenges to implementing this law in agriculture (Gülçubuk, 2017).

This study aims to examine the statistics on work accidents, occupational diseases, and temporary and permanent workday losses in the "Plant and Animal Production Sector" in Türkiye over the five years from 2018 to 2022 and to contribute to the policies to be produced on occupational health and safety by making some recommendations based on the data obtained from these statistics.

While occupational health and safety (OHS) in agriculture has been explored in previous studies, there remains a significant gap in the literature regarding comprehensive, up-to-date statistical analysis specific to Türkiye’s plant and animal production sectors. Most existing research focuses broadly on OHS risks without providing detailed temporal data on work accidents, occupational diseases, and lost workdays across multiple years. This study fills that gap by offering an in-depth analysis of recent OHS data from 2018 to 2022, which includes year-over-year comparisons that highlight trends and emerging risks specific to Türkiye’s agricultural sector. The originality of this research lies in its focus on quantifying and examining OHS incidents in a sector marked by high rates of informal and seasonal employment, which presents unique challenges. By identifying specific risk factors and trends within the agricultural sector, this study contributes actionable insights to policymakers, aiming to improve safety regulations and reduce work-related accidents and diseases in agriculture. Furthermore, the study’s findings provide a foundation for future research on effective OHS policies tailored to the needs of Türkiye’s agricultural workforce.

This article is structured as follows: The introduction section provides general information about the agricultural sector in Turkey, its workers and occupational health and safety risks in the sector. The materials and methods section describes in detail the materials and methods outlining the data sources and statistical approaches used to analyze occupational accidents, occupational diseases and lost work days in the agricultural sector in Turkey. The results and discussion section presents the results of this analysis, including trends in accident frequency, mortality rates, occupational disease incidence and temporary disability days in different years. The section also places these findings in the context of occupational health and safety (OHS) practices, compares them with international standards and identifies critical gaps in current practices. Finally, the conclusion section concludes with policy recommendations aimed at improving OSH in the agricultural sector in Turkey, including the need for enhanced safety protocols, regular health screenings and improved training programs for agricultural workers.

## 2. Materials and Methods

According to the Social Insurance and General Health Insurance Law No. 5510, work accidents and occupational diseases only cover insured individuals (Social Security and General Health Insurance Law, 2006). Therefore, this study utilised the annual statistics of the T.C. Social Security Institution (SGK) as the data source. The "statistics on work accidents and occupational diseases," "statistics on work incapacity periods," and "insured and workplace statistics" from the 2018-2022 period in the SGK annual statistics were examined. Attempts were made to analyse work accidents, occupational diseases, and work incapacity days for insured workers in Türkiye's Plant and Animal Production Sector. The statistical data used in this study were obtained according to the NACE Rev.2 classification. In the NACE classification, the main category is defined as 'Agriculture, forestry, hunting, and related service activities.' However, our study focuses specifically on the subcategory 'Crop and animal production.' The data used do not include statistics from the 'Hunting and related service activities' subcategory but instead cover only the statistics related to crop and animal production activities. Therefore, the OHS data analyzed in this study provide specific information related to the agriculture and livestock subsector. Due to the classification of employees as 4-a and 4-b under the Social Insurance and General Health Insurance Law No. 5510 after 2017, the five years from 2018 to 2022 include the total of these two groups. Definitions related to the concepts examined in this study are given below.

### *2.1.* Work Accident

Accidents occurring under the following conditions are considered occupational accidents.

a) Incidents occurring while the insured is at the workplace, causing immediate or later physical or mental harm to the insured,

b) Incidents occurring outside the workplace while performing tasks or duties assigned by the employer, causing immediate or later physical or mental harm to the insured,

c) Incidents occurring while the insured working for an employer is sent to another place outside the workplace for a task, causing immediate or later physical or mental harm to the insured when the main job is not being performed,

d) Incidents occurring during the time allowed for breastfeeding by insured female workers, causing immediate or later physical or mental harm to the insured,

e) Incidents occur while the insured is commuting to and from where the job is performed using a vehicle provided by the employer, causing immediate or later physical or mental harm to the insured (Social Security and General Health Insurance Law, 2006).

### *2.2.* Work Accident Frequency Rate

Work Accident Frequency Rate (WAFR) indicates how many insured individuals out of every 100 who work full-time have experienced a work accident. The formula is as follows.

$$WAFR = IKS \, / \, (Total\ Working\ Hours)\ x\ 225{,}000 \quad (1)$$

IKS represents the number of insured individuals who have experienced a work accident. In contrast, Total Working Hours represent 2,250 hours (considering a standard work year of 45 hours per week and 50 weeks per year for a full-time worker) and the total number of workers. The coefficient of 225,000 is calculated based on the assumption of 100 insured individuals working full-time for 45 hours per week and 50 weeks per year (Akyüz et al., 2016; Arıtan & Ataman, 2017).

### *2.3.* Death Resulting from a Work Accident

According to the definition adopted by the European Statistics on Accidents at Work (ESAW) project, "fatal work accidents are accidents that cause the death of the insured worker within one year" (Erginel & Toptancı, 2017).

Occupational Disease refers to temporary or permanent diseases or physical or mental disabilities experienced by the insured due to repeated reasons related to the nature of the job or the conditions of its execution (Social Security and General Health Insurance Law, 2006).

Incapacity for Work refers to when the insured cannot work due to a work accident. Temporary incapacity for work represents the number of days the insured cannot work due to a work accident, occupational disease, illness, and maternity conditions, as indicated in the reports by doctors or health boards authorised by SGK. Permanent incapacity for work represents the number of insured individuals whose ability to earn a livelihood has decreased by at least 10% due to a work accident or occupational disease, as determined by health boards of healthcare providers authorised by SGK and confirmed by the SGK health board (Social Security and General Health Insurance Law, 2006).

Total Incapacity for Work Duration consists of the temporary incapacity period due to a work accident or occupational disease, the days lost due to death from a work accident or occupational disease, and the days lost due to becoming permanently incapable of working.

## 3. Results and Discussion

In Türkiye, the total number of workplaces in the Plant and Animal Production Sector increased from 17,216 in 2018 to 19,802 in 2022, an increase of approximately 15%. The number of workplaces in the Plant and Animal Production Sector by year is shown in Table 1 (SGK, 2024).

**Table 1.** Number of workplaces in the plant and animal production sector by year.

| Years | Permanent | Temporary | Public | Private | Total |
|---|---|---|---|---|---|
| 2022 | 19,508 | 294 | 820 | 18,982 | 19,802 |
| 2021 | 18,575 | 292 | 799 | 18,068 | 18,867 |
| 2020 | 17,638 | 288 | 800 | 17,126 | 17,926 |
| 2019 | 16,911 | 282 | 635 | 16,558 | 17,193 |
| 2018 | 16,863 | 353 | 740 | 16,476 | 17,216 |

In parallel with this increase in the number of workplaces in the Plant and Animal Production Sector, the total number of insured workers increased from 103,608 in 2018, with 32,659 being female workers and 70,949 being male workers, to 123,990 in 2022, with 42,979 being female workers and 81,011 being male workers. Over the five years, workers increased by approximately 19.6%. The number of workers in the Plant and Animal Production Sector by year is shown in Table 2 (SGK, 2018, 2019, 2020, 2021, 2022).

**Table 2.** Number of employees in the plant and animal production sector by year.

| Years | Permanent | Temporary | Public | Private | Male | Female | Total |
|---|---|---|---|---|---|---|---|
| 2022 | 118,617 | 5,373 | 11,937 | 112,053 | 81,011 | 42,979 | 123,990 |
| 2021 | 111,606 | 4,297 | 10,673 | 105,230 | 76,529 | 39,374 | 115,903 |
| 2020 | 108,417 | 4,996 | 10,709 | 102,704 | 75,363 | 38,050 | 113,413 |
| 2019 | 101,278 | 3,591 | 8,564 | 96,305 | 71,925 | 32,944 | 104,869 |
| 2018 | 98,691 | 4,917 | 9,887 | 93,721 | 70,949 | 32,659 | 103,608 |

In the Plant and Animal Production Sector, 13,993 work accidents occurred over the five-year period from 2018 to 2022, 96 of which were fatal. The highest number of work accidents during this period was recorded in 2022, with 3,114 work accidents, 19 of which resulted in death. The number of work and fatal work accidents by year in the Plant and Animal Production Sector is shown in Table 3 (SGK, 2018, 2019, 2020, 2021, 2022).

**Table 3.** Number of employees involved in work accidents and fatal work accidents in the plant and animal production sector by year.

| Years | Number of Work Accidents | Number of Fatal Work Accidents |
|---|---|---|
| 2022 | 3,114 | 19 |
| 2021 | 3,060 | 19 |
| 2020 | 2,452 | 15 |
| 2019 | 2,758 | 18 |
| 2018 | 2,609 | 25 |

The work accident frequency rate, calculated per 100 full-time workers, was the lowest at 2.1 in 2020 and the highest at 2.6 in 2021. The work accident frequency rates by year in the Plant and Animal Production Sector are shown in Table 4 (SGK, 2018, 2019, 2020, 2021, 2022).

**Table 4.** Frequency rates of work accidents in the plant and animal production sector by year.

| Years | Frequency Rate of Work Accidents |
|---|---|
| 2022 | 2.5 |
| 2021 | 2.6 |
| 2020 | 2.1 |
| 2019 | 2.6 |
| 2018 | 2.5 |

In the Plant and Animal Production Sector, 13 workers contracted occupational diseases over the five years from 2018 to 2022. None of these occupational diseases resulted in death. The highest number of occupational diseases was recorded in

2018, with 5 cases. The number of workers contracting occupational and fatal occupational diseases per year in the Plant and Animal Production Sector is shown in Table 5 (SGK, 2018, 2019, 2020, 2021, 2022).

**Table 5.** Number of employees with occupational diseases and fatal occupational diseases in the plant and animal production sector by year.

| Years | Number of Occupational Diseases | Number of Fatal Occupational Diseases |
|---|---|---|
| 2022 | 2 | 0 |
| 2021 | 1 | 0 |
| 2020 | 1 | 0 |
| 2019 | 4 | 0 |
| 2018 | 5 | 0 |

In the Plant and Animal Production Sector, workers received 133,008 days of temporary incapacity reports over the five years from 2018 to 2022, with 128,037 days treated as outpatients and 4,971 days hospitalised. The highest number of days of incapacity, 32,767 days, occurred in 2021 when the COVID-19 pandemic was intensely experienced in Türkiye. The number of days of temporary incapacity due to work accidents by year in the Plant and Animal Production Sector is shown in Table 6 (SGK, 2018, 2019, 2020, 2021, 2022).

**Table 6.** Number of temporary disability days due to work accidents in the plant and animal production sector by year.

| Years | Outpatient Treatment | Inpatient Treatment | Total |
|---|---|---|---|
| 2022 | 28,843 | 974 | 29,817 |
| 2021 | 31,662 | 1,105 | 32,767 |
| 2020 | 22,790 | 711 | 23,501 |
| 2019 | 25,371 | 1,016 | 26,387 |
| 2018 | 19,371 | 1,165 | 20,536 |

A total of 2,700,296 work accidents occurred in Türkiye between 2018 and 2022, resulting in the deaths of 8,480 workers. During the same period, 5,901 workers were diagnosed with occupational diseases, and 48 workers died due to these diseases (SGK, 2018, 2019, 2020, 2021, 2022). According to the SGK data for 2022, which alone recorded 588,823 work accidents and 1,517 fatal work accidents, approximately one work accident occurs every 60 seconds in Türkiye, and four workers die each day due to these accidents.

In this study, data on occupational health and safety in the agricultural sector in Türkiye between 2018 and 2022 were examined, and the current situation in the sector was assessed in light of the findings. The results indicate that OHS practices in the agricultural sector are inadequate and need improvement.

Work accident rates remained relatively steady between 2018 and 2022. While a partial decrease was observed in 2020 (2.16%), the rate rose again to 2.64% in 2021. This fluctuation may be related to restrictions and a reduced workforce in agricultural activities during the COVID-19 pandemic in 2020. In 2021, as pandemic restrictions were lifted and work intensity increased, accident rates also rose.

Although the rate of fatal work accidents showed slight fluctuations over the years, it remained between 0.01% and 0.02%. While the limited number of fatal accidents suggests relatively low incidence, it also highlights the hazardous nature of agricultural activities and the need for further improvements in safety measures.

The rate of occupational diseases remains at very low levels (ranging from 0.00% to 0.0048%). However, these low rates may not fully reflect the health risks in agricultural activities. In particular, diseases related to pesticide exposure, heavy physical workload, and ergonomic issues may be underdiagnosed or unreported. Therefore, it is important to conduct more frequent health screenings and raise awareness regarding occupational diseases in the sector.

The rate of temporary incapacity days per employee also varies by year. The highest rate of incapacity days, 0.28%, was recorded in 2021. This increase indicates that health conditions and workload related to the intense period of the COVID-19 pandemic led to a rise in the rate of employees taking sick leave.

The analysis results suggest that stronger occupational health and safety measures are needed to reduce accident rates and prevent occupational diseases in the sector. Expanding training programs, tightening safety protocols, and conducting regular health screenings can help mitigate safety and health risks in the sector. Given the seasonal and physical challenges inherent in agricultural activities, these measures are essential to maintaining a sustainable work environment.

## 4. Conclusion

This study analyzes the status of occupational health and safety (OHS) in Türkiye’s agricultural sector by examining work accidents, occupational diseases, and temporary incapacity days. The high prevalence of informal and seasonal employment in the agricultural sector creates a risky environment for worker safety and health.

The findings of this study exhibit certain similarities and differences when compared to existing literature. Previous studies, such as Bakırcı (2011) and Seyhan and Seyhan (2015), emphasize that occupational accidents in the agricultural sector are predominantly associated with risks like machinery use, pesticide exposure, and heavy physical workload. These findings align with the primary causes of occupational accidents observed in our study during the 2018-2022 period. For instance, the prevalence of accidents involving tractors and other agricultural machinery corroborates the conclusions drawn in earlier research.

Furthermore, the more recent data presented in our study highlights the trends in occupational accidents over the years, particularly emphasizing the impact of the COVID-19 pandemic on lost workdays. The increase in temporary incapacity days during the pandemic period underscores the influence of pandemic-specific conditions on occupational safety in the agricultural sector.

In the literature, studies like Gülçubuk (2017) and Yalçın et al. (2016) argue that seasonal and informal employment significantly complicates the implementation of occupational health and safety (OHS) measures in this sector. Similarly, our findings identify the high proportion of seasonal workers and limitations in access to social security as key structural challenges restricting the effectiveness of OHS practices.

Additionally, our study contributes to the literature by addressing the frequently reported inadequacies in diagnosing occupational diseases in the agricultural sector (e.g., Bakırcı, 2011; Seyhan & Seyhan, 2015). It suggests that the low reported rates of occupational diseases between 2018 and 2022 may be linked to underreporting and a lack of awareness.

The results indicate that current OHS measures are insufficient to effectively reduce work accidents and improve the diagnosis of occupational diseases in the sector. Key measures that should be implemented include expanding OHS training programs for workers, increasing safety education regarding the use of agricultural machinery, and conducting regular health screenings. Additionally, stricter enforcement of safety protocols and regular inspections are essential to prevent work accidents and occupational diseases. Improving access to essential facilities such as accommodation, hygiene, and healthcare services for seasonal workers is also critical for enhancing workplace health and safety in the sector.

For future research, it would be beneficial to conduct detailed analyses across different subfields within agriculture using larger datasets. Field studies on the causes of work accidents and risk analyses based on the demographic characteristics of sector employees would provide valuable insights for improving OHS policies. Such studies will contribute to establishing a sustainable work environment in the agricultural sector and enhancing OHS standards.

In conclusion, improving OHS in the agricultural sector is critical to protecting workers' health and well-being and ensuring sustainability. Utilising larger data sets in the future and examining the different sub-sectors in detail will contribute to developing more comprehensive and effective OHS policies.

## Conflict of Interest

The author has no conflict of interest to declare.

## References

Akyüz, K. C., Yıldırım, İ., Tugay, T., Akyüz, İ., & Gedik, T. (2016). Orman ürünleri sanayi sektöründe iş kazası istatistiklerine genel bir bakış. *Düzce University Faculty of Forestry Journal of Forestry*, *12*(2), 66-79. (In Turkish)

Arıtan, A. E., & Ataman, M. (2017). Kaza oranları hesaplamalarıyla iş kazası analizi. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, *17*(1), 239-246. https://doi.org/10.5578/fmbd.51762 (In Turkish)

Bakırcı, N. (2011). Tarımda çalışanların sağlığı ve güvenliği. *TTB Mesleki Sağlık ve Güvenlik Dergisi*, *11*(39), 7-13. (In Turkish)

Demir, M. (2015). Mevsimlik tarım işçilerinin sosyal güvenlik haklarına ilişkin değerlendirmeler ve öneriler. *Çalışma ve Toplum*, *1*(44), 177-194. (In Turkish)

Engin, T. (2014). *6331 sayılı kanun çerçevesinde iş sağlığı ve güvenliği hizmetlerinin desteklenmesi* (Master's thesis, Uludağ University). (In Turkish)

Erginel, N., & Toptancı, Ş. (2017). İş kazası verilerinin olasılık dağılımları ile modellenmesi. *Journal of Engineering Sciences and Design*, *5*, 201-212. https://doi.org/10.21923/jesd.20116 (In Turkish)

Gülçubuk, B. (2017). 6331 Sayılı İş Sağlığı ve Güvenliği Kanunu tarımda çalışanlar için bir şey ifade eder mi. *International Journal of Social Sciences and Education Research*, *3*(2), 573-582. (In Turkish)

Seyhan, S., & Seyhan, G. (2015). Tarım sektöründe iş sağlığı ve güvenliği. *Mühendislik Bilimleri ve Tasarım Dergisi*, *3*(3), 549-552. (In Turkish)

SGK. (2018). *2018 yılı istatistik bilgisi*. https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4 (In Turkish)

SGK. (2019). *2019 yılı istatistik bilgisi*. https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4 (In Turkish)

SGK. (2020). *2020 yılı istatistik bilgisi*. https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4 (In Turkish)

SGK. (2021). *2021 yılı istatistik bilgisi*. https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4 (In Turkish)

SGK. (2022). *2022 yılı istatistik bilgisi*. https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4 (In Turkish)

SGK. (2024). *SGK veri uygulaması*. https://veri.sgk.gov.tr/ (In Turkish)

Social Security and General Health Insurance Law. (2006). *Republic of Türkiye, Law no: 5510*. https://www.mevzuat.gov.tr/mevzuatmetin/1.5.5510.pdf (In Turkish)

Solmaz, E. (2023). Türkiye ekonomisinin sektörel analizi. In O. Bahar & M. Avcı (Eds.), *Tarım, ormancılık, balıkçılık sektörü ve gelişimi* (pp. 7-40). Efe Akademi Yayınları. (In Turkish)

Suvla, R. Ş. (1949). *Genel ekonomi dersleri*. İstanbul Üniversitesi Yayınları. (In Turkish)

TÜİK. (2023). *İşgücü istatistikleri, 2023*. https://data.tuik.gov.tr/Bulten/Index?p=Isgucu-Istatistikleri-2023-53521#:~:text=2023 (In Turkish)

Yalçın, G. E., Yazıcı, E., Kara, F. Ö., İpekçioğlu, Ş., & Yalçın, M. (2016). *Tarımda iş kazaları ve hastalıkları*. XII. Ulusal Tarım Ekonomisi Kongresi. Isparta. (In Turkish)

*Journal of Agricultural Production* (2024), *5*(4), 255-264 https://doi.org/10.56430/japro.1565531

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSIP

https://prensip.gen.tr

RESEARCH ARTICLE

# Impact of *Mesorhizobium ciceri* Inoculation on Symbiotic Nitrogen Fixation of Various Chickpea (*Cicer arietinum* L.) Cultivars

Yasin Altuntaş • Erdal Elkoca✉ • Selçuk Kodaz

Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye

ARTICLE INFO

**Article History**
Received: 12.10.2024
Accepted: 29.11.2024
First Published: 31.12.2024

**Keywords**
Chickpea
Cultivar
*Mesorhizobium ciceri*
Symbiotic nitrogen fixation

ABSTRACT

In this study, conducted in pots under outdoor conditions, the aim was to determine the symbiotic performance of 10 registered chickpea cultivars (Akça, Aksu, Arda, Aslanbey, Bayram, Botan, Cevdetbey, Göktürk, Ilgaz and Yazıcı) inoculated with *Mesorhizobium ciceri*. Inoculation significantly increased the average nodule fresh weights, nodule dry weights, shoot height, shoot fresh weights, shoot dry weights, root fresh weights, root dry weights, chlorophyll content, N% and total N compared to the un-inoculated control. The shoot fresh weight, N% and total N significantly correlated with nodule weight. Additionally, chlorophyll content is highly correlated with N%. The magnitude of response to inoculation differed significantly among cultivars, except shoot height and root fresh weight. The amount of fixed N per plant by the cultivars varied significantly between 0 (Ilgaz) and 23.5 mg (Akça). Akça, Arda, Aslanbey, Cevdetbey, Göktürk and Yazıcı were the most compatible cultivars based on the amount of fixed N. These results show that inoculation efficiency and nitrogen fixation in chickpea can be increased by selecting compatible cultivars.

**Please cite this paper as follows:**

Altuntaş, Y., Elkoca, E., & Kodaz, S. (2024). Impact of *Mesorhizobium ciceri* inoculation on symbiotic nitrogen fixation of various chickpea (*Cicer arietinum* L.) cultivars. *Journal of Agricultural Production*, *5*(4), 255-264. https://doi.org/10.56430/japro.1565531

## 1. Introduction

Nitrogen is the most limiting nutrient for crop production in many agricultural areas worldwide (Fageria & Baligar, 2005). The atmosphere, which contains about 78% nitrogen gas, is the main source of nitrogen on Earth. Although nitrogen is abundant in the atmosphere as a gas, it cannot be taken up and utilized directly by plants. Plants can only take nitrogen from the soil in the reduced form of ammonium and nitrate (Gul et al., 2014; Li et al., 2013). Today, most of the nitrogen is applied to cropping systems in the form of industrially produced nitrogen fertilizers, which causes various ecological problems worldwide (J. P. Verma et al., 2013). Increasing fertilizer costs and the negative effects of chemical fertilizers on the environment have forced the search for alternative sources in terms of plant nutrition. In this context, biological nitrogen fixation, a process in which elemental atmospheric nitrogen is converted into organic forms by symbiotic and asymbiotic microorganisms possessing nitrogenase enzymes, has gained great importance as an alternative nitrogen source to chemical fertilizers (Elkoca et al., 2008). These microorganisms, which have nitrogenase enzymes, convert gaseous nitrogen into a form that can be taken up by plants and provide an important nitrogen gain biologically (Bohlool et al., 1992; Elkoca et al., 2010; Mohammadi & Sohrabi, 2012).

Chickpea is the second most produced edible legume plant in the world after dry beans with 14.8 million hectares of cultivation area and 18.1 million tonnes of production. In

✉ Correspondence
E-mail address: eelkoca@atauni.edu.tr

Türkiye, it ranks first among edible legumes with 456 thousand hectares of cultivation area and 580 thousand tonnes of production (FAO, 2022). Chickpea, a member of the legume family, can fix atmospheric nitrogen to the soil symbiotically through *Rhizobium* bacteria (Kantar et al., 2007). *Mesorhizobium ciceri* is the bacterium that forms nodules and fixes nitrogen in chickpea. This bacterium is specific to chickpea and does not show inoculation affinity with any member of the cross-inoculation groups (Kantar et al., 2007; Rupela & Saxena, 1987). Therefore, there is a need for inoculation with effective strains in soils where chickpea has not been previously grown or do not contain effective bacterial strains (El Hadi & Elsheikh, 1999; Gul et al., 2014; Somasegeran et al., 1988; D. P. S. Verma, 1984).

Nodule formation and nitrogen fixation in legumes are influenced by both bacterial and host plant genes (Buttery et al., 1997; Danso et al., 1987; Davis et al., 1985; Goyal et al., 2021; D. P. S. Verma, 1984). Therefore, even if the bacteria used in inoculation are effective and environmental conditions are suitable for nitrogen fixation, the amount of nitrogen fixed is limited if the bacteria and the host are not compatible. Genotypic variation among cultivars has a significant impact on nodulation and nitrogen fixation in chickpea as in many legume species (Bhuiyan et al., 2009; Pandey et al., 2018; Priyadarsini et al., 2017; Roy et al., 2018). Thus, it is crucial to identify chickpea cultivars with high nodulation and nitrogen fixation potential. While many chickpea cultivars have been developed in our country in recent years, there has been insufficient research on the response of these cultivars to bacterial inoculation and their nitrogen fixation potential. In light of this information, this study aims to determine the effect of *Mesorhizobium ciceri* inoculation on nodulation, nitrogen fixation and plant growth parameters of some chickpea cultivars.

## 2. Materials and Methods

The experiment was conducted in 2-liter pots under outdoor conditions in the Field Crops Department of Atatürk University, Erzurum. The pots were filled with a mixture of sifted field soil, peat and sand in a ratio of 1:1:1. Some properties of the experimental soil are presented in Table 1. In the study, 10 registered chickpea cultivars (Akça, Aksu, Arda, Aslanbey, Bayram, Botan, Cevdetbey, Göktürk, Ilgaz, Yazıcı) were used as seed material. Bacterial culture (*Mesorhizobium ciceri*) in peat was obtained from the Central Research Institute of Soil, Fertilizer and Water Resources, Ankara.

**Table 1.** Characteristics of the experimental soil used in the study.

| Contents | Value | Contents | Value |
|---|---|---|---|
| pH | 7.27 | Mg (cmol/kg) | 11.99 |
| EC (µmhos/cm) | 81.45 | Na (cmol/kg) | 1.16 |
| Organic matter (%) | 0.78 | B (ppm) | 0.03 |
| $CaCO_3$ (%) | 1.99 | Cu (ppm) | 0.53 |
| Total N (ppm) | 5.14 | Fe (ppm) | 0.59 |
| P (ppm) | 33.55 | Zn (ppm) | 0.16 |
| K (cmol/kg) | 2.28 | Mn (ppm) | 0.21 |
| Ca (cmol/kg) | 14.05 | Cl (ppm) | 2.59 |

The research was conducted using a completely randomized design with 3 replications. The responses of chickpea cultivars to *Mesorhizobium ciceri* inoculation were compared to an un-inoculated control treatment. The un-inoculated control treatment was sown first to prevent bacterial contamination. In the inoculation treatment, bacterial culture in peat ($1.1 \times 10^5$ cfu/g) was applied to seeds soaked in a 10% glucose solution. The inoculation was performed in the shade and the inoculated seeds were immediately sown. Five seeds were planted in each pot and three plants were retained per pot after emergence. Pots were irrigated with tap water as needed. Sowing took place on June 29, 2024 and plants were harvested 42 days after emergence. Daily minimum and maximum temperatures were recorded throughout the experimental period, ranging between 7 and 30.6ºC (Figure 1).

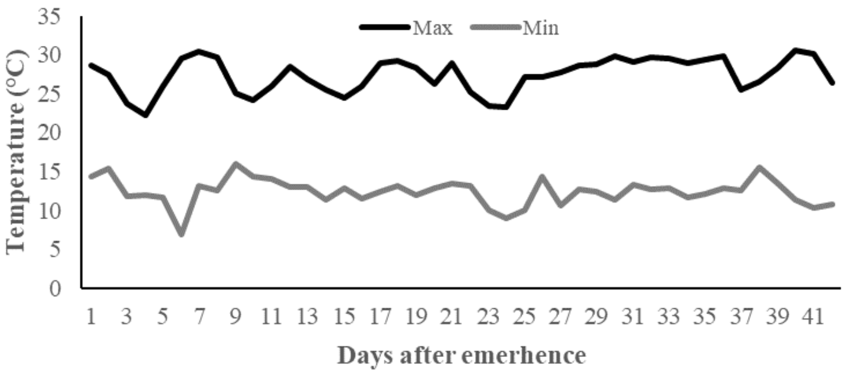


**Figure 1.** The minimum and maximum air temperatures recorded during the experimental period.

### *2.1.* Data Collection

A portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) was utilized to assess leaf greenness as an indicator of chlorophyll content of chickpea cultivars just before harvesting. SPAD measurements were conducted on 10 fully expanded leaves in each pot and averaged. The relative leaf chlorophyll content was represented in SPAD units with “1” indicated very light green pigmentation (chlorotic) and “50” indicated very dark green pigmentation.

Plants were harvested 42 days after emergence. The root and shoot parts were separated after washing the roots thoroughly with tap water. Nodules on roots were removed and the fresh weights of nodules, shoots and roots per plant were determined. Dry weight parameters were recorded after drying the root, shoot and nodule samples in an oven at 65°C for 24 hours. Plant nitrogen content (N%) was determined using the Kjeldahl method. Total N per plant (shoot dry weight x N%) and the fixed N (total N in inoculated plants – total N in un-inoculated plants) were calculated (Elkoca et al., 2015; Öğütçü et al., 2008).

### *2.2.* Statistical Analysis

The data was subjected to analysis of variance using the MSTATC Statistical Package (version 1.4, Michigan State University, MI, USA) and means were separated according to Duncan’s multiple range test at $p<0.05$.

## 3. Results and Discussion

### *3.1.* Effect of Inoculation

In this study, an average of 69.3 mg of fresh and 9.7 mg of dry nodule weight per plant was determined in the un-inoculated control treatment, serving as evidence of the presence of native rhizobial population in the soil (Table 2). The success of inoculation depends on the ability of the introduced rhizobium bacteria to survive and colonize by competing with the native bacterial population in the soil (Bordeleau & Prevost, 1994). When inoculation is successful, significant increases in plant dry matter production and total nitrogen amounts are observed in chickpea (Öğütçü et al., 2008; Pandey et al., 2018; Solaiman et al., 2010). Therefore, increases in these parameters as a result of rhizobial inoculation are considered among the most important indicators of nitrogen fixation (Beck, 1992; Hardarson & Danso, 1993). In our study, *Mesorhizobium ciceri* inoculation provided significant increases in all parameters examined in chickpea as an average of the cultivars (Tables 2 and 3). These results demonstrate that the bacteria used in the inoculation could compete with the native bacterial population in the soil, indicating successful inoculation.

### *3.2.* Effect of Cultivar

Infection of root hairs, nodule development and the resulting amount of nitrogen fixed are influenced by host plant genes (Dwivedi et al., 2015; Perret et al., 2000; D. P. S. Verma, 1984). Therefore, different genotypes and cultivars within the same legume species may react differently to inoculation. As a result of the genotypic effect, significant differences may occur between cultivars in terms of the parameters examined in bacterial inoculation studies (Di Bonito et al., 1990; Farid & Navabi, 2015; Pandey et al., 2018). In this study, significant differences were also detected among the cultivars in terms of nodulation, plant growth parameters, chlorophyll content, N% and total N (Tables 2 and 3).

Ilgaz had no nodule formation, while Aksu had the highest nodule fresh and dry weights (434.4 and 60.2 mg plant$^{-1}$, respectively). On the other hand, Aslanbey, Bayram, Botan and Cevdetbey with nodule dry weight ranging between 27.8 and 37.1 mg plant$^{-1}$ were in the second highest group. The highest shoot fresh and dry weight values were recorded in Akça, Aslanbey, Cevdetbey and Aksu (Table 2). Meanwhile, Arda, Cevdetbey, Göktürk, Aslanbey and Ilgaz were the leading cultivars in root fresh weight, with the highest root dry weight determined in Akça and Aslabey (Table 3). The chlorophyll contents of Göktürk, Cevdetbey and Aksu were higher than the other cultivars. Plant nitrogen content varied considerably between 2.07% (Ilgaz) and 2.53% (Aslanbey) according to cultivars. The highest total N per plant was determined in Akça (69.4 mg) and Aslanbey (73.7 mg), followed by Aksu, Botan, Cevdetbey and Yazıcı with amounts ranging between 56.2 mg and 60.8 mg (Table 3). In other inoculation studies, it was also reported that significant differences were found among genotypes in terms of nodulation, plant growth, chlorophyll and nitrogen content, indicating that the host factor is an important determinant of the parameters investigated (Bidlack et al., 2001; Hossain et al., 2018; Hungria & Bohrer, 2000; Keneni et al., 2012; Ndiaye et al., 2000).

### *3.3.* Effect of Inoculation x Cultivar Interaction

In this study, chickpea cultivars generally performed better in the inoculated treatment than in the un-inoculated treatment. However, there was significant variation among cultivars in response to *Mesorhizobium ciceri* inoculation. Except for shoot height and root fresh weight, the inoculation-cultivar interaction had a significant effect on all parameters examined (Tables 2 and 3).

**Table 2.** The effects of *Mesorhizobium ciceri* inoculation on the nodulation, shoot height, shoot fresh and dry matter production of chickpea cultivars.

| | **Nodule fresh weight (mg plant$^{-1}$)** | **Nodule dry weight (mg plant$^{-1}$)** | **Shoot height (cm)** | **Shoot fresh weight (g)** | **Shoot dry weight (g)** |
|---|---|---|---|---|---|
| ***Inoculation (I)*** | | | | | |
| Un-inoculated (I-) | 69.3 b | 9.7 b | 29.5 b | 8.41 b | 2.14 b |
| Inoculated (I+) | 249.8 a | 35.7 a | 32.0 a | 9.78 a | 2.56 a |
| ***Cultivar (C)*** | | | | | |
| Akça | 88.8 de | 16.3 c | 34.2 ab | 10.62 a | 2.94 a |
| Aksu | 434.8 a | 60.2 a | 32.7 b | 9.22 b | 2.31 c |
| Arda | 61.2 de | 8.8 cd | 24.1 d | 7.35 d | 1.79 e |
| Aslanbey | 215.3 c | 28.0 b | 34.4 ab | 10.67 a | 2.90 a |
| Bayram | 197.2 c | 27.8 b | 28.8 c | 7.90 cd | 2.04 d |
| Botan | 283.3 b | 37.1 b | 28.4 c | 9.83 ab | 2.57 b |
| Cevdetbey | 179.3 c | 28.6 b | 29.8 c | 9.45 b | 2.46 bc |
| Göktürk | 40.3 ef | 5.2 d | 25.9 d | 7.80 cd | 1.92 de |
| Ilgaz | 0.0 f | 0.0 d | 33.4 ab | 8.77 bc | 2.38 bc |
| Yazıcı | 95.0 d | 15.0 c | 35.9 a | 9.32 b | 2.31 c |
| ***I x C*** | | | | | |
| Akça (I-) | 51.3 f-i | 6.7 hi | 33.5 | 9.67 b-f | 2.60 bc |
| Akça (I+) | 126.3 ef | 26.0 ef | 34.9 | 11.57 a | 3.27 a |
| Aksu (I-) | 202.7 d | 29.2 de | 30.3 | 8.43 e-h | 2.22 def |
| Aksu (I+) | 667.0 a | 91.1 a | 35.1 | 10.00 a-e | 2.41 b-e |
| Arda (I-) | 10.0 hi | 1.0 i | 23.6 | 6.00 i | 1.49 h |
| Arda (I+) | 112.3 efg | 16.7 e-h | 24.7 | 8.70 d-h | 2.09 ef |
| Aslanbey (I-) | 110.0 efg | 14.0 f-i | 33.2 | 10.00 a-e | 2.65 b |
| Aslanbey (I+) | 320.7 c | 42.0 cd | 35.6 | 11.33 ab | 3.15 a |
| Bayram (I-) | 121.3 ef | 15.3 fgh | 27.5 | 7.57 ghi | 1.98 fg |
| Bayram (I+) | 273.0 c | 40.3 cd | 30.1 | 8.23 fgh | 2.10 ef |
| Botan (I-) | 122.7 ef | 17.2 e-i | 27.9 | 9.33 c-g | 2.42 b-e |
| Botan (I+) | 444.0 b | 57.0 b | 28.8 | 10.33 a-d | 2.71 b |
| Cevdetbey (I-) | 42.0 ghi | 5.0 hi | 26.6 | 8.67 d-h | 2.24 def |
| Cevdetbey (I+) | 316.7 c | 52.3 bc | 33.1 | 10.23 a-d | 2.68 b |
| Göktürk (I-) | 0.0 i | 0.0 i | 24.7 | 7.13 hi | 1.71 gh |
| Göktürk (I+) | 80.7 e-h | 10.4 ghi | 27.2 | 8.47 e-h | 2.13 ef |
| Ilgaz (I-) | 0.0 i | 0.0 i | 33.6 | 9.30 c-g | 2.47 bcd |
| Ilgaz (I+) | 0.0 i | 0.0 i | 33.2 | 8.23 fgh | 2.29 c-f |
| Yazıcı (I-) | 32.7 hi | 8.7 ghi | 34.4 | 7.97 fgh | 2.02 fg |
| Yazıcı (I+) | 157.3 de | 21.3 efg | 37.4 | 10.67 abc | 2.60 bc |
| *CV (%)* | *26.2* | *32.8* | *6.9* | *10.0* | *7.5* |
| | | | | | |
| Source | F-test | | | | |
| *I* | ** | ** | ** | ** | ** |
| *C* | ** | ** | ** | ** | ** |
| *I x C* | ** | ** | ns | * | * |

* and ** significant at 0.05 and 0.01 levels, respectively; ns, not significant.
Mean values with the same letter in the column are not statistically different at $p<0.05$.

**Table 3.** The effects of *Mesorhizobium ciceri* inoculation on the root fresh and dry matter production, chlorophyll, nitrogen content and total nitrogen amount of chickpea cultivars.

| | **Root fresh weight (g)** | **Root dry weight (mg)** | **Chlorophyll (SPAD)** | **Nitrogen content (%)** | **Total N (mg plant$^{-1}$)** |
|---|---|---|---|---|---|
| ***Inoculation (I)*** | | | | | |
| Un-inoculated (I-) | 13.3 b | 1.41 b | 37.0 b | 2.19 b | 48.1 b |
| Inoculated (I+) | 15.2 a | 1.66 a | 41.7 a | 2.50 a | 64.1 a |
| ***Cultivar (C)*** | | | | | |
| Akça | 13.2 abc | 1.75 ab | 39.2 bc | 2.35 bc | 69.4 a |
| Aksu | 12.5 bc | 1.28 de | 40.6 ab | 2.42 ab | 56.3 b |
| Arda | 16.6 a | 1.50 bcd | 39.9 bc | 2.39 abc | 43.1 c |
| Aslanbey | 15.7 ab | 1.91 a | 36.5 c | 2.53 a | 73.7 a |
| Bayram | 11.0 c | 1.23 e | 39.8 bc | 2.26 c | 46.2 c |
| Botan | 12.6 bc | 1.40 cde | 37.6 bc | 2.36 bc | 60.8 b |
| Cevdetbey | 16.0 ab | 1.66 abc | 40.9 ab | 2.42 ab | 60.1 b |
| Göktürk | 15.9 ab | 1.52 bcd | 43.3 a | 2.40 abc | 46.4 c |
| Ilgaz | 15.4 ab | 1.66 abc | 36.9 c | 2.07 d | 49.1 c |
| Yazıcı | 14.1 abc | 1.46 cde | 39.0 bc | 2.42 ab | 56.2 b |
| ***I x C*** | | | | | |
| Akça (I-) | 12.0 | 1.34 cde | 35.6 efg | 2.22 def | 57.6 c-f |
| Akça (I+) | 14.5 | 2.16 a | 42.7 abc | 2.48 abc | 81.1 a |
| Aksu (I-) | 11.6 | 1.21 de | 37.6 c-g | 2.16 def | 48.0 fg |
| Aksu (I+) | 13.3 | 1.35 cde | 43.7 a | 2.67 a | 64.5 bcd |
| Arda (I-) | 15.1 | 1.39 b-e | 37.2 d-g | 2.24 def | 33.4 i |
| Arda (I+) | 18.0 | 1.61 bcd | 42.6 abc | 2.53 ab | 52.8 efg |
| Aslanbey (I-) | 13.9 | 1.65 bc | 34.6 fg | 2.35 bcd | 62.4 b-e |
| Aslanbey (I+) | 17.5 | 2.16 a | 38.4 b-g | 2.70 a | 85.0 a |
| Bayram (I-) | 10.4 | 1.10 e | 37.0 d-g | 2.23 def | 44.2 gh |
| Bayram (I+) | 11.6 | 1.37 cde | 42.7 abc | 2.29 cde | 48.2 fg |
| Botan (I-) | 12.2 | 1.30 cde | 34.4 g | 2.21 def | 53.6 efg |
| Botan (I+) | 13.0 | 1.49 b-e | 40.7 a-d | 2.51 ab | 68.0 b |
| Cevdetbey (I-) | 15.1 | 1.63 bc | 42.2 abc | 2.25 def | 50.4 fg |
| Cevdetbey (I+) | 16.9 | 1.68 bc | 39.6 a-f | 2.60 a | 69.7 b |
| Göktürk (I-) | 14.9 | 1.54 bcd | 42.9 ab | 2.14 def | 36.3 hi |
| Göktürk (I+) | 16.9 | 1.49 b-e | 43.7 a | 2.65 a | 56.5 def |
| Ilgaz (I-) | 14.2 | 1.52 bcd | 33.5 g | 2.04 f | 50.4 fg |
| Ilgaz (I+) | 16.5 | 1.79 b | 35.2 efg | 2.10 ef | 47.8 fg |
| Yazıcı (I-) | 13.6 | 1.40 b-e | 35.3 efg | 2.26 def | 45.2 gh |
| Yazıcı (I+) | 14.7 | 1.52 bcd | 42.6 abc | 2.58 a | 67.3 bc |
| *CV (%)* | *19.3* | *13.3* | *6.7* | *5.1* | *10.0* |
| | | | | | |
| Source | F-test | | | | |
| *I* | ** | ** | ** | ** | ** |
| *C* | * | ** | ** | ** | ** |
| *I x C* | ns | * | * | * | ** |

* and ** significant at 0.05 and 0.01 levels, respectively; ns, not significant.
Mean values with the same letter in the column are not statistically different at $p<0.05$.

The nodulation process is tightly regulated by both host and rhizobia genetics (Goyal et al., 2021; Wang et al., 2018). Significant increases in nodulation are observed when compatible bacteria x cultivar associations are achieved (Rupela & Saxena, 1987). This indicates that specific expression of both plant and bacterial genes is necessary for successful nodulation and nitrogen fixation. The inoculation x cultivar interaction was also significant for nodule weight in this study. The highest nodule fresh and dry weights per plant (667.0 and 91.1 mg, respectively) were determined in the inoculated Aksu, significantly higher than the other inoculated cultivars. On the other hand, Ilgaz could not produce nodules in either the un-inoculated or inoculated treatment. Compared to the un-inoculated control, the inoculation treatment significantly increased the nodule dry weight of cultivars except Göktürk, Ilgaz and Yazıcı (Table 2). The highest and lowest shoot fresh and dry weights were recorded in inoculated Akça and un-inoculated Arda, respectively. The response of cultivars to inoculation in terms of shoot dry weight was higher in Aslanbey, Cevdetbey, Göktürk, Akça, Yazıcı and Arda. These cultivars showed 18.9%, 19.6%, 24.6%, 25.8%, 28.7% and 40.3% increase in shoot dry weight compared to the un-inoculated control, respectively. In contrast, the inoculation treatment did not increase shoot dry weight in Ilgaz (Table 2).

The highest root dry weight (2.16 mg plant$^{-1}$) was found in inoculated Akça and Aslanbey, which was significantly higher than all other inoculated and un-inoculated cultivars. The lowest root dry weight (1.1 mg plant$^{-1}$) was recorded in the un-inoculated Bayram (Table 3). Similar shoot dry weight, significant differences were observed in root dry weight among cultivars in terms of their response to inoculation. Inoculation did not increase root dry weight in Cevdetbey and Göktürk compared to the un-inoculated control. Aslanbey and Akça exhibited the most significant response to inoculation, increasing root dry weight by 30.9% and 61.2%, respectively, compared to the un-inoculated control (Table 3). Consistent with our findings, other studies conducted on chickpea, have also shown that the interaction between inoculation and cultivar has a significant impact on nodulation and plant dry matter production (Bhuiyan et al., 2009; Di Bonito et al., 1990; Eusuf Zai et al., 1999).

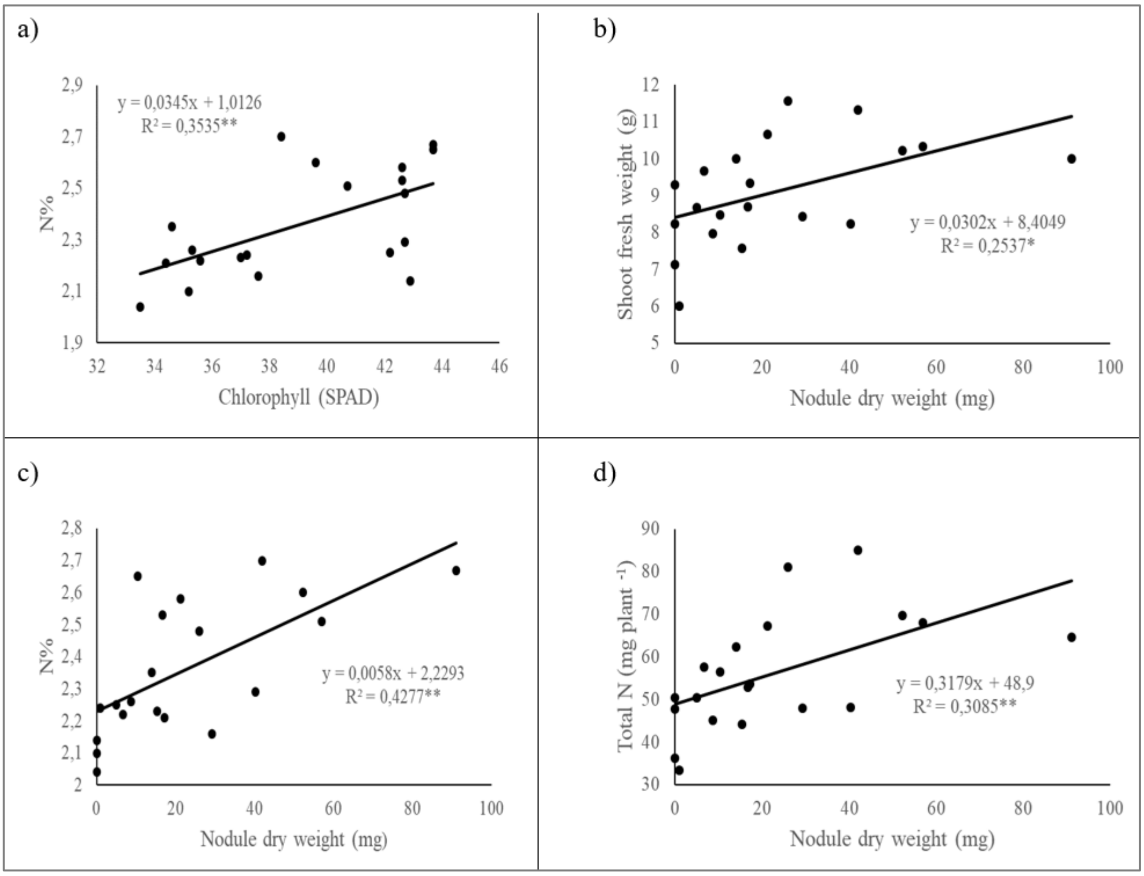


**Figure 2.** Relationships of chlorophyll and nodule dry weight with shoot fresh weight, N% and total N. * and ** significant at 0.05 and 0.01 levels, respectively; n= 20.

Nitrogen is an essential component of chlorophyll production in plants (Mendoza-Tafolla et al., 2019). In case of nitrogen deficiency, plants become light green, while the amount of chlorophyll increases and plant color becomes darker depending on the increase in the amount of nitrogen supplied to the plant (Kumawat et al., 2000; Odabaş & Gülümser, 2001).

Therefore, chlorophyll content is considered one of the indicators of plant nitrogen content and nitrogen fixation (Hoque et al., 1999; Wood et al., 1992). In this study, the average chlorophyll content of the cultivars significantly increased from 37.0 SPAD in the un-inoculated control to 41.7 SPAD in the inoculation treatment, indicating that inoculation provides nitrogen to the plant (Table 3). The high correlation coefficient (r= 0.59**) between chlorophyll content and N% confirmed this phenomenon (Figure 2a). The highest and lowest chlorophyll contents were measured in inoculated Aksu (43.7 SPAD) and un-inoculated Ilgaz (33.5 SPAD), respectively. However, cultivars exhibited different responses to the inoculation treatment in terms of chlorophyll content. The inoculation treatment significantly increased the amount of chlorophyll in Akça, Aksu, Arda, Bayram, Botan and Yazıcı compared to the un-inoculated control. In other cultivars, the increases in chlorophyll content in relation to inoculation were not statistically significant (Table 3).

In rhizobium inoculation studies, increases in plant nitrogen percentage and total nitrogen content are the most important indicators of nitrogen fixation. Rhizobia-host compatibility is crucial in increasing plant nitrogen content and thus ensuring effective nitrogen fixation (Kantar et al., 2007; Santalla et al., 2001; Solaiman et al., 2007). This study observed significant variation in the effect of bacterial inoculation on nitrogen percentage, total and fixed nitrogen depending on the cultivars. The nitrogen percentage ranged from 2.04% (un-inoculated Ilgaz) to 2.70% (inoculated Aslanbey) and total N per plant ranged from 33.4 mg (un-inoculated Arda) to 85.0 mg (inoculated Aslanbey). The impact of inoculation on nitrogen percentage and total nitrogen varied significantly among the cultivars, similar to chlorophyll content. Except for Bayram and Ilgaz, the inoculation treatment significantly increased nitrogen percentage and total nitrogen in all cultivars compared to the un-inoculated control (Table 3). Akça, Arda, Aslanbey, Cevdetbey, Göktürk and Yazıcı, which fixed between 19.3 and 23.5 mg nitrogen per plant, exhibited the highest response to rhizobial inoculation. In contrast, Ilgaz did not fix any nitrogen, while Bayram fixed a considerably low amount of nitrogen (4 mg plant$^{-1}$) (Table 4). Other researchers have also reported that the interaction between cultivar and bacteria significantly effects the amount of nitrogen fixed in chickpea (Beck, 1992; Elkoca et al., 2015; Solaiman et al., 2007).

**Table 4.** The amount of fixed N by the chickpea cultivars inoculated with *Mesorhizobium ciceri*.

| **Cultivars** | **Fixed N (mg plant$^{-1}$)** |
|---|---|
| Akça | 23.5 a |
| Aksu | 16.5 bc |
| Arda | 19.4 abc |
| Aslanbey | 22.6 ab |
| Bayram | 4.0 d |
| Botan | 14.4 c |
| Cevdetbey | 19.3 abc |
| Göktürk | 20.2 abc |
| Ilgaz | 0.0 d |
| Yazıcı | 22.1 ab |
| *CV (%)* | *21.6* |

Mean values with the same letter are not statistically different at $p < 0.05$.

Correlation coefficients revealed significantly relationship between shoot fresh weight, N% and total N with nodule weight (Table 5 and Figure 2 b,c,d,). Many researchers, including Bhuiyan et al. (2009), Öğütçü et al. (2010) and Solaiman et al. (2010) have reported similar positive correlations between nodulation and the parameters examined when effective nodules are formed. However, the amount of nitrogen fixed did not show a correlation with nodule weight (Table 5). For instance, Bayram with 40.3 mg nodule dry weight per plant only fixed 4 mg of nitrogen, while Akça and Göktürk with lower nodule dry weight per plant (26.0 and 10.4 mg, respectively), fixed larger amounts of nitrogen (23.5 and 20.2 mg, respectively) (Tables 2 and 4). These results indicate that nodule efficiency is more crucial than nodule weight in nitrogen fixation. Similar findings have been reported by other researchers such as Elkoca et al. (2015), Kipe-Nolt and Giller (1993) and Ögütçü et al. (2008).

**Table 5.** Correlation coefficients between nodulation and examined parameters.

| | **SH** | **SFW** | **SDW** | **RFW** | **RDW** | **Chlor.** | **N%** | **Total N** | **Fixed N** |
|---|---|---|---|---|---|---|---|---|---|
| **NFW** | 0.30 $^{ns}$ | 0.48* | 0.37 $^{ns}$ | -0.12 $^{ns}$ | -0.01 $^{ns}$ | 0.39 $^{ns}$ | 0.64** | 0.53* | 0.11 $^{ns}$ |
| **NDW** | 0.34 $^{ns}$ | 0.50* | 0.40 $^{ns}$ | -0.10 $^{ns}$ | 0.03 $^{ns}$ | 0.41 $^{ns}$ | 0.65** | 0.56** | 0.14 $^{ns}$ |

∗ and ∗∗ significant at 0.05 and 0.01 levels, respectively; ns: Not significant; n = 20.
SH: Shoot height; SFW: Shoot fresh weight; SDW: Shoot dry weight; RFW: Root fresh weight; RDW: Root dry weight.

## 4. Conclusion

The results of this study showed that the response of chickpea to *rhizobium* inoculation varied significantly among cultivars and that symbiotic nitrogen gain in chickpea can be increased by selecting compatible cultivars. Akça, Arda, Aslanbey, Cevdetbey, Göktürk and Yazıcı are the most compatible cultivars, considering the amount of nitrogen fixed. It would be useful to confirm the nitrogen fixation efficiency of chickpea cultivars used in this research conducted under pot conditions with field trials in future studies.

## Acknowledgment

This article was prepared from Yasin Altuntaş's Master's Thesis.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Beck, D. P. (1992). Yield and nitrogen fixation of chickpea cultivars in response to inoculation with selected rhizobial strains. *Agronomy Journal*, *84*(3), 510-516. https://doi.org/10.2134/agronj1992.00021962008400030029x

Bhuiyan, M. A. H., Khanam, D., Ullah, M. H., & Alam, M. M. (2009). Effect of inoculation and varietal interactions of chickpea at southern region of Bangladesh. *Bulletin of the Institute Tropical Agriculture*, *Kyushu University*, *32*(1), 17-23. https://doi.org/10.11189/bita.32.17

Bidlack, J. E., Rao, S. C., & Demezas, D. H. (2001). Nodulation, nitrogenase activity, and dry weight of chickpea and pigeon pea cultivars using different *Bradyrhizobium* strains. *Journal of Plant Nutrition*, *24*(3), 549-560. https://doi.org/10.1081/PLN-100104979

Bohlool, B., Ladha, J. K., Garrity, D. P., & George, T. (1992). Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant and Soil*, *141*, 1-11. https://doi.org/10.1007/BF00011307

Bordeleau, L. M., & Prevost, D. (1994). Nodulation and nitrogen fixation in extreme environments. *Plant and Soil*, *161*, 115-125. https://doi.org/10.1007/BF02183092

Buttery, B. R., Park, S-J., & van Berkum, P. (1997). Effects of common bean (*Phaseolus vulgaris* L.) cultivar and rhizobium strain on plant growth, seed yield and nitrogen content. *Canadian Journal of Plant Science*, *77*, 347-351. https://doi.org/10.4141/P96-088

Danso, S. K. A., Hera, C., & Douka, C. (1987). Nitrogen fixation in soybean as influenced by cultivar and Rhizobium strain. *Plant and Soil*, *99*, 163-174. https://doi.org/10.1007/BF02370163

Davis, T. M., Foster, K. W., & Phillips, D. A. (1985). Nodulation mutants in chickpea. *Crop Science*, *25*(2), 345-348. https://doi.org/10.2135/cropsci1985.0011183X002500020033x

Di Bonito, R., Porreca, G., & Mannelli, S. (1990). Competition for nodulation of *Rhizobium* spp. (*Cicer arietinum* L.) inoculated on different cultivars of chickpea. *Symbiosis*, *9*(1-3), 405-409.

Dwivedi, S. L., Sahrawat, K. L., Upadhyaya, H. D., Mengoni, A., Galardini, M., Bazzicalupo, M., Biondi, E. G., Hungria, M., Kaschuk, G., Blair, M. W., & Ortiz, R. (2015). Advances in host plant and Rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. *Advances in Agronomy*, *129*, 1-116. https://doi.org/10.1016/bs.agron.2014.09.001

El Hadi, E. A., & Elsheikh, E. A. E. (1999). Effect of *Rhizobium* inoculation and nitrogen fertilization on yield and protein content of six chickpea (*Cicer arietinum* L.) cultivars in marginal soils under irrigation. *Nutrient Cycling in Agroecosystems*, *54*, 57-63. https://doi.org/10.1023/A:1009778727102

Elkoca, E., Kantar, F., & Sahin, F. (2008). Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *Journal of Plant Nutrition*, *31*, 157-171. https://doi.org/10.1080/01904160701742097

Elkoca, E., Turan, M., & Dönmez, M. F. (2010). Effects of single, dual and triple inoculations with *Bacillus subtilis*, *Bacillus megaterium* and *Rhizobium leguminosarum* bv. *phaseoli* on nodulation, nutrient uptake, yield and yield parameters of common bean (*Phaseolus vulgaris* L. cv. 'Elkoca-05'). *Journal of Plant Nutrition*, *33*, 2104-2119. https://doi.org/10.1080/01904167.2010.519084

Elkoca, E., Kocli, T., Gunes, A., & Turan, M. (2015). The symbiotic performance and plant nutrient uptake of certain nationally registered chickpea (*Cicer arietinum* L.) cultivars of Turkey. *Journal of Plant Nutrition*, *38*, 1427-1443. https://doi.org/10.1080/01904167.2014.983123

Eusuf Zai, A. K., Solaiman, A. R. M., & Ahmed, J. U. (1999). Response of some chickpea varieties to *Rhizobium* inoculation in respect to nodulation, biological nitrogen fixation and dry matter yield. *Bangladesh Journal of Microbiology*, *16*(2), 135-144.

Fageria, N. K., & Baligar, V. C. (2005). Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*, *88*, 97-185. https://doi.org/10.1016/S0065-2113(05)88004-6

FAO. (2022). *Crops and livestock products*. Retrieved Jun 27, 2024, from https://www.fao.org/faostat/en/#data/QCL

Farid, M., & Navabi, A. (2015). $N_2$ fixation ability of different dry bean genotypes. *Canadian Journal of Plant Science*, *95*, 1243-1257. https://doi.org/10.4141/cjps-2015-084

Goyal, R. K., Mattoo, A. K., & Schmidt, M. A. (2021). Rhizobial–host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. *Frontiers in Microbiology*, *12*, 669404. https://doi.org/10.3389/fmicb.2021.669404

Gul, R., Khan, H., Khan, N. U., & Khan, F. U. (2014). Characterization of chickpea germplasm for nodulation and effect of rhizobium inoculation on nodules number and seed yield. *The Journal of Animal & Plant Sciences*, *24*(5), 1421-1429.

Hardarson, G., & Danso, S. K. A. (1993). Methods for measuring biological nitrogen fixation in grain legumes. *Plant and Soil*, *152*, 19-23. https://doi.org/10.1007/BF00016330

Hoque, M. F., Sattar, M. A., & Dutta, R. K. (1999). Nodulation, chlorophyll and leghaemoglobin content of soybean as affected by *Bradyrhizobium japonicum* and sulphur molybdenum supplementation. *Bangladesh Journal of Botany*, *28*(1), 47-52.

Hossain, Z., Wang, X., Hamel, C., & Gan, Y. (2018). Nodulation and nitrogen accumulation in pulses vary with species, cultivars, growth stages, and environments. *Canadian Journal of Plant Science*, *98*(3), 527-542. https://doi.org/10.1139/cjps-2017-0114

Hungria, M., & Bohrer, T. R. J. (2000). Variability of nodulation and dinitrogen fixation capacity among soybean cultivar. *Biology and Fertility of Soils*, *31*, 45-52. https://doi.org/10.1007/s003740050622

Kantar, F., Hafeez, F. Y., Shivakumar, B. G., Sundaram, S. P., Tejera, N. A., Aslam, A., Bano, A., & Raja, P. (2007). Chickpea: *Rhizobium* management and nitrogen fixation. In S. S. Yadav, R. J. Redden, W. Chen & B. Sharma (Eds.), *Chickpea breeding and management* (pp. 179-192). CAB International. https://doi.org/10.1079/9781845932138.008

Keneni, G., Bekele, E., Assefa, F., Imtiaz, M., Debele, T., Dagne, K., & Getu, E. (2012). Phenotypic diversity for symbio-agronomic characters in Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions. *African Journal of Biotechnology*, *11*(63), 12634-12651. https://doi.org/10.5897/AJB12.428

Kipe-Nolt, J. A., & Giller, K. E. (1993). A field evaluation using the $^{15}N$ isotope dilution method of lines of *Phaseolus vulgaris* L. bred for increased nitrogen fixation. *Plant and Soil*, *152*, 107-114. https://doi.org/10.1007/BF00016339

Kumawat, S. M., Dhakar, L. L., & Maliwal, P. L. (2000). Effect of irrigation regimes and nitrogen on yield, oil content and nutrient uptake of soybean (*Glycine max*). *Indian Journal of Agronomy*, *45*(2), 361-366.

Li, S. X., Wang, Z. H., & Stewart, B. A. (2013). Responses of crop plants to ammonium and nitrate N. *Advances in Agronomy*, *118*, 205-397. https://doi.org/10.1016/B978-0-12-405942-9.00005-0

Mendoza-Tafolla, R. O., Juarez-Lopez, P., Ontiveros-Capurata, R. E., Sandoval-Villa, M., Iran, A. T., & Alejo-Santiago, G. (2019). Estimating nitrogen and chlorophyll status of romaine lettuce using SPAD and at LEAF Readings. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47*(3), 751-756. https://doi.org/10.15835/nbha47311525

Mohammadi, K., & Sohrabi, Y. (2012). Bacterial biofertilizers for sustainable crop production: A review. *Journal of Agricultural and Biological Science*, *7*(5), 307-316.

Ndiaye, M. A. F., Spencer, M. M., & Gueye, M. (2000). Genetic variability in dinitrogen fixation between cowpea [*Vigna unguiculata* (L.) Walp] cultivars determined using the nitrogen-15 isotope dilution technique. *Biology and Fertility of Soils*, *32*, 318-320. https://doi.org/10.1007/s003740000254

Odabaş, M. S., & Gülümser, A. (2001). An investigation on the effects of yield, yield components and chlorophyll amounts of the leaves of different nitrogen sources in bean (*Phaseolus vulgaris* L.). *Anadolu Tarım Bilimleri Dergisi*, *16*(1), 42-47.

Öğütçü, H., Algur, O. F., Elkoca, E., & Kantar, F. (2008). The determination of symbiotic effectiveness of *Rhizobium* strains isolated from wild chickpeas collected from high altitudes in Erzurum. *Turkish Journal of Agriculture and Forestry*, *32*, 241-248.

Öğütçü, H., Kasımoğlu, C., & Elkoca, E. (2010). Effects of rhizobium strains isolated from wild chickpeas on the growth and symbiotic performance of chickpeas (*Cicer arietinum* L.) under salt stress. *Turkish Journal of Agriculture and Forestry*, *34*, 361-371. https://doi.org/10.3906/tar-0904-34

Pandey, R. P., Srivastava, A. K., Gupta, V. K., O'Donovan, A., & Ramteke, P. W. (2018). Enhanced yield of diverse varieties of chickpea (*Cicer arietinum* L.) by different isolates of *Mesorhizobium ciceri*. *Environmental Sustainability*, *1*, 425-435. https://doi.org/10.1007/s42398-018-00039-9

Perret, X., Staehelin, C., & Broughton, W. J. (2000). Molecular basis of symbiotic promiscuity. *Microbiology and Molecular Biology Reviews*, *64*, 180-201. https://doi.org/10.1128/mmbr.64.1.180-201.2000

Priyadarsini, L., Singh, P. K., Chatterjee, C., Sadhukhan, R., & Biswas, T. (2017). Estimation of genetic variability of nodulation characters and their association with different agromorphic characters and yield in chickpea (*C. arietinum* L.). *International Journal of Current Microbiology and Applied Sciences*, *6*(9), 1928-1935. https://doi.org/10.20546/ijcmas.2017.609.236

Roy, A., Ghosh, S., & Kundagrami, S. (2018). Nodulation pattern and its association with seed yield in chickpea (*Cicer arietinum* L.) germplasms. *Indian Journal of Agricultural Research*, *53*(2), 172-177. https://doi.org/10.18805/IJARe.A-5162

Rupela, O. P., & Saxena, M. C. (1987). Nodulation and nitrogen fixation in chickpea. In M. C. Saxena & K. B. Singh (Eds.), *The chickpea* (pp. 191-206). CAB International.

Santalla, M., Amurrio, J. M., Ron, J. M., & De, A. M. (2001). Symbiotic interactions between *Rhizobium leguminosarum* strains and elite cultivars of *Pisum sativum* L. *Journal of Agronomy and Crop Science*, *187*(1), 59-68. https://doi.org/10.1046/j.1439-037X.2001.00502.x

Solaiman, A. R. M., Hossain, D., & Rabbani, M. G. (2007). Influence of *Rhizobium* inoculant and mineral nitrogen on some chickpea varieties. *Bangladesh Journal of Microbiology*, *24*(2), 146-150. https://doi.org/10.3329/bjm.v24i2.1261

Solaiman, A. R. M., Talukder, M. S., & Rabbani, M. G. (2010). Influence of some *Rhizobium* strains on chickpea: Nodulation, dry matter yield and nitrogen uptake. *Bangladesh Journal of Microbiology*, *27*(2), 61-64. https://doi.org/10.3329/bjm.v27i2.9174

Somasegaran, P., Hoben, H. J., & Gurgun, V. (1988). Effects of inoculation rate, rhizobial competition, and nitrogen fixation in chickpea. *Agronomy Journal*, *80*(1), 68-73. https://doi.org/10.2134/agronj1988.00021962008000010016x

Verma, D. P. S. (1984). Involvement of specific host genes in symbiotic nitrogen fixation. In V. L. Chopra, B. C. Joshi, R. P. Sharma & H. C. Bansal (Eds.), *Genetics: New frontiers, volume II recombinant DNA technology* (pp. 227-239). Oxford & IBH Publishing Co.

Verma, J. P., Yadav, J., Tiwari, K. N., & Kumar, A. (2013). Effect of indigenous *Mesorhizobium* sp. and plant growth promoting rhizobacteria on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). *Ecological Engineering*, *51*, 282-286. https://doi.org/10.1016/j.ecoleng.2012.12.022

Wang, Q., Liu, J., & Zhu, H. (2018). Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions. *Frontiers in Plant Science*, *9*, 313. https://doi.org/10.3389/fpls.2018.00313

Wood, C. W., Reeves, D. W., Duffield, R. R., & Edmisten, K. L. (1992). Field chlorophyll measurements for evaluation of corn nitrogen status. *Journal of Plant Nutrition*, *15*(4), 487-500. https://doi.org/10.1080/01904169209364335

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

PRENSIP

https://prensip.gen.tr

RESEARCH ARTICLE

# Altitude-Dependent Variation in Chemical Composition of Essential Oil of *Origanum acutidens* (Hand-Mazz.) Ietswaart

Furkan Coban[1]✉ • Hakan Ozer[1] • Ramazan Cakmakci[2]

[1]Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye
[2]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crops, Çanakkale/Türkiye

ARTICLE INFO

**Article History**
Received: 30.10.2024
Accepted: 02.12.2024
First Published: 31.12.2024

**Keywords**
Altitude
Carvacrol
Essential oil
*Origanum acutidens*
*p*-cymene

ABSTRACT

Altitude significantly influences the yield and composition of essential oils in medicinal plants, with *Origanum acutidens* (Hand-Mazz.) Ietswaart, an endemic species in Eastern Anatolia, Türkiye, showing noticeable variations. Known for its traditional medicinal uses and aromatic qualities, this species was studied at three different altitudes (1150, 1650, and 2150 m) in the Eastern Black Sea Region. The results showed that essential oil yield increased with altitude, with yields at 0.75%, 0.86%, and 1.03% at each altitude, respectively. Key components of the oil, carvacrol and *p*-cymene, also varied with altitude. Carvacrol content increased significantly from 38.30% to 58.76% as altitude increased, while *p*-cymene content decreased from 35.47% to 17.12%. These results suggest that higher altitudes, which provide conditions like lower temperature, reduced air pressure, and higher UV exposure, stimulate secondary metabolite production in *O. acutidens*. It is recommended that further research be conducted to explore this plant's chemical diversity across varied topography and climate conditions.

**Please cite this paper as follows:**

Coban, F., Ozer, H., & Cakmakci, R. (2024). Altitude-dependent variation in chemical composition of essential oil of *Origanum acutidens* (Hand-Mazz.) Ietswaart. *Journal of Agricultural Production*, *5*(4), 265-271. https://doi.org/10.56430/japro.1576344

## 1. Introduction

Türkiye is home to a diverse array of plant species harvested for purposes such as food, medicine, and various other applications, thereby contributing significantly to both domestic and foreign markets. Among these, members of the Lamiaceae family, which includes approximately 220 genera and 4,000 species globally, are of particular importance. Türkiye hosts 48 genera and 873 species from this family, showcasing the country's rich botanical diversity (Güner et al., 2012). Within this family, plants colloquially referred to as "thyme" are especially valued for their medicinal and aromatic properties. These plants, from genera like *Thymus*, *Origanum*, *Satureja*, *Tymbra*, and *Coridothymus*, are widely used across Türkiye, often known by various local names depending on the region. Notably, a large portion of the Lamiaceae species in Türkiye is endemic, with endemicity rates of 44.2% for the family, 65.2% for *Origanum*, and 52.6% for *Thymus*, emphasizing Türkiye's role as a gene center for these genera (Baser et al., 1997; Davis, 1970; Tumen et al., 1995), (Figure 1). Many taxa belonging to the genus *Origanum*, commonly known as oregano, are widely used in folk medicine for the treatment of ailments such as headaches, dizziness, cough, flu, gastrointestinal diseases, bronchitis, high cholesterol, diabetes,

✉ Correspondence
E-mail address: furkan.coban@atauni.edu.tr

abdominal pain, hypertension, and toothache (Fidan et al., 2020).

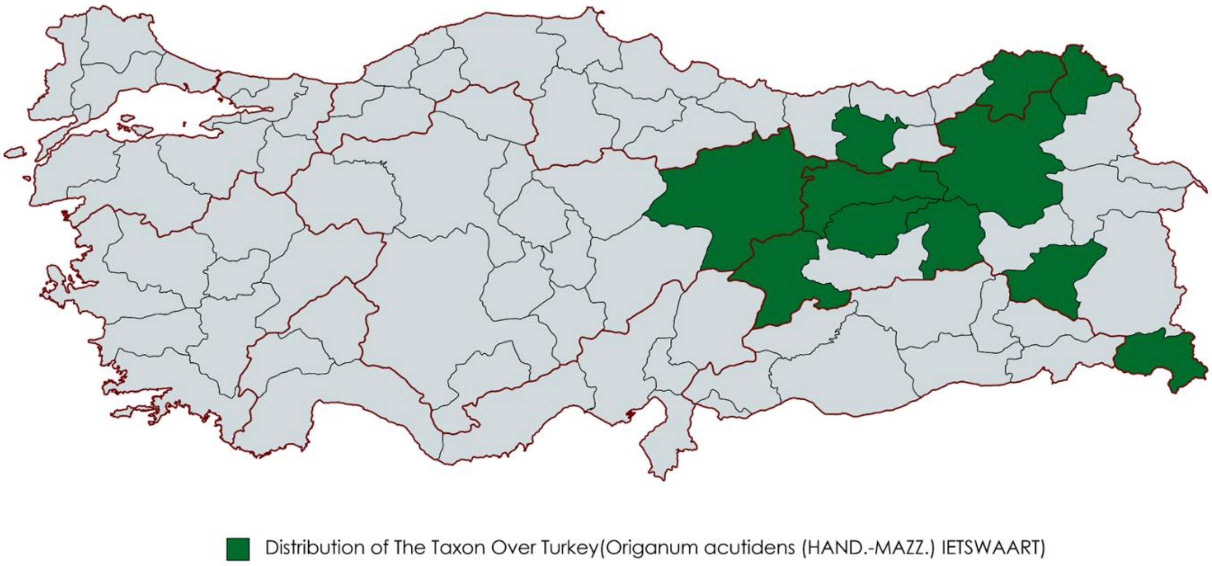


**Figure 1.** Geographic distribution of *Origanum acutidens* in Türkiye (TÜBİVES, 2023).

One noteworthy member of this family is *Origanum acutidens*, a perennial herb endemic to Eastern Anatolia Region in Türkiye (Figure 1). Flowering from July to August in bright pink to white hues, this species thrives on sun-exposed slopes with calcareous or limestone substrates, requiring minimal moisture. *O. acutidens* appealing aroma has made it a candidate for ornamental use in urban landscaping and rock gardens, while its fragrant and disease-protective properties have led to its adoption in the food industry as a natural flavoring and preservative. The essential oil of *O. acutidens* is particularly rich in carvacrol, a phenolic compound known for its antimicrobial properties, which, along with other components like *p*-cymene and thymol, provides a potent, natural alternative to synthetic preservatives (Burt, 2004). This oil has demonstrated significant antibacterial efficacy, notably against strains like *Salmonella typhimurium* and *Escherichia coli*, making it a promising natural antiseptic for applications in health and hygiene sectors (Cosge et al., 2009). As a high-altitude plant endemic to Eastern Anatolia, *O. acutidens* exemplifies the traditional medicinal uses associated with *Origanum* species, which have historically been applied as sedatives, diuretics, digestive aids, and antiseptics (Köse et al., 2021).

Thymol and carvacrol are often confused due to their similar structures; however, these two compounds have different properties and effects. Especially in medical and industrial applications, it is important not to mix up thymol and carvacrol to ensure the correct compound is used (De Vincenzi et al., 2004; Ultee et al., 2002). One of the primary pathways for carvacrol synthesis is through the conversion of *p*-cymene, a compound with the formula $C_{10}H_{14}$. This transformation can occur via different mechanisms. Isomerization allows *p*-cymene to serve as an intermediate, where structural rearrangement of atoms leads to the formation of carvacrol. Oxidation is another pathway; exposure to oxygen or oxidative conditions within the plant modifies *p*-cymene structure, enabling the formation of carvacrol. Additionally, enzymatic reactions in essential oil-producing plant cells play a crucial role, as specific enzymes can catalyze the transformation of *p*-cymene into carvacrol during the plant's natural metabolic processes. These pathways underscore the biochemical complexity behind essential oil composition in plants (Marchese et al., 2016) (Figure 2).

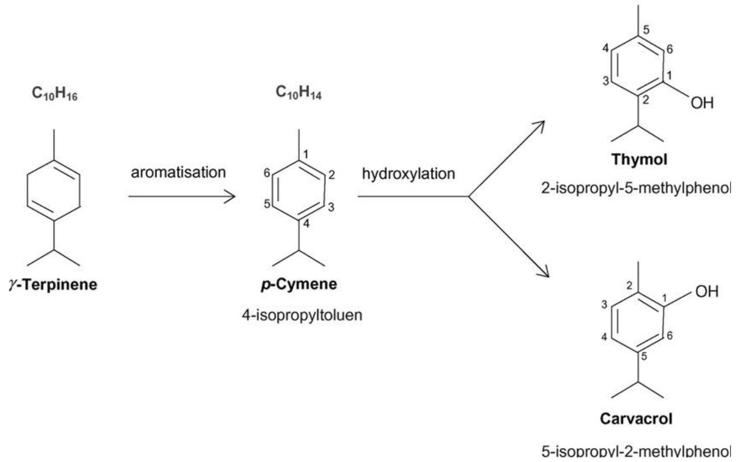


**Figure 2.** Pathways for the synthesis of carvacrol from *γ*-terpinene via *p*-cymene (Taherian et al., 2009).

The composition of essential oils is influenced by numerous factors that determine the chemical profiles and quality of these oils in various plant species. These factors include the geographical location of the plant, climatic conditions, soil composition, and the altitude at which the plant grows (Hussain et al., 2008; Talebi et al., 2019). Altitude plays a critical role in determining the quality and yield of medicinal plants by impacting their biochemical composition, growth rate, and adaptability (Khalil et al., 2020; Mahdavi et al., 2013). For species like *Origanum acutidens*, these altitude-related variations are particularly significant, as changes in altitude can influence essential oil content, concentration of active compounds, and overall plant morphology. Variations in the composition of essential oils with altitude are often complex and depend on multiple interacting factors, including specific plant species, geographic location, climate conditions, soil composition, and the altitude range itself.

Given this context, the present study seeks to investigate how different altitudinal levels influence the essential oil yield and composition in the aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart during its flowering period. This research will examine three distinct altitudes 1150, 1650, and 2150 meters in the Eastern Black Sea Region of Türkiye, aiming to capture how variations in altitude affect the plants chemical profile. By understanding these differences, the study will contribute valuable insights into the medicinal and commercial applications of *O. acutidens*. This information could inform optimal harvesting conditions for maximizing essential oil yield and quality, aiding in the sustainable and efficient use of this valuable plant in industries ranging from food preservation to natural medicine.

## 2. Materials and Methods

### *2.1.* Plant Materials

Aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart were collected from Baba Mountain, İspir, Erzurum, Türkiye, during the flowering season in 2023 at three different altitudes (1150, 1650, and 2150 m) (Figure 3). The samples were collected simultaneously on the same date to ensure consistency, as the content and yield of samples collected at different times may vary. The collected plant material was dried in the shade, ground in a grinder equipped with a 2 mm diameter mesh, and stored. The taxonomic identification of the plant material was verified by Dr. Ali Kandemir, a senior plant taxonomist from the Department of Biology at Erzincan University, Erzincan, Türkiye. Additionally, herbarium specimens were prepared and deposited in the herbarium of the Department of Field Crops, Atatürk University, under the accession numbers Org2023008, Org2023009, and Org2023010, corresponding to the collection sites in ascending order of altitude.

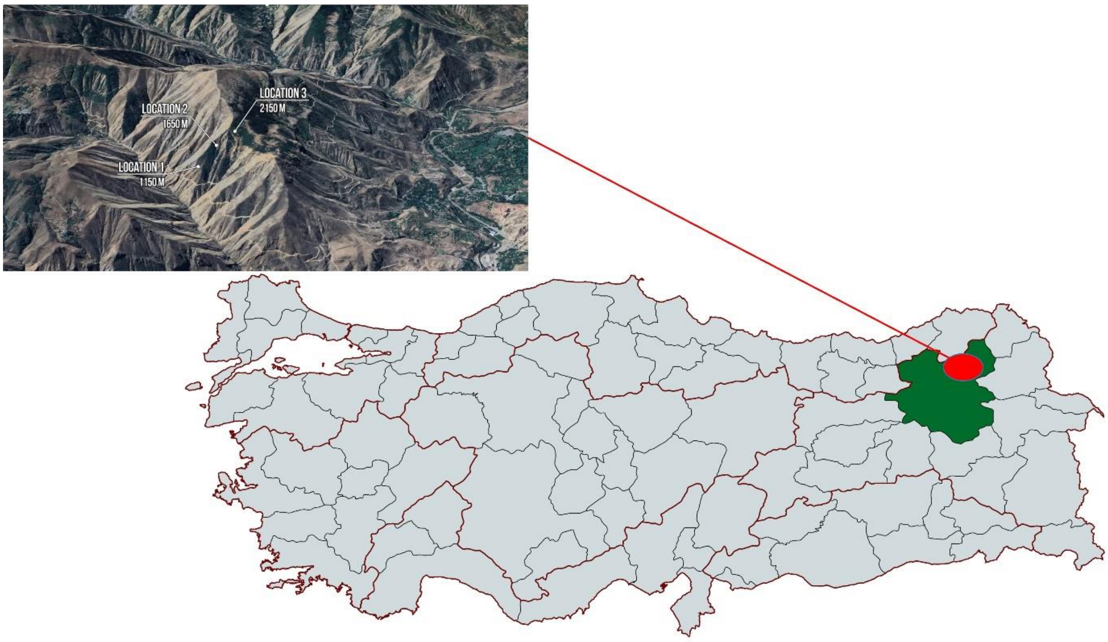


**Figure 3.** Collection sites of *Origanum acutidens* at different altitudes on Baba Mountain, İspir, Erzurum, Türkiye.

### *2.2.* Essential Oil Extraction

The air-dried aerial parts of the plant samples were extracted via hydrodistillation using a Clevenger-type apparatus for a duration of 3 hours. The extraction process was repeated three times to ensure reproducibility. The resulting essential oil was then dehydrated using anhydrous sodium sulfate, filtered, and stored at +4°C until further testing and analysis.

### *2.3.* GC Analysis Conditions

Essential oil analysis was conducted on a Thermofinnigan Trace GC/A1300 system equipped with an SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set to 220°C. The temperature program started at 50°C, increased to 150°C at 3°C/min, held isothermal for 10 minutes, and then raised to 250°C at 10°C/min. Diluted samples (1/100, v/v in methylene chloride) of 1.0 µL were manually injected in splitless mode. Quantitative data were obtained from the FID area percentage.

### *2.4.* GC-MS Analysis Conditions

The essential oils were analyzed using a Thermofinnigan Trace GC/Trace DSQ/A1300 system (E.I. Quadrupole) with an SGE-BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Detection was carried out via electron ionization at 70 eV, with helium as the carrier gas at 1 mL/min. Injection and MS transfer line temperatures were 220°C and 290°C, respectively. The temperature program was set from 50°C to 150°C at 3°C/min, held for 10 minutes, and then increased to 250°C at 10°C/min. A 1.0 µL volume of diluted sample (1/100, v/v in methylene chloride) was injected in splitless mode. Component identification was based on retention times, mass spectra comparison with Wiley7N and TRLIB databases, and literature data. Identification was further validated by matching relative retention indices with non-polar phase values reported in the literature (Adams, 2007).

## 3. Results and Discussion

The essential oil (EO) yield of *Origanum acutidens* varied significantly with altitude, indicating that altitude plays a role in enhancing oil production in this plant. At 1150 m, the essential oil yield was recorded at 0.75 ± 0.01%, increasing to 0.86 ± 0.02% at 1650 m, and reaching a peak of 1.03 ± 0.02% at 2150 m (Table 1). Increasing altitude positively impacts essential oil (EO) yield, mainly due to environmental stresses such as elevated UV radiation, lower temperatures, and fluctuating light intensity. These conditions stimulate the production of secondary metabolites, including essential oils, in plants. The heightened UV exposure at higher altitude promotes the production of protective compounds with antioxidant properties, such as phenolics and terpenoids (Chrysargyris et al., 2020). These compounds enhance the plants defense against UV-induced oxidative stress, thereby boosting EO yield and effectiveness. Temperature fluctuations in mountainous areas also influence EO composition, specifically by promoting the biosynthesis of oxygenated monoterpenes, which are crucial for aroma and medicinal properties (Chrysargyris et al., 2020; Zhang et al., 2021). Furthermore, studies on medicinal plants indicate that both light and temperature stress enhance EO yield and alter antioxidant activity, emphasizing the critical role of altitude in plant bioactivity (Thoma et al., 2020). The EO yield we obtained, ranging from 0.75% to 1.03%, aligns closely with the findings reported by Karagöz et al. (2022), who identified EO yields within the range of 0.42% to 1.13%. This consistency suggests that our results fall within the expected yield range under similar conditions, supporting the reliability of our findings in the context of established research.

Carvacrol and *p*-cymene are two primary monoterpene compounds that play a crucial role in the chemical composition of plant essential oils. However, the concentrations of these compounds vary depending on the altitude at which the plants grow and the environmental stress factors they experience. As observed in the study, *p*-cymene is found in high levels at moderate altitudes (1150-1650 m), while its concentration decreases at higher elevations (2150 m). In contrast, the Carvacrol content shows a significant increase with altitude; at 1150 m, the Carvacrol level is 38.30%, which rises to 41.58% at 1650 m and further to 58.76% at 2150 m (Table 1).

**Table 1.** Chemical profiling of essential oil of *Origanum acutidens* from different altitudes of Eastern Black Sea Region, Türkiye.

| Constituents | RI* | Composition (%) | | | Method of identification |
|---|---|---|---|---|---|
| | | Altitude | | | |
| | | 1150m | 1650m | 2150m | |
| *α*-thujene | 924 | 0.87 | 3.07 | 0.70 | GC-MS- RI |
| *α*-pinene | 932 | 3.08 | - | 2.18 | GC-MS- RI |
| camphene | 946 | 1.30 | 0.47 | 0.79 | GC-MS- RI |
| *β*-pinene | 974 | - | 0.32 | 0.41 | GC-MS- RI |
| myrecene | 988 | 3.05 | 4.19 | 3.28 | GC-MS- RI |
| *α*-phellandrene | 1002 | - | 0.61 | 0.44 | |
| *δ*-3-Carene | 1008 | 0.55 | - | - | |
| *α*-terpinene | 1014 | 1.80 | 3.38 | 1.78 | GC-MS- RI |
| *p*-cymene | 1020 | 35.47 | 22.75 | 17.12 | GC-MS- RI |
| trans-*β*-ocimene | 1044 | - | 0.35 | - | GC-MS- RI |
| *γ*-terpinene | 1054 | 5.53 | 15.89 | 7.69 | GC-MS- RI |
| cis-sabinenehydrate | 1065 | 0.43 | 0.22 | 0.30 | MS- RI |
| terpinolene | 1086 | 0.36 | - | 0.13 | GC-MS- RI |
| linalool | 1095 | 2.45 | - | 0.71 | GC-MS- RI |
| borneol | 1165 | 2.46 | 0.55 | 1.88 | GC-MS- RI |
| terpinen-4-ol | 1174 | 0.76 | 0.83 | 0.95 | GC-MS- RI |
| *α*-terpineol | 1186 | 0.57 | 0.33 | 0.44 | GC-MS- RI |
| cis-dihydrocarvone | 1191 | - | - | 0.13 | |
| carvocrol methyl ether | 1241 | - | 2.58 | 0.09 | |
| ascaridole | 1234 | - | - | 0.14 | |
| thymol | 1289 | 0.16 | 2.13 | 0.36 | GC-MS- RI |
| carvacrol | 1298 | 38.30 | 41.58 | 58.76 | GC-MS- RI |
| carvacrol acetate | 1370 | - | 0.26 | - | |
| geranyl acetate | 1379 | 0.39 | - | 0.14 | GC-MS- RI |
| *β*-caryphyllene | 1417 | 2.12 | 0.49 | 1.39 | GC-MS- RI |
| aromadendrene | 1439 | - | - | 0.19 | GC-MS- RI |
| EO yield | | 0.75 ± 0.01 | 0.86 ± 0.02 | 1.03 ± 0.02 | |
| Total | | 99.65 | 100 | 100 | |

*RI, retention index: Compounds listed in order of elution from a BPX5 MS column.

The carvacrol content of *Origanum acutidens* and its related species exhibits considerable variability across different regions of Türkiye, with documented values ranging between 49.4% and 87.0%. This variation is closely associated with geographical and environmental factors that significantly influence the composition of essential oils (Baser et al., 1997; Cosge et al., 2009; Gulec et al., 2014; Kordali et al., 2008; Sökmen et al., 2004). Among these factors, altitude emerges as a critical determinant, shaping the biosynthesis of major constituents such as carvacrol and *p*-cymene.

High-altitude regions expose plants to unique environmental stressors, including increased UV radiation and lower temperatures. These conditions stimulate the production of secondary metabolites, particularly phenolic compounds, which play a vital role in enhancing the plant's defense mechanisms. The increase in carvacrol content at higher altitudes is attributed to the upregulation of the mevalonate (MVA) pathway, which governs the biosynthesis of terpenoids and related compounds (Khoshbakht et al., 2020; Pirigharnaei et al., 2012; Tsoumani et al., 2022). Furthermore, the conversion of precursor molecules, such as *p*-cymene, into carvacrol is believed to intensify in response to these stressors (Hosseini et al., 2024).

These adaptive biosynthetic responses are not merely survival mechanisms but also illustrate the complex relationship between environmental factors and the chemical profile of oregano essential oils. Understanding these dynamics provides a basis for optimizing cultivation strategies aimed at enhancing carvacrol content. For instance, deliberate exposure to moderate environmental stress, as observed at higher altitudes, may be strategically utilized to improve the therapeutic and commercial value of oregano essential oils.

## 4. Conclusion

This study demonstrates that altitude significantly affects the essential oil yield and composition of *Origanum acutidens* (Hand-Mazz.) Ietswaart, an endemic species to Türkiye's Eastern Anatolia Region. As altitude increases, there is a corresponding increase in essential oil yield and notable shifts in chemical composition, particularly in carvacrol and *p*-cymene content. Higher altitudes stimulate the production of carvacrol while reducing *p*-cymene levels, likely due to increased UV radiation, lower temperatures, and reduced atmospheric pressure. These environmental factors activate biosynthetic pathways such as the mevalonate (MVA) pathway, enhancing the production of secondary metabolites that strengthen the plant's defense mechanisms. The findings suggest that by strategically cultivating *O. acutidens* at higher altitudes, it may be possible to optimize carvacrol levels, thereby improving its antimicrobial potency and commercial value for applications in food preservation, medicine, and natural products.

Further research on the influence of altitude and other environmental factors across various topographies and climates could provide deeper insights into the adaptive mechanisms of *O. acutidens*. This knowledge could inform more sustainable harvesting and cultivation practices, enhancing the quality and yield of essential oils from this valuable species.

## Acknowledgment

This study was presented as an oral presentation by Furkan Çoban at the ISEO 2023 (International Symposium on Essential Oils) in Italy and was published as an abstract in the conference proceedings. We extend our gratitude to the symposium organizers and participants for their valuable feedback, which contributed significantly to the refinement of this work.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectroscopy*. Allured Publishing Corporation.

Baser, K. H. C., Tümen, G., & Duman, H. (1997). Essential oil of *Origanum acutidens* (Hand.-Mazz.) Ietswaart. *Journal of Essential Oil Research*, *9*(1), 91-92. https://doi.org/10.1080/10412905.1997.9700721

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, *94*(3), 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chrysargyris, A., Mikallou, M., Petropoulos, S., & Tzortzakis, N. (2020). Profiling of essential oils components and polyphenols for their antioxidant activity of medicinal and aromatic plants grown in different environmental conditions. *Agronomy*, *10*(5), 727. https://doi.org/10.3390/agronomy10050727

Cosge, B., Turker, A., Ipek, A., & Gurbuz, B. (2009). Chemical compositions and antibacterial activities of the essential oils from aerial parts and corollas of *Origanum acutidens* (Hand.-Mazz.) Ietswaart, an endemic species to Turkey. *Molecules*, *14*(5), 1702-1712. https://doi.org/10.3390/molecules14051702

Davis, P. H. (1970). *Flora of Turkey and the East Aegean islands*. Edinburgh University Press.

De Vincenzi, M., Stammati, A., De Vincenzi, A., & Silano, M. (2004). Constituents of aromatic plants: Carvacrol. *Fitoterapia*, *75*(7-8), 801-804. https://doi.org/10.1016/j.fitote.2004.05.002

Fidan, M., Teğin, İ., Erez, M. E., Pınar, S. M., & Eroğlu, H. (2020). Etnobotanik amaçlı kullanılan *Origanum acutidens* bitkisinin toplam fenolik-flovonoid içeriği, fenolik bileşikleri ve element analizi. *Academic Platform-Journal of Engineering and Science*, *8*(1), 49-55. https://doi.org/10.21541/apjes.510659 (In Turkish)

Gulec, A. K., Erecevit, P., Yuce, E., Arslan, A., Bagci, E., & Kirbag, S. (2014). Antimicrobial activity of the methanol extracts and essential oil with the composition of endemic *Origanum acutidens* (Lamiaceae). *Journal of Essential Oil Bearing Plants*, *17*(2), 353-358. https://doi.org/10.1080/0972060X.2014.884770

Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M. T. (2012). *Türkiye bitkileri listesi (damarlı bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. (In Turkish)

Hosseini, N., Ghorbanpour, M., & Mostafavi, H. (2024). Habitat potential modelling and the effect of climate change on the current and future distribution of three

*Thymus* species in Iran using MaxEnt. *Scientific Reports*, *14*, 3641. https://doi.org/10.1038/s41598-024-53405-5

Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*, *108*(3), 986-995. https://doi.org/10.1016/j.foodchem.2007.12.010

Karagöz, H., Hosseinpour, A., Karagöz, F. P., Cakmakci, R., & Haliloglu, K. (2022). Dissection of genetic diversity and population structure in oregano (*Origanum acutidens* L.) genotypes based on agro-morphological properties and start codon targeted (SCoT) markers. *Biologia*, *77*, 1231-1247. https://doi.org/10.1007/s11756-021-00989-2

Khalil, N., El-Jalel, L., Yousif, M., & Gonaid, M. (2020). Altitude impact on the chemical profile and biological activities of *Satureja thymbra* L. essential oil. *BMC Complementary Medicine and Therapies*, *20*, 186. https://doi.org/10.1186/s12906-020-02982-9

Khoshbakht, T., Karami, A., Tahmasebi, A., & Maggi, F. (2020). The variability of thymol and carvacrol contents reveals the level of antibacterial activity of the essential oils from different accessions of *Oliveria decumbens*. *Antibiotics*, *9*(7), 409. https://doi.org/10.3390/antibiotics9070409

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., & Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. *Bioresource Technology*, *99*(18), 8788-8795. https://doi.org/10.1016/j.biortech.2008.04.048

Köse, Y. B., Saltan, N., & Kürkçüoğlu, M. (2021). SPME/GC-MS analysis of volatile organic compounds from *Origanum acutidens* (Hand.-Mazz.) Ietsw.—An endemic species in Turkey. *Natural Volatiles and Essential Oils*, *8*(2), 18-26. https://doi.org/10.37929/nveo.909788

Mahdavi, M., Jouri, M. H., Mahmoudi, J., Rezazadeh, F., & Mahzooni-Kachapi, S. S. (2013). Investigating the altitude effect on the quantity and quality of the essential oil in *Tanacetum polycephalum* Sch.-Bip. *Chinese Journal of Natural Medicines*, *11*(5), 553-559. https://doi.org/10.1016/S1875-5364(13)60100-4

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., & Nabavi, S. M. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry*, *210*, 402-414. https://doi.org/10.1016/j.foodchem.2016.04.111

Pirigharnaei, M., Zare, S., Heidary, R., Khara, J., & Emamali Sabzi, R. (2012). Determination and comparing of essential oil components in wild and cultivated population of *Thymus kotschyanus* Boiss. and Hohen. *African Journal of Plant Science*, *6*(2), 89-95. https://doi.org/10.5897/AJPS11.243

Sökmen, M., Serkedjieva, J., Daferera, D., Gulluce, M., Polissiou, M., Tepe, B., & Sokmen, A. (2004). In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. *Journal of Agricultural and Food Chemistry*, *52*(11), 3309-3312. https://doi.org/10.1021/jf049859g

Taherian, A. A., Babaei, M., Vafaei, A. A., Jarrahi, M., Jadidi, M., & Sadeghi, H. (2009). Antinociceptive effects of hydroalcoholic extract of *Thymus vulgaris*. *Pakistan Journal of Pharmaceutical Sciences*, *22*(1), 83-89.

Talebi, S. M., Nohooji, M. G., Yarmohammadi, M., Khani, M., & Matsyura, A. (2019). Effect of altitude on essential oil composition and on glandular trichome density in three *Nepeta* species (*N. sessilifolia*, *N. heliotropifolia*, and *N. fissa*). *Mediterranean Botany*, *40*(1), 81-93. https://doi.org/10.5209/MBOT.59730

Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., & Deerberg, G. (2020). Effects of light on secondary metabolites in selected leafy greens: A review. *Frontiers in Plant Science*, *11*, 497. https://doi.org/10.3389/fpls.2020.00497

Tsoumani, E. S., Kosma, I. S., & Badeka, A. V. (2022). Chemometric screening of oregano essential oil composition and properties for the identification of specific markers for geographical differentiation of cultivated Greek oregano. *Sustainability*, *14*(22), 14762. https://doi.org/10.3390/su142214762

TÜBİVES. (2023). *Turkish plants data service (TÜBİVES) version 2.0 beta*. Retrieved Aug 12, 2023, from http://www.tubives.com/

Tumen, G., Baser, K. H. C., Kirimer, N., & Ozek, T. (1995). Essential oil of *Origanum saccatum* P.H. Davis. *Journal of Essential Oil Research*, *7*(2), 175-176. https://doi.org/10.1080/10412905.1995.9698493

Ultee, A., Bennik, M. H. J., & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, *68*(4), 1561-1568. https://doi.org/10.1128/AEM.68.4.1561-1568.2002

Zhang, S., Zhang, L., Zou, H., Qiu, L., Zheng, Y., Yang, D., & Wang, Y. (2021). Effects of light on secondary metabolite biosynthesis in medicinal plants. *Frontiers in Plant Science*, *12*, 781236. https://doi.org/10.3389/fpls.2021.781236

http://agriprojournal.com
JOURNAL OF AGRICULTURAL PRODUCTION
ISSN: 2757-6620
https://prensip.gen.tr

RESEARCH ARTICLE

# Farm Management and Socio-Economic Structure of Cattle Enterprises in Eastern Anatolia: A Case Study of Selim District, Kars Province

Veysel Fatih Özdemir • Oğuzhan Tarhan • Bahri Bayram✉

Atatürk University, Faculty of Agriculture, Department of Animal Husbandry, Erzurum/Türkiye

ARTICLE INFO

**Article History**
Received: 30.07.2024
Accepted: 24.12.2024
First Published: 31.12.2024

**Keywords**
Cattle enterprises
Eastern Anatolia
Farm management
Selim District
Socio-economic structure

ABSTRACT

This study investigated the socio-economic characteristics of cattle farms in the Selim district of Kars province. A face-to-face survey was conducted with 350 cattle farm owners selected by random sampling method. Frequency analysis was used to interpret the data obtained. The majority of the farmers in the district were above 40 years of age, while the share of farmers younger than 40 years was only 19.7%. The share of the farmers with primary school (34.4%) or secondary school (33.5%) education was considerably high. A large majority of the farmers in the district had over (68.5%) 20 years of cattle farming experience while only 6.0% had less than 10 years of experience. It was found that 76.8% of the enterprises in the district had 5 or more people in the family. Crossbreds of European and indigenous cattle breeds were quite common (72.3%) in the district. Simmental was determined to be the most commonly raised European breed (44.8%). The 30.9% of enterprises had cattle between 20-30. The majority (50.6%) of the enterprises had 10-20 lactating cows. It was found that 50.9% of the farmers engage in both meat and milk production in their enterprises, while 48.0% engage solely in milk production. It was determined that 52.0% of the enterprise owners in the district were members of associations and cattle breeders' association was the most preferred (90%) among the farmers. High feed prices (98.3%) and diseases (33.1%) were indicated as the major problems by the farmers. In addition, credit support (88.3%), supply of quality breeding stock animals (34%), veterinary services (39.4%) and support in marketing of the products obtained (25.1%) were the main expectations of the farmer from the government.

**Please cite this paper as follows:**

Özdemir, V. F., Tarhan, O., & Bayram, B. (2024). Farm management and socio-economic structure of cattle enterprises in Eastern Anatolia: A case study of Selim District, Kars Province. *Journal of Agricultural Production*, *5*(4), 272-282. https://doi.org/10.56430/japro.1524942

## 1. Introduction

The livestock sector, which has a strategic position in Türkiye, is the main source of livelihood in developing regions and contributes to meeting the demand for protein of animal origin. The sector also provides raw materials for many industries, especially dairy, meat, leather, textiles, pharmaceuticals and cosmetics, and contributes to national income, employment as well as exports (Ergün & Bayram, 2021). Cattle farming is one of the most important sub-sectors of the livestock sector in Türkiye. According to current statistics, approximately 92.9% of total raw milk and 70.1% of the total red meat production in Türkiye is obtained from cattle (TURKSTAT, 2024). The Eastern Anatolian region in particular plays an important role in Türkiye's livestock sector. About 37.53% of the country pastures and meadow area is located in this region (Okcu, 2020). This region is home to over 19 per cent of the total cattle population of the country. According to actual data, the cattle population of the eastern Anatolia region is 3073955 (TURKSTAT, 2024). Kars province ranks 2$^{nd}$ in the region and 4$^{th}$ in the country with 615279 cattle population as compared to other provinces of

✉ Correspondence
E-mail address: bbayram@atauni.edu.tr

Türkiye. Kars is a highland province with an altitude of 1750 meters, and livestock farming plays an important role in the economy of the province. The abundance of grasslands, meadows, pastures and plateaus, which are of great importance for cattle farming, has made cattle farming one of the main economic activities of the province from the past to the present. The province has a total of 8 districts, and after the central district, Selim district has the highest cattle population. The number of cattle in the district is 107140 and increasing every year (Figure 1). Cattle farming has a very special place in the economy of the district, providing a livelihood for the majority of the population. The district is home to 17 per cent of the province's cattle population (TURKSTAT, 2024).

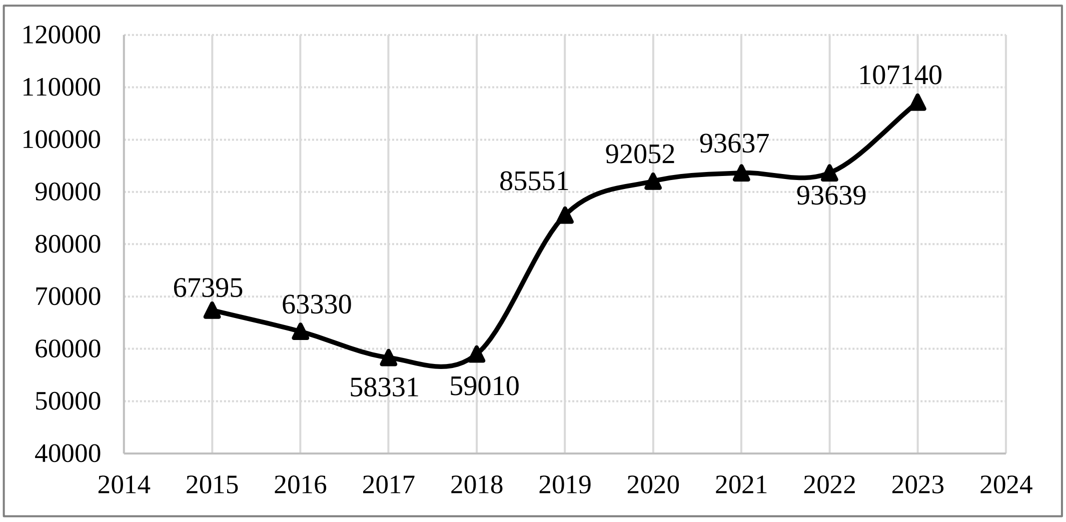


**Figure 1.** Changes in the number of cattle in Selim district over the years (TURKSTAT, 2024).

Despite the increase in the number of cattle, the population of the district has decreased from 24204 in 2015 to 21178 in 2023. Cattle production in the district faces several problems and challenges. The decline in the district's population and the increase in the average age of livestock farmers are among the most significant. Majority of the cattle enterprises in the district is small or medium scale. In order to ensure the development and sustainability of the farming sector, economic and social status in these enterprises should be taken into consideration (Diler et al., 2022). Improving the economic and social conditions of farmers will help them adapt to future challenges and maintain their operations over the long term. In order to achieve this, it is very important to reveal the problems of farmers in the region. This study aimed to determine the farm management and socio-economic structure of cattle enterprises in the Selim district of Kars province, identify the existing problems, and provide solutions to achieve a sustainable cattle farming.

## 2. Materials and Methods

The study has been approved by Atatürk University, Agricultural Faculty Ethics Committee Chairmanship in 2024 (Decision no: 2024/1).

The research involved administering questionnaires to 350 cattle enterprise owners, randomly selected from 3925 cattle enterprises in the Selim district of Kars province. Face-to-face surveys were conducted with these 350 enterprise owners.

The data obtained were transferred to Microsoft Office Excel. Numerical and proportional values were obtained by frequency analysis in descriptive statistics in the statistical program SPSS 20.0v. The numerical and proportional values were used to create graphs and interpret the results. In determining the sample size, the following method was utilized (Arıkan, 2007).

$$n = \frac{N.t^2.p.q}{(N-1).D^2+t^2.p.q} \quad (1)$$

n = minimum number of necessary samples

N = population size

D = acceptable or desired sampling error (5%)

t = table value

p = the rate to be calculated (0.5)

q = 1-p

$$n = \frac{3925.(1.96)^2.0.5.(1-0.5)}{(3925-1).0.05^2+(1.96)^2.0.5.(1-0.5)} = 350$$

## 3. Results and Discussion

The distribution of enterprise owners by age is shown in Figure 2. It was found that 3.7% of the farmers were less than 30 years old, 16.0% were between 31-40 years old, 42.0% were between 41-50 years old, 28.9% were between 51-60 years old and 9.4% were 61 years old and above. The average age of the cattle farmers in Selim district is predominantly falls between

41-50 years old. Our results indicate that the interest of the younger population (<30 age group) in animal farming is not at the desired level. Similar results have been reported in other studies conducted in other parts of Türkiye. For instance, Diler et al. (2022) reported that the average age of cattle farmers in İspir district of Erzurum was 55.2 years. In Eyyübiye district of Şanlıurfa Province, 41.5% of enterprise owners were determined to be between 46-55 years of age (Doğanay & Yanar, 2023). Additionally, the average age of the cattle farmers was reported to be 47.6 years in Hatay province and 44.0 years in Aksaray province (Paksoy & Bulut, 2020; Tapkı et al., 2018). These results suggest that the average age of the cattle farmers in Türkiye is increasing and younger generations don't find cattle farming attractive. This trend is not unique for Türkiye. Mzingula (2019) found that the majority (43.3%) of the cattle farmers in Tanzania were between 40-59 years of age. The average age of the cattle breeders was reported to be 47 years in Finland (Sahlström et al., 2014) and 48 years in Nigeria (Saleh, 2018). Together, these results indicate a global trend of ageing cattle farmers, with younger generations less interested in the profession.

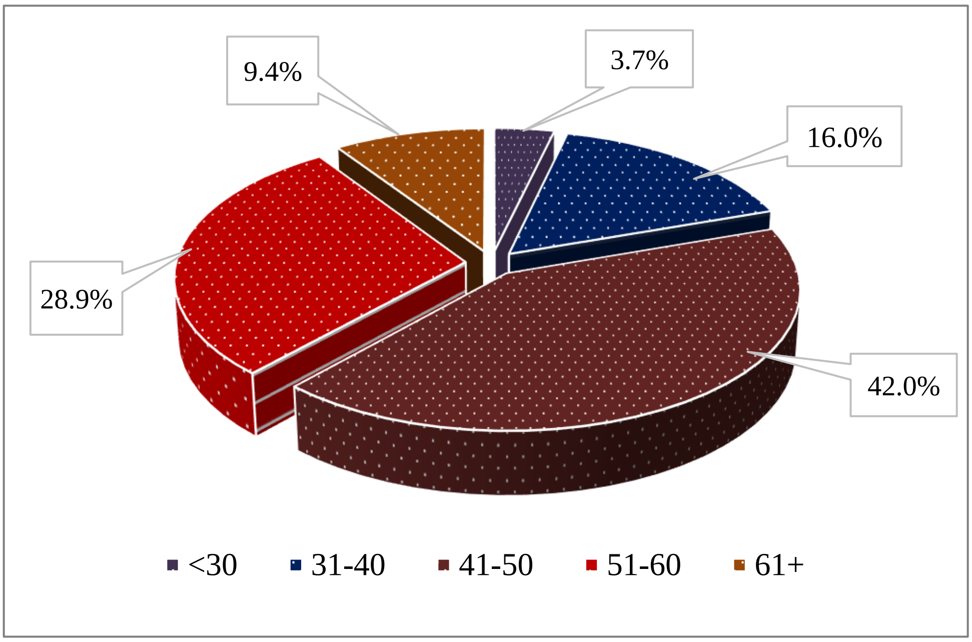


**Figure 2.** Age of enterprise owners (years).

It was found that 1.4% of the enterprise owners in the district were illiterate, 34.4% were primary school graduates, 33.5% were secondary school graduates, 28.9% were high school graduates and 1.7% were university graduates (Figure 3). The majority of the farm owners in Selim district were primary school graduates, and the rates of primary and secondary school graduates (34.4% and 33.5%, respectively) were noticeably high while the share of university graduates was considerably low. Studies conducted in the eastern regions of Türkiye revealed that the education level of farmers is lower and is not in desired level. For instance, the share of primary school graduates was 51.7% and university graduates was 0.8% among farmers in Çatak, Özalp and Erciş districts of Van province (Terin & Ateş, 2010). Similarly, the percentage of cattle farmers who are primary school graduates was reported as 55.6% in Kars province (Tilki et al., 2013). Majority of the cattle farmers (54.2%) in Giresun province was primary school graduates (Tugay & Bakır, 2009). Additionally, in Diyarbakır Province 43% of the dairy cattle farmers were primary school graduates and 13% were illiterate, while only 5% were university graduate (Tutkun et al., 2017). On the other hand, in Tekirdağ province, the share of high school and university graduates was reported to be 15% and 14% respectively (Soyak et al., 2007). Education level of the farmers is highly important in terms of economic efficiency of cattle enterprises. It was observed that the majority of the farmers in Selim district of Kars province had low level of education. Higher education levels among farmers correlate with increased profitability of their enterprises (Tilki et al., 2013).

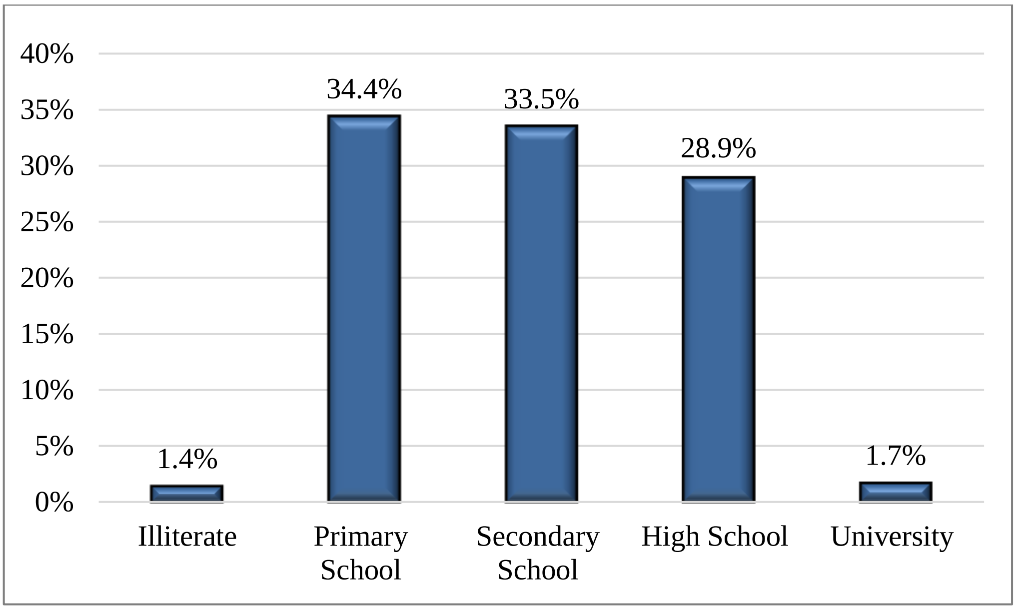


**Figure 3.** Education status of the farmers.

In Selim district of Kars province 6.0% of the farm owners had less than 10 years of experience, while 25.4% had 10-20 years, 37.1% had 21-30 years, 27.1% had 31-40 years, and 4.3% had more than 40 years of cattle farming experience. Majority of the farmers in the district had over (68.5%) 20 years of cattle raising experience while only 6.0% had less than 10 years of experience. Similarly, Bakan and Aydın (2016) reported that the average cattle farming experience of farmers were 24.3 years in Ağrı province. While 82.5% of the cattle farmers has over 20 years of cattle farming experience in Tekkeköy district of Samsun province (Kaygısız & Özkan, 2021). Similarly, in Kars province the average cattle farming experience of farmers were found to be 30.2 years (Tilki et al., 2013). Additionally, Duguma et al. (2012) found that 50% of the cattle farmers in Ethiopia had 15 years of experience. However, in Nigeria 62% of farmers were reported to have less than 10 years of cattle breeding experience (Saleh, 2018). While in South Africa 41.4% of the farmers had over 21 years of farming experience (Grobler et al., 2008). The experience among cattle farmers in Selim district was determined to be higher compared to similar studies. However, despite this apparently advantageous situation, the high proportion of older farmers with low levels of education in the district is a disadvantage and a serious threat to the sustainability of cattle farming in the district.

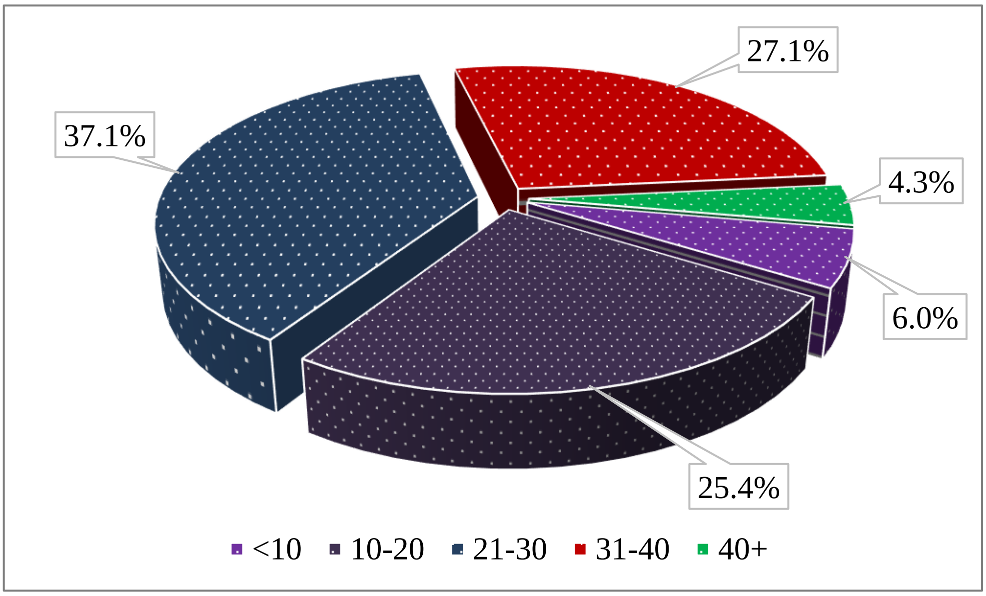


**Figure 4.** Farming experience (years).

In the current study we also instigated the family populations of the enterprises in the district. It was determined that the majority (76.8%) of the farms had 5 or more individuals in the family. Only 23.2% of the farms had less than 5 individuals in the family. 63.1% of the farms had 5-7 people in the family (Figure 5). Similarly, Güler et al. (2016) found that the average family population in Hınıs district of Erzurum was 5.2 in the cattle enterprises. While majority of the enterprises in Kahramanmaraş province had 3-5 people in the family (Kaygısız et al., 2010). In İspir district of Erzurum over 70% of the farmers had 3-5 people in the family (Diler et al., 2022). Similarly, majority of the enterprises in Karaçoban district of Erzurum was determined to have 4 (15.2%), 5 (23.5%) and 6 (20.6%) individuals in their families (Yanar et al., 2024). In the studies conducted in Western Kenya and Uganda number of individuals in the farmer's families were reported to be 8 and 8.7 respectively (Ahikiriza et al., 2021; Amimo et al., 2011). The family population of the enterprises is important for the sustainability and production capacity of the enterprises. Based on the findings, it was observed that the average number of family members in enterprises in the district is sufficient when compared to other regions, which is promising for the continuity of the enterprises.

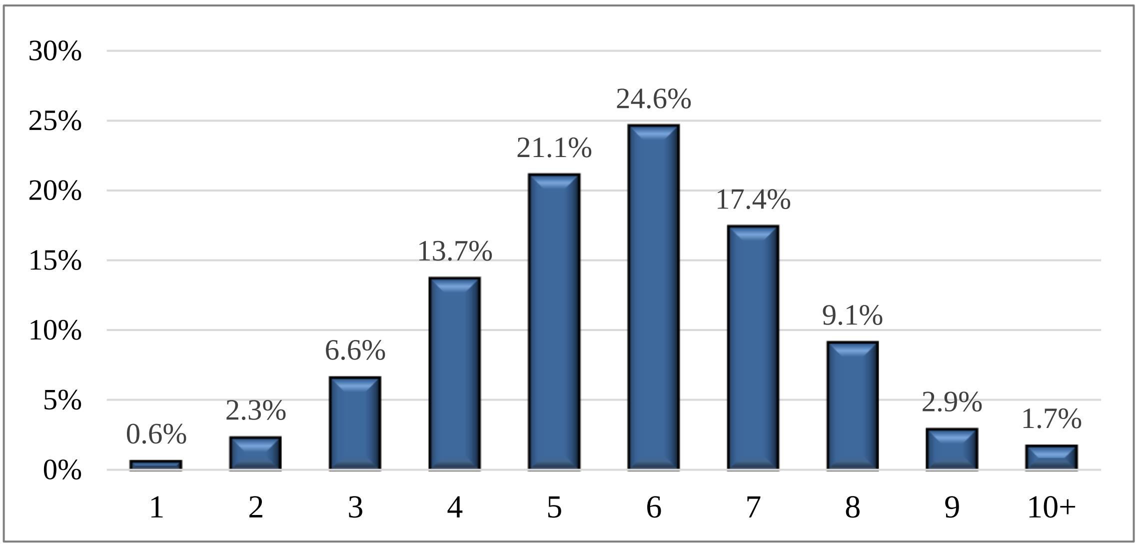


**Figure 5.** Number of family members.

Majority of the enterprises had crossbreds in their enterprises (72.3%). Simmental was the most preferred (44.8%) European cattle breed by the farmers. The proportions of Brown Swiss and indigenous cattle breeds were 22.6% and 38.0%, respectively. Holstein Friesian was the least preferred (2.6%) cattle breed among the cattle farmers in the district (Figure 6). Similarly, in Eyyübiye district of Şanlıurfa province Simmental was the most widely raised European cattle breed (59.3%). However, indigenous breeds were raised only in 5.2% of the enterprises (Doğanay & Yanar, 2023). Moreover, in Giresun province the majority of the cattle raised in the farms were crossbreds (71.1%), and 23.6% of the cattle were indigenous breeds (Tugay & Bakır, 2006). Şeker et al. (2012) reported that 46.9%, 37.2% and 15.9% of the farmers reared indigenous, crossbreds and European breeds respectively in cattle farms located in Muş province. On the other hand, 71% of the cattle farmers in Eastern Mediterranean raised European dairy cattle breeds in their farms while the share of crossbreds was 19% (Boz, 2013).

In the questionnaire the farmers were also asked about the reasons why they had chosen these breeds. The majority of farmers (65.4%) stated that they had chosen these breeds because they were adapted to the conditions of the region and 38.6% of farmers cited being more resistant to diseases as the reason. On the other hand, 35.7% of them (farmers keeping European breeds or their crossbreds) stated that their reason for choosing these breeds was their high yield (Figure 6). In a similar study carried out in Eyyübiye district of Şanlıurfa province, where the proportion of indigenous breeds is much lower, the majority of farmers (55.6%) stated that they chose their breeds because of their high yield (Doğanay & Yanar, 2023). In comparison with the other regions, the preference for indigenous breeds or their crossbreds with European breeds is higher in the region. The main reason for this result is the harsh climatic and geographical conditions of the region. The adaptation of European breeds in this region is very difficult. The second reason is that cattle farming in the district is largely based on traditional methods. The stables and rearing conditions are more suitable for indigenous cattle breeds. Therefore, most of the breeders prefer to cross these breeds with indigenous breeds, which are highly adapted to the conditions of the region.

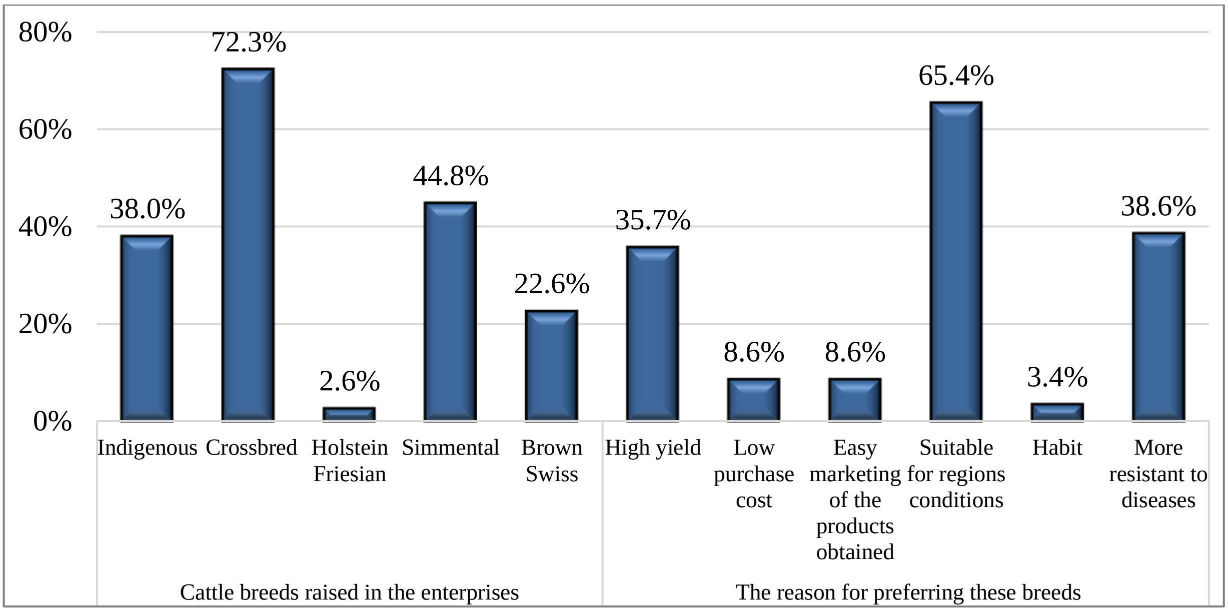


**Figure 6.** Cattle breeds raised in the enterprises and the reason for preferring these breeds.

The distribution of cattle, cows and heifers (female cattle that have not yet given their first birth) on the enterprises is shown in Figure 7. In Selim district, 6.0% of the enterprises had between 1-10 cattle, 26.3% had between 10-20, 30.9% had between 20-30, 19.1% had between 30-40, 10.3% had between 40-50, 7.4% had 51 or more cattle (Figure 7). The majority of enterprises had between 20 and 30 cattle (30.9%). The majority of farms (50.6%) also had between 10 and 20 lactating cows in the barn. While 89% of the farms had less than 10 heifers, only 11% of them had 10 or more heifers. In a similar study conducted in the Gönen district of Balıkesir province, researchers found that the average number of lactating cows was 7.14 (Y. Özdemir et al., 2021). Average number of cattle in the cattle enterprises in Iğdır province was reported as 20.9 head (Yılmaz et al., 2020). In addition, the average number of cattle in Çankırı province was reported to be 30 and 220 in traditional and modern farms, respectively (Kaba & Çanakcı, 2020). In Kahramanmaraş province, the number of cattle in the enterprises was between 1-5 in 52% of the enterprises and 6-10 in 26% of the enterprises (Kaygısız et al., 2010). Additionally, the average number of lactating cows in the enterprises in Torul district of Gümüşhane province was reported as 9.2 by C. Y. Özdemir et al. (2023). In Selim district, majority of the enterprises are determined to be middle sized with 21-30 cattle and 10-20 lactating cows.

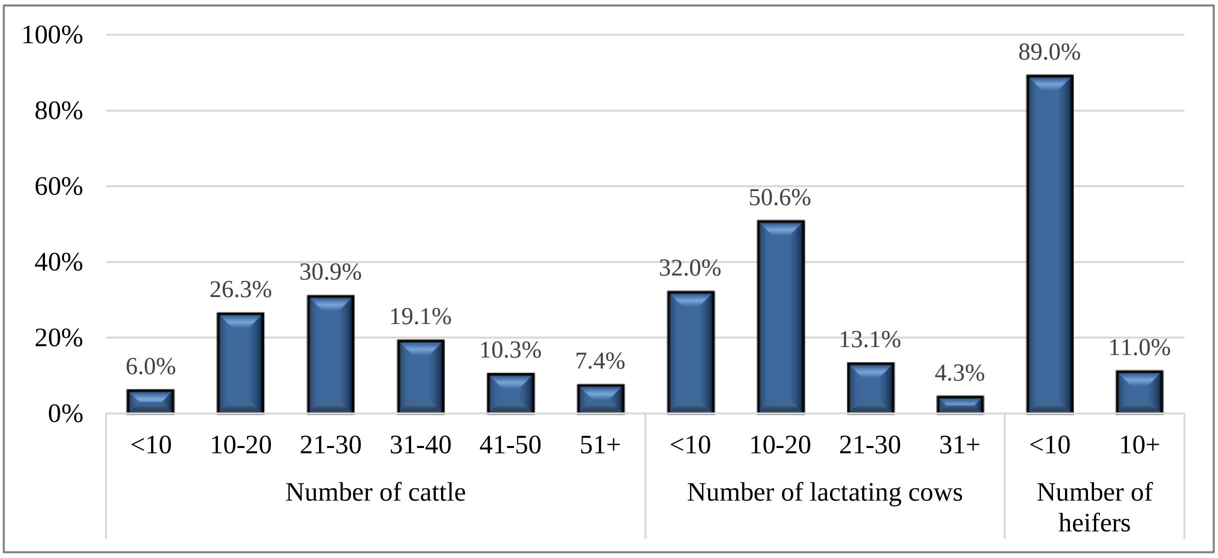


**Figure 7.** Distribution of cattle, lactating cows, and heifers by number in the district.

It was found that 50.9% of the farmers engage in both meat and milk production in their enterprises, while 48.0% prefers milk production and sell their male calves at early ages. Only 1.1% of the farmers engage solely in meat production (Table 1). In a similar study conducted in Hınıs district of Erzurum province Güler et al. (2016) reported that 94.0% of the farmers engaged in both meat and milk production. Similarly, in Muş province 79.2% of the cattle farmers were determined to focus on combined rearing system (meat and milk production) in their enterprises (Şeker et al., 2012). In a similar study conducted in Southern Nigeria, Ahaotu et al. (2013) found that 77.5% of the farmers engaged solely in milk production. In Türkiye, meat production farms are not common. Thus, meat production is based mostly on the males of dairy cattle or multipurpose cattle breeds. As in the country, the preference of multipurpose cattle is widespread in Selim district of Kars province.

It was determined that 12% of the enterprises in the district had 1, 52.3% had 2, 24.9% had 3, 7.4% had 4 and 3.4% had 5 working person. Majority of the farms had 2 or 3 employees. Similarly, Diler et al. (2022) reported that 43.7% and 33.5% of the farms had 2 and 3 employees respectively in the farms in İspir district of Erzurum. Additionally, in Hınıs district of Erzurum Güler et al. (2016) found that majority of the farms had 2 or 3 employees. On the other hand, Daş et al. (2014) indicated that majority of the farms in Bingöl province had 3-5 people. Since the big share of the farms in the district is small or middle scale, only family members were working in the farms. It was found that in 92.7% of the farms, only family members were working. Only 3.2% of the enterprises had external employees, while in 4.1% of the farms both workers and family members engaged in the care of animals (Table 1). Similarly, Ünalan et al. (2013) found that 92% of the cattle enterprises relied solely in family labor in Niğde province. Additionally, the share of farms using solely family members in the cattle enterprises was 96.6% in İspir district of Erzurum (Diler et al., 2022).

**Table 1.** Distribution of farm types, number of employees, and labor source.

| **Type of Cattle Farming** | **Frequency** | **Percentage (%)** |
|---|---|---|
| Meat Production | 4 | 1.1% |
| Milk Production | 168 | 48.0% |
| Meat and Milk Production | 178 | 50.9% |
| **Total** | **350** | **100.0%** |
| **Number of employees working in the cattle farm** | | |
| 1 | 42 | 12.0% |
| 2 | 183 | 52.3% |
| 3 | 87 | 24.9% |
| 4 | 26 | 7.4% |
| 5+ | 12 | 3.4% |
| **Total** | **350** | **100.0%** |
| **Labor Source** | | |
| Only family labor | 292 | 92.7% |
| Worker | 10 | 3.2% |
| Family labor and worker | 13 | 4.1% |
| **Total** | **315** | **100.0%** |

It was determined that 52.0% of the enterprise owners in the district were members of associations and 48.0% were not (Figure 8). In a similar study conducted in Tekkeköy district of Samsun revealed that 62.5% of the cattle farmers were member of milk producer's union (Kaygısız & Özkan, 2021). Furthermore, 75% of the farmers were members of any unions or cooperatives in Tokat province (Çallı, 2016), while only 30.0% of the enterprise owners were members of any agricultural cooperative in eastern Mediterranean region (Boz, 2013). In İspir province of Erzurum, only 27.9% of the cattle farmers had membership to agriculture related associations (Diler et al., 2022). Among the farmers who were members of an association, 90% were members of a cattle breeders' association, 7.2% were members of a village cooperative, and 2.8% were members of other associations. In a similar study, Özyürek et al. (2014) found that in Çayırlı district of Erzincan province 55.0% of the farmers were not members of any organization, while 13% were members of cattle breeders' association and 32.0% were members of other agricultural cooperatives. Such organizations play a crucial role in providing services to farmers and producers, including facilitating access to markets and enabling small enterprises to create network. In the district, the reason for the membership to unions or cooperatives was either to meet agricultural input needs or to benefit from agricultural supports.

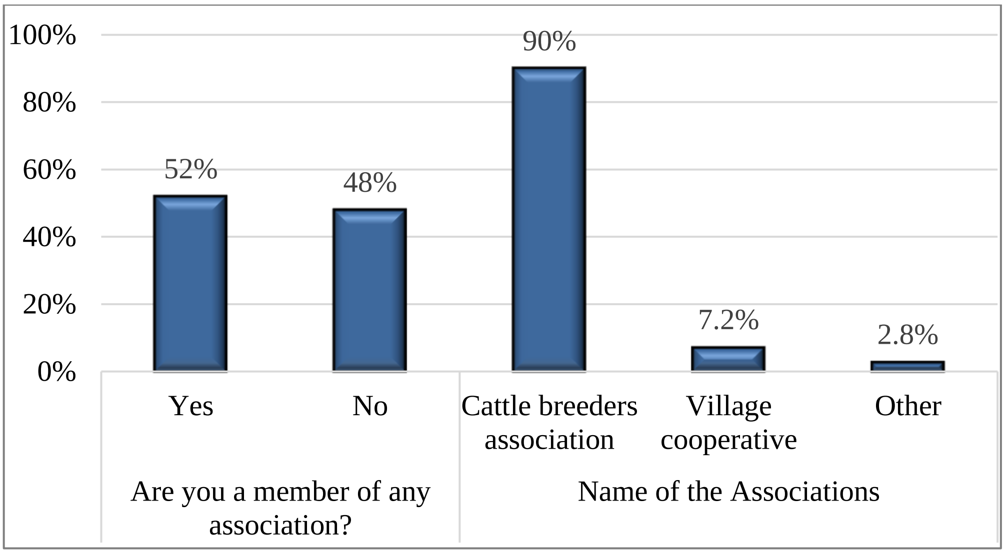


**Figure 8.** Association membership status in the district.

Respondents were also asked whether or not they were satisfied with cattle farming. The majority of the farmers (66.5%) indicated that they found cattle farming fulfilling. While 33.5% were not satisfied with this activity. Cattle farming was the only economic activity of the 84.3% of the enterprise owners. Only a small proportion of the enterprises (15.7%) had other occupation in addition to cattle farming (Table 2). Cattle farming was reported by 91.1% of enterprise owners as the main source of income. In a similar study conducted in İspir district of Erzurum, 41.6% of the farmers were found to have other occupations in addition to cattle farming (Diler et al., 2022). In Muş and Sivas provinces the share of enterprise owners having additional occupations to cattle farming were reported to be 48% and 37%, respectively (Hozman & Akçay, 2016; Şeker et al., 2012). Similarly, in Hınıs district of Erzurum only 17% of the cattle farmers were reported to have additional occupation by Koçyiğit et al. (2016).

Based on the respondents' answers, high feed prices stand out as the biggest problem facing farmers in the district. Almost all farmers (98.3%) reported that feed is expensive. Diseases were also cited as a major problem by 33.1% of farmers. In addition, 22.6%, 20.6% and 17.4% of the farmers, respectively, indicated that difficulty in accessing feed, lack of market and difficulty in handling cattle were among the problems faced in this activity. Only 9.7% of farmers replied that cattle farming was not profitable (Table 2). High feed prices have been identified as the main problem in many studies conducted in different parts of Türkiye. In Giresun province 93.7% (Tugay & Bakır, 2009), and in Ödemiş district of İzmir 78.3% (Koyubenbe, 2005) of the farmers indicated high feed prices as the main problem. Similarly, Akbay et al. (2023) reported that high feed prices were among the main problems faced by dairy cattle enterprises in Türkiye.

Participants were also asked about their expectations from the governments. A vast majority of the farmers (88.3%) indicated that credit support was their main expectation from the government. Veterinary services (39.4%), supply of quality breeding stock (34%) and marketing support (25.1%) were also among the main expectations of farmers in the district (Table 2). Only 10% of the farmers replied that they expected educational programs from the government. Similarly, Şeker et al. (2012) found that the major expectations of the farmers were credit support with reasonable conditions (42.7%), veterinary services (29.1%) and support in marketing the products (19.1%) in Muş province. On the other hand, only 2.7% of farmers stated that they expected technical educational programs. In addition, credit support (67.6%) and supply of quality breeding stock (53.1%) were reported as the main expectations of farmers in Giresun province (Tugay & Bakır, 2009).

**Table 2.** Information about the cattle farming practices, satisfaction, and challenges.

| **Do you find cattle farming satisfying?** | **Frequency** | **Percentage (%)** |
|---|---|---|
| Yes | 232 | 66.5% |
| No | 117 | 33.5% |
| **Total** | **349** | **100.0%** |
| **Do you have any other occupation?** | | |
| Yes | 55 | 15.7% |
| No | 295 | 84.3% |
| **Total** | **350** | **100.0%** |
| **Why do you engage in cattle farming?** | | |
| Main source of livelihood | 319 | 91.1% |
| Habit | 14 | 4.0% |
| Contribution to family income | 118 | 33.7% |
| Just to meet my families need for animal products | 14 | 4.0% |
| **What are the main problems you face in cattle farming** | | |
| Feed is expensive | 344 | 98.3% |
| Feed is hard to obtain | 79 | 22.6% |
| Diseases | 116 | 33.1% |
| Cattle are difficult to care for | 61 | 17.4% |
| Market insufficiency | 72 | 20.6% |
| It is not profitable | 34 | 9.7% |
| **What are your expectations from the government?** | | |
| Credit support | 309 | 88.3% |
| Supply of quality breeding stock animals | 119 | 34.0% |
| Educational programs | 35 | 10.0% |
| Marketing support | 88 | 25.1% |
| Veterinary services | 138 | 39.4% |

## 4. Conclusion

This study investigated the socio-economic characteristics of cattle farms in the Selim district of Kars province. The majority of the farmers in the district were above 40 years of age, while the share of farmers younger than 40 years was only 19.7%. Younger generation’s interest in cattle farming in the district is determined to be insufficient. This situation seems to be a significant threat for the future of cattle farming in the district. The enterprises in the district were mostly small and medium scale family type. The level of education of the farmers in the district was considerably low. The majority of the farmers were primary school (34.4%) or secondary school graduate (33.5%). On the other hand, the experience level of the farmers was significantly higher. However, this is also a major problem for the district since a large number of farmers are elderly and have limited education. This situation poses a significant challenge to the sustainability of livestock production in the district, as it may limit the ability to adapt to new practices and technologies that are essential for long-term viability. For this reason, the active participation of young and educated entrepreneurs in agricultural activities is crucial both for the future and sustainability of cattle farming in the district. The family population of the enterprises is important for the sustainability and production capacity of the enterprises. It was found that 76.8% of the enterprises in the district had 5 or more people in the family which is sufficient when compared to other regions. This situation seems promising for the continuity of cattle farming in the district. It was found that 48% of the farms in the district are not members of any agricultural organization. In addition, a number of challenges such as high feed prices, diseases and market shortages are threatening livestock production in the district as stated by the farmers. Increasing the activities of agricultural unions and associations to overcome these problems would increase farmers' interest in such organizations. The majority of farmers indicated that their main expectations from the government were credit support, veterinary services, marketing support and the supply of quality breeding stock. The government can play a more active role to address the challenges faced by farmers and meet their expectations by improving market and veterinary services, providing loans or feed incentives as well as supplying high quality breeding stock cattle in the region. However, prioritizing the activities and initiatives of cattle breeders' associations and other agricultural unions over reliance on government support alone could also help to solve the problems of the cattle farmers. These associations can offer educational programs, technical assistance, and shared resources such as group purchasing, access to shared equipment, or cooperative marketing strategies to improve production efficiency, providing targeted and specialized support according to the needs of breeders.

## Compliance with Ethical Standards

The study has been approved by Atatürk University, Agricultural Faculty Ethics Committee Chairmanship in 2024 (Decision no: 2024/1).

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Ahaotu, E. O., Madubiuke, F. N., & Ifut, O. J. (2013). Smallholder dairy production in Southern Nigeria: Production, management and milk quality problems. *International Journal of Agriculture and Biosciences*, *2*(2), 76-81.

Ahikiriza, E., Wesana, J., Gellynck, X., Van Huylenbroeck, G., & Lauwers, L. (2021). Context specificity and time dependency in classifying sub-Saharan Africa dairy cattle farmers for targeted extension farm advice: The case of Uganda. *Agriculture*, *11*(9), 836. https://doi.org/10.3390/agriculture11090836

Akbay, C., Çetinkaya, S., & Akbay, F. (2023). Türkiye'de coğrafi bölgelere göre süt sığırcılığı işletmelerinde yem bitkisi üretim durumu. *Turkish Journal of Agricultural and Natural Sciences*, *10*(4), 1156-1166. https://doi.org/10.30910/turkjans.1285087 (In Turkish)

Amimo, J. O., Thumbi, S., Inyangala, B., Junga, J., & Mosi, R. (2011). Socioeconomic characteristics and perceptions of cattle keepers and constraints to cattle production in western Kenya. *Livestock Research for Rural Development*, *23*(6).

Arıkan, R. (2007). *Araştırma teknikleri ve rapor hazırlama*. Asil Yayın Dağıtım. (In Turkish)

Bakan, Ö., & Aydın, R., (2016). Ağrı ili süt sığırcılığı işletmelerinin sosyo-ekonomik özellikleri. *Atatürk University Journal of Agricultural Faculty*, *47*(2), 113-122. (In Turkish)

Boz, İ. (2013). Doğu Akdeniz Bölgesi'nde süt sığırcılığı yapan işletmelerin yapısı, sorunları ve çözüm önerileri. *KSÜ Doğa Bilimleri Dergisi*, *16*(1), 24-32. (In Turkish)

Çallı, A. (2016). *Türkiye'de uygulanan hayvancılık destekleme politikalarının Tokat ili hayvancılık işletmeleri açısından değerlendirilmesi* (Master's thesis, Gaziosmanpaşa University). (In Turkish)

Daş, A., İnci, H., Karakaya, E., & Şengül, A. (2014). Bingöl ili damızlık sığır yetiştiricileri birliğine bağlı sığırcılık işletmelerinin mevcut durumu. *Turkish Journal of Agricultural and Natural Sciences*, *1*(3), 421-429. (In Turkish)

Diler, A., Özdemir, V. F., Aydın, R., Yanar, M., Koçyiğit, R., & Tosun, M. (2022). Socio-economic structure of cattle enterprises in northeast Anatolia region: An example of İspir county of Erzurum Province. *Black Sea Journal of Agriculture*, *5*(2), 150-159. https://doi.org/10.47115/bsagriculture.1069977

Doğanay, S., & Yanar, M. (2023). Güney Doğu Anadolu Bölgesi sığırcılık işletmelerinin sosyo-ekonomik yapısı: Şanlıurfa ili merkez Eyyubiye ilçesi örneği. *Journal of Animal Science and Economics*, *2*(1), 13-21. https://doi.org/10.5152/JASE.2023.1165020 (In Turkish)

Duguma, B., Kechero, Y., & Janssens, G. P. (2012). Survey of major diseases affecting dairy cattle in Jimma town, Oromia, Ethiopia. *Global Veterinaria*, *8*(1), 62-66.

Ergün, O. F., & Bayram, B. (2021). Türkiye'de hayvancılık sektöründe yaşanan değişimler. *Bahri Dağdaş Hayvancılık Araştırma Dergisi*, *10*(2), 158-175. (In Turkish)

Grobler, S., Scholtz, M., Bester, J., Mamabolo, J., & Ramsay, K. (2008). Dairy production systems in the emerging and communal sectors of South Africa: Results from a structured survey. *Applied Animal Husbandry & Rural Development*, *1*, 25-30

Güler, O., Aydın, R., Yanar, M., Diler, A., Koçyiğit, R., & Avcı, M. (2016). Erzurum ili Hınıs ilçesi sığırcılık işletmelerinin sosyo-ekonomik yapısı. *Alinteri Journal of Agriculture Science*, *30*(1), 27-37. (In Turkish)

Hozman, S. B., & Akçay, H. (2016). Sivas ili damızlık sığır yetiştiricileri birliğine üye süt sığırcılığı işletmelerinin bazı teknik ve ekonomik özellikleri. *Tarım Ekonomisi Dergisi*, *22*(1), 57-65. (In Turkish)

Kaba, H., & Çanakcı, M. (2020). Geleneksel ve modern büyükbaş hayvancılık işletmelerinin tarımsal yapı ve mekanizasyon özelliklerinin kıyaslanması: Çankırı İli Örneği. *COMU Journal of Agriculture Faculty*, *8*(2), 367-378. https://doi.org/10.33202/comuagri.722392 (In Turkish)

Kaygısız, A., Tümer, R., Orhan, H., & Vanlı, Y. (2010). Kahramanmaraş ili süt sığırcılık işletmelerinin yapısal özellikleri 4. işletmecilerin sosyal ve kültürel durumları. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *41*(1), 39-44. (In Turkish)

Kaygısız, A., & Özkan, İ. (2021). Samsun Tekkeköy ilçesindeki süt sığırcılık işletmelerinin yapısal özellikleri ve hijyen koşulları. *Harran Tarım ve Gıda Bilimleri Dergisi*, *25*(2), 225-233. https://doi.org/10.29050/harranziraat.879606 (In Turkish)

Koçyiğit, R., Diler, A., Yanar, M., Güler, O., Aydın, R., & Avcı, M. (2016). Süt sığırcılığı işletmelerinde hayvan sağlığı, veteriner sağlık hizmetleri ve yetiştirici memnuniyeti: Erzurum ili Hınıs ilçesi örneği. *Türk Tarım ve Doğa Bilimleri Dergisi*, *3*(1), 24-32. (In Turkish)

Koyubenbe, N. (2005). İzmir İli Ödemiş İlçesinde Süt Sığırcılığının Geliştirilmesi Olanakları Üzerine Bir Araştırma. Hayvansal Üretim Dergisi. 46(1): 8-13. (In Turkish)

Mzingula, E. P. (2019). Socio-economic impacts of smallholder dairy cattle farming on livelihood in Sunga ward of Lushoto district, Tanzania. *International Journal of Economics and Management*, *1*(2), 41-49.

Okcu, M. (2020). Türkiye ve Doğu Anadolu Bölgesi çayır-mer'a alanları, hayvan varlığı ve yem bitkileri tarımının mevcut durumu. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *51*(3), 321-330. https://doi.org/10.17097/ataunizfd.708884 (In Turkish)

Özdemir, Y., Kınıklı, F., & Engindeniz, S. (2021). Süt sığırcılığı işletmelerinin yapısal özellikleri ve sorunları üzerine bir araştırma: Balıkesir'in Gönen ilçesi örneği. *Turkish Journal of Agricultural and Natural Sciences*, *8*(4), 1001-1011. https://doi.org/10.30910/turkjans.938077 (In Turkish)

Özdemir, C. Y., Kaygısız, A., & Aydın, R. (2023). Gümüşhane ili Torul ilçesi süt sığırcılığı işletmelerinin sosyo-ekonomik durumu. *Palandöken Journal of Animal Sciences Technology and Economics*, *2*(2), 38-43. https://doi.org/10.5152/JASE.2023.1162878 (In Turkish)

Özyürek, S., Koçyiğit, R., & Tüzemen, N. (2014). Erzincan ilinde süt sığırcılığı yapan işletmelerin yapısal özellikleri: Çayırlı ilçesi örneği. *Tekirdağ Ziraat Fakültesi Dergisi*, *11*(3), 19-26. (In Turkish)

Paksoy, M., & Bulut, O. D. (2020). Aksaray ilinde süt sığırcılığı yapan kooperatif ortaklarının sosyo-ekonomik özellikleri ve kooperatif - ortak ilişkilerinin incelenmesi. *International Journal of Agricultural and Wildlife Sciences*, *6*(2), 252-262. https://doi.org/10.24180/ijaws.684674 (In Turkish)

Sahlström, L., Virtanen, T., Kyyrö, J., & Lyytikäinen, T. (2014). Biosecurity on Finnish cattle, pig and sheep farms-results from a questionnaire. *Preventive Veterinary Medicine*, *117*(1), 59-67. https://doi.org/10.1016/j.prevetmed.2014.07.004

Saleh, M. (2018). Socio-economic characteristics of dairy cattle farmers in Northern Nigeria. *Journal of Agricultural Extension*, *22*(1), 266-276. https://doi.org/10.4314/jae.v22i1.26S

Şeker, İ., Tasalı, H., & Güler, H. (2012). Muş ilinde sığır yetiştiriciliği yapılan işletmelerin yapısal özellikler. *Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi*, *26*(1), 9-16. (In Turkish)

Soyak, A., Soysal, M. İ., & Gürcan, E. K. (2007). Tekirdağ ili süt sığırcılığı işletmelerinin yapısal özellikleri ve bu işletmelerdeki siyah alaca süt sığırlarının çeşitli morfolojik özellikleri üzerine bir araştırma. *Tekirdağ Ziraat Fakültesi Dergisi*, *4*(3), 297-305. (In Turkish)

Tapkı, N., Tapkı, İ., Dağıstan, E., Selvi, M. H., Kaya, A., Güzey, Y. Z., Demirtaş, B., & Çelik, A. D. (2018). Hatay ili damızlık sığır yetiştiricileri birliği üyesi işletmelerin sosyo-ekonomik özellikleri. *Hayvansal Üretim*, *59*(1), 25-32. https://doi.org/10.29185/hayuretim.410517 (In Turkish)

Terin, M., & Ateş, H. Ç. (2010). Çiftçilerin örgütlenme düzeyi ve örgütlerden beklentileri üzerine bir araştırma: Van ili örneği. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, *47*(3), 265-274. (In Turkish)

Tilki, M., Sarı, M., Aydın, E., Işık, S., & Aksoy, A. R. (2013). Kars ili sigir işletmelerinde barinaklarin mevcut durumu ve yetiştirici talepleri: I. mevcut durum. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *19*(1), 109-116. https://doi.org/10.9775/kvfd.2012.7282 (In Turkish)

Tugay, A., & Bakır, G. (2006). Giresun yöresindeki süt sığırcılığı işletmelerinin ırk tercihleri ve barınakların yapısal durumu. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *37*(1), 39-47. (In Turkish)

Tugay, A., & Bakır, G. (2009). Giresun yöresindeki süt sığırcılığı işletmelerinin yapısal özellikleri. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, *40*(1), 37-47. (In Turkish)

TURKSTAT. (2024). Turkish Statistical Institute. https://www.tuik.gov.tr/

Tutkun, M., Denli, M., & Sessiz, A. (2017). Diyarbakır ili süt sığırcılığı işletmelerinin yapısal durum analizi. Turkish Journal of Agriculture-Food Science and Technology, 5(5), 476-483. https://doi.org/10.24925/turjaf.v5i5.476-483.933 (In Turkish)

Ünalan, A., Serbester, U., Çınar, M., Ceyhan, A., Akyol, E., Şekeroğlu, A., Erdem, T., & Yılmaz, S. (2013). Niğde ili süt sığırcılığı işletmelerinin mevcut durumu, başlıca sorunları ve çözüm önerileri. *Türk Tarım -Gıda Bilim ve Teknoloji Dergisi*, *1*(2), 67-72. https://doi.org/10.24925/turjaf.v1i2.67-72.34

Yanar, M., Koçyiğit, R., Diler, A., Aydın, R., Bayram, B., Yüksel, S., Özdemir, V. F., Ergün, O. F., & Tiryaki, A. (2024). Socio-economic structure of cattle enterprises operating in Karaçoban county of Erzurum province. *Journal of Animal Science and Economics*, *3*(1), 1-7. https://doi.org/10.5281/zenodo.10730987

Yılmaz, İ., Kaylan, V., & Yanar, M. (2020). Iğdır ili büyükbaş hayvan yetiştiriciliğinin yapısal analizi. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *10*(1), 684-693. https://doi.org/10.21597/jist.567366 (In Turkish)

http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

# Microplastic Levels in Water and Sediment of Karaçomak Dam Lake (Kastamonu, Türkiye)

Yiğit Taştan[1] • Adem Yavuz Sönmez[2]

[1]Kastamonu University, Faculty of Engineering and Architecture, Department of Environmental Engineering, Kastamonu/Türkiye
[2]Kastamonu University, İnebolu Vocational School, Department of Transportation Services, Kastamonu/Türkiye

ARTICLE INFO

**Article History**
Received: 30.11.2024
Accepted: 25.12.2024
First Published: 31.12.2024

**Keywords**
Karaçomak Dam Lake
Plastic
Pollution
Sediment
Water

ABSTRACT

This paper investigates the microplastic (MP) abundance and morphology in Karaçomak Dam Lake, a freshwater source used for irrigation and drinking water, located in Kastamonu, Türkiye. Water and sediment samples were collected during January, April, July and October 2023 from two stations determined on the lake. MPs obtained from the samples were counted and classified according to their sizes, shapes (fiber, fragment, film, microbead) and colors. Results showed that the mean MP abundance in the water samples was 3206 particle/m$^3$, while it was 180 particle/kg dry weight in the sediment samples. The most frequent MP type was fiber in surface water samples and fragment in sediment samples. Predominant colors were blue and black for surface water and sediment, respectively. Majority of the MPs found were small-sized MPs (<1 mm). MP abundance was the highest in winter season, followed by autumn, spring and summer, respectively. The seasonal differences were probably driven by rainfall and the anthropogenic activities around the lake. We concluded that the values obtained in this study are moderate in comparison with the literature data. Although it was inferred that sources of MP are domestic waste, agriculture and recreational activities, it is recommended to conduct more comprehensive studies to better understand the sources of MP pollution in Karaçomak Dam Lake.

**Please cite this paper as follows:**

Taştan, Y., & Sönmez, A. Y. (2024). Microplastic levels in water and sediment of Karaçomak Dam Lake (Kastamonu, Türkiye). *Journal of Agricultural Production*, *5*(4), 283-294. https://doi.org/10.56430/japro.1593885

## 1. Introduction

Plastics are widely used worldwide due to their lightness, durability, corrosion resistance and low electrical and thermal conductivity (Wagner et al., 2014). This use has led to intensive production and has become one of the biggest threats to the environment in recent years. These plastics, which are discarded into nature from various sources or have limited recycling possibility, undergo a series of degradation processes and turn into microplastics (MPs), defined as those with a diameter of less than 5 mm (Andrady, 2011). MPs can spread widely to all surrounding environments (i.e., soil, water and atmosphere) through various ways (Peeken et al., 2018; Sun et al., 2021).

MPs can be dispersed into the aquatic ecosystem through natural means such as wind, water currents, turbulence and oceanographic effects (e.g., physical properties of water and wave movements) (Ballent et al., 2012; Turra et al., 2014). In addition to being transported naturally, they can also enter the aquatic ecosystem as a result of human activities. MPs directly or indirectly mix into the aquatic ecosystems as a result of

Correspondence
E-mail address: ytastan@kastamonu.edu.tr

unfiltered discharge of domestic or industrial wastewater (Ryan et al., 2009). The circulation in aquatic ecosystems can cause critical pollution problems. A significant portion of these ecosystems consists of freshwater ecosystems used for drinking water, irrigation, fishing and energy production (Şahin & Zeybek, 2019). While most researchers focus on the presence of microplastic pollution in the seas, the reality of microplastics in freshwater systems has received less attention (Wong et al., 2020). However, studies show that microplastics also constitute a significant pollution burden in freshwater ecosystems (Egessa et al., 2020).

The examination of freshwater resources in Türkiye in terms of MP pollution has gained momentum in recent years (Atamanalp et al., 2022a, 2022b, 2023; Böyükalan & Yerli, 2023; Çullu et al., 2021, Erdoğan, 2020; Gedik & Atasaral, 2022; Gündoğdu et al, 2023; Kankılıç et al., 2023; Mutlu et al., 2024; Tavşanoğlu et al., 2020; Turhan, 2022). However, more data are required and more freshwater sources need to be monitored to better understand the nature of MP pollution and determine the current status. Furthermore, seasonal investigations are limited to only a few studies. Seasonal determination will help evaluate the trends in MP pollution.

Therefore, this study aims to fill the data gaps regarding MP pollution in Türkiye's freshwater ecosystems and to contribute to the creation of an infrastructure for future monitoring studies by qualitatively and quantitatively determining the microplastic load of the Karaçomak Dam Lake, which is used as a source of drinking and irrigation water for Kastamonu and is also an important recreational fishing area, and examining its seasonal changes.

## 2. Materials and Methods

### *2.1.* Study Area and Field Sampling

Karaçomak is a dam lake, which was built for irrigation, flood control and drinking water supply purposes, located on the Karaçomak Creek within the borders of Kastamonu province in the Western Black Sea region, at the coordinates of 41°19′08″N, 33°44′41″E (Figure 1). Its height from the river bed is 49.00 m, the lake's volume at normal water level is 23.10 $hm^3$, and the lake's area at normal water level is 1.43 $km^2$. It provides irrigation service to an area of 2,596 hectares and 3 $hm^3$ of drinking water per year (DSİ, 2016).

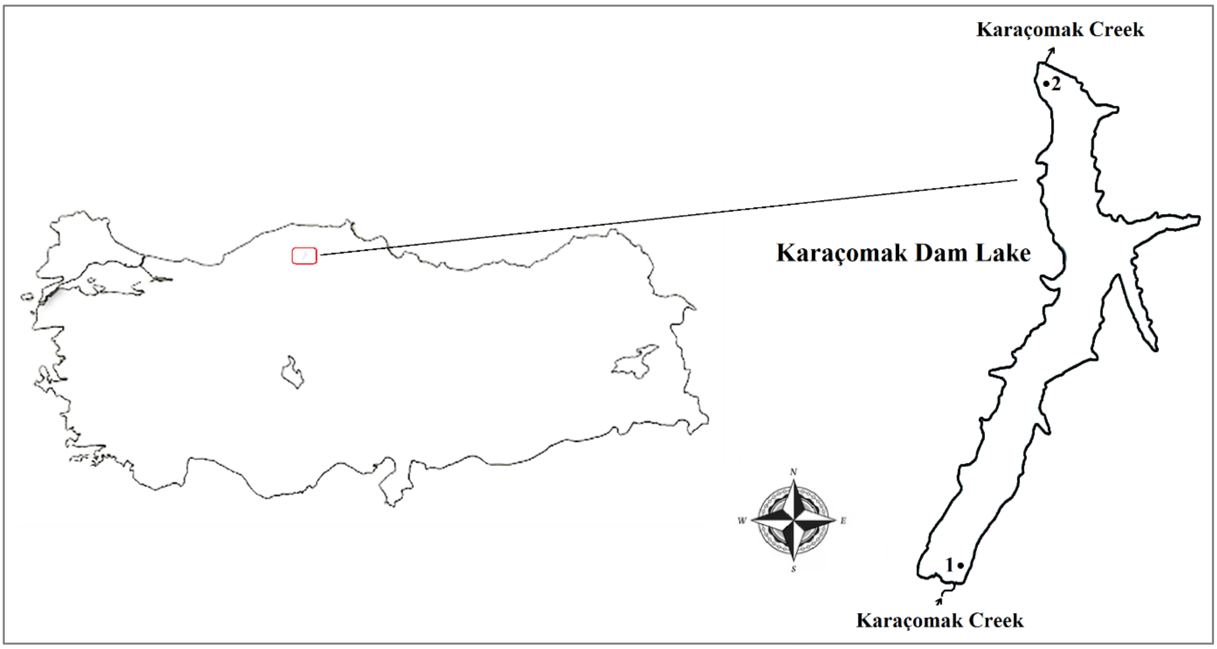


**Figure 1.** Location of the investigated lake and the sampling stations.

Surface water and sediment samplings were carried out on January, April, July and October 2023. Two stations were determined on the dam lake and samplings were carried out from these stations. The 1st station was the Karaçomak Creek's entrance to the dam and the 2nd station was the dam's outlet (Figure 1).

Surface water and sediment sampling was carried out according to Baldwin et al. (2016), Bergmann et al. (2017) and Gray et al. (2018) with some modifications. For surface water sampling, forty liters of surface water sample (0-30 cm depth) was collected from each station with a large pre-cleaned stainless-steel bucket. The collected water was passed through a stainless-steel sieve (45 µm mesh size). All particles remaining on the sieve were carefully taken into a 1-L glass bottle containing deionized water. At each sampling interval, the sampling bucket was carefully cleaned with pre-filtered deionized water to prevent cross-contamination. At each sampling, a new sieve was used.

Sediment samples (at least 1 kg) were collected from each station using a stainless-steel Van Veen Grab sediment sampler. The samples were put into pre-cleaned glass bottles and transported to the laboratory along with the water samples immediately.

### *2.2.* Laboratory Analysis

Wet peroxide oxidation was used to detect the presence of microplastics in surface water samples (Masura et al., 2015). Water samples were transferred to a conical flask and 30 ml of $H_2O_2$ was added to the samples. Then, organic substances were removed with hydrogen peroxide in an incubator at 50°C for 72 hours (Nuelle et al., 2014). Afterward, this mixture was passed through a 0.45 µm mesh-sized filter (47 mm ⌀) and left to dry for 24 h at room temperature (Tavşanoğlu et al., 2020).

Sediment samples were transferred to 1-L glass beakers covered with aluminum foil and placed in an oven at 50°C for 48 h to dry (Yuan et al., 2019). After drying, 500 g of the sediment samples were weighed on a precision scale and placed in 1-L beakers, and 1 L of 1.2 ppm NaCl solution was added to the samples and then mixed for 5 min (Di & Wang, 2018). The samples were left to settle for 24 h. Afterwards, the upper part was transferred to a separate beaker, taking care not to suspend the sediment samples that settled to the bottom. This process was repeated three times. Then, 100 mL of 30% $H_2O_2$ was added to this beaker. The beakers were covered with aluminum foil and kept in an oven at 50 °C for 72 h. After this period, the liquid in the beaker was removed from the oven and filtered through a 0.45 µm mesh-sized filter (47 mm ⌀) and left to dry for 24 h at room temperature.

### *2.3.* Identification of Microplastics

MPs were counted and classified under a stereomicroscope (ZEISS Stemi 508) at up to 250x magnification, and the number of MPs determined was calculated as particles per $m^3$ water ($p/m^3$) in water samples and as particles per kg dry weight (p/kg) in sediment samples. Unfortunately, we were unable to further characterize MPs with an instrument, such as FT-IR or µ-Raman spectroscopy. For this reason, while identifying MPs, a set of rules were followed. The items, which lack cellular structures, have uniform width and display artificial color (Barrows et al., 2018; Hidalgo-Ruz et al., 2012; Weideman et al., 2019), were recorded as MPs. Although it has been recommended to use spectroscopy to confirm MP type when available, it has been proposed that visual inspection with a microscope is a sufficient tool for quantification of MPs bigger than 50 µm (Kotar et al., 2022).

### *2.4.* Quality Assurance and Quality Control

Utmost attention was paid to prevent contamination of samples and filters. Nitril gloves and cotton lab coats were worn throughout all procedures. All reagents used were pre-filtered through 0.45 µm mesh-sized filters. Three blank filters were arbitrarily left in different places in the lab throughout the laboratory work to detect airborne contamination. Also, three blank samples were prepared following the same procedure for water samples with the pre-filtered distilled water to identify the efficacy of the filtration and cleanliness of the equipment. None of the materials used were plastic. All equipment were rinsed with pre-filtered distilled water three times before use and in between samples. Equipment and samples were covered with aluminum foil when not in use.

### *2.5.* Statistical Analysis

MP numbers between stations and seasons were compared with Kruskal-Wallis test. Mann-Whitney U test was employed to determine different groups. All analyses were carried out using SPSS (IBM) version 23.0. Five percent confidence interval was preferred.

## 3. Results

### *3.1.* Microplastic Abundance

MP values detected in water and sediment samples taken from two stations in the Karaçomak Dam for different seasons are given in Table 1. No MP was detected in blank samples throughout the study.

**Table 1.** MP abundance in water and sediment samples.

| Sample | Season | Station | Mean | Range |
|---|---|---|---|---|
| Water ($p/m^3$) | Winter | 1st | 3925 ± 1082 | 3025-5125 |
| | | 2nd | 3733 ± 401 | 3350-4150 |
| | Spring | 1st | 3458 ± 472 | 3050-3975 |
| | | 2nd | 2592 ± 101 | 2500-2700 |
| | Summer | 1st | 2850 ± 250 | 2600-3100 |
| | | 2nd | 2825 ± 139 | 2700-2975 |
| | Autumn | 1st | 3367 ± 76 | 3300-3450 |
| | | 2nd | 2900 ± 246 | 2700-3175 |
| Sediment (p/kg) | Winter | 1st | 236 ± 22 | 216-260 |
| | | 2nd | 257 ± 28 | 230-286 |
| | Spring | 1st | 152 ± 17 | 136-170 |
| | | 2nd | 159 ± 16 | 144-176 |
| | Summer | 1st | 133 ± 11 | 122-144 |
| | | 2nd | 125 ± 12 | 114-138 |
| | Autumn | 1st | 210 ± 11 | 200-222 |
| | | 2nd | 169 ± 23 | 146-192 |

### *3.2.* Morphological Features of Microplastics

The classification graphs of MPs detected in water and sediment samples collected from different seasons in terms of their size, color and shape are presented in Figures 2, 3 and 4, respectively.

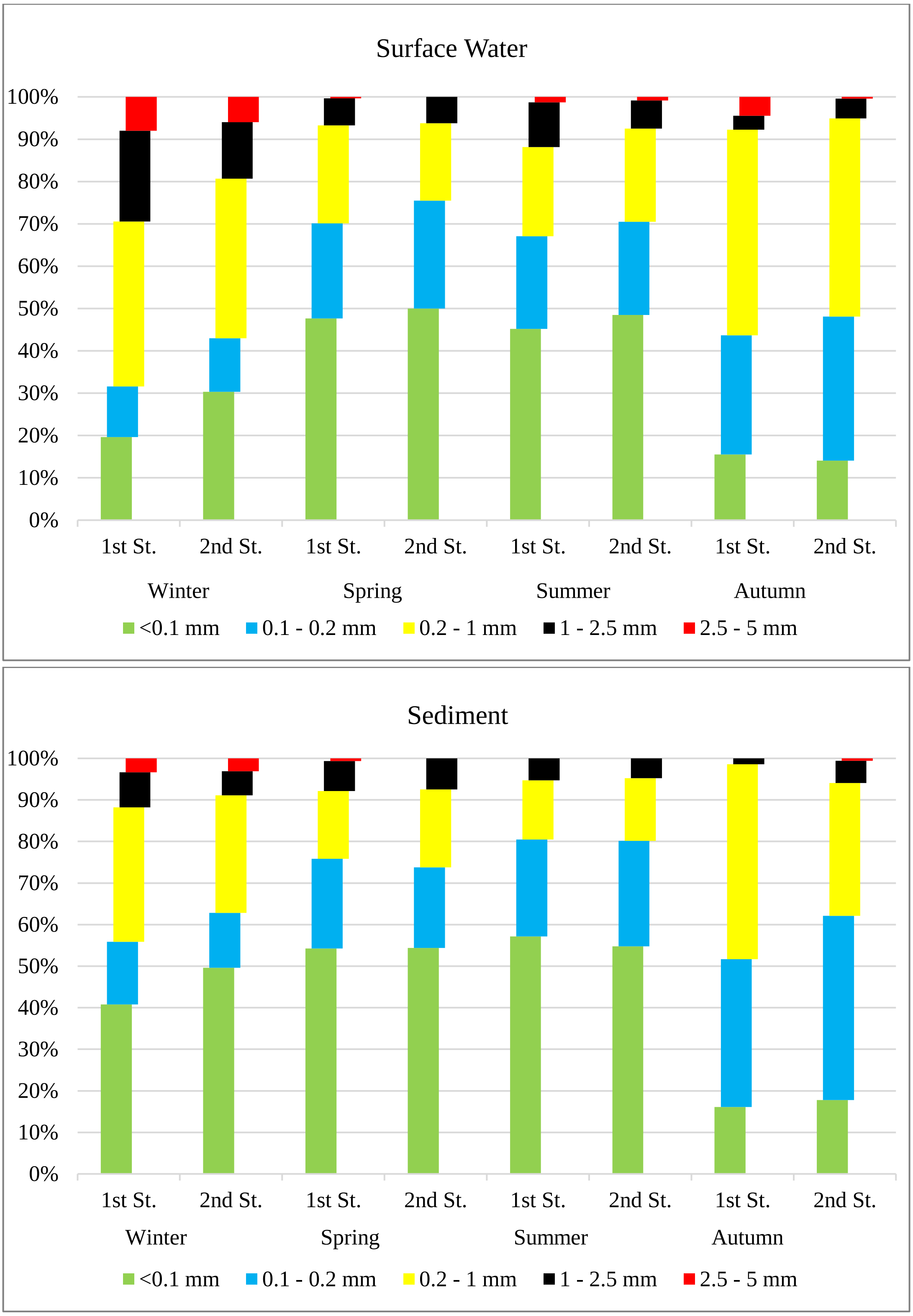


**Figure 2.** Percentages of MPs detected in surface water and sediment samples in different seasons according to their size ranges.

When the distributions according to sizes were examined, the predominantly detected MP groups in surface water in autumn and winter seasons were microplastics between 0.2-1 mm, while microplastics with sizes <0.1 mm were more frequent in other seasons. There was a statistical difference between all seasons in terms of microplastic sizes (*p<0.05*). In sediment samples, microplastic sizes measured in autumn and winter seasons were statistically higher than in other seasons (*p<0.05*).

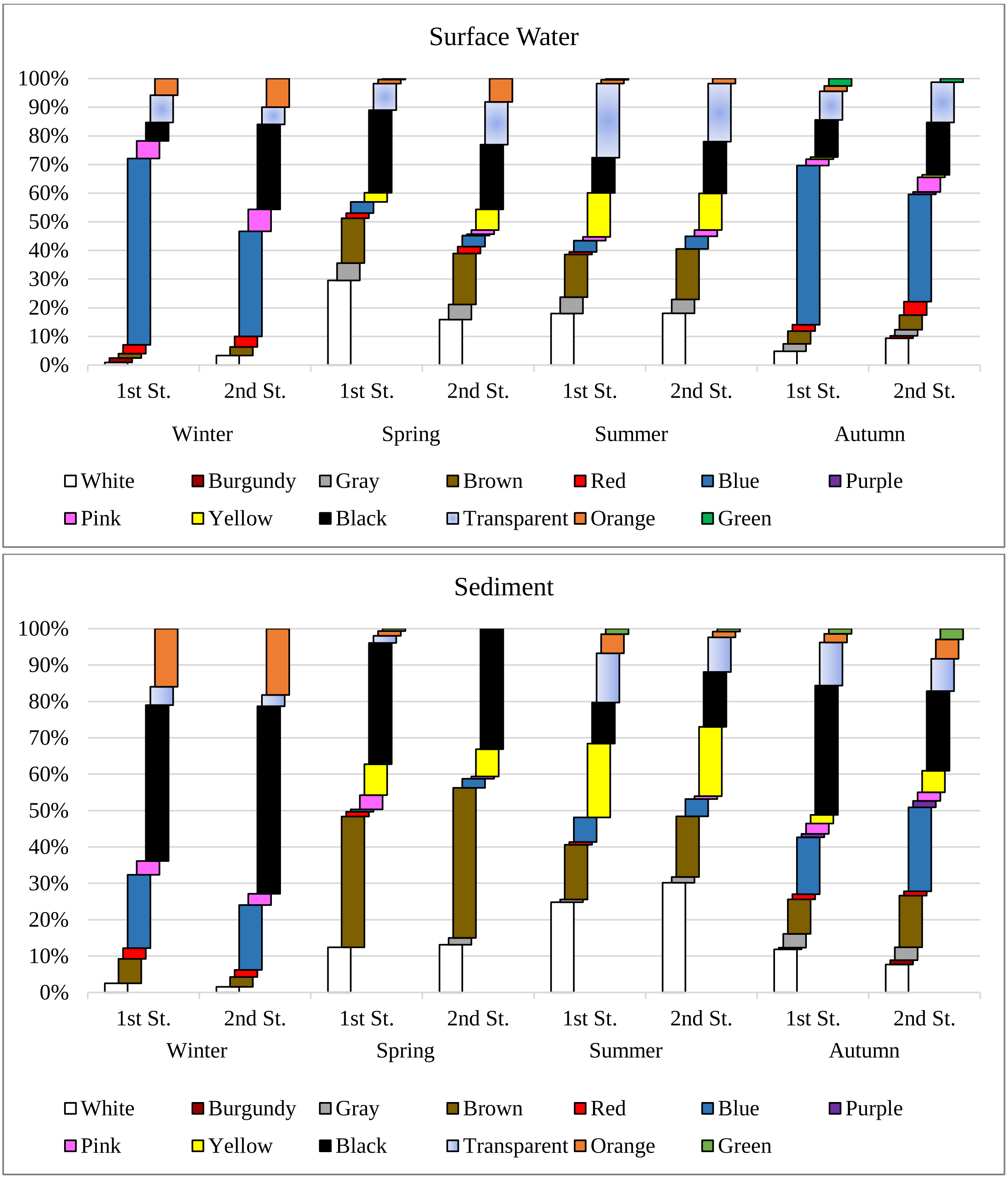


**Figure 3.** Percentages of MPs detected in water and sediment samples according to their colors.

The classification of microplastics in surface water and sediment samples based on their colors is given in Figure 3. According to the findings, blue colored microplastics were dominant in the water samples in the autumn and winter seasons, while white, brown, black and transparent colored microplastics were distributed proportionally close in the spring and summer seasons. In the sediment, black was dominant in the winter, while white, brown, black and partly yellow colors were distributed in the stations in other seasons.

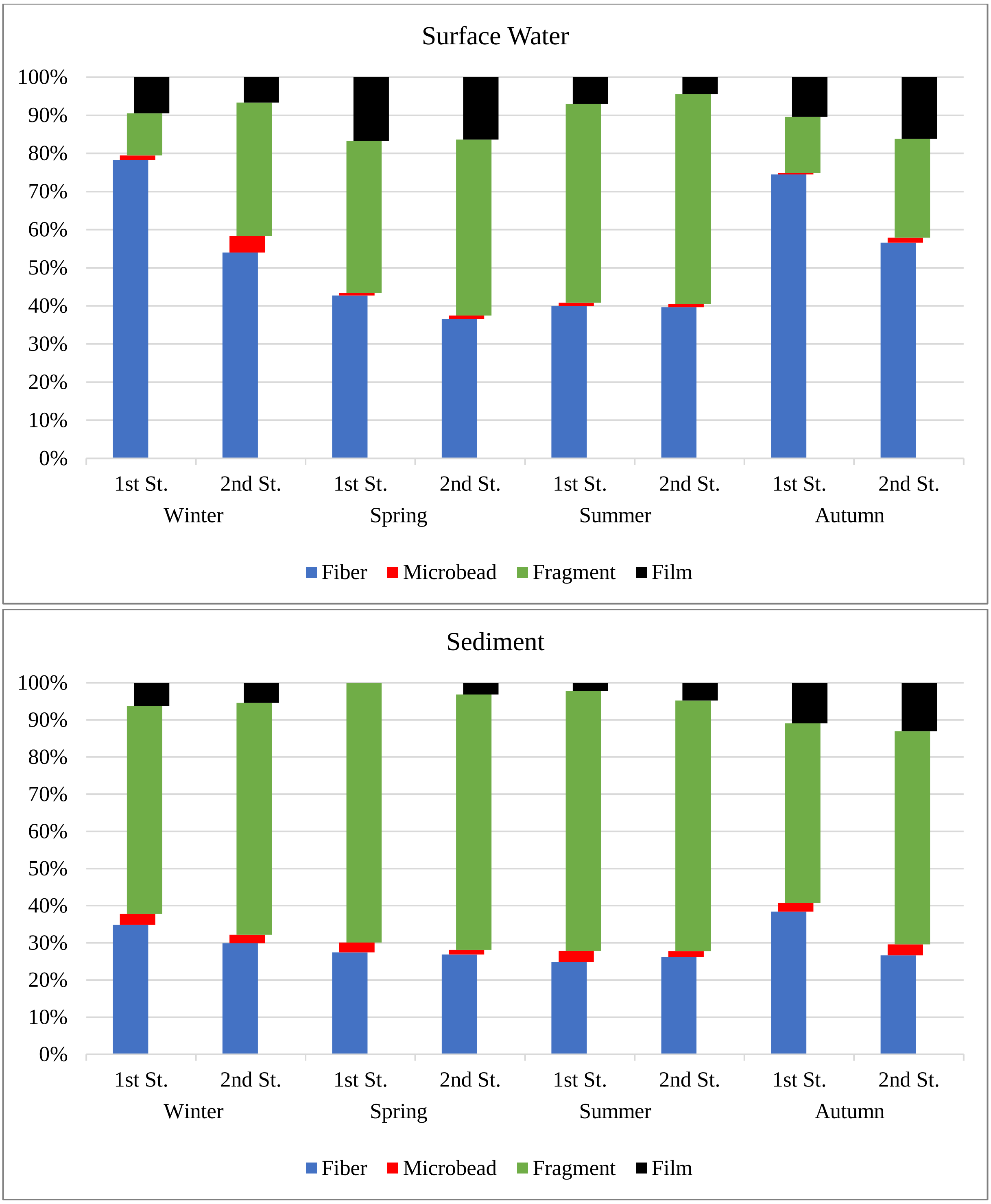


**Figure 4.** Percentages of MPs detected in water and sediment samples according to their types.

When the distribution of microplastics detected in water and sediment samples was examined according to their shapes, it was observed that fibers were usually predominant in water samples, whereas the most common MP type in sediment samples were fragments.

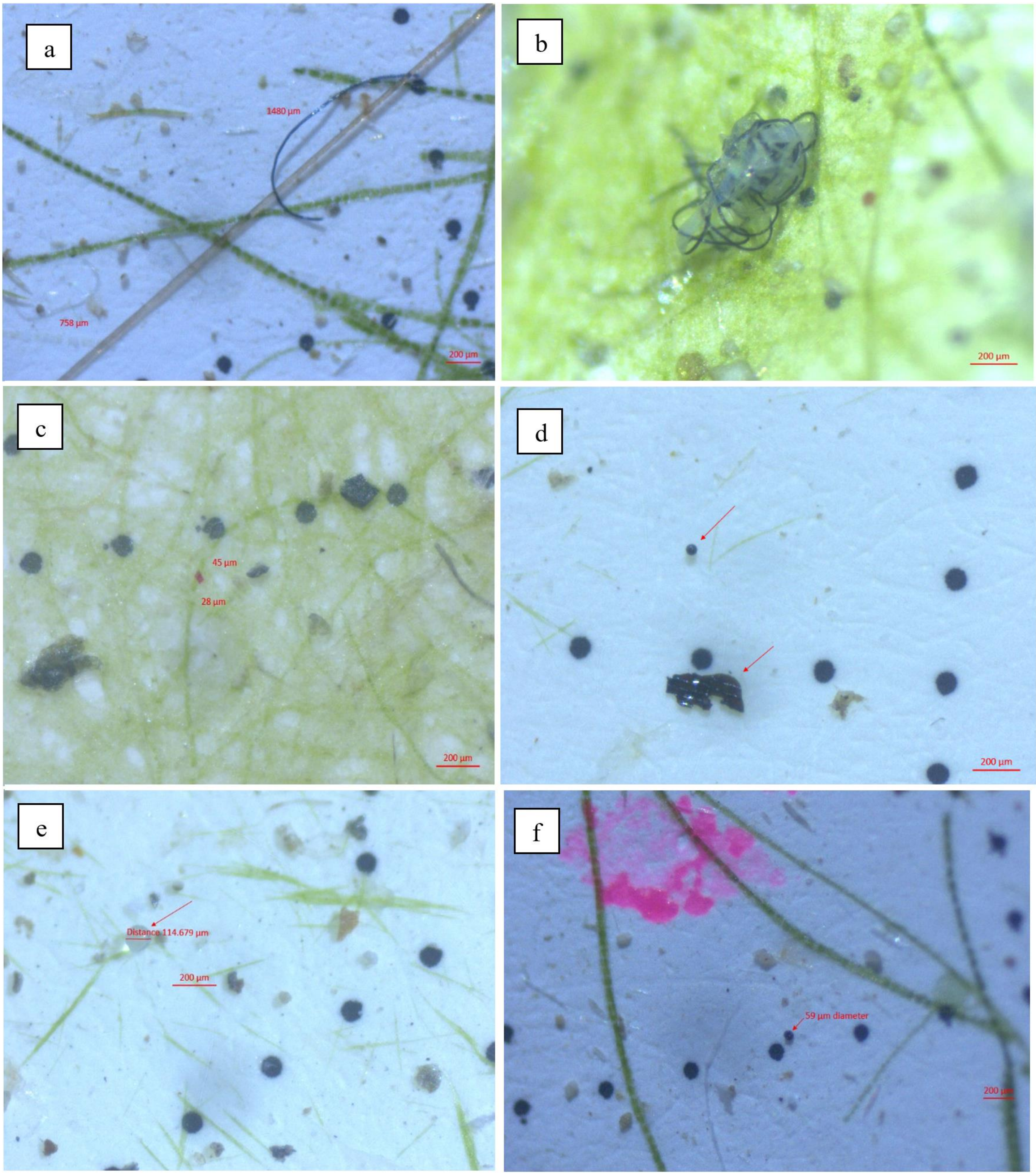


**Figure 5.** Photographs of MP types detected in surface water and sediment samples. (a): Two fibers; (b): An entangled fiber; (c): A fragment; (d): A microbead and a film; (e): A microbead; (f): A fragment.

## 4. Discussion

MPs are defined as plastic particles smaller than 5 mm. Some of these MPs are produced as small on purpose, while others are formed as a result of the breakdown of large plastics under the influence of sun, temperature, wind and waves, and can be transported to water resources either by direct disposal or by factors such as rain, wind, stormwater, etc. (NOAA, 2024). MPs have been detected in many water sources and MP pollution has become a major problem on a global scale today because MPs pose a health risk to both aquatic life and other wild animals and humans who use the respective water resource (Zolotova et al., 2022). Therefore, it is utmost importance to monitor water sources and determine MP pollution levels. Based on this, this study aimed to determine the amounts and types of MPs in Karaçomak Dam and a total of 3523 MPs were counted in the water and sediment samples obtained. It was observed that the average number of MPs in water was 3829 p/m$^3$ in winter, 3133 p/m$^3$ in autumn, 3025 p/m$^3$ in spring and 2837.5 p/m$^3$ in summer. Similarly, an average of 247 p/kg of MPs were detected in the sediment in winter, 189 p/kg in autumn, 156 p/kg in spring and 129 p/kg in summer. Other studies on lakes and dams have yielded varying results. For example, it was determined that the surface water of the Three Gorges Dam in China contained an average of 4703 p/m$^3$ and its sediment contained an average of 82 p/kg MPs (Di & Wang, 2018). Another similar research found that the surface water of Lake Taihu contained between 3400 and 25800 p/m$^3$ MPs (Su et al., 2016). It has been reported that the surface water of Sürgü Dam contains an average of 157 p/m$^3$ MPs (Turhan, 2022). Significantly less than these results, MPs detected in the waters of Chiusi and Bolsena lakes were in the range of 2.68-3.36 p/m$^3$ and 0.82-4.42 p/m$^3$, respectively (Fischer et al., 2016). As can be inferred from these data, the MP numbers in different water sources can significantly vary. Although the sampling method could influence this difference, it is primarily attributed to the level of anthropogenic pressure that the relevant water source is under (Ma et al., 2024; Quesadas-Rojas et al., 2021). Furthermore, one should note that we were unable to use spectrometry to characterize the MPs. For this reason, the numbers reported in this paper may be slightly overestimated.

It was determined that the majority of MPs in Karaçomak Dam water were fibers (54%). Likewise, fibers were reported as the dominant MP type in many different freshwater sources, including Sürgü Dam (Turhan, 2022), a treatment plant in Scotland (Blair et al., 2019), Süreyyabey Dam (Tavşanoğlu et al., 2020), Pearl River (Lin et al., 2018) and Manas River Basin (G. Wang et al., 2020). Plastic fibers mixed into water bodies can originate from many products. The main sources of these are fishing gear, textile products, industrial activities and domestic waste (Dris et al., 2015; Horton et al., 2017; Hu et al., 2020). On the other hand, in this study, it was determined that the most common MP type in sediment samples was particles (61%). Similar to these results, it has been reported that particle-type MPs are predominant in Lakes Erie and Ontario (Mason et al., 2020), a wastewater treatment plant in China (Lv et al., 2019) and the Nakdong River (Eo et al., 2019). Particle MPs found in water resources can originate from various plastic products such as widely used plastic bottles, toys, decorative products, building products, etc. (Lv et al., 2019). In this study, it was determined that the film and microbead-type MPs were the least abundant. While 11% of MPs in water were films and 1% were microbeads, it was observed that 6% of MPs in sediment were films and 2% were microbeads. Films are generally formed as a result of the breakdown of plastic bags and packaging wastes (Nor & Obbard, 2014). The main source of microbeads is personal care products (Cheung & Fok, 2016). These results show that Karaçomak Dam is under the pressure of MP pollution from many different products and that these are predominantly composed of secondary MPs.

It was determined that most of the MPs in the water samples obtained from Karaçomak Dam Lake were blue (29%) and were followed by black MPs (19%). In sediment samples, it was observed that the majority consisted of black (33%) and brown (16%) MPs. Color is an important parameter in MPs because MPs can be perceived as food by aquatic creatures or water birds due to their colors and can be ingested (Hidalgo-Ruz et al., 2012). Furthermore, due to the variety of pollutants affecting water resources, MPs with different colors can be detected in different water bodies. For example, there are studies reporting that MP samples are predominantly transparent (Tavşanoğlu et al., 2020), white (Li et al., 2018), black (Lam et al., 2020) or blue (Weideman et al., 2019). Color can also give an idea about for how long MP pollution has been persistent because MPs that have been in water for a long time tend to fade (yellowing) due to oxidation (Hidalgo-Ruz et al., 2012). However, in this study, white-yellow MPs were detected in very small amounts and the observed MPs typically had bright and vivid colors. This indicates that the MP pollution in Karaçomak Dam may be relatively recent.

When classified based on their sizes, MPs sized 0.2-1 mm and smaller than 0.1 mm were detected in the water samples at the same rate (33%). These were followed by MPs sized 0.1-0.2 mm with 22%. In the sediment samples, the predominant MP size was MPs smaller than 0.1 mm with 42%, while MPs sized 0.2-1 mm (27%) came second, and MPs size 0.1-0.2 mm (24%) came third. On the other hand, MPs sized 1-2.5 mm (10% in water, 6% in sediment) and 2.5-5 mm (3% in water, 1% in sediment) were found in much smaller amounts. According to these results, it is possible to say that the majority of MPs in Karaçomak Dam Lake are small-sized. Consistent with these findings, it has been reported that most MPs in both water and sediment in Three Gorges Dam are small in size (Di & Wang, 2018). MPs perceived as food and ingested by aquatic organisms may cause internal abrasions, physical obstruction or damage to the endocrine system through the release of toxic components (Wright et al., 2013; Wright & Kelly, 2017). MPs can be taken into the body of fish not only by being swallowed but also via water passing through the gills and skin. The small sizes of the MPs detected in this study may facilitate this intake. Therefore, considering the high MP concentrations and small sizes, it is possible to infer that MP pollution may pose a risk for the populations in the Karaçomak Dam Lake. Karaçomak Dam is also the drinking water source of Kastamonu. With this feature, it has the potential to threaten human health.

The abundance of MP levels in Karaçomak Dam was determined in winter, autumn, spring and summer seasons, respectively. It was determined that the amount of MP increased in autumn, peaked in winter, and started to decrease in spring and reached the lowest amount in summer. This situation can be attributed to the amount of rainfall during the year, the area where the dam is located, the surrounding agricultural and animal husbandry activities, and the fact that the dam lake is also a good fishing and recreational area. Indeed, many studies have shown that MP concentrations in water can vary seasonally, and heavy rainfall, stormwater and melting snow can transport MPs from land to water (Ross et al., 2023; J. Wang et al., 2022). Moreover, it is an unequivocal fact that human activities such as urban life, agriculture, fishing and tourism can also affect the amounts of MPs in water resources (Zhao et al., 2024).

## 5. Conclusion

Ultimately, the microplastic data obtained from the study conducted in the Karaçomak Dam Lake are similar to other studies conducted in Türkiye and the world lakes in terms of quantity and quality, and are at average levels. However, this is insufficient to draw a conclusion on whether the dam lake is rich or poor in plastic, under a risk/pressure or not since there is no reference regarding how much MPs cause pollution in the waters and to what extent yet. On the other hand, it is not possible to clearly demonstrate how much of it is ingested by inhabitant wildlife in the lake or how much of it poses a risk to the end consumers. Therefore, more studies and comprehensive research are needed. Based on this, studies should be carried out to determine the source of MP pollution in the Karaçomak Dam, reduce and prevent it. Furthermore, the MP levels in tap waters should be investigated in future studies as the dam provides drinking water to Kastamonu.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, *62*(8), 1596-1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Atamanalp, M., Kokturk, M., Kırıcı, M., Ucar, A., Kırıcı, M., Parlak, V., Aydın, A., & Alak, G. (2022a). Interaction of microplastic presence and oxidative stress in freshwater fish: A regional scale research, east Anatolia of Türkiye (Erzurum & Erzincan & Bingöl). *Sustainability*, *14*(19), 12009. https://doi.org/10.3390/su141912009

Atamanalp, M., Köktürk, M., Parlak, V., Ucar, A., Arslan, G., & Alak, G. (2022b). A new record for the presence of microplastics in dominant fish species of the Karasu River Erzurum, Turkey. *Environmental Science and Pollution Research*, *29*, 7866-7876. https://doi.org/10.1007/s11356-021-16243-w

Atamanalp, M., Kokturk, M., Gündüz, F., Parlak, V., Ucar, A., Alwazeer, D., & Alak, G. (2023). The use of zebra mussel (*Dreissena polymorpha*) as a sentinel species for the microplastic pollution of freshwater: The case of Beyhan Dam Lake, Turkey. *Sustainability*, *15*(2), 1422. https://doi.org/10.3390/su15021422

Baldwin, A. K., Corsi, S. R., & Mason, S. A. (2016). Plastic debris in 29 Great Lakes tributaries: Relations to watershed attributes and hydrology. *Environmental Science & Technology*, *50*(19), 10377-10385. https://doi.org/10.1021/acs.est.6b02917

Ballent, A., Purser, A., de Jesus Mendes, P., Pando, S., & Thomsen, L. (2012). Physical transport properties of marine microplastic pollution. *Biogeosciences Discussions*, *9*, 18755-18798. https://doi.org/10.5194/bgd-9-18755-2012

Barrows, A. P. W., Cathey, S. E., & Petersen, C. W. (2018). Marine environment microfiber contamination: Global patterns and the diversity of microparticle origins. *Environmental Pollution*, *237*, 275-284. https://doi.org/10.1016/j.envpol.2018.02.062

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M. B., & Gerdts, G. (2017). High quantities of microplastic in Arctic deep-sea sediments from the HAUSGARTEN observatory. *Environmental Science & Technology*, *51*(19), 11000-11010. https://doi.org/10.1021/acs.est.7b03331

Blair, R. M., Waldron, S., & Gauchotte-Lindsay, C. (2019). Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. *Water Research*, *163*, 114909. https://doi.org/10.1016/j.watres.2019.114909

Böyükalan, S., & Yerli, S. V. (2023). Microplastic pollution at different trophic levels of freshwater fish in a variety of Türkiye's lakes and dams. *Turkish Journal of Fisheries and Aquatic Sciences*, *23*(11), TRJFAS23747. https://doi.org/10.4194/TRJFAS23747

Cheung, P. K., & Fok, L. (2016). Evidence of microbeads from personal care product contaminating the sea. *Marine Pollution Bulletin*, *109*(1), 582-585. https://doi.org/10.1016/j.marpolbul.2016.05.046

Çullu, A. F., Sönmez, V. Z., & Sivri, N. (2021). Microplastic contamination in surface waters of the Küçükçekmece Lagoon, Marmara Sea (Turkey): Sources and areal distribution. *Environmental Pollution*, *268*(Part B), 115801. https://doi.org/10.1016/j.envpol.2020.115801

Di, M., & Wang, J. (2018). Microplastics in surface waters and sediments of the Three Gorges Reservoir, China. *Science of the Total Environment*, *616*, 1620-1627. https://doi.org/10.1016/j.scitotenv.2017.10.150

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., & Tassin, B. (2015). Microplastic contamination in an urban area: A case study in Greater Paris. *Environmental Chemistry*, *12*(5), 592-599. https://doi.org/10.1071/EN14167

DSİ. (2016). *Karaçomak Barajı*. http://www2.dsi.gov.tr/baraj/detay.cfm?BarajID=51 (In Turkish)

Egessa, R., Nankabirwa, A., Ocaya, H., & Pabire, W. G. (2020). Microplastic pollution in surface water of Lake Victoria. *Science of the Total Environment*, *741*, 140201. https://doi.org/10.1016/j.scitotenv.2020.140201

Eo, S., Hong, S. H., Song, Y. K., Han, G. M., & Shim, W. J. (2019). Spatiotemporal distribution and annual load of microplastics in the Nakdong River, South Korea. *Water Research*, *160*, 228-237. https://doi.org/10.1016/j.watres.2019.05.053

Erdoğan, Ş. (2020). Microplastic pollution in freshwater ecosystems: A case study from Turkey. *Su Ürünleri Dergisi*, *37*(3), 213-221. https://doi.org/10.12714/egejfas.37.3.02

Fischer, E. K., Paglialonga, L., Czech, E., & Tamminga, M. (2016). Microplastic pollution in lakes and lake shoreline sediments–a case study on Lake Bolsena and Lake Chiusi (central Italy). *Environmental Pollution, 213*, 648-657. https://doi.org/10.1016/j.envpol.2016.03.012

Gedik, K., & Atasaral, Ş. (2022). The microplastic pattern in Turkish lakes: Sediment and bivalve samples from Çıldır Lake, Almus Dam Lake, and Kartalkaya Dam Lake. *Turkish Journal of Zoology*, *46*(5), 397-408. https://doi.org/10.55730/1300-0179.3093

Gray, A. D., Wertz, H., Leads, R. R., & Weinstein, J. E. (2018). Microplastic in two South Carolina Estuaries: Occurrence, distribution, and composition. *Marine Pollution Bulletin*, *128*, 223-233. https://doi.org/10.1016/j.marpolbul.2018.01.030

Gündoğdu, S., Kutlu, B., Özcan, T., Büyükdeveci, F., & Blettler, M. C. (2023). Microplastic pollution in two remote rivers of Türkiye. *Environmental Monitoring and Assessment*, *195*, 791. https://doi.org/10.1007/s10661-023-11426-z

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environmental Science & Technology*, *46*(6), 3060-3075. https://doi.org/10.1021/es2031505

Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, *586*, 127-141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Hu, D., Zhang, Y., & Shen, M. (2020). Investigation on microplastic pollution of Dongting Lake and its affiliated rivers. *Marine Pollution Bulletin*, *160*, 111555. https://doi.org/10.1016/j.marpolbul.2020.111555

Kankılıç, G. B., Koraltan, İ., Erkmen, B., Çağan, A. S., Çırak, T., Özen, M., Seyfe, M., Altındağ, A., & Tavşanoğlu, Ü. N. (2023). Size-selective microplastic uptake by freshwater organisms: Fish, mussel, and zooplankton. *Environmental Pollution*, *336*, 122445. https://doi.org/10.1016/j.envpol.2023.122445

Kotar, S., McNeish, R., Murphy-Hagan, C., Renick, V., Lee, C. F. T., Steele, C., Lusher, A., Moore, C., Minor, E., Schroeder, J., Helm, P., Rickabaugh, K., De Frond, H., Gesulga, K., Lao, W., Munno, K., Thornton Hampton, L. M., Weisberg, S. B., Wong, C. S., Amarpuri, G., & Rochman, C. M. (2022). Quantitative assessment of visual microscopy as a tool for microplastic research: Recommendations for improving methods and reporting. *Chemosphere*, *308*(Part 3), 136449. https://doi.org/10.1016/j.chemosphere.2022.136449

Lam, T. W. L., Ho, H. T., Ma, A. T., & Fok, L. (2020). Microplastic contamination of surface water-sourced tap water in Hong Kong—a preliminary study. *Applied Sciences*, *10*(10), 3463. https://doi.org/10.3390/app10103463

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., & Zeng, E. Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. *Water Research*, *142*, 75-85. https://doi.org/10.1016/j.watres.2018.05.034

Lin, L., Zuo, L. Z., Peng, J. P., Cai, L. Q., Fok, L., Yan, Y., Li, H. X., & Xu, X. R. (2018). Occurrence and distribution of microplastics in an urban river: a case study in the Pearl River along Guangzhou City, China. *Science of the Total Environment*, *644*, 375-381. https://doi.org/10.1016/j.scitotenv.2018.06.327

Lv, X., Dong, Q., Zuo, Z., Liu, Y., Huang, X., & Wu, W. M. (2019). Microplastics in a municipal wastewater treatment plant: Fate, dynamic distribution, removal efficiencies, and control strategies. *Journal of Cleaner Production*, *225*, 579-586. https://doi.org/10.1016/j.jclepro.2019.03.321

Ma, H., Chao, L., Wan, H., & Zhu, Q. (2024). Microplastic pollution in water systems: Characteristics and control methods. *Diversity*, *16*(1), 70. https://doi.org/10.3390/d16010070

Mason, S. A., Daily, J., Aleid, G., Ricotta, R., Smith, M., Donnelly, K., Knauff, R., Edwards, W., & Hoffman, M. J. (2020). High levels of pelagic plastic pollution within the surface waters of Lakes Erie and Ontario. *Journal of Great Lakes Research*, *46*(2), 277-288. https://doi.org/10.1016/j.jglr.2019.12.012

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). *Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments*. NOAA Technical Memorandum NOS-OR&R-48.

Mutlu, T., Minaz, M., Baytaşoğlu, H., & Gedik, K. (2024). Monitoring of microplastic pollution in sediments along the Çoruh River Basin, NE Türkiye. *Journal of Contaminant Hydrology*, *263*, 104334. https://doi.org/10.1016/j.jconhyd.2024.104334

NOAA. (2024). *What are microplastics?* https://oceanservice.noaa.gov/education/tutorial-coastal/marine-debris/md04.html

Nor, N. H. M., & Obbard, J. P. (2014). Microplastics in Singapore's coastal mangrove ecosystems. *Marine Pollution Bulletin*, *79*(1-2), 278-283. https://doi.org/10.1016/j.marpolbul.2013.11.025

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution*, *184*, 161-169. https://doi.org/10.1016/j.envpol.2013.07.027

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., & Gerdts, G. (2018). Arctic sea ice is an important temporal sink and means of transport for microplastic. *Nature Communications*, *9*, 1505. https://doi.org/10.1038/s41467-018-03825-5

Quesadas-Rojas, M., Enriquez, C., & Valle-Levinson, A. (2021). Natural and anthropogenic effects on microplastic distribution in a hypersaline lagoon. *Science of the Total Environment*, *776*, 145803. https://doi.org/10.1016/j.scitotenv.2021.145803

Ross, M. S., Loutan, A., Groeneveld, T., Molenaar, D., Kroetch, K., Bujaczek, T., Kolter, S., Moon, S., Huynh, A., Khayam, R., Franczak, B. C., Camm, E., Arnold, V. I., & Ruecker, N. J. (2023). Estimated discharge of microplastics via urban stormwater during individual rain events. *Frontiers in Environmental Science*, *11*, 1090267. https://doi.org/10.3389/fenvs.2023.1090267

Ryan, P. G., Moore, C. J., Van Franeker, J. A., & Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1999-2012. https://doi.org/10.1098/rstb.2008.0207

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in Taihu lake, China. *Environmental Pollution*, *216*, 711-719. https://doi.org/10.1016/j.envpol.2016.06.036

Sun, J., Zhu, Z. R., Li, W. H., Yan, X., Wang, L. K., Zhang, L., Jin, J., Dai, X., & Ni, B. J. (2021). Revisiting microplastics in landfill leachate: Unnoticed tiny microplastics and their fate in treatment works. *Water Research*, *190*, 116784. https://doi.org/10.1016/j.watres.2020.116784

Şahin, S. K., & Zeybek, M. (2019). Diversity and species composition of the macrobenthic invertebrates in Sürgü stream (Malatya, Turkey). *Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *10*(1), 60-67. https://doi.org/10.29048/makufebed.529074

Tavşanoğlu, Ü. N., Başaran Kankılıç, G., Akca, G., Çırak, T., & Erdoğan, Ş. (2020). Microplastics in a dam lake in Turkey: Type, mesh size effect, and bacterial biofilm communities. *Environmental Science and Pollution Research*, *27*, 45688-45698. https://doi.org/10.1007/s11356-020-10424-9

Turhan, D. Ö. (2022). Evaluation of microplastics in the surface water, sediment and fish of Sürgü Dam Reservoir (Malatya) in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(7), TRJFAS20157. http://doi.org/10.4194/TRJFAS20157

Turra, A., Manzano, A. B., Dias, R. J. S., Mahiques, M. M., Barbosa, L., Balthazar-Silva, D., & Moreira, F. T. (2014). Three-dimensional distribution of plastic pellets in sandy beaches: Shifting paradigms. *Scientific Reports*, *4*, 4435. https://doi.org/10.1038/srep04435

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A. D., Winther-Nielsen, M., & Reifferscheid, G. (2014). Microplastics in freshwater ecosystems: What we know and what we need to know. *Environmental Sciences Europe*, *26*, 12. https://doi.org/10.1186/s12302-014-0012-7

Wang, G., Lu, J., Tong, Y., Liu, Z., Zhou, H., & Xiayihazi, N. (2020). Occurrence and pollution characteristics of microplastics in surface water of the Manas River Basin, China. *Science of the Total Environment*, *710*, 136099. https://doi.org/10.1016/j.scitotenv.2019.136099

Wang, J., Bucci, K., Helm, P. A., Hoellein, T., Hoffman, M. J., Rooney, R., & Rochman, C. M. (2022). Runoff and discharge pathways of microplastics into freshwater ecosystems: A systematic review and meta-analysis. *Facets*. https://doi.org/10.1139/facets-2022-0140

Weideman, E. A., Perold, V., & Ryan, P. G. (2019). Little evidence that dams in the Orange–Vaal River system trap floating microplastics or microfibres. *Marine Pollution Bulletin*, *149*, 110664. https://doi.org/10.1016/j.marpolbul.2019.110664

Wong, J. K. H., Lee, K. K., Tang, K. H. D., & Yap, P. S. (2020). Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. *Science of the Total Environment*, *719*, 137512. https://doi.org/10.1016/j.scitotenv.2020.137512

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483-492. https://doi.org/10.1016/j.envpol.2013.02.031

Wright, S. L., & Kelly, F. J. (2017). Plastic and human health: A micro issue? *Environmental Science & Technology*, *51*(12), 6634-6647. https://doi.org/10.1021/acs.est.7b00423

Yuan, W., Liu, X., Wang, W., Di, M., & Wang, J. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicology and Environmental Safety*, *170*, 180-187. https://doi.org/10.1016/j.ecoenv.2018.11.126

Zhao, W., Li, J., Liu, M., Wang, R., Zhang, B., Meng, X. Z., & Zhang, S. (2024). Seasonal variations of microplastics in surface water and sediment in an inland river drinking water source in southern China. *Science of The Total Environment*, *908*, 168241. https://doi.org/10.1016/j.scitotenv.2023.168241

Zolotova, N., Kosyreva, A., Dzhalilova, D., Fokichev, N., & Makarova, O. (2022). Harmful effects of the microplastic pollution on animal health: A literature review. *PeerJ*, *10*, e13503. https://doi.org/10.7717/peerj.13503

## AIMS & SCOPE

*Journal of Agricultural Production* aims to create an environment for researchers to introduce, share, read, and discuss recent scientific progress and adopts the policy of providing open access to the readers who may be interested in recent scientific developments. *Journal of Agricultural Production* publishes papers in the categories of research articles, review articles, short communications, and letters to the Editor.

Research areas include but not limited to:

- Agriculture
- Aquaculture
- Fisheries
- Ecology
- Environmental sciences
- Horticulture
- Crop protection
- Bio systems
- Landscape
- Agriculture and aquaculture economics
- Agriculture and aquaculture biotechnology
- Soil science and plant nutrition
- Husbandry
- Basic sciences

And closely related life sciences, as well as engineering topics.

## AUTHOR GUIDELINES

Manuscripts must be submitted to the journal in electronic version through online submission system at https://dergipark.org.tr/en/pub/japro following the Author Guidelines of the journal.

**Types of Paper**

Research articles, review articles, short communications, and letters to the Editor.

- Research articles: original full-length research papers which have not been published previously and should not exceed 7500 words or 25 manuscript pages (including tables and figures).
- Review articles: on topical subjects and up to 10000 words or 25 manuscript pages (including tables and figures).
- Short communications: describing work that may be of a preliminary nature or a case report; preferably no more than 3000 words or 10 manuscript pages (including tables and figures).
- Letters to the Editor: should be included on matters of topical interest and not exceeding 2000 words or 10 manuscript pages (including tables and figures).

**Preparation of Manuscripts**

Papers must be written in English. Prepare your text using a word-processing software and save in ".doc" or ".docx" formats. Manuscripts must be structured in the following order:

- ***Title Page File***
  - Title (Concise and informative. Avoid abbreviations and formulae)
  - Author names and affiliation addresses (Full names should be given, no abbreviations. The corresponding author should be identified with an asterisk. Each affiliation address should include institution, faculty/school, department, city, and country)
  - ORCID numbers for all authors.
  - Corresponding author's e-mail, telephone number, and address
  - Acknowledgements (if applicable. Keep these to the absolute minimum)
  - Compliance with Ethical Standards
    - ❖ Conflict of Interest Statement
    - ❖ Statement on the Welfare of Animals (if applicable)
    - ❖ Statement of Human Rights (if applicable)
- ***Main File***
  - Title
  - Abstract (Should be between 100 and 350 words. References and abbreviations should be avoided)
  - Keywords (Minimum 3, Maximum 6 keywords)
  - Introduction
  - Materials and Methods
  - Results
  - Discussion (Can be combined with Results section if appropriate)
  - Conclusion
  - References
  - Table(s) with caption(s) (on appropriate location in the text)
  - Figure(s) with caption(s) (on appropriate location in the text)
  - and appendices (if any)

**Manuscript Formatting**

Use a 12-point Times New Roman font, including the references, table headings and figure captions, double-spaced and with 25 mm margins on all sides of A4 size paper throughout the manuscript. The text should be in single-column format.

- Each page must be numbered with Arabic numerals, and lines must be continuously numbered from the start to the end of the manuscript.
- Use italics for emphasis.
- Use only SI (international system) units.
- Use "dot" for decimal points.
- Use italics for species name.

**References**

*Journal of Agricultural Production* uses APA style (7th edition). Accordingly, authors must format their references as per the guidelines below. Please ensure that each reference cited in the text is also presented in the reference list. Authors should always supply DOI or URL of the work cited if available.

***In-text citation (Narrative):***

…The results of Bliss (2022) support…

…Sönmez and Taştan (2020) indicated that…

…According to the method of Öztürk et al. (2021)…

***In-text citation (In parenthesis):***

…It was found to be isometric (Öztürk, 2018)…

…is highly susceptible to diseases (Doma & Craig, 2019)…

…have been studied (Kale et al., 2020)…

***Two or more works in the same parenthesis:***

…extremely toxic for the environment (Sönmez, 2018, 2019; Öztürk et al., 2020a; Kadak & Taştan, 2021)…

***Citation in the reference list:***

References should be listed first alphabetically and then further sorted chronologically at the end of the article. The citation of all references should conform to the following examples:

***Article:***

Lastname, N., Lastname, M., & Lastname, O. (Year). Title of the work. *Title of the Journal*, *Volume*(Issue), Page numbers. DOI

Tort, S. (1998). Stress and immune modulation in fish. *Developmental & Comparative Immunology*, *35*(12), 1366-1375. https://doi.org/ 10.1016/j.dci.2011.07.002

Kasumyan, A. O., & Døving, K. B. (2003). Taste preferences in fishes. *Fish and Fisheries*, *4*(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x

Özçelik, H., Taştan, Y., Terzi, E., & Sönmez, A. Y. (2020). Use of onion (*Allium cepa*) and garlic (*Allium sativum*) wastes for the prevention of fungal disease (*Saprolegnia parasitica*) on eggs of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*, *43*(10), 1325-1330. https://doi.org/10.1111/jfd.13229

***Article by DOI (early access):***

Salem, M. O. A., Salem, T. A., Yürüten Özdemir, K., Sönmez, A. Y., Bilen, S., & Güney, K. (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. *Fish Physiology and Biochemistry*. https://doi.org/10.1007/s10695-021-00962-5

***Book:***

Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Publisher.

Oidtmann, K., Xao, Q., & Lloyd, A. S. (2018). *The food need by the year 2050*. Elsevier.

***Book Chapter:***

Lastname, N., Lastname, M., & Lastname, O. (Year). Title of the chapter. In N. N. Lastname, A. Lastname & B. Lastname (Eds.), *Title of the book* (pp. Page numbers). Publisher.

Pickering, A. D. (1993). Growth and stress in fish production. In G. A. E. Gall & H. Chen (Eds.), *Genetics in Aquaculture* (pp. 51-63). Elsevier. https://doi.org/10.1016/b978-0-444-81527-9.50010-5

***Dissertation or Thesis:***

Lastname, N. (Year). *Title of dissertation/thesis* (Doctoral dissertation/Master's thesis, Name of Institution).

Sönmez, A. Y. (2011). *Karasu ırmağında ağır metal kirliliğinin belirlenmesi ve bulanık mantıkla değerlendirilmesi* (Doctoral dissertation, Atatürk University).

Taştan, Y. (2018). *Tatlısu kerevitindeki (Astacus leptodactylus) siyah solungaç hastalığı etkeni mantar Fusarium oxysporum'un PCR yöntemi ile teşhisi* (Master's thesis, Akdeniz University).

***Conference Proceedings:***

Lastname, N., Lastname, M., & Lastname, O. (Year). *Title of the work*. Title of the Conference. City.

Ken, A., & Kumar, S. (2020). *A new statistical model for fuzzy logic evaluation*. 3rd International Congress on Statistics. İstanbul.

***Institution Publication:***

Institution name. (Year). *Title of the work*. URL

FAO. (2020). *Fishery and aquaculture statistics 2018*. http://www.fao.org/3/cb1213t/CB1213T.pdf

***Internet Source:***

Lastname, N. (Year). *Title of the work*. Retrieved May 15, 2020, from URL

Perreault, L. (2019). *The future of agriculture: Polyculture*. Retrieved January 12, 2020, from https://www.agriculture.com

**Table(s)**

Tables, numbered in Arabic, should be in separate pages with a short descriptive title at the top. Place footnotes to tables below the table body and indicate them with superscript lowercase letters (or asterisks for significance values and other statistical data).

**Figure(s)**

All illustrations should be labelled as 'Figure' and numbered in consecutive Arabic numbers, Figure 1, Figure 2 etc. in the text. If panels of a figure are labelled (a, b, etc.) use the same case when referring to these panels in the text. Figures are recommended to be in electronic formats such as PNG, JPEG, TIFF (min. 300 dpi). All figures or tables should be presented in the body of the text.

**Online Manuscript Submission**

Authors are requested to submit manuscripts via the journal's online submission system at https://dergipark.org.tr/en/pub/japro

**Submission Checklist**

- Author Guidelines of the journal has been read and adhered

- **Title Page File**
  - Title
  - Full names, e-mails, and affiliation addresses of all authors
  - ORCID numbers of all authors
  - Corresponding author's e-mail, telephone number, and address
  - Ethical statements

- **Main File**
  - Continuous page numbers
  - Continuous line numbers
  - Blinded document (no personal information is present)
  - Title
  - Abstract (100-350 words)
  - Keywords (3-6 keywords)
  - All figures and tables are numbered and cited in order in text
  - Completeness and accuracy of the references have been checked
  - References have been edited as per the Journal guidelines

**Publication Frequency**

The journal includes original scientific articles on a variety of different subjects in English and is being published twice a year in June and December.

**Price Policy**

*Journal of Agricultural Production* requests article submission fee. This fee is collected from all articles sent to the journal regardless of the acceptance/rejection decision.

Authors will receive an email regarding the payment upon submission. Authors should make the payment after this email. The evaluation process will be initiated once the payment is completed.

Submission Fee: 50$ (For Turkish Authors: 1000 TL)

Editorial Processing Charge: Free

Article Processing Charge (or any other post-acceptance fee): Free.

The articles are open access and freely available to all readers.

## PUBLICATION ETHICS

Details about Publication Ethics can be found at https://dergipark.org.tr/en/pub/japro/policy

## ETHICAL GUIDELINES FOR THE USE OF ANIMALS IN RESEARCH

Details can be found at https://dergipark.org.tr/en/pub/japro/policy

## OPEN ACCESS POLICY

Details can be found at https://dergipark.org.tr/en/pub/japro/page/13659

## REVIEW PROCESS

**Double-Blind Review and Evaluation Process**

Double-Blind Review is a method applied for publishing scientific publications with the highest quality. This method forms the basis of an objective evaluation of scientific studies and is preferred by many scientific journals.

The views of referees have a decisive place in the publication quality of a Journal. *Journal of Agricultural Production* uses the double-blind review method, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process, in the evaluation process of all studies. For this reason, the authors are asked to blind their manuscripts before submitting. All the studies submitted to *Journal of Agricultural Production* are evaluated by double-blind review method according to the following steps.

***1. Initial Evaluation Process***

The studies submitted to *Journal of Agricultural Production* are first evaluated by the editor. At this stage, studies that are not in line with the aim and scope of the journal, are weak in terms of language and narrative rules in English, contain scientifically critical mistakes, are not original worthy, and cannot meet publication policies are rejected. Authors of rejected studies will be notified within one month at the latest from the date of submission. Eligible studies are sent to the field editor to which the study is relevant for pre-evaluation.

***2. Pre-Evaluation Process***

In the pre-evaluation process, the field editors examine the studies, introduction and literature, methods, findings, results, evaluation and discussion sections in detail in terms of journal publication policies, scope and authenticity of study. Study which is not suitable as a result of this examination is returned to the author with the field editor's evaluation report within four weeks at the latest. The studies which are suitable for the journal are passed to the referee process.

***3. Referee Process***

The studies are sent to the referees according to their content and the expertise of the referees. The field editor examining the study may propose at least two referees from the pool of *Journal of Agricultural Production* Advisory Board or referee pool according to their field of expertise or may propose a new referee appropriate to the field of study. The editors evaluate the referee's suggestions coming from the field editor and the studies are submitted to the referees. Referees are obliged to guarantee that they will not share any process or document about the study they are evaluating.

***4. Referee Evaluation Process***

The period given to the referee for the evaluation process is 15 days. Proposals for corrections from referees or editors must be completed by the authors within 1 month according to the "correction instruction". Referees can decide on the suitability of the study by reviewing the corrections and may also request multiple corrections if necessary.

**Referee Reports**

Referee evaluations are based in general on the originality of the studies, the method used, and the conformity with the ethical rules, the consistent presentation of the findings and results, and the examination of the literature.

This review is based on the following elements:

*1. Introduction and Literature:* The evaluation report contains the presentation and purpose of the problem addressed in the study, the importance of the topic, the scope of the relevant literature, the timeliness and the originality of the study.

*2. Methodology:* The evaluation report includes information on the suitability of the method used, the choice and characteristics of the research group, validity and reliability, as well as on the data collection and analysis process.

*3. Findings:* The evaluation report includes opinions on the presentation of the findings obtained in the frame of the method, the correctness of the analysis methods, the aims of the research and the consistency of the findings, the presentation of the required tables, figures and images and the conceptual evaluation of the tests used.

*4. Evaluation and discussion:* The evaluation report includes the opinion on the subject based on findings, relevance to research questions and hypotheses, generalizability and applicability.

*5. Conclusion and suggestions:* The evaluation report contains the opinion on the contributions to the literature, future studies and recommendations for the applications in the area.

*6. Style and narration:* The evaluation report includes compatibility of the title with the content, appropriate use of English in the study, refers and references in accordance with the language of the study and APA rules.

*7. Overall evaluation:* The evaluation report contains opinion on the authenticity of the study as a whole, its contribution to the educational literature and the applications in the area. The journal considers that scientists should avoid research which kills or damages any species of animal which, using IUCN criteria, is regarded as threatened or is listed as such in a Red Data Book appropriate for the geographic area concerned. In accordance with this view, papers based on such research will not be accepted by the Journal, unless the work had clear conservation objectives.

**Plagiarism Detection**

In agreement with publishing policies of *Journal of Agricultural Production*, plagiarism check is required for each study that has undergone the "Review Process". The Turnitin plagiarism checker software is used for plagiarism detection.

**Proofs**

Proof documents will be sent to the corresponding authors via e-mail. Proofs should be checked immediately and responses should be returned back within 15 working days. It is the responsibility of the authors to check carefully the proofs. No changes will be allowed after this stage.

# DISCLAIMER

The Publisher and Editors cannot be held responsible for errors or any consequences arising from the use of information contained in this journal; the views and opinions expressed do not necessarily reflect those of the Publisher and Editors.

Editor or members of the editorial board are not responsible for the author's opinions and manuscript contents. Authors are responsible for the ethical originality of and possible errors in their manuscripts.

This journal is available online at https://dergipark.org.tr/en/pub/japro

Kindly visit website to search the articles and register for table of contents e-mail alerts.

# LICENCE



# INDEXING

*Journal of Agricultural Production* is currently indexed by

- TRDİZİN
- EBSCO
- CABI
- ROAD-Directory of Open Access Scholarly Resources
- Google Scholar
- Crossref

# PUBLISHER

Gökhan ARSLAN
Atatürk University
agriprojournal@gmail.com
Yakutiye mah. Atatürk Üni. Su Ürünleri Fakültesi, Merkez/Erzurum, TÜRKİYE

# PUBLISHER SUPPORT

PRENSIP

Prensip Publishing and Consultancy Ind. Trade. Co. Ltd.
Kuzeykent mah. Orgeneral Atilla Ateş Paşa cad. No:15 CD, İç Kapı No: 31, Merkez/Kastamonu, 37150, TÜRKİYE
https://prensip.gen.tr
info@prensip.gen.tr

JOURNAL OF AGRICULTURAL PRODUCTION

PRENSIP