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Original article

Agro-morphological traits and some bacterial leaf pathogens susceptibility in local super tomato genotypes

Yerel süper domates genotiplerinde tarımsal morfolojik özellikler ve bazı bakteriyel yaprak patojenlerine duyarlılık

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ABSTRACT

This study aimed to characterize the morphological traits of Super tomato genotypes grown widely in Iğdir plain and to determine the reaction of the Super tomato genotype to bacterial diseases caused by *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*). Super tomato genotypes were collected from 20 different regions in the harvest season in 2021-2022. Morphological and physiological measurements in the laboratory were taken on tomato genotypes to characterize plant traits. Also, pot experiments were carried out in a plant growth chamber to assess the reaction of Super tomato to infection by *Pst* and *Xep*. Data on plant morphology and growth were obtained, including measurements of plant heights (136.9-88.7 cm), root lengths (69.0-46.3 cm), stem diameters (2.17-1.52 cm), plant fresh weights (596-426 g), plant dry weights (127.6-94.0 g), root fresh weights (74.5 to 51.8 g), root dry weights (24.3-11.9 g), yield per plant (4717.3-2906.5 g), mean fruit weight (385.2-223.7 g), fruit length (55.5-50.3 mm), and mean fruit diameter (96.0-81.1 mm). In terms of the physiological properties of tomato fruits, the water-soluble dry matter content ranged from 4.55% to 4.11%, fruit juice pH from 4.69 to 4.43, titratable acidity from 3.16 to 2.93 mval 100 ml⁻¹, vitamin C content from 26.63 to 17.80 mg/100 g, lycopene content from 2951.1 to 2629.5 ng/μl, and β-carotene content from 272.55 to 228.82 ng/μl. Additionally, pot experiments demonstrated that the super tomato genotype exhibited moderate susceptibility to both *Pst* and *Xep* infections, with disease severity index (DSI) of 2.4 and 2.2, respectively.

INTRODUCTION

Tomato (*Solanum lycopersicum* L., Solanaceae) ranks among the most economically important cultivated plants globally, exhibiting high production, consumption, and trade

(Keskin and Gül 2004). Türkiye is the world's third-largest tomato producer, contributing 6.99% of the global output. According to FAO (2022) data, Türkiye produced 13 million

tons of tomatoes annually, trailing only China (68.2 million tons) and India (20.7 million tons). Tomato cultivation in Türkiye is versatile, with production occurring in the field and controlled environments such as greenhouses.

Based on Turkish Statistical Institute (TUIK) data from 2022, most tomatoes (61.2%) were cultivated for direct consumption as table tomatoes. Processing tomatoes (paste tomatoes) constituted the remaining 38.8% of production. Notably, 32% of the table tomato crop was grown in greenhouses. Tomato production in Türkiye utilizes sophisticated agricultural practices. Furthermore, the industry has undergone a structural change, moving from a predominantly small-scale, family-based model to a larger, corporate structure characterized by advanced agricultural practices. Despite these agricultural improvements, the risk of crop damage or destruction caused by pests, diseases, and adverse weather conditions has escalated. Such challenges lead to considerable tomato quality and quantity losses within our country (Yucel et al. 2008).

Tomatoes are infected by over 200 pathogens, with bacterial species from the genera *Xanthomonas*, *Pseudomonas*, *Clavibacter*, *Pectobacterium*, and *Dickeya* posing significant economic losses to tomato cultivation (Horuz and Serin 2024). Pathogenic bacteria belonging to the genera *Pseudomonas* (Canzoniere et al. 2021, Silvera-Pérez et al. 2023) and *Xanthomonas* (Abrahamian et al. 2021, Osdaghi et al. 2017) have been reported to cause substantial economic losses in tomato cultivation. Bacterial speck, caused by *Pseudomonas syringae* pv. tomato (Okabe) Young, Dye & Wilkie, and bacterial spot, a complex disease primarily caused by *Xanthomonas euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans* (Jones et al.) Constantin et al., *X. hortorum* pv. *gardneri*, and *X. vesicatoria*, are among the most economically devastating bacterial diseases affecting global tomato production (Canzoniere et al. 2021, Constantin et al. 2016, Jones et al. 2004, Morinière et al. 2020).

The symptoms of these two diseases first appear on the leaves, as the disease progresses, lesions spread to the stems, petioles, and flowers. Yield reductions may also occur due to reduced photosynthetic capacity of infected leaves, leaf drops, flower drops, and fruit lesions. Bacterial speck is more severe in cool, moist conditions, while bacterial spot symptoms generally appear more severe in warm, wet weather (Ji et al. 2006). Current strategies for controlling both diseases are known to be of limited effectiveness. Cultural practices alone cannot adequately control diseases and are generally not adopted by commercial growers. Copper compounds, alone or often combined with the ethylene bisdithiocarbamate (EBDC) fungicides, were the primary method of disease control in tomato cultivation.

However, the widespread development of copper resistance among pathogen populations has rendered these treatments increasingly ineffective in many regions. Furthermore, the potential risks associated with pesticide residues have stimulated research into alternative or supplementary disease management strategies (Lai et al. 2021, McLeod et al. 2017, Potnis et al. 2015).

Host plant resistance offers a cost-effective and efficient strategy for disease management (Zhan et al. 2014). Despite the potential benefits, bacterial spot-resistant tomato cultivars are currently commercially unavailable. Breeding programs have encountered difficulties in developing varieties with sufficient resistance (Sharma and Bhattarai 2019). This is primarily due to the pathogen's rapid evolution and the complex nature of quantitative resistance (Qiao et al. 2020). Several wild and cultivated tomato plants exhibit resistance to the bacterial speck. Most studies suggest that this trait is inherited simply (Yang and Francis 2007). Breeding tomatoes for genetic resistance to bacterial diseases is a crucial and promising endeavour (Kozik and Sobiczewski 2007).

Biodiversity is a broad term that includes the variety of life on Earth, from wild species to cultivated plants. This diversity also encompasses the genetic resources that form the foundation of our planet's genetic pool (Dal et al. 2017). Türkiye ranks among the world's leading countries regarding genetic resources and diversity. The sustainability of plant diversity depends on the effective discovery, collection, and conservation of genetic resources.

A single plant species can exhibit significant variation through its varieties and genotypes. Therefore, it's crucial to conserve plant genetic resources and to identify the varieties with the widest diversity (Gross et al. 2006, Karagöz et al. 2010, Karataş et al. 2017). In other words, to fully utilize genetic resources, it's essential to understand the range of variation within plant species (Bode et al. 2013, Che et al. 2003).

Local genotypes were formed by a process of continuous selection for superior qualities tailored to the region, coupled with successive generations of breeding and the influence of natural selection (Dal et al. 2017). For breeding studies, local varieties are crucial, and they can be successfully crossed with cultivated varieties (Eser et al. 2005). Plant breeders extensively utilize morphological and agronomic data to characterize and assess plant genetic diversity. Modern plant science involves collecting genotypes of various plant species, identifying similarities to eliminate redundancies, and creating focused core collections for breeding programs. Breeders must thoroughly understand their genetic material, including morphological, phenological, and agronomic

traits (Madakbaş and Ergin 2011). Physiological and molecular advances have made substantial contributions to accurate variety classification. Iğdır plain's low altitude and high temperatures contribute to its status as one of Türkiye's largest microclimatic regions. Both cultivated crops and native plants are represented in abundance within this area. Tomatoes are the leading edible vegetable in Iğdır plain, with an annual production of 35.217 tons cultivated across approximately 8.915 decares (TUIK 2022). Roughly one-third of the region's tomato production is attributed to the Super tomato genotype, a locally cultivated large-fruited variety renowned for its exceptional flavour and aroma (Özden and Akbaba 2023).

This study characterized the morphological traits of the Super tomato genotypes, a commercially prominent variety cultivated extensively in the Iğdır plain. Additionally, this study assessed the susceptibility of the local Super tomato genotype to both bacterial speck and bacterial spot diseases caused by *P. syringae* pv. *tomato* and *X. euvesicatoria* pv. *perforans*, respectively.

MATERIALS AND METHODS

Plant material

Super tomato plant samples were examined during the peak harvest season of 2021-2022 (June-July). Samples were collected from 20 different regions. Measurements were taken from 5 plants within each area, and the results were averaged. Testing was conducted at the Vegetable and Seed Physiology Laboratory of Iğdır University. Also, to evaluate the Super tomato genotype's response to pathogens, the H2274 variety was included as a comparative control.

Bacterial material

P. syringae pv. *tomato* DO24 (Akbaba et al. 2023) and *X. euvesicatoria* pv. *perforans* XCV2 (Akbaba et al. 2025, in press), previously characterized strains from the Iğdır University phytopathology laboratory, were used as pathogens in this study.

Collection of plant samples

Using portable scales, uprooted plants were immediately weighed in the field to minimize water loss. Weighted plant samples were transported to the laboratory between damp coarse filter paper to maintain moisture. For further analysis, the plant samples were transported in cool bags to the lab (Mumtaz et al. 2021). The identical procedure was applied to fruit samples. Morphological and physiological measurements were taken from tomato samples.

Morphological and physiological analysis

Plants were chosen to reflect the typical characteristics

of their respective regions. Five plants were examined per region.

- *Plant height (PH)*: The height of each uprooted plant was determined by measuring the distance from the root collar to the plant apex using a meter, and the results were recorded in centimeters.
- *Root length (RL)*: Root damage was minimized during the field studies. The roots were extracted by cutting at the root collar, thoroughly cleaned in water, and then measured from the root collar to the radicle tip using a meter to determine root length in centimeters.
- *Stem diameter (SD)*: The mean diameter of the main stem was determined using a digital caliper and recorded in millimeters.
- *Plant fresh weight (PFW) and Dry weight (PDW)*: The plants were cut at the crown region with a knife, and the upper part of the plant was weighed on a coarse scale in grams. The plants with calculated fresh weight were dried in ventilated ovens at 65 °C for 6 hours, and then the dry weights of the plants were calculated.
- *Root fresh weight (RFW) and dry weight (RDW)*: The plants removed from the field were cut at the crown region using a knife, and the root part of the plant was weighed on a coarse scale in grams. The roots with calculated fresh weight were dried in ventilated ovens at 65 °C for 6 hours, and then the dry weights of the roots were calculated.
- *Yield per plant (Y)*: Fruits were harvested at the pink-red stage of maturity. The total weight of fruits per plant was recorded in grams to calculate yield.
- *Fruit weight (FW)*: To calculate average fruit weight, 10 fruit samples were taken to represent the plants in that region for each replicate and weighed. The mean value was recorded in grams.
- *Fruit length (FL)*: To calculate average fruit length, 10 fruit samples were taken to represent the plants in that region for each replicate and measured longitudinally with the help of a digital caliper. The mean value was recorded in millimeters.
- *Fruit diameter (FD)*: To calculate average fruit diameter, 10 fruit samples were taken to represent the plants in that region for each replicate and measured from the equatorial parts with the help of a digital caliper. The mean value was recorded in millimeters.
- *Water-soluble dry matter content (Brix%)*: Five fruits per replicate were homogenized. The homogenate was filtered using Whatman No. 4 filter paper. The (Brix%) of the

obtained fruit juice was measured with a refractometer (Digital Abbe Refractometer).

- *Fruit juice pH measurement (pH)*: The pH of the fruit juices prepared for the water-soluble dry matter measurement was measured using a pH electrode (Titrette).
- *Titrateable acidity (TA)*: It was determined by titrating 10 ml of fruit juice with a 0.1 N sodium hydroxide (NaOH) solution until the pH value reached 8.1 using a pH meter. The results were calculated as citric acid based on the amount of base (NaOH) used and expressed as a percentage (Cemeröglü 1992).
- *Determination of ascorbic acid (Vitamin C)*: 100 g of the fruit sample was mixed with an equal amount of 2% oxalic acid solution and homogenized. Then, 30 g of this sample was taken and diluted to 100 ml with 2% oxalic acid solution. After vortexing the samples, they were filtered. From the filtered samples, 10 ml was taken and titrated with a 2,6-dichlorophenolindophenol solution until a pink color appeared. The amount of ascorbic acid was calculated using the formula below (Cemeröglü 1992).

$$\text{Ascorbic acid (mg/100g)} = V \times F \times 100 \times W \quad (2)$$

V: The amount of 2,6-dichlorophenolindophenol solution used in the titration (ml)

F: The factor of the 2,6-dichlorophenolindophenol solution, i.e., the amount of ascorbic acid (mg) equivalent to 1 ml of this solution

W: The amount of sample contained in the filtrate used in the titration (g)

- *Determination of lycopene and β -carotene*: 1 g tomato samples were homogenized for 5 minutes with a 16 ml mixture of acetone (4:6) in a homogenizer. The hexane phase at the top of the prepared extraction was collected using a micropipette, and readings were taken at wavelengths of 663, 645, 505, and 453 nm using a spectrophotometer. After completing the readings, the amounts of lycopene and β -carotene were calculated according to Nagata and Yamashita (1992) and the results were expressed as mg/100g.

Experimental design for the control and pathogen treatments

In-pot experiments were conducted in the growth chamber. Sterile peat was used as a growth medium in 0.38 liter disposable thermoform pots (8x8x9 cm). Seeds were sown in these pots and placed in the growth chamber. Throughout the experiment, tomato plants were maintained in a controlled environment (for approximately 45 days)

with a 16-hour light, 8-hour dark photoperiod, 60% relative humidity, and a day/night temperature regime of 24 °C and 20 °C, respectively. Tomato seedlings were inoculated with pathogenic bacteria at the 3-5 leaf stage (day 25). The experimental design consisted of a randomized complete block arrangement with five replications, each containing a single plant. This experiment was replicated twice.

Bacterial strain cultivation, inoculum preparation, and disease assessment

In this study, Pst strain DO24 and Xep strain XCV2 were used as pathogenic bacterial strains. Bacterial cultures stored at -80 °C were streaked onto Nutrient Agar (NA) in petri dishes and incubated at 24 ± 2 °C for 48–72 h. The grown bacterial colonies were stored at 4 °C for subsequent experiments. Inoculum for tomato seedlings, prepared by suspending bacterial colonies in sterile distilled water from NA medium growth for 48-72 hours at 24 ± 2 °C. Bacterial suspensions were adjusted to OD600nm: 0.2 for Pst and 0.1 for Xep (approximately 108 and 107 CFU/ml, respectively) using spectrometry. One or two drops of Tween 20 surfactant were added to the bacterial suspensions. Plants were inoculated by spraying bacterial suspensions onto the undersides of fully developed leaves. To maintain high humidity, the treated seedlings were placed in polyethylene bags and the environment was adjusted to a relative humidity of 80-90%. Following a 48-hour incubation period in polyethylene bags, the plants were transferred back to the growth chamber, where they were maintained at approximately 60% relative humidity. Tomato seedlings were treated with distilled water as the negative group (-), and those inoculated solely with the pathogenic bacterial suspension were considered the positive control group (+). Standard variety H2274 was used as a control for comparing Super tomato disease resistance. Fourteen days after inoculation with Pst, disease severity was evaluated using a modified Chambers and Merriman (1975) disease rating 0-4 scale (0= no lesions, 1= 1-10 lesions, 2= 11-20 lesions, 3= 21-40 lesions, 4= 40 and more for per plant) (Ekici and Baştaş 2014). The disease symptoms were evaluated on a scale of 0-4 based on the scale described by Al-Dahmani et al. (2003) with a slight modification on the 21st day after Xep's inoculation. The modified scale is as follows: 0= symptomless, 1= one to five lesions per leaflet, 2 = many lesions and some coalesced lesions, 3 = coalesced lesions and some necrotic leaflets, and 4 = dead leaflets. The DSI value was calculated from the sum of the data classified by scale values obtained from five replicates (two experiments n=10) divided by the replication number for each cultivar (Eenink 1981). Ekici and Baştaş (2014) described 5 resistance classes

following: Resistant; R (DSI:0), Moderately Resistant; MR (DSI:1), Moderately Susceptible; MS (DSI: 2), Susceptible; S (DSI:3) and Highly Susceptible; HS (DSI:4).

Plant growth analysis

For both disease-inoculated and control plants, leaf number (LN), plant fresh weight (PFW), plant dry weight (PDW), root fresh weight (RFW), and root dry weight (RDW) were determined using previously described methods. Chlorophyll concentration (CC) was measured using a portable chlorophyll meter (Minolta SPAD-502+) (Fischer 2001). Chlorophyll content was determined by taking three replicate measurements from the lower, middle, and upper leaves of each plant under bright afternoon light conditions (14:00-16:00). The average SPAD value for each plant was calculated by combining data from the three-leaf positions and the replicate measurements.

The efficacy (%) of pathogen infection on growth parameters of different tomato cultivars is also calculated according to Abbott's formula* (Akbaş et al. 2009) as follows:

*Efficacy (%) = $(C - T) / C \times 100$ Where C refers to the measurement of the control (-), and T refers to the measurement of the relevant treatment (*Pst* or *Xep* for this study).

Statistical analysis

The experimental groups were compared using one-way variance analysis coupled with Duncan's multiple range test ($P < 0.05$) (SPSS 26 Package program).

RESULTS

Morphological outputs

Examination of Super tomato genotypes collected from 20 different regions within the Iğdır plain revealed substantial variation in vegetative characteristics. Plant height (PH) exhibited regional variation, ranging from 136.9 to 88.7 cm across regions. The Akyumak region exhibited the maximum plant height, whereas the Bayraktutan region displayed the minimum plant height value. Root length (RL) exhibited variation among genotypes, with measurements spanning from 69.0 cm to 46.3 cm. Similar to the pattern observed for plant height, the Akyumak region displayed the maximum root length value, whereas Taşburun, Yüzbaşılar, Özdemir, and Bayraktutan regions exhibited the minimum root length values. The stem diameter (SD) varied between 2.17 cm and 1.52 cm. The largest stem diameter values were measured in the Akyumak, Obaköy, Yaycı and Küllük regions, while the smallest stem diameter values were determined in the Taşburun, Yüzbaşılar, Özdemir, Hakmehmet and Bayraktutan regions (Table 1).

In addition to length measurements, plant fresh and dry weights were determined. Plant fresh weight (PFW) ranged from 596 g to 426 g. Akyumak had the highest fresh weight, while Bayraktutan had the lowest. Plant dry weights (PDW) varied between 127.6 and 94.0 among the regions. The highest PDW was measured in Obaköy, Akyumak and Küllük, and the lowest in Taşburun, Yüzbaşılar, Özdemir and Bayraktutan. Root fresh weights (RFW) ranged from 74.5 g to 51.8 g. Akyumak and Obaköy had the highest root fresh weight, while Taşburun had the lowest. Root dry weights (RDW) ranged from 24.3 g to 11.9 g. Akyumak and Obaköy had the highest DWW values, while Taşburun, Hakmehmet, and Bayraktutan had the lowest (Table 1).

Fruit characteristics exhibited significant variation among regions. The average yield (per plant) varied between the sites from 4717.3 g to 2906.5 g. Akyumak had the highest yield, while Bayraktutan had the lowest. Average fruit weights (FW) varied between 385.2-223.7 g. The highest fruit weight was determined in the Akyumak and Alikamerli regions, while the lowest was determined in the Bayraktutan region (Table 2). Fruit lengths (FL) varied between 55.5 and 50.3 mm depending on the region. The Küllük region had the highest fruit length, while the Hakveyis region had the lowest. Fruit diameters (FD) varied between 81.1 and 96.0 mm depending on the region. Fruit diameter (MD) was highest in the Akyumak, Obaköy, Yaycı, and Alikamerli regions, but lowest in the Taşburun, Mirhanlı, and Bayraktutan regions (Table 2).

Physiological outputs

In addition to yield parameters, some physiological properties of the fruits were measured. Water-soluble dry matter content (Brix%) ranged from 4.11% to 4.55% across regions. Evcı and Obaköy had the highest water-soluble dry matter content (Brix%), while Alikamerli, Kasımcan, Kuzugüden, and Bayraktutan had the lowest. Fruit pH ranged from 4.43 to 4.69. The Yaycı region had the highest pH value, while Taşburun and Mirhanlı regions had the lowest. Titratable fruit acidity (TA) ranged from 2.93 to 3.16 mval 100 ml⁻¹. The Özdemir region had the highest titratable acidity, while the Taşburun region had the lowest (Table 3). There is a wide variation in vitamin C (VitC) content between regions. Vitamin C (VitC) content ranged from 17.80 to 26.63 mg/100g. The Küllük region had the highest vitamin C content, while the Zülfikar region had the lowest. Lycopene content ranged from 2629.5 to 2951.1 ng/µl. The Evcı and the Obaköy regions had the highest lycopene content, while the Hakmehmet region had the lowest. Beta-carotene (β-carotene) content ranged from 228.82 to 272.55

Table 1. Vegetative growth parameters (plant height, root length, stem diameter, fresh and dry weights of plant and root) of Super tomato genotypes across different regions

Genotype	PH (cm)	RL (cm)	SD (cm)	PFW (g)	PDW (g)	RFW (g)	RDW(g)
Taşburun	91.1 lm ±2.8	46.9 j ±0.7	1.52 h ±0.017	444.4 m ±3.9	95.6 h ±0.9	51.8 j ±0.8	12.6 g ±0.4
Mirhanlı	104.5 i ±0.6	50.9 i ±0.6	1.68 fg ±0.015	494.7 i ±2.1	102.1 g ±1.0	60.5 fg ±0.4	15.5 f ±0.2
Zülfikar	111.8 gh ±0.8	53.5 fg ±0.8	1.79 de ±0.015	520.6 fg ±1.6	105.5 fg ±0.4	64.7 de ±1.2	17.9 de ±0.4
Evcı	115.8 ef ±1.3	58.6 d ±0.8	1.80 de ±0.023	522.4 fg ±2.9	107.5 ef ±1.1	69.2 c ±0.4	19.3 cd ±0.5
Pinazar	98.8 j ±0.4	51.9 g-i ±0.4	1.61 gh ±0.021	477.8 j ±1.5	102.4 g ±1.4	56.0 hi ±1.3	15.2 f ±0.3
Melekli	113.9 fg ±0.9	55.7 e ±0.5	1.76 d-f ±0.024	524.8 f ±1.9	105.5 fg ±0.6	68.3 cd ±1.0	18.2 d ±0.3
Akyumak	136.9 a ±0.8	69.0 a ±0.1	2.14 a ±0.045	596.0 a ±3.6	125.6 a ±0.6	74.3 a ±0.5	22.9 a ±0.9
Enginalan	117.1 e ±1.1	61.5 c ±0.4	1.85 cd ±0.021	529.6 f ±2.2	110.2 de ±0.3	69.8 bc ±0.8	21.1 b ±0.4
Yüzbaşılar	95.0 k ±0.8	46.6 j ±0.8	1.54 h ±0.047	453.4 l ±2.0	94.0 h ±0.8	55.3 h-j ±0.3	16.0 f ±0.1
Özdemir	93.1 kl ±0.4	47.6 j ±0.9	1.57 h ±0.039	468.3 k ±3.6	97.6 h ±2.6	55.1 ij ±4.0	15.1 f ±0.2
Hakveysi	109.2 h ±0.6	51.2 hi ±0.6	1.71 ef ±0.018	515.4 g ±2.4	111.4 de ±0.9	63.7 ef ±0.7	16.5 ef ±0.3
Obaköy	130.9 b ±0.4	67.4 a ±1.2	2.17 a ±0.052	575.0 c ±1.7	125.2 a ±0.6	74.5 a ±0.9	24.3 a ±1.3
Yaycı	122.7 cd ±0.6	64.8 b ±0.3	2.13 a ±0.038	559.8 d ±2.6	117.5 bc ±1.2	69.5 c ±0.8	20.6 bc ±0.4
Alikamerli	121.0 d ±0.8	62.6 bc ±0.2	1.97 b ±0.042	559.5 d ±2.9	118.7 b ±1.0	66.4 c-e ±0.4	19.4 cd ±0.2
Kasımcan	117.4 e ±0.2	60.9 c ±0.8	1.93 bc ±0.042	542.0 e ±1.7	114.0 cd ±0.8	65.3 de ±0.6	19.0 d ±0.3
Kuzugüden	105.7 i ±0.9	53.2 gh ±1.3	1.60 gh ±0.026	521.8 fg ±2.0	108.5 ef ±0.5	63.1 ef ±1.0	15.5 f ±0.3
Hakmehmet	102.8 i ±0.7	49.8 i ±0.3	1.57 h ±0.025	481.1 j ±3.9	101.8 g ±1.1	58.9 gh ±0.5	13.0 g ±0.3
Küllük	124.3 c ±1.0	63.8 b ±0.8	2.13 a ±0.026	584.5 b ±3.5	127.6 a ±1.1	73.1 ab ±0.8	20.7 bc ±0.3
Çarıklı	112.0 gh ±0.8	55.4 ef ±1.1	1.76 d-f ±0.018	503.4 h ±2.2	105.2 fg ±1.4	65.5 de ±1.0	18.2 d ±0.4
Bayraktutan	88.7 m ±1.2	46.3 j ±0.5	1.57 h ±0.012	426.0 n ±5.3	96.6 h ±4.0	53.0 ij ±0.9	11.9 g ±0.7
Mean	110.63	55.88	1,79	515.02	108.63	63.90	17.64

Means with different letters in the same column denote significant difference at P <0.05. The error bars represent ± SEM. ns: non-significant.

Table 2. Yield (per plant), fruit weight, length, and diameter of Super tomato genotypes across different regions

Genotype	Y (g)	FW (g)	FL (mm)	FD (mm)
Taşburun	3069.9 j ±34.0	249.4 k ±1.8	51.2 gh ±0.6	81.1 g ±0.2
Mirhanlı	3562.7 h ±13.1	306.9 gh ±1.8	52.7 e ±0.1	81.0 g ±0.3
Zülfikar	4276.7 de ±25.6	319.1 f ±1.6	52.4 ef ±0.2	87.7 e ±1.1
Evcı	4479.7 bc ±23.4	337.7 de ±6.9	55.1 ab ±0.1	91.7 c ±0.3
Pinazar	3323.1 i ±17.5	288.3 i ±4.0	51.0 gh ±0.3	84.1 f ±0.3
Melekli	4239.4 e ±21.5	329.2 e ±3.0	52.5 e ±0.4	93.5 b ±0.2
Akyumak	4717.3 a ±34.2	385.2 a ±5.8	54.0 cd ±0.4	96.0 a ±0.3
Enginalan	4319.2 de ±10.8	340.6 d ±5.1	53.2 de ±0.2	91.5 c ±0.3
Yüzbaşılar	3101.9 j ±8.1	270.0 j ±0.9	52.7 e ±0.3	83.2 f ±0.5
Özdemir	3147.2 j ±27.4	277.8 j ±1.2	51.4 fg ±0.3	83.2 f ±0.1
Hakveyis	4261.7 de ±29.1	314.0 fg ±1.2	50.3 h ±0.5	89.5 d ±0.5
Obaköy	4531.2 b ±19.3	364.7 bc ±4.5	54.6 a-c ±0.4	96.4 a ±0.3
Yaycı	4326.9 d ±13.8	369.2 b ±0.6	54.3 bc ±0.3	96.0 a ±0.5
Alikamerli	4346.0 d ±47.4	382.3 a ±3.6	53.9 cd ±0.1	95.8 a ±0.1
Kasımcın	4073.5 f ±33.9	359.7 bc ±2.5	54.7 a-c ±0.3	94.0 b ±0.1
Kuzugüden	4096.3 f ±21.9	313.3 fg ±1.3	52.3 ef ±0.1	89.6 d ±0.5
Hakmehmet	3771.7 g ±35.2	303.0 h ±2.5	51.2 gh ±0.3	83.6 f ±0.2
Küllük	4450.5 c ±32.1	355.0 c ±2.8	55.5 a ±0.2	93.7 b ±0.9
Çarıklı	4316.2 de ±18.5	316.0 fg ±1.1	54.1 cd ±0.4	91.2 c ±0.3
Bayraktutan	2906.5 k ±30.0	223.7 l ±2.8	50.6 gh ±0.4	81.6 g ±0.3
Mean	3965.87	320.25	52.88	89.22

Means with different letters in the same column denote significant difference at $P < 0.05$. The error bars represent \pm SEM. ns: non-significant.

Table 3. Regional distribution of water-soluble dry matter content, pH, titratable fruit acidity, vitamin C, Lycopene and β -Carotenoid contents in Super tomato fruits

Genotype	Brix (%)	pH	TA (mval 100 ml ⁻¹)	VitC (mg 100 g ⁻¹)	Lycopene (ng/ μ l)	β -carotene (ng/ μ l)
Taşburun	4.36 de ± 0.017	4.43 l ± 0.038	2.93 h ± 0.012	20.95 h ± 0.61	2648.1 hi ± 14.2	232.49 j ± 0.4
Mirhanlı	4.37 d ± 0.015	4.43 l ± 0.012	3.04 ef ± 0.007	19.62 jk ± 0.15	2684.4 h ± 4.1	239.22 i ± 0.4
Zülfikar	4.22 f ± 0.015	4.53 i-k ± 0.018	3.04 ef ± 0.006	17.80 n ± 0.09	2793.7 ef ± 6.5	240.20 i ± 0.9
Evcı	4.60 a ± 0.015	4.62 cd ± 0.015	3.08 cd ± 0.001	25.61 b ± 0.20	2951.1 a ± 9.4	271.82 a ± 0.7
Pinazar	4.20 f ± 0.015	4.50 k ± 0.012	3.05 ef ± 0.009	18.19 mn ± 0.22	2757.1 fg ± 23.7	248.82 gh ± 0.2
Melekli	4.35 de ± 0.003	4.59 d-g ± 0.003	3.11 b ± 0.007	22.61 fg ± 0.29	2908.3 bc ± 4.7	265.07 b ± 0.5
Akyumak	4.44 bc ± 0.018	4.57 e-h ± 0.012	2.97 g ± 0.012	24.33 d ± 0.17	2862.0 d ± 12.1	262.32 c ± 0.6
Enginalan	4.46 b ± 0.026	4.55 g-i ± 0.015	2.95 gh ± 0.003	25.61 b ± 0.25	2939.0 ab ± 2.9	272.55 a ± 1.4
Yüzbaşılar	4.35 de ± 0.003	4.51 jk ± 0.009	3.09 b-d ± 0.007	20.05 ij ± 0.05	2734.9 g ± 9.3	232.42 j ± 0.7
Özdemir	4.31 e ± 0.012	4.53 i-k ± 0.001	3.16 a ± 0.010	18.94 kl ± 0.28	2668.1 hi ± 18.9	228.82 k ± 0.6
Hakveyis	4.25 f ± 0.015	4.61 c-e ± 0.012	3.02 f ± 0.023	22.82 ef ± 0.42	2853.4 d ± 20.6	253.41 e ± 0.3
Obaköy	4.55 a ± 0.015	4.64 bc ± 0.007	3.10 bc ± 0.009	25.09 bc ± 0.10	2946.3 a ± 7.6	265.48 b ± 0.6
Yaycı	4.44 bc ± 0.024	4.69 a ± 0.003	3.04 ef ± 0.003	24.52 cd ± 0.08	2864.0 d ± 11.3	253.29 e ± 0.7
Alikamerli	4.14 g ± 0.032	4.55 g-i ± 0.012	3.11 b ± 0.012	20.62 hi ± 0.08	2784.6 ef ± 9.7	259.83 d ± 0.2
Kasımcan	4.12 g ± 0.026	4.56 f-i ± 0.012	3.06 de ± 0.003	20.65 hi ± 0.10	2806.3 e ± 5.3	248.08 h ± 0.1
Kuzugüden	4.13 g ± 0.017	4.66 ab ± 0.009	2.93 h ± 0.007	20.19 ij ± 0.34	2882.4 cd ± 2.1	250.90 f ± 0.5
Hakmehmet	4.20 f ± 0.033	4.57 e-h ± 0.003	3.04 ef ± 0.006	22.03 g ± 0.26	2629.5 i ± 15.4	250.53 fg ± 0.5
Küllük	4.38 cd ± 0.015	4.67 ab ± 0.007	2.97 g ± 0.015	26.63 a ± 0.13	2903.2 bc ± 3.2	259.67 d ± 0.1
Çarıklı	4.45 b ± 0.020	4.61 c-e ± 0.003	3.04 ef ± 0.018	23.35 e ± 0.13	2772.1 e-g ± 8.6	247.79 h ± 0.4
Bayraktutan	4.11 g ± 0.003	4.54 h-k ± 0.013	3.09 b-d ± 0.003	18.75 lm ± 0.07	2661.1 hi ± 23.2	252.33 ef ± 0.5
Mean	4,32	4,57	3,04	21,92	2802,49	251,75

Means with different letters in the same column denote significant differences at $P < 0.05$. The error bars represent \pm SEM. ns: non-significant.

ng/μl across regions. The Evci and the Enginalan regions had the highest beta-carotene content, while the Özdemir region had the lowest (Table 3).

Reaction of the Super tomato genotype to Pst and Xep

The Super tomato genotype (Küllük) and H2274 cultivar were evaluated for disease severity in this study 14 days after Pst and 21 days after Xep inoculation (Figure 1).



Figure 1. Disease symptoms in Super tomato genotype following *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*) inoculation: a: control (-), b: Pst (+), c: bacterial speck on tomato, d: control (-), e: Xep (+), f: bacterial spot on tomato

H2274 and the Super tomato genotype both exhibited moderate susceptibility to Pst infection (DSI: 2.7 and 2.4, respectively). However, H2274 demonstrated moderate resistance to Xep infection (DSI: 1.3), while the Super tomato remained moderately susceptible (DSI: 2.2) (Table 4).

Effects of pathogens on plant growth parameters

It was investigated the impact of *Pst* and *Xep* on tomato plant growth parameters, including leaf number (LN), plant and root fresh (PFW, RFW) and dry weights (PDW, RDW), and chlorophyll content (C) in this study. Both Super tomato and H2274 genotypes exhibited significant reductions in plant growth parameters following Pst infection compared to healthy controls. Super tomato displayed decreases of 8.06% (LN), 28.03% (SFW), 35.25% (SDW), 39.77% (RFW), 50.57% (RDW), and 9.37% (C) at a disease index of 2.4. H2274 showed more severe reductions, with decreases of 20.31% (LN), 50.86% (SFW), 35.75% (SDW), 42.17% (RFW), 57.21% (RDW), and 1.4% (C) at a disease index of 2.7 (Table 5). Super tomato genotypes infected with Xep (disease index: 2.2) exhibited significant reductions in leaf number (1.32%), plant fresh weight (18.06%), plant dry weight (46.18%), root fresh weight (41.28%), root dry weight (25.98%), and chlorophyll content (0.95%) compared to healthy controls. H2274 plants with a disease index of 1.3 showed decreases in SDW (1.61%) and RDW (2.46%), but increases in LN (1.35%), SFW (13.63%), RFW (21.11%), RDW (2.46%), and C (2.67%) (Table 6).

DISCUSSION

This study examined the plant characteristics of Super tomato genotypes collected from 20 regions in the Iğdır Plain. In the study conducted in Iğdır Plain, when parameters such as plant height, root length, and stem diameter were examined, higher plant development values were reached in the central regions of the plain. These values were lower in the eastern, northern and western parts of the plain. Plant weights (SFW, SDW, RFW, RDW) were higher in the central and southern than in other parts of the plain (Table 1). Healthy plants typically exhibit optimal vegetative growth and root development. Özenç and Şen (2017) reported tomato plant heights ranging from 193 cm to 156 cm in their study. In the other study, Tezcan et al. (2022) found plant height, stem diameter, and root projection area to be 35-20 cm, 12.3-9.1 mm, and 1405-322 cm², respectively. Super tomato

Table 4. Disease severity index (DSI) values of tested tomato genotypes/varieties and number of plants in infection class (0-4)

Pathogens	Genotype	Number of plants in infection class					DSI	Resistance classes*
		0	1	2	3	4		
<i>Pst</i> strain	Süper			6	4		2.4	Moderately Susceptible
DO24	H2274			3	7		2.7	Moderately Susceptible
<i>Xep</i> strain	Süper			8	2		2.2	Moderately Susceptible
XCV2	H2274		7	3			1.3	Moderately Resistant

* Resistance classes described by Ekici and Baştaş (2014); *0: Resistant; 1: Moderately Resistant, 2: Moderately Susceptible, 3: Susceptible, 4: Highly Susceptible

Table 5. Effects of *Pseudomonas syringae* pv. *tomato* (Pst) inoculation on tomato plant growth parameters, including leaf number (LN), plant and root fresh (SFW, RFW) and dry weights (SDW, RDW), and chlorophyll content (C)

Genotype	Pathogens*	LN (Adet)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	C (g)
Super	<i>Pst</i> strain DO24	5.7	4.06	0.24	0.32	0.02	25.45
	C (-)	6.2	5.64	0.37	0.53	0.04	28.08
	Efficacy (%) **	-8.06	-28.03	-35.25	-39.77	-50.57	-9.37
H2274	<i>Pst</i> strain DO24	5.1	4.17	0.24	0.38	0.02	24.67
	C (-)	6.4	8.49	0.37	0.66	0.04	25.02
	Efficacy (%) **	-20.31	-50.86	-35.75	-42.17	-57.21	-1.4

* Data were collected 14 days post-inoculation. Results represent the mean of 10 observations (5 replicates x 2 experiments).

** A negative sign (-) was used to indicate a reduction in plant parameters compared to the pathogen-free control.

Table 6. Effects of *Xanthomonas euvesicatoria* pv. *perforans* (Xep) inoculation on tomato plant growth parameters, including leaf number (LN), plant and root fresh (SFW, RFW) and dry weights (SDW, RDW), and chlorophyll content (C)

Genotype	Pathogens*	LN (Adet)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	C (g)
Süper	<i>Xep</i> strain XCV2	7.70	12.11	0.57	0.64	0.07	30.31
	C (-)	7.60	14.78	1.06	1.08	0.10	30.60
	Etki (%) **	1.32	-18.06	-46.18	-41.28	-25.98	-0.95
H2274	<i>Xep</i> strain XCV2	7.50	15.32	0.73	1.04	0.07	26.88
	C (-)	7.40	13.48	0.75	0.86	0.07	26.18
	Etki (%) **	1.35	13.63	-1.61	21.11	-2.46	2.67

* Data were collected 21 days post-inoculation. Results represent the mean of 10 observations (5 replicates x 2 experiments).

** A negative sign (-) was used to indicate a reduction in plant parameters compared to the pathogen-free control.

genotypes cultivated on the Iğdır plain exhibit significantly larger fruit and more robust, expansive plants compared to those reported in the literature.

While commercial producers prioritize hybrid tomatoes for yield and quality, consumers increasingly favor local genotypes due to perceived taste, naturalness, and support for local producers. Our research findings show that the average yield per plant is 3965.9 g. In the literature, yield values per plant in tomatoes vary considerably. According to Özbay and Ateş (2015), this value is between 7.02-2.44 kg, while Tosun and Aktaş (2022) reported that this range is 4.89-1.20 kg. Our results indicated that the Super tomato is a medium-yielding genotype with consistent yield performance, as reflected by the narrow range between maximum (4717.3 g) and minimum (2906.5 g) yields. Additionally, the average fruit weight across all regions was determined to be 320.3 g. Researchers reported that fruit weights of tomato genotypes varied between 118.5-55.3 g (Paksoy 2003), 332.45 -18.18 g (Turhan and Şeniz 2009), 324.25-15.5 g (Aoun et al. 2013), 58.67-22.33 g (Kathayat et al. 2015), 112.50-47.16 g (Singh and Goswami 2015) and 529.56-60.22 g (Tosun and

Aktaş 2022). Based on our findings, the Iğdır Super tomato genotype can be characterized by its relatively large fruit size. Fruit diameters (FD) among Super tomato genotypes ranged from 96.0 mm to 81.1 mm across different regions. Aydın and Aktaş (2023) reported that fruit length in cherry and cocktail tomato genotypes varied between 57.59 mm and 23.42 mm, while fruit diameter ranged from 52.1 mm to 18.1 mm. Super tomato genotypes generally produced large, beef-type fruit with diameters exceeding length. This is a common characteristic of large-fruited species (Renna et al. 2019).

The Brix level is a crucial factor in classifying tomato varieties as either table or industrial. Our results indicate a regional average Brix of 4.32%, classifying this as a table variety. The Iğdır Super tomato's market share is diminishing due to its excessive juiciness and rapid spoilage resulting from its delicate skin. Previous studies on tomato Brix ratios reported a range of 8.6-3.6% (Hanson et al. 2004), 4.36-3.96% (Giorio et al. 2007), 5.98-4.36% (Al-Aysh et al. 2012), 6.03-3.50% (Pal et al. 2018), 4.71-3.12% (Raj et al. 2018), and 4.91-2.50% (Tosun and Aktaş 2022).

The pH level of fruit juice significantly impacts its overall flavor profile, contributing to both taste and aroma perception. Previous studies have reported pH values for tomatoes ranging from 4.58 to 4.37 (Figueiredo et al. 2017), 4.6 to 4.1 (Liu et al. 2017), and 4.49 to 4.24 (Peixoto et al. 2018). Our findings align with previous research, indicating a slightly acidic fruit juice pH range of 4.69-4.43.

Tomatoes are rich in vitamin C, whose content can fluctuate based on various factors, and are also a significant source of the antioxidant pigments lycopene and beta-carotene, responsible for their color and known for their phenolic properties. These phytochemicals may vary widely depending on cultivar, cultivation methods, and environmental conditions. Aydın and Aktaş (2023) reported vitamin C, lycopene, and β -carotene contents in tomato fruits ranging from 60.0-4.9 mg/100 g, 18.6-0.31 mg/100 g, and 6.29-0.75 mg/100 g, respectively. Average vitamin C, lycopene, and β -carotene contents in tomato fruits are reported to range from 67-15 mg/100 g, 25-0.5 mg/100 g, and 6.2-0.3 mg/100 g, respectively (Felföldi et al. 2022, Renna et al. 2019). Compared to literature values, Super tomato genotypes from the Iğdır plain exhibited typical vitamin C levels but were notably high in lycopene and β -carotene.

Our study investigated the vegetative characteristics of the local Super tomato genotype and evaluated its susceptibility to bacterial spot and speck diseases. The local genotype of Super tomato exhibited moderate susceptibility to *Xep*, whereas the H2274 variety demonstrated moderate resistance. A field study in Mysore, India, screened 20 tomato cultivars for resistance to bacterial spot disease caused by *X. axonopodis* pv. *vesicatoria*. Cultivars were categorized as highly resistant (Safal), resistant (Indam, Vignesh, Rasi, Pradhan, Naveen, Pioneer seeds), susceptible (Rukshita, Marglobe, PKM-1, Rohini, SCL-4, Utsav, Leadbeter, Arka vikas), and highly susceptible (Madanapalli, Heemsona, Vajra, Amar, Golden). These twenty cultivars exhibited varying levels of resistance to the pathogen. This variation correlated with the activation of cinnamyl alcohol dehydrogenase (CAD), a key enzyme in plant defence. The researchers emphasized CAD's direct role in lignification and its contribution to bacterial spot resistance (Umesha and Kavitha 2011). Tomato varieties with complete resistance to bacterial spot disease remain elusive. Breeding programs have had limited success in developing acceptably resistant cultivars (Sharma and Bhattarai 2019), primarily due to the emergence of new pathogen strains that overcome existing resistance genes and the complex genetic nature of resistance (Hutton et al. 2010).

Both the local Super tomato genotype and the H2274 variety exhibited moderate susceptibility to infection by *Pst*. A study conducted in the Aegean region of Türkiye reported varying bacterial spot resistance levels among tomato varieties. Marmara and 144 were classified as highly resistant, Beril and Selin as moderately resistant, Dorit and 5656 as susceptible, and Newton as highly susceptible (Bakır et al. 2012). An investigation of 50 tomato varieties cultivated in Türkiye's Mediterranean and Central Anatolia regions reported 15 carrying the *Pto* gene, conferring resistance to *Pst*. These varieties include T-6, Kutlu, OD-8, Impala, H2274, 144, Gülhan, OD-5, Gözde, T-3, Erdem, Ebia, Konya, Çiğdem, and Natura sırk. Despite carrying the *Pto* resistance gene, the H2274 variety exhibits susceptibility to *Pst* (Ekici and Baştaş 2014). Kozik (2002) observed numerous necrotic lesions on tomato varieties possessing the *Pto* gene. Previous findings indicate that tomato resistance to *Pst* is a complex trait controlled by multiple genes rather than a single gene (Roberts 2002). The study also assessed the impact of disease agents on tomato plant growth, examining parameters such as leaf numbers, plant and root fresh and dry weights, and chlorophyll content.

The moderately susceptible Super tomato genotype exhibited significant reductions in leaf number, plant mass, root biomass, and chlorophyll content following both pathogen infections. The H2274 variety, being moderately susceptible to *Pst* to our findings, experienced significant reductions in leaf number, plant, and root weight following pathogen infection. However, the H2274 variety's moderate resistance to *Xep* mitigated its negative impact on plant growth parameters. These findings demonstrate a clear correlation between varietal resistance levels and their effect on plant growth parameters when challenged by pathogen infection. These diseases alter the host's physiology, biochemistry, and structure, resulting in changes in plant phenotypes (e.g., decreased photosynthetic capacity of diseased foliage, defoliation, flower abortion, and fruit lesions). Ultimately, they result in yield reductions of susceptible varieties due to the damage caused to plants and fruits (Reis Pereira et al. 2023).

Plant genetic resources serve as a crucial repository for genes conferring resistance to diseases and pests, enabling the development of superior crop cultivars (Salgotra and Chauhan 2023). Local plant genotypes such as Super tomatoes constitute a valuable genetic reservoir for developing crop varieties with enhanced disease and pest resistance, as well as increased yield. This study will contribute significantly to tomato breeding programs in terms of developing productive, disease-resistant and consumer-preferred varieties. Further studies should be conducted in the field or in the greenhouse to confirm these findings.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmada Iğdır Ovası'nda yaygın olarak yetiştirilen Süper domates genotiplerinin morfolojik özelliklerinin karakterize edilmesi ve Süper domates genotipinin *Pseudomonas syringae* pv. *tomato* (*Pst*) ve *Xanthomonas euvesicatoria* pv. *perforans* (*Xep*)'in neden olduğu bakteriyel hastalıklara reaksiyonunun belirlenmesi amaçlanmıştır. Süper domates genotipleri, 2021-2022 hasat sezonunda 20 farklı bölgeden toplanmıştır. Bitki özelliklerini karakterize etmek için domates genotiplerinden laboratuvarında morfolojik ve fizyolojik ölçümler alınmıştır. Ayrıca, Süper domatesin *Pst* ve *Xep* enfeksiyonuna reaksiyonunu değerlendirmek için bitki yetiştirme odasında saksı denemeleri gerçekleştirilmiştir. Analizlerin sonucunda, bitki morfolojisi ve büyümesine ilişkin veriler elde edilmiştir. Bu veriler arasında bitki boyları (136.9-88.7 cm), kök uzunlukları (69.0-46.3 cm), gövde çapları (2.17-1.52 cm), bitki taze ağırlıkları (596-426 g), bitki kuru ağırlıkları (127.6-94.0 g), kök taze ağırlıkları (74.5-51.8 g), kök kuru ağırlıkları (24.3-11.9 g), bitki başına verim (4717.3-2906.5 g), ortalama meyve ağırlığı (385.2-223.7 g), meyve uzunluğu (55.5-50.3 mm) ve ortalama meyve çapı (96.0-81.1 mm) ölçümleri yer almıştır. Domates meyvelerinin fizyolojik özellikleri açısından, suda çözünür kuru madde içeriği %4.55 ile %4.11 arasında, meyve suyu pH'ı 4.69 ile 4.43 arasında, titrasyon asitliği 3.16 ile 2.93 mval 100 ml⁻¹ arasında, C vitamini içeriği 26.63 ile 17.80 mg/100 g arasında, likopen içeriği 2951.1 ile 2629.5 ng/µl arasında ve β-karoten içeriği 272.55 ile 228.82 ng/µl arasında değişmiştir. Ayrıca, saksı denemeleri, Süper domates genotipinin hem *Pst* hem de *Xep* enfeksiyonlarına karşı orta düzeyde duyarlılık gösterdiğini, hastalık şiddeti endekslerinin (DSI) sırasıyla 2.4 ve 2.2 olduğunu göstermiştir.

Anahtar kelimeler: Süper domates, bakteriyel leke, bakteriyel benek, *Xep*, *Pst*, Iğdır.

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Original article

Foliar diseases of barley and wild barleys in Siirt Province, Türkiye

Siirt ilinde arpa ve yabani arpalarda görülen yaprak hastalıkları

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ABSTRACT

In May 2023, surveys were conducted in the barley growing areas of the Central district, Baykan, Kurtalan, Pervari, Şirvan, Eruh, and Tillo districts of Siirt province, Türkiye. Leaf diseases occurring in barley and wild barley (*Hordeum spontaneum*, *Hordeum bulbosum*) plants were determined. On barley plants, net and spot forms of net blotch disease caused by *Pyrenophora teres* f. *teres*, and *Pyrenophora teres* f. *maculata*, powdery mildew caused by *Blumeria graminis* f. sp. *hordei*, scald caused by *Rhynchosporium commune*, barley stripe caused by *Pyrenophora graminea* and spot blotch disease caused by *Cochliobolus sativus* were detected. On wild barley plants, the net form of net blotch disease caused by *Pyrenophora teres* f. *teres*, spot form of net blotch disease caused by *Pyrenophora teres* f. *maculata*, powdery mildew caused by *Blumeria graminis* f. sp. *hordei*, scald caused by *Rhynchosporium commune*, barley stripe caused by *Pyrenophora graminea* and brown rust disease caused by *Puccinia hordei* were found.

INTRODUCTION

Barley (*Hordeum vulgare*) is a cool-season cereal resistant to cold and arid conditions, and it can be grown even in nutrient-poor soils (Mathre 1997). Barley, a cereal with high feed value, is primarily used in animal nutrition. Additionally, barley is utilized in human nutrition and malt production (Geçit 2016). Barley is a significant cereal crop in Turkish agriculture and has been cultivated in Anatolia for a considerable period. After wheat, barley is the most commonly planted cereal in Turkish agriculture. Türkiye is one of the important gene centers of both barley and wild barleys (Karakaya et al. 2016a, 2020, Kün 1996). In 2022, barley was planted in 7350, 2400, 9000, 1500, 2620,

11260, and 200 decares of land in Siirt Central district and Baykan, Kurtalan, Pervari, Şirvan, Eruh and Tillo districts, respectively (TÜİK 2024).

Barley plants are affected by various abiotic and biotic disease agents (Mathre 1982). It is crucial to identify these diseases that reduce yield and quality and take necessary measures. In studies conducted in Türkiye, various barley disease agents such as *Pyrenophora teres* (anamorph: *Drechslera teres*), *Rhynchosporium commune* (formerly named as *R. secalis* (Zaffarano et al. 2011)), *Pyrenophora graminea* (anamorph: *Drechslera graminea*), *Puccinia hordei*, *Cochliobolus sativus*,

and *Blumeria graminis* f. sp. *hordei* (*Erysiphe graminis* f. sp. *hordei*) were identified (Saraç Sivrikaya et al. 2019, 2021). One of the wild barley species, *Hordeum spontaneum* is naturally grown in the Fertile Crescent region (Karakaya et al. 2016a) and it has been reported as an important disease-resistance source (Çelik and Karakaya 2017). *Hordeum bulbosum*, also common in the region, is in the barley secondary gene pool (Karakaya et al. 2020, Ruge-Wehling and Wehling 2014, Saraç Sivrikaya et al. 2023). In this study, 46 barley fields, 95 naturally grown *H. spontaneum* populations, and 40 naturally grown *H. bulbosum* populations in Siirt province of Türkiye were examined for the presence of foliar diseases.

MATERIALS AND METHODS

In May 2023, a survey was conducted in Siirt province, Türkiye to determine the presence of diseases on *H. vulgare*, *H. bulbosum*, and *H. spontaneum*. A total of 46 barley fields were examined, with 11, 7, 10, 2, 6, and 10 fields surveyed in Siirt Central district, Baykan, Kurtalan, Pervari, Şirvan, and Eruh, respectively, for the presence of foliar diseases. At each barley field, at least 100 plants were inspected, except for Tillo district, which was not sampled due to its small barley cultivation area. The sampling method used was systematic sampling (Aktaş 2001). Additionally, 95 *H. spontaneum* populations and 40 *H. bulbosum* populations were inspected. Twenty-eight, 10, 9, 12, 16, 12, and 8 *H. spontaneum* populations in Siirt Central district, Baykan, Kurtalan, Pervari, Şirvan, Eruh and Tillo districts were investigated, respectively. Eight, 5, 6, 3, 13, and 5 *H. bulbosum* populations in Siirt Central district, Baykan, Kurtalan, Pervari, Şirvan, and Tillo were investigated, respectively. At each location, at least 50 *H. spontaneum* plants and at least 30 *H. bulbosum* plants were inspected. No samples were taken from the Eruh district, which has a small *H. bulbosum* population.

Wild barley and barley plants were visually inspected for foliar diseases in the field (Mathre 1997, Zaffarano et

al. 2011). Disease severity was assessed using Saari and Prescott's (1975) 0-9 scale, which is designed to evaluate the intensity of foliar diseases in wheat but can also be used for barley foliar diseases. On this scale, 0 means no infection, and 9 means severe infection, with 5 representing disease development up to the midpoint of the plant. This scale allows for the quick assessment of a large number of plants. In our study, we used these scale values as disease severity values. For suspected samples, isolations were made from the diseased leaf samples, and inocula were prepared and sprayed onto susceptible Bülbül 89 seedlings under greenhouse conditions as explained in Çelik Oğuz and Karakaya (2017 a,b). When calculating the average incidence, fields with and without disease were evaluated together.

RESULTS AND DISCUSSION

Inoculations were performed on the susceptible Bülbül 89 cultivar to confirm the leaf symptoms suspected to be caused by *P. teres* forms and *Cochliobolus sativus*. After inoculation with the suspected fungal cultures, we observed typical net form of net blotch, spot form of net blotch, and spot blotch symptoms. In the barley surveys conducted in Siirt province, both forms of net blotch disease caused by *P. teres* f. *maculata* and *P. teres* f. *teres*, scald caused by *R. commune*, barley stripe caused by *P. graminea*, powdery mildew caused by *B. graminis* f. sp. *hordei*, and spot blotch disease caused by *C. sativus* were found in barley plants (Tables 1-3). Out of the 46 barley fields examined in Siirt province, the following diseases were observed: *Blumeria graminis* f. sp. *hordei* in 24 fields, *P. teres* f. *maculata* in 19 fields, *R. commune* in 17 fields, *P. graminea* in 15 fields, *P. teres* f. *teres* in 14 fields, and *C. sativus* in 3 fields. Powdery mildew and the spot form of net blotch were the most commonly observed diseases in the fields, followed by scald.

Table 1. Barley (*Hordeum vulgare*) foliar diseases observed in Siirt province, Türkiye. For disease severity, a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum vulgare</i>)	<i>Pyrenophora teres</i> f. <i>teres</i>			<i>Pyrenophora teres</i> f. <i>maculata</i>			<i>Rhynchosporium commune</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	11	3	1.45%	3-5(3.66)	5	11.27%	1-5(3.8)	2	0.45%	3(3)
Baykan	7	1	0.14%	3(3)	4	0.86%	1-3(2.5)	5	4.42%	3-5(4.2)
Kurtalan	10	5	1.1%	3(3)	6	0.8%	3(3)	1	0.3%	3(3)
Pervari	2	2	1%	3(3)	1	0.5%	3(3)	2	2.5%	3(3)
Şirvan	6	1	0.17%	3(3)	1	0.5%	5(5)	3	1.66%	3(3)
Eruh	10	2	0.5%	3(3)	2	0.3%	3(3)	4	0.9%	1-3(2)

Table 2. Barley (*Hordeum vulgare*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum vulgare</i>)	<i>Pyrenophora teres f. teres</i>			<i>Pyrenophora teres f. maculata</i>			<i>Rhynchosporium commune</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	11	3	2.73%	3-5(3.66)				4	0.45%	-
Baykan	7	4	0.86%	3(3)	-	-	-	4	0.57%	-
Kurtalan	10	5	3%	3-5(3.8)	-	-	-	4	0.8%	-
Pervari	2	2	1.5%	3(3)	-	-	-	1	1%	-
Şirvan	6	6	2.16%	3-5(3.33)	-	-	-	-	-	-
Eruh	10	4	1%	3(3)	-	-	-	2	0.4%	-

Table 3. Barley (*Hordeum vulgare*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum vulgare</i>)	<i>Cochliobolus sativus</i>		
		Field	Incidence (%)	Severity (mean)
Central	11	3	20%	5-7(5.66)
Baykan	7	-	-	-
Kurtalan	10	-	-	-
Pervari	2	-	-	-
Şirvan	6	-	-	-
Eruh	10	-	-	-

Disease severity values ranged from 3 to 5 for *P. teres f. teres*, *R. commune*, and *B. graminis f. sp. hordei*. For *P. teres f. maculata* and *C. sativus*, disease severity values were between 1-5 and 5-7, respectively.

The net form of net blotch was observed in all examined Siirt districts except the Tillo district, from which no sample was taken. This disease was most prevalent in the Central district (1.45%). The spot form of net blotch was observed in all districts, with the highest incidence observed in the Central district (11.27%). Scald was also observed in all districts, with the highest incidence found in the Baykan district (4.42%). Powdery mildew was seen in all districts, with an incidence of 2.73% in the central district and 2.16% in the Şirvan district, while being lower in other districts. Barley stripe was observed in all districts except the Şirvan district, with a low intensity of the disease. Spot blotch was observed only in the central district (20%). Additionally, no disease was observed in one barley field in the Eruh district.

Rhynchosporium commune was observed in 57 populations, *B. graminis f. sp. hordei* in 54 populations, *P. teres f. teres*

in 13 populations, *P. teres f. maculata* in 11 populations, *P. graminea* in 11 populations, and *P. hordei* in 2 populations out of 95 *H. spontaneum* populations examined in Siirt province (Tables 4 and 5). Scald and powdery mildew were the most common diseases encountered in *H. spontaneum* populations. Disease severity values varied between 3-5 for *P. teres f. teres*, *P. teres f. maculata*, and *R. commune*, 5-7 for *C. sativus*, 3 for *P. hordei*, and 1-5 for *B. graminis f. sp. hordei*. The net form of net blotch was observed in all districts except Baykan, Kurtalan, and Tillo districts. This disease was most prevalent in the Central district (5.75%). The spot form of net blotch was observed in all districts except in Baykan, Şirvan, and Tillo districts. The highest incidence of this disease was recorded in Siirt Central district (5.17%). Scald was observed in all districts, with the highest incidence found in the Kurtalan district (17.77%). Powdery mildew was observed in all districts. It was most common in the Tillo district (8.75%). Barley stripe was observed in all districts except the Baykan and Kurtalan districts, with a low incidence. Brown rust was seen only in the Baykan and Şirvan districts. No disease was observed in 2, 3, 2, 2, and 2 *H. spontaneum* populations in the Central district and Eruh, Şirvan, Pervari, and Kurtalan districts.

In Siirt province, out of 40 *H. bulbosum* populations examined, the following diseases were observed: *B. graminis f. sp. hordei* in 18 populations, *R. commune* in 4 populations, *P. teres f. teres* in 3 populations, *P. hordei* in 1 population, *P. teres f. maculata* in 1 population, and *P. graminea* in 1 population (Tables 6 and 7). The most common diseases were powdery mildew and scald. Disease severity values in these populations were 3 for *P. teres f. teres*, *P. teres f. maculata*, *R. commune*, and *P. hordei*, and varied between 1 and 5 for *B. graminis f. sp. hordei*. The net form of net blotch was observed in the Central district, Kurtalan, and Tillo districts. The spot form of net blotch was only seen in the

Table 4. Wild barley (*Hordeum spontaneum*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum spontaneum</i>)	<i>Pyrenophora teres f. teres</i>			<i>Pyrenophora teres f. maculata</i>			<i>Rhynchosporium commune</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	28	7	5.75%	3-5(3.42)	8	5.17%	3-5(4.25)	16	0.04%	3-5(3.62)
Baykan	10	-	-	-	-	-	-	7	6.8%	3-5(3.57)
Kurtalan	9	-	-	-	1	0.11%	3(3)	2	17.77%	3-5(3.57)
Pervari	12	2	0.17%	3(3)	1	0.83%	3(3)	9	2.5%	3-5(3.44)
Şirvan	16	1	0.06%	3(3)	-	-	-	9	2.5%	3-5(3.44)
Eruh	12	3	0.42%	3(3)	1	0.83%	3(3)	6	2.08%	3-5(3.33)
Tillo	8	-	-	-	-	-	-	8	7.75%	3-5(3.25)

Table 5. Wild barley (*Hordeum spontaneum*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum spontaneum</i>)	<i>Blumeria graminis f. sp. hordei</i>			<i>Puccinia hordei</i>			<i>Pyrenophora graminea</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	28	15	4.07%	3-5(3.4)	-	-	-	1	0.04%	-
Baykan	10	6	2.6%	3-5(3.33)	1	0.1%	3(3)	-	-	-
Kurtalan	9	6	2.11%	1-3(2.66)	-	-	-	-	-	-
Pervari	12	3	0.33%	3(3)	-	-	-	1	0.83%	-
Şirvan	16	9	1.63%	3-5(3.44)	1	0.06%	3(3)	2	0.13%	-
Eruh	12	7	1.42%	1-3(2.42)	-	-	-	1	0.17%	-
Tillo	8	8	8.75%	3(3)	-	-	-	6	3.25%	-

Baykan district. Scald was observed in the Central district, Pervari, Şirvan, and Tillo districts. Brown rust was observed only in Baykan district, and barley stripe was present only in Tillo district. The incidence of these diseases was recorded as low. Powdery mildew was observed in all districts except Baykan. It was most common in Şirvan (2.62%), Kurtalan (2.5%), and Pervari (2.33%) districts. No disease was observed in 4, 1, 7, 5, and 4 *H. bulbosum* populations in the Central district and Tillo, Şirvan, Kurtalan, and Baykan districts. The only disease found in Siirt barley fields that was not present in the *H. spontaneum* and *H. bulbosum* populations in Siirt province was spot blotch. Additionally, a small amount of brown rust was discovered in the populations of *H. spontaneum* and *H. bulbosum*, which was not seen in *H. vulgare* fields.

A survey of barley fields in the Eskişehir province of Türkiye revealed the presence of net blotch, brown rust, barley stripe, powdery mildew, scald, stem rust, and smut diseases. Among these, net blotch and scald were the most

common (Çelik and Karakaya 2015). In a study by Karakaya et al. (2016a), it was found that scald was the most common disease in populations of *H. spontaneum* grown in Gaziantep, Şanlıurfa, Diyarbakır, Şırnak, Mardin, Siirt, Kilis, and Hatay provinces of Türkiye. Following scald disease, powdery mildew and net blotch were also prevalent. Additionally, brown rust, barley stripe, loose smut, and semi-loose smut were observed in the populations of *H. spontaneum*. However, nine *H. spontaneum* populations were found to be disease-free. Özdemir et al. (2017) identified both forms of net blotch, scald, barley stripe, powdery mildew, yellow rust, stem rust, and barley brown rust in barley fields in Kırıkkale province, with net blotch and scald being the most prevalent diseases. In the Çubuk district of Ankara, Türkiye, İlgen et al. (2017) reported the presence of net blotch (both forms), barley stripe, scald, yellow rust, powdery mildew, stem rust, and barley brown rust diseases in the barley fields they investigated. In the Bala district of Ankara, Ertürk et al. (2018) reported the presence of net and spot forms of net

Table 6. Wild barley (*Hordeum bulbosum*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum bulbosum</i>)	<i>Pyrenophora teres f. teres</i>			<i>Pyrenophora teres f. maculata</i>			<i>Rhynchosporium commune</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	8	1	0.13%	3(3)	-	-	-	1	0.13%	3(3)
Baykan	5	-	-	-	1	0.6%	3(3)	-	-	-
Kurtalan	6	1	0.17%	3 (3)	-	-	-	-	-	-
Pervari	3	-	-	-	-	-	-	1	0.33%	3(3)
Şirvan	13	-	-	-	-	-	-	1	0.23%	3(3)
Tillo	8	1	0.6%	3(3)	-	-	-	1	1%	3(3)

Table 7. Wild barley (*Hordeum bulbosum*) foliar diseases observed in Siirt province, Türkiye. For disease severity a 0-9 scale developed by Saari and Prescott (1975) was used

District	Total field (<i>Hordeum bulbosum</i>)	<i>Blumeria graminis f. sp. hordei</i>			<i>Puccinia hordei</i>			<i>Pyrenophora graminea</i>		
		Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)	Field	Incidence (%)	Severity (mean)
Central	8	3	0.37%	3(3)	-	-	-	-	-	-
Baykan	5	-	-	-	1	0.2%	3(3)	-	-	-
Kurtalan	6	1	2.5%	3 (3)	-	-	-	-	-	-
Pervari	3	3	2.33%	3-5(3.66)	-	-	-	-	-	-
Şirvan	13	7	2.62%	3-7(3.85)	-	-	-	-	-	-
Tillo	8	4	1.8%	1-3(2)	-	-	-	1	0.2%	-

blotch, barley stripe, scald, and powdery mildew in the barley fields they surveyed. The most common diseases found were the spot and net forms of net blotch. Saraç Sivrikaya et al. (2019) conducted a study in the Elazığ province of Türkiye and found both forms of net blotch, powdery mildew, scald, barley brown rust, and barley stripe in barley fields. Both forms of net blotch were prevalent, with scald being the next most common disease. In the Bingöl province of Türkiye, Karakaya et al. (2020) investigated the diseases affecting *H. bulbosum* plants. They found that five *Hordeum bulbosum* populations showed no disease symptoms, while other populations exhibited symptoms of spot form of net blotch, powdery mildew, brown rust, and scald. Saraç Sivrikaya et al. (2021) identified the leaf diseases that occurred in *H. vulgare* fields and *H. spontaneum* populations in the Batman province and surrounding areas of Türkiye. They found scald, both forms of net blotch, spot blotch, powdery mildew, brown rust, and barley stripe. Except for barley stripe and spot blotch, the same diseases were also found in *H. spontaneum* populations. Barley scald was the most commonly seen disease in both barley and wild barley. Among the Siirt barley fields and both wild barley populations, barley stripe disease

was observed. In a study conducted in Central Anatolia, Türkiye, barley stripe disease was found in 40% of the fields surveyed (Karakaya et al. 2016b). Saraç Sivrikaya et al. (2023) identified the barley and wild barley diseases present in the Şanlıurfa province of Türkiye. They observed both forms of net blotch, barley stripe, and scald. Seidi Arslan et al. (2024) identified the barley diseases present in Edirne, Türkiye. The most prevalent disease in Edirne was the net form of barley net blotch, followed by scald, barley brown rust, spot form of barley net blotch, powdery mildew, and spot blotch. During our survey of barley fields in Siirt, we observed both forms of net blotch, scald, powdery mildew, barley stripe, and spot blotch diseases. Powdery mildew was the most common disease, followed by the spot form of net blotch. We also encountered spot blotch disease in our barley survey. Among *H. spontaneum* populations, scald and powdery mildew were the most common diseases. In *H. bulbosum* populations, powdery mildew and scald were the most common diseases. In wild barley plants, we found *P. hordei* in 3 populations, but this pathogen was not detected in the Siirt barley fields.

In this study, 46 fields of *H. vulgare*, 95 populations of naturally-grown *H. spontaneum*, and 40 populations of naturally-grown *H. bulbosum* in the Siirt province of Türkiye were examined for foliar diseases. Powdery mildew was the most common disease among the barley fields. It was followed by the spot form of net blotch, scald, barley stripe, net form of net blotch, and spot blotch. Among the *H. spontaneum* populations, scald was the most common disease, followed by powdery mildew, the net form of net blotch, the spot form of net blotch, barley stripe, and barley brown rust. In the populations of *H. bulbosum*, powdery mildew was the most common disease, followed by barley scald, the net form of net blotch, the spot form of net blotch, barley stripe, and barley brown rust. Powdery mildew, net blotch, and scald were commonly found in the barley fields and wild barley populations that were surveyed in Türkiye. These diseases have been reported by other researchers (Çelik and Karakaya 2015, Ertürk et al. 2018, İlgen et al. 2017, Karakaya et al. 2014, 2016a, 2020, Özdemir et al. 2017, Saraç Sivrikaya et al. 2019, 2021, 2023, Seidi Arslan et al. 2024). It is necessary to develop control methods for these diseases. Populations of wild barleys with no diseases under natural conditions could be used in breeding studies for disease resistance after testing under controlled and field conditions.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Siirt ilinin Merkez ilçe, Başkan, Kurtalan, Pervari, Şirvan, Eruh ve Tillo ilçelerinin arpa yetiştirilen alanlarında ve yabancı arpa (*Hordeum spontaneum*, *Hordeum bulbosum*) popülasyonlarında 2023 yılı mayıs ayında sürveyler yapılmış, arpa ve yabancı arpa bitkilerinde görülen yaprak hastalıkları belirlenmiştir. Arpa bitkilerinde *Pyrenophora teres* f. *teres* ve *Pyrenophora teres* f. *maculata*'nın sebep olduğu Arpa ağbenek hastalığının ağ ve nokta formları, *Blumeria graminis* f.sp. *hordei*'nin sebep olduğu Külleme hastalığı, *Rhynchosporium commune*'nin sebep olduğu Arpa yaprak lekesi hastalığı, *Pyrenophora graminea*'nın sebep olduğu Arpa çizgili yaprak lekesi ve *Cochliobolus sativus*'un sebep olduğu Yaprak lekesi hastalığı tespit edilmiştir. Yabancı arpa bitkilerinde ise *Pyrenophora teres* f. *teres*'in sebep olduğu Arpa ağbenek hastalığının ağ formu ve *Pyrenophora teres* f. *maculata*'nın sebep olduğu Arpa ağbenek hastalığının nokta formu, *Blumeria graminis* f. sp. *hordei*'nin sebep olduğu Külleme hastalığı, *Rhynchosporium*

commune'nin sebep olduğu Arpa yaprak lekesi hastalığı, *Pyrenophora graminea*'nın sebep olduğu Arpa çizgili yaprak lekesi ve *Puccinia hordei*'nin sebep olduğu Kahverengi pas hastalığı tespit edilmiştir.

Anahtar kelimeler: Siirt, *Hordeum vulgare*, *Hordeum spontaneum*, *Hordeum bulbosum*, arpa yaprak hastalıkları

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Original article

Efficacy of some fungicides against *Septoria pistaciarum* Caracc. on Pistacia species in Türkiye

Pistacialarda sorun olan *Septoria pistaciarum* Caracc.'a karşı bazı fungusitlerin etkinliklerinin belirlenmesi

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ABSTRACT

Pistachio (*Pistacia vera* L.) is considered a strategic agricultural product due to its high nutritional value and demand in international markets. With the expansion of production areas in recent years, pistachio cultivation has increased, while the management of pest and diseases comes to the fore. *Septoria* leaf spot disease caused by *Septoria pistaciarum* Caracc. is an important fungal pathogen causing leaf spots on pistachio trees. In this study, the efficacy of fungicides from different groups on ten isolates obtained from different Pistacia species was investigated. In the inhibition of mycelial radial growth of the pathogen fungus in vitro, the highest level of inhibition was 85.15% at a dose of 1.25 ml l⁻¹ with azoxystrobin + difenoconazole, while the lowest level of inhibition was 10.30% at a dose of 0.3 ml l⁻¹ with dodine. In the study carried out in pistachio trees contaminated with *S. pistaciarum* in Karabük province, it was observed that the highest biological activity of boscalid + pyraclostrobin fungicide at a dose of 75 g/100 l of water was 85.81%, and azoxystrobin + tebuconazole fungicide at a dose of 75 ml/100 l of water provided 83.80% disease control. The fungicide with the active ingredient prochloraz (33.44%) showed the lowest biological activity in the control of *Septoria* leaf spot disease. These results provide important data in terms of disease control by revealing the effectiveness rates of fungicides used in the control of *Septoria* leaf spot disease in pistachio cultivation.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is a species of Anacardiaceae family and has an economically important place among non-wood forest products in Türkiye. Although there are 11 species in the genus *Pistacia*, only *P. vera* is grown commercially. Other *Pistacia* species, naturally grown, are commonly used by grafting for pistachio production (Ak

and Kaşka 1998). Although there has been a significant increase in pistachio production in Türkiye in recent years, this increase in production has been accompanied by the spread of some diseases and this has become an important limiting factor in production.

Although the diseases that limit pistachio production vary according to countries, *Phytophthora* species (Trouillas et al. 2022) *Verticillium dahliae* (Hadizadeh and Banhashemi 2007) and some *Fusarium* species (Aydm et al. 2023, Ören et al. 2023); and fungal diseases of aerial origin are *Botryosphaeria dothidea*, *Alternaria alternata* (Michailides et al. 1994), and *Septoria* species (Crous et al. 2013, Gusella et al. 2024, Sarpkaya 2014). These diseases cause serious damage to leaves, fruits, and roots and reduce both product quality and yield.

Septoria leaf spot disease is recognized as one of the major foliar diseases of pistachio in all pistachio-growing countries in the Mediterranean basin. The typical main symptom of the disease is irregular dark brown leaf spots with a diameter of 2 mm developing on the leaf surface. As the disease progresses, these spots may cover the entire surface of the compound leaves. Subsequently, the affected areas become chlorotic and then brown and necrotic. In necrotic lesions, spore beds of the pathogen (pycnidia) develop, which can be seen as black dots on the brown background of the lesion. Pycnidia produce abundant conidia under high humidity conditions and cottony white mycelium masses may develop on the lesions under humid conditions. Although not common, black spots also develop on fruits and petioles. Severe outbreaks of the disease combined with periods of high drought cause necrosis, premature

defoliation, and reduced tree vigour (Gusella et al. 2024, López-Moral et al. 2022). Traditionally, the disease has been associated with three fungal species belonging to the genus *Septoria*: *S. pistaciae*, *S. pistaciarum*, and *S. pistacina* (Chitzanidis 1956). New information on the molecular characterisation of Septoria-like *Pistacia* species pathogens has reported *S. pistaciarum* and *S. pistaciae* as true *Septoria* species. However, *S. pistacina* was reassigned to the genus *Pseudocercospora* and renamed *Pseudocercospora pistacina* (Chitzanidis 1956; Crous et al. 2013). After the disease agent was definitively differentiated in our country (Crous et al. 2013), it was determined that it also caused disease in wild *Pistacia* species.

Especially climate change and modern agricultural practices cause diseases to occur more frequently and severely, therefore, effective control of these diseases in production processes gains great importance. Pathogenic characterisation of the disease has not been carried out in our country and most importantly, its control is not known. Detailed study of Septoria leaf spot disease and identification of the disease is very important in terms of control and continuity of pistachio production. In this study, for the sustainability of pistachio production and control of the disease, fungicides from different groups used in the control of similar diseases (called 'Karazenk') were tested and their *in vitro* and *in vivo* effects were performed to be determined.

Table 1. *Septoria pistaciarum* isolates used in the study

Isolate Codes	District	Location	North	East	Altitude	Sampling Date	Host
S-M-14	Merkez	Bulak Mezarlığı (Aykol Aile Kab.)	41°14'48"	32°39'33"	505	24.08.2021	<i>P. atlantica</i>
S-M-15	Merkez	Bulak Mezarlığı (Ahmet Uysal)	41°14'48"	32°39'32"	507	25.08.2021	<i>P. vera</i>
S-M-37	Merkez	Bulak Mezarlığı (Yol Kenarı)	41°14'50"	32°39'31"	508	06.09.2021	<i>P. vera</i>
S-M-43	Merkez	Bulak Mezarlığı (Gazi Üniv. Emekli Öğr.)	41°14'49"	32°39'33"	504	07.09.2021	<i>P. vera</i>
S-S-53	Safranbolu	Mezarlık (Canbülbul Aile Mezarlığı)	41°14'29"	32°41'47"	470	12.09.2021	<i>P. vera</i>
S-S-62	Safranbolu	Mezarlık (Emine Köklü)	41°14'29"	32°41'46"	470	14.09.2021	<i>P. atlantica</i>
S-S-63	Safranbolu	Mezarlık (Şevki Sazık)	41°14'29"	32°41'47"	470	16.09.2021	<i>P. atlantica</i>
S-S-76	Safranbolu	Mezarlık	41°14'27"	32°41'46"	470	26.09.2021	<i>P. atlantica</i>
S-S-81	Safranbolu	Mezarlık	41°14'26"	32°41'46"	469	19.09.2021	<i>P. terebinthus</i>
01-M	Adana/ Karaisalı	Çakmak Köyü Murtluca Mevkii	-	-	260	22.09.2022	<i>P. terebinthus</i>

MATERIALS AND METHODS

Plant and fungal material

The fungal material used in the project study was 9 isolates of *Septoria pistaciarum* obtained in the TUBITAK-2209-A (Project No: 1919B012005296) titled 'Investigation of Septoria Leaf Spotting Agents which is a Problem in Pistachio and Other Pistacia species in Karabük Province' previously carried out at Karabük University Faculty of Forestry. In addition, leaves infected with *S. pistaciarum* were collected from wild *P. terebinthus* trees in Çakmak neighbourhood of Karaisalı District of Adana Province and used as material (Table 1).

The studies were carried out in the laboratories of Karabük University, Faculty of Forestry. Potato Dextrose Agar (PDA) was used for fungal cultures, adding streptomycin sulphate to prevent bacterial contamination.

The active ingredients and ratios, trade names, formulation forms, and doses of the fungicides used for the control of the disease are given in Table 2.

and inoculated on PDA. For the isolation of the fungus, the single spore isolation method proposed by Sarpkaya (2014) was used. According to this method, spore suspension was obtained by scraping the pycnidium beds on the leaves in sterile 10 ml distilled water. The spore suspension was diluted 1/10 and the dilution process was continued until $2-3 \times 10^6$ conidia were obtained. 30-40 µl of the final suspension was added to each Petri dish and spread on the medium with a Drigalski spatula.

In vitro fungicide efficacy experiments

The PDA medium was sterilised in an autoclave and then placed in a water bath to cool to 45 °C. The fungicides listed in Table 2 were added at the recommended doses to each 90 mm diameter Petri dish and then 20 ml of fungicidal PDA was poured into each Petri dish. They were allowed to cool and become semi-solid in a sterile environment. Four Petri dishes were used for each dose, while four Petri dishes without fungicide were prepared as a control (Alberoni et al. 2005, Avenot and Michailides 2007). The experiments were carried out by a randomised experimental design.

Table 2. Fungicides used in the study

Active Substance and Ratio	Commercial Name	Formulation	Doses (dose/ml)
Boscalid + Pyraclostrobin (26.7%+6.7%)	Signum	WG (Suda Eriyen Granül)	0.3 mg, 0.6 mg, 1.2 mg, 2 mg
Azoxystrobin + Tebuconazole (120 g/l + 200 g/l)	Azimut	SC (Süspansiyon Konsantre)	0.2 µl, 0.4 µl, 0.75 µl, 1.25 µl
Prochloraz (450 g/l)	Tommi	EC (Emülsiyon Konsantre)	0.3 µl, 0.6 µl, 1 µl, 1.5 µl
Isopyrazam + Difenconazole (100 g/l + 40 g/l)	Embrelia	SC (Süspansiyon Konsantre)	0.2 ml, 0.4 ml, 0.8 ml, 1 ml
Dodine (65%)	İzolprex	WP (Islanabilir Toz)	0.3 ml, 0.6 ml, 1 ml, 1.5 ml

After *in vitro* studies, the doses found to be effective were used in field trials on 30-year-old pistachio trees in the Safranbolu district of Karabük province.

Isolation method of fungi

Nine of the fungal isolates used in the study were obtained from previous studies which were stored in the Phytopathology laboratories of Karabük University, Faculty of Forestry before. An isolate was obtained from infected *Pistacia terebinthus* leaves. To ensure the culture continuity of the long-term stored samples, the isolates stored at -20 °C were kept in a sterile cabinet for 6 hours after they were removed. Then, small pieces were taken with a sterile scalpel

From the cultures grown in the incubator for 21 days, mycelial discs with a diameter of 5 mm were taken from the active growth zone using a Koch borer and placed in the middle of the Petri dishes. Petri dishes to which no fungicide was added were used as a negative control group. They were incubated in the dark in incubators maintained at 24 ± 1 °C.

At the end of the weekly controls, the mycelial radial growth of the fungal cultures was measured from four directions with the help of a ruler on the 28th day, and the experiment was terminated. After radial growth measurements, the samples were carefully separated from the medium and no medium residue was left on the surface. The wet weights of the mycelium were weighed on a precision balance

and recorded. For the determination of dry weights, the samples were wrapped in aluminium foil, placed in an oven, and dried in an oven at 60 °C for 45 min according to the method of Yılmaz and Çolak (2008) and then weighed on a precision balance to determine the mycelium dry weights of the isolate groups.

Field fungicide efficacy determination studies

Fungicide efficacy trials against Septoria leaf spot disease were carried out on 40-year-old pistachio trees naturally infected with *S. pistaciarum* in Safranbolu in Karabük. The treatments were carried out after the natural infection of pistachio trees. Since there is no registered fungicide against Septoria leaf spot disease of pistachio in our country, the study was carried out with the fungicides listed in Table 2. Fungicide efficacy was evaluated using three different doses obtained from studies on inhibition of mycelial growth (Table 3). Fungicide applications were made under 20 atm pressure with the help of a motorised atomiser (Palmera PA-768) with 25 l capacity and conical nozzle type and 4.8 l of water was used for each tree. The first applications were made twice, the first on May 17th, 2023 and the second on June 7th, 2023. Evaluations and counts were carried out after 90 days.

Evaluation of results and statistical analyses

In *in vitro* fungicide efficacy trials, percentage efficacy was calculated by applying Abbott's (1925) formula to the results obtained from mycelial radial growth, mycelial wet weight, and mycelial dry weight measurements. The experiment was planned according to random plots experimental design with five replicates and each replicate was arranged to contain three Petri dishes. Data were analysed by the ANOVA method and differences between means were determined by LSD (Least Square Differences) test ($p < 0.05$). All statistical analyses were performed using JMP 14.3.0 software.

Fungicide efficacy on plants was experimented according to the randomised block design with 3 replicates and 1 tree in each replicate. Symptom development from the leaves collected on the plants 90 days after the treatments were counted according to the scale values specified in Table 4 and fungicide efficacy was determined. No fungicide was applied to the control group plants.

Table 3. Fungicides and doses used in the field against Septoria leaf spot disease

Active Substances	Formulation	Doses (dose/100 l water)		
		1 st dose	2 nd dose	3 rd dose
Boscalid + Pyraclostrobin	WG	20 gr	40 gr	75 gr
Azoxystrobin + Tebuconazole	SC	10 ml	30 ml	75 ml
Prochloraz	EC	20 ml	50 ml	100 ml
Isopyrazam + Difenconazole	SC	20 ml	40 ml	80 ml
Dodine	WP	20 ml	50 ml	100 ml

Table 4. Karazenk (*Pseudocercospora pistacina*) disease Evaluation Scale* (Anonymous, 2024a)

Scales	Description
0	No spot
1	Spot rates on compound leaves till 20%
2	Spot rates on compound leaves between 21-40%
3	Spot rates on compound leaves between 41-60%
4	Spot rates on compound leaves between 61-80%
5	Spot rates on compound leaves more than 80%

* Since Septoria leaf spot disease is not included in the Agricultural Control Technical Instruction specified by the General Directorate of Agricultural Research and Policies of the Ministry of Agriculture and Forestry, it was evaluated according to the evaluation scale of Karazenk disease, which causes damage as leaf spot disease.

RESULTS

Efficacy of fungicides on mycelial radial growth of S. pistaciarum

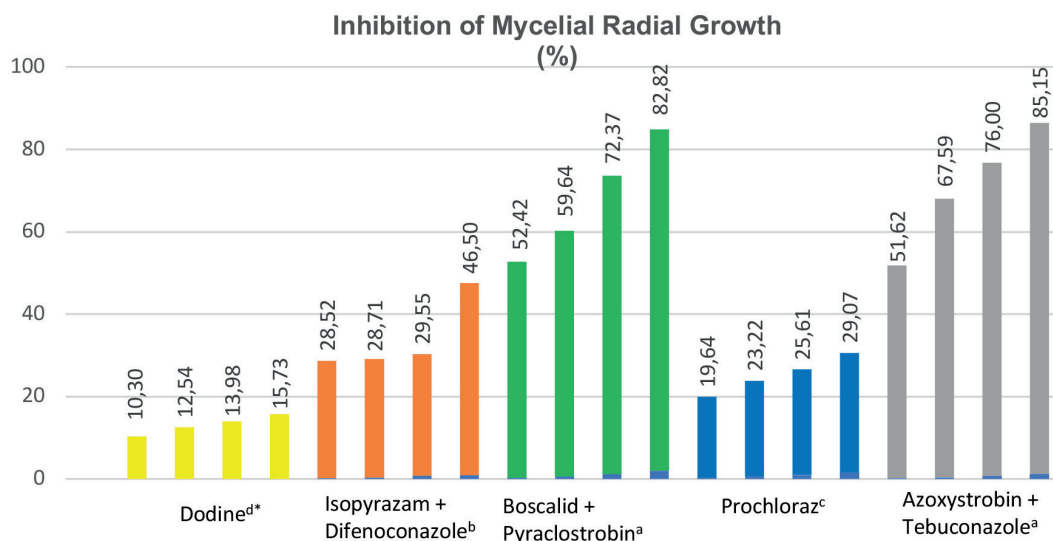
The effects of the selected fungicides on the mycelial radial growth of 10 *S. pistaciarum* isolates are presented in Table 5. As the fungicide doses increased, the percentage inhibition of mycelial radial growth of all isolates increased. The highest mean inhibition was observed at the dose of azoxystrobin + tebuconazole fungicide, 1.25 ml l⁻¹ with 85.15% and at the dose of boscalid + pyraclostrobin fungicide, 2 g l⁻¹ with 82.82%. The lowest inhibition was observed at 0.3 ml l⁻¹ dose of dodine (10.30%) and 0.3 ml l⁻¹ dose of prochloraz (19.64%) (Table 6).

In the statistical analysis, the fungicides containing the active substances azoxystrobin + tebuconazole and boscalid + pyraclostrobin were in the same group with the highest effect, while the fungicide with the active substance dodine showed the lowest effect in inhibiting the mycelial development of the pathogen fungus *in vitro* (Figure 1).

conducted with isopyrazam + difenoconazole fungicide, the average mycelial dry weights of the isolates obtained at 0.2, 0.4, 0.8 and 1 ml l⁻¹ doses were 0.50, 0.46, 0.42, and 0.39 g, respectively. The mean mycelial dry weights of boscalid + pyraclostrobin fungicide at 0.3, 0.6, 1.2, and 2 ml l⁻¹ doses were 0.47, 0.44, 0.42 and 0.39 g, respectively. For prochloraz, 0.30, 0.25, 0.20, and 0.18 g mycelial dry weight was obtained at the doses (0.3, 0.6, 1, 1.5 ml l⁻¹), respectively. The average mycelial dry weights of the isolates were 0.38, 0.31, 0.24, and 0.18 g for azoxystrobin + tebuconazole fungicide (0.2, 0.4, 0.75 and 1.25 ml l⁻¹), respectively.

Biological activity of fungicides on Septoria leaf blight disease under field conditions

The results of fungicide efficacy trials against *Septoria* leaf spot disease of pistachio trees are given in Table 7. A statistically significant difference was observed between the biological efficacies of the fungicides used in the study. The fungicides with the highest control efficacy were boscalid + pyraclostrobin and azoxystrobin + difenoconazole, while the lowest efficacy was obtained from prochloraz.



* There is a statistical difference between different letters following the same line (LSD test, P<0,05).

Figure 1. Mycelial radial growth inhibition of some fungicides tested against some selected isolates of *Septoria pistaciarum* (%)

Efficacy of fungicides on mycelial dry weight of S. pistaciarum

The effects of fungicides on the mycelial dry weight of 10 *S. pistaciarum* isolates are given in Table 6. Mycelial dry weight of all isolates decreased with increasing doses of fungicides. Mycelial weights of 0.48, 0.45, 0.41, and 0.38 g were obtained at the doses (0.3, 0.6, 1, and 1.5 ml l⁻¹) of the isolates in the study with dodine fungicide. In the study

In the statistical analysis of the doses of fungicides, there was no difference between the averages of biological activity percentages. However, an increase in the efficacy of all fungicides was observed with increasing doses. In the study conducted with boscalid + pyraclostrobin, disease control was achieved at 59.75%, 70.03%, and 85.81% at doses of 20, 40 and 75 g/100 l, respectively. In the trials with azoxystrobin

Table 5. Mycelial radial growth inhibition of some fungicides tested against some selected isolates of *S. pistaciarum* according to doses (%)

Fungicides	Doses (dose/L)	Inhibition of Mycelial Radial Growth Inhibition by Isolates (%)										Mean of Inhibition (%)
		SS-62	SS-53	SS-81	SS-63	SM-14	SM-37	SM-82	SM-43	SM-15	01-M	
Dodine	0.3 ml	18.47	7.63	17.02	7.98	0.59	25.82	16.67	0.82	0.99	7.04	10.30
Dodine	0.6 ml	23.98	8.47	18.10	12.91	1.35	26.17	19.05	2.60	1.05	11.71	12.54
Dodine	1 ml	24.36	11.28	19.04	16.59	2.32	26.29	21.26	2.43	1.12	15.07	13.98
Dodine	1.5 ml	24.62	12.11	20.30	21.15	3.98	28.23	23.34	4.09	1.38	18.05	15.73
Isopyrazam + Difenonazole	0.2 ml	30.98	28.53	31.52	27.25	25.49	26.96	24.31	32.55	27.40	30.20	28.52
Isopyrazam + Difenonazole	0.4 ml	29.39	29.48	30.29	31.59	30.40	27.99	24.82	26.25	31.50	25.36	28.71
Isopyrazam + Difenonazole	0.8 ml	29.86	30.35	31.88	37.09	22.28	29.57	38.59	20.79	34.30	20.83	29.55
Isopyrazam + Difenonazole	1 ml	39.92	30.39	54.44	56.02	54.44	30.43	66.04	45.38	42.50	45.45	46.50
Boscalid + Pyraclostrobin	0.3 g	75.53	70.64	64.86	61.80	34.42	38.08	33.69	41.73	56.82	46.62	52.42
Boscalid + Pyraclostrobin	0.6 g	84.14	73.93	66.76	71.66	48.15	38.54	42.76	52.76	67.97	49.68	59.64
Boscalid + Pyraclostrobin	1.2 g	86.18	75.83	78.50	73.88	67.53	46.76	85.21	68.10	78.97	62.75	72.37
Boscalid + Pyraclostrobin	2 g	91.15	85.12	89.28	86.12	85.12	59.41	91.13	84.91	80.02	75.97	82.82
Prochloraz	0.3 ml	31.02	59.32	25.00	6.98	4.45	14.48	13.26	5.83	12.24	23.85	19.64
Prochloraz	0.6 ml	32.33	59.62	25.74	10.86	9.86	16.05	17.55	20.83	13.80	25.53	23.22
Prochloraz	1 ml	33.82	59.97	27.19	14.23	12.96	16.68	18.91	29.81	15.59	26.91	25.61
Prochloraz	1.5 ml	34.88	60.65	28.73	17.68	16.69	18.50	28.81	39.47	17.44	27.85	29.07
Azoxystrobin + Tebuconazole	0.2 ml	68.01	48.95	59.36	35.37	69.92	62.21	35.01	22.29	54.79	60.29	51.62
Azoxystrobin + Tebuconazole	0.4 ml	79.41	73.69	69.12	47.43	79.75	74.54	58.37	51.08	72.11	70.41	67.59
Azoxystrobin + Tebuconazole	0.75 ml	87.34	75.08	72.89	58.40	87.96	76.02	69.51	68.97	82.35	81.46	76.00
Azoxystrobin + Tebuconazole	1.25 ml	88.05	87.08	85.55	70.39	91.29	95.97	74.53	76.15	91.02	91.48	85.15

Table 6. Mycelial dry weights according to the doses of some fungicides tested against some selected isolates of *Septoria pistaciarum* (g)

Fungicides	Doses (dose/L)	Mycelial Dry Weights According to Isolates (g)										Means of Myce- lial Dry Weights (g)		
		SS-62	SS-53	SS-81	SS-63	SM-14	SM-37	SM-82	SM-43	SM-15	01-M			
Dodine	0.3 ml	0.49	0.42	0.56	0.49	0.34	0.47	0.55	0.51	0.48	0.49	0.48	0.49	0.48
Dodine	0.6 ml	0.44	0.37	0.53	0.44	0.30	0.44	0.54	0.49	0.46	0.45	0.46	0.45	0.45
Dodine	1 ml	0.39	0.32	0.44	0.42	0.30	0.39	0.51	0.45	0.45	0.42	0.45	0.42	0.41
Dodine	1.5 ml	0.34	0.27	0.42	0.39	0.29	0.35	0.50	0.41	0.45	0.39	0.45	0.39	0.38
Isopyrazam + Difenocanazole	0.2 ml	0.32	0.52	0.59	0.56	0.48	0.56	0.47	0.56	0.49	0.40	0.49	0.40	0.50
Isopyrazam + Difenocanazole	0.4 ml	0.27	0.49	0.57	0.52	0.45	0.53	0.46	0.55	0.46	0.34	0.46	0.34	0.46
Isopyrazam + Difenocanazole	0.8 ml	0.25	0.44	0.54	0.49	0.29	0.51	0.41	0.54	0.38	0.33	0.38	0.33	0.42
Isopyrazam + Difenocanazole	1 ml	0.21	0.41	0.52	0.48	0.25	0.47	0.40	0.51	0.32	0.31	0.32	0.31	0.39
Boscalid + Pyraclostrobin	0.3 g	0.53	0.47	0.48	0.48	0.40	0.41	0.47	0.51	0.49	0.47	0.49	0.47	0.47
Boscalid + Pyraclostrobin	0.6 g	0.47	0.41	0.45	0.42	0.39	0.39	0.45	0.50	0.48	0.46	0.48	0.46	0.44
Boscalid + Pyraclostrobin	1.2 g	0.46	0.38	0.41	0.40	0.35	0.37	0.41	0.49	0.46	0.44	0.46	0.44	0.42
Boscalid + Pyraclostrobin	2 g	0.42	0.37	0.37	0.36	0.32	0.33	0.40	0.43	0.44	0.43	0.44	0.43	0.39
Prochloraz	0.3 ml	0.52	0.39	0.27	0.14	0.17	0.38	0.35	0.27	0.22	0.25	0.22	0.25	0.30
Prochloraz	0.6 ml	0.32	0.31	0.26	0.14	0.16	0.32	0.25	0.27	0.20	0.22	0.20	0.22	0.25
Prochloraz	1 ml	0.25	0.24	0.22	0.12	0.15	0.30	0.22	0.21	0.13	0.20	0.13	0.20	0.20
Prochloraz	1.5 ml	0.23	0.16	0.18	0.11	0.14	0.25	0.21	0.21	0.11	0.18	0.11	0.18	0.18
Azoxystrobin + Tebuconazole	0.2 ml	0.16	0.47	0.38	0.44	0.40	0.43	0.32	0.40	0.40	0.40	0.40	0.40	0.38
Azoxystrobin + Tebuconazole	0.4 ml	0.15	0.38	0.30	0.34	0.33	0.34	0.26	0.32	0.28	0.40	0.28	0.40	0.31
Azoxystrobin + Tebuconazole	0.75 ml	0.14	0.30	0.20	0.26	0.26	0.23	0.22	0.27	0.17	0.39	0.17	0.39	0.24
Azoxystrobin + Tebuconazole	1.25 ml	0.10	0.20	0.15	0.18	0.19	0.12	0.17	0.19	0.08	0.37	0.08	0.37	0.18

+ difenoconazole, 10, 30, and 75 ml/100 l water doses of the fungicide were used and the efficacy levels obtained were 57.13%, 68.08%, and 83.80%, respectively. For isopyrazam + difenoconazole fungicide, efficacy levels of 42.44%, 58.26%, and 65.62% were obtained at 20, 40, and 80 ml/100 l water doses, respectively. Dodine provided 39.60%, 46.42%, and 52.29% disease control at doses of 20, 50 and 100 ml/100 l water, respectively. Prochloraz showed biological efficacy of 33.44%, 39.90%, and 48.30% in trials with 20, 50, and 100 ml/100 l water doses, respectively (Table 7).

Table 4. Biological efficacy results of different doses of fungicides used under field conditions in controlling Septoria leaf blight disease

Fungicides	Doses (100 l)	Effect (%)
Boscalid + Pyraclostrobin ^a	20 g	59.75
	40 g	70.03
	75 g	85.81
Azoxystrobin + Tebuconazole ^a	10 ml	57.13
	30 ml	68.08
	75 ml	83.80
Prochloraz ^d	20 ml	33.44
	50 ml	39.90
	100 ml	48.30
Isopyrazam +Difenoconazole ^b	20 ml	42.44
	40 ml	58.26
	80 ml	65.62
Dodine ^c	20 ml	39.60
	50 ml	46.42
	100 ml	52.29

DISCUSSION

Pistachio production in the world is carried out by direct garden establishment or grafting of wild *Pistacia species* and there are many wild *Pistacia species* in our country (Ak and Kaşka 1998). Species of the genus *Septoria* cause leaf spots on pistachios and other Pistaceae, prevent the plant from photosynthesizing, and cause premature leaf fall, resulting in yield losses. In the studies carried out with *Septoria-like* fungi in our country, the causal organisms were clearly separated from each other, and the causal agent of Karazenk disease in pistachio was redefined as *Pseudocercospora pistacina*, while it was revealed that *Septoria pistaciarum* causes leaf spots not only in pistachio but also in other Pistacias (Crous et al. 2013, Sarpkaya 2014).

Although there are many effective fungicides in the control of 'Karazenk' disease, which is widespread in pistachio

production areas in the Southeast region, studies on the control of *Septoria* leaf spot disease caused by *S. pistaciarum* are limited. Call and Matheron (1994) reported that all fungicides reduced the severity of the disease in field studies conducted in the United States of America with chlorothalonil, benomyl, and copper hydroxide.

The most effective fungicides were boscalid + pyraclostrobin and azoxystrobin + difenoconazole group fungicides in the *in vitro* mycelial radial growth inhibition study with *S. pistaciarum* isolates used in the study. Pappas et al. 2010, in a study conducted with 36 isolates of *Septoria pyricola*, found that fungicides containing boscalid and azoxystrobin were highly effective. In *in vitro* fungicide efficacy studies carried out against *S. petroselini*, which is a problem in parsley, it was found that fungicide containing tebuconazole was highly effective in inhibiting mycelial development. Erdurmuş et al. (2024) determined the susceptibility levels of the isolates to azoxystrobin, tebuconazole, and mancozeb by radial growth test in their study on the determination of fungicide susceptibilities against *Alternaria alternata*, early leaf blight of tomato and determined that some isolates were highly susceptible to the mentioned fungicides.

In the fungicide efficacy experiment conducted in pistachio orchards contaminated with natural *S. pistaciarum* in field studies, boscalid + pyraclostrobin and azoxystrobin + difenoconazole fungicides were found to be highly effective compared to the others. On the other hand, the lowest efficacy was observed with prochloraz fungicide. Fungicides with this active ingredient are widely used in many crops in the world with licences (Anonymous 2024b). However, since *Septoria* leaf spot disease is not defined in the agricultural control technical instructions of our country, there is no registration.

Pistachio areas in our country have increased by 37% in the last decade and although most of the production is carried out in the Southeastern Anatolia Region, pistachio cultivation is also increasing in the Aegean, Mediterranean, Marmara and Central Anatolia regions (İlikçiöğlü 2022). With the expansion of production in different regions, different diseases may occur in pistachios. *Septoria* leaf spot disease is seen as an important factor that will limit pistachio production. The data obtained from this study have an important place in controlling the disease.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Antep fıstığı (*Pistacia vera* L.), yüksek besin değerine sahip olması ve uluslararası pazarlarda da talep görmesi nedeniyle stratejik bir tarım ürünü olarak kabul edilir. Son yıllarda üretim alanlarının genişlemesiyle birlikte, Antep fıstığı yetiştiriciliği artarken, hastalık ve zararlılarla mücadele ön plana çıkmaktadır. *Septoria pistaciarum* Caracc.'ın, sebep olduğu Septoria yaprak lekeli hastalığı Antep fıstığı ağaçlarında yaprak lekeleri oluşturan önemli bir fungal patojendir. Bu çalışmada farklı *Pistacia* türlerinden elde edilen on izolat üzerinde farklı gruptan yer alan fungusitlerin etkinlikleri araştırılmıştır. Patojen fungusun *in vitro* miseliyal radial gelişiminin inhibe edilmesinde azoxystrobin + difenoconazole etken maddeli fungusitin 1.25 ml l⁻¹ dozunda %85.15 düzeyinde en yüksek oranda gelişimin engellendiği, dodine'in 0.3 ml l⁻¹ dozunda fungal gelişimin %10.30 ile en düşük düzeyde inhibe edildiği görülmüştür. Karabük ilinde *S. pistaciarum* ile bulaşık Antep fıstığı ağaçlarında yürütülen çalışmada boscalid + pyraclostrobin etken maddeli fungusitin 75 g/100 l su dozunda %85.81 oranında en yüksek biyolojik etkinliği gösterdiği, azoxystrobin + tebuconazole etken maddeli fungusitin 75 ml/100 l su dozunda %83.80 düzeyinde hastalık kontrolü sağladığı görülmüştür. Septoria yaprak lekeli hastalığının kontrolünde en düşük biyolojik etkinliği prochloraz (%33.44) etken maddeli fungusit göstermiştir. Bu sonuçlar, Antep fıstığı yetiştiriciliğinde Septoria yaprak lekeli hastalığı ile mücadelede kullanılan fungusitlerin etkililik oranlarını ortaya koyarak, hastalık kontrolü açısından önemli veriler sunmaktadır.

Anahtar kelimeler: *Septoria pistaciarum*, Antep fıstığı, fungusit, hastalık kontrolü

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Original article

Detection of harmful Curculionoidea (Insecta: Coleoptera) species in stone fruit trees of Kahramanmaraş, Adıyaman, Gaziantep provinces (Türkiye)

Kahramanmaraş, Adıyaman ve Gaziantep illeri sert çekirdekli meyve ağaçlarında zararlı Curculionoidea (Insecta: Coleoptera) türlerinin tespiti

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ABSTRACT

In this study, Curculionoidea (Insecta: Coleoptera) species were determined in Kahramanmaraş, Adıyaman and Gaziantep provinces 2022, in almond (*Amygdalus communis* L.), apricot (*Prunus armenica* L.), cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.), peach (*Prunus persica* L.), plum (*Prunus domestica* L.) trees. Visual inspection method, shoot removal method and impact method were applied to determine the species. Surveys were carried out weekly (once a week for the specified provinces) from the beginning of March to the end of October, considering the flowering periods of fruit trees. Based on the results, two species from Curculionoidea superfamily to the Rhynchitidae family; *Tatianaerhynchites aequatus* (Linnaeus, 1767), *Epihynchites (Colonnellinius) smyrnensis* (Desbrochers des Loges, 1869) and 11 species belonging to the Curculionidae family; *Anthonomus (Anthonomus) pyri* Boheman, 1843, *Tychius (Tychius) picirostris* (Fabricius, 1787), *Tychius (Tychius) brevisculus* Desbrochers, 1873, *Smicronyx (Smicronyx) jungermanniae* Reich, 1797, *Ceutorhynchus assimilis* Paykull, 1792, *Ceutorhynchus picitarsis* Gyllenhal, 1837, *Lixus (Dilixellus) vilis* (Rossi, 1790), *Polydrusus (Eustolus) ponticus* Faust, 1888, *Sitona macularius* (Marsham, 1802), *Sitona lineellus* (Bonsdorff 1785), *Mylocerus damascenus* Miller, 1861 a total of 13 species were identified. Among these species, *S. lineellus*, *C. assimilis*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *T. aequatus* were determined to be the first record for Kahramanmaraş province, *T. aequatus*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae* were determined to be the first record for Gaziantep province and *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae*, *L. vilis* were determined to be the first record for Adıyaman province.

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INTRODUCTION

Türkiye has many fruit species, genetic resources, and natural distribution areas since it has different climate characteristics and geographical locations (Polat and

Kazankaya 2020). Stone fruits are located in the Rosales order in the Prunoideae subfamily of the Rosaceae family (Özçağırın et al. 2003). These fruits have economic value

in the temperate climate regions of the world (Şimşek et al. 2020). One of the most critical problems in fruit growing is plant protection problems. Insects are indispensable pests in orchards due to the number of species they have. Insects belonging to the superfamily Curculionoidea are the most important pests that cause economic damage to stone fruits. Most individuals belonging to Curculionoidea are polyphagous (Borror et al. 1989). More than one species can be found on parts of plants such as roots, stems, leaves, or fruits, and both larvae and adults of the same species can cause damage to the same plant (Mihajlova 1978). The studies conducted in our country by Lodos (1960) reported that *Polydrosus roseiceps* Pes. (Coleoptera: Curculionidae) is a polyphagous pest whose damage was observed in apples, apricots, plums, sour cherries, and cherries. They stated that the damage caused by *P. roseiceps* on almond trees in Elazığ and Mardin provinces was significant (Bolu and Özgen 2005, 2009, Maçan 1986). Bolu et al. (2005) stated that pistachio, almond, and cherry are the most important fruit species grown in the provinces of the GAP area. They identified 12 species of pistachio trees belonging to the Curculionoidea superfamily. Özbek (2016), in their study in Eskişehir, they determined that *Otiorhynchus ovalipennis* Boheman (1842) (Coleoptera: Curculionidae: Entiminae) fed on sour cherry and cherry trees. The study indicated that the feeding started from the leaf edges and the damaged leaves were broken irregularly. Öztürk and Ulusoy (2014) determined the damage type, damage rate and mechanical control effectiveness of *Polydrusus ponticus* Faust (Coleoptera: Curculionidae) in apricots in their study carried out in Malatya apricot orchards. Kahramanmaraş, Adıyaman, and Gaziantep provinces have more advantages than stone fruit growing provinces due to their location and climate conditions. The low effect of late spring frosts is especially important for almond and apricot cultivation. In this study, species belonging to the Curculionoidea superfamily that cause economic damage to fruits in stone fruit orchards were detected in almond, apricot, cherry, sour cherry, peach and plum.

MATERIALS AND METHODS

This study was carried out in 2022 in Kahramanmaraş, Adıyaman, and Gaziantep provinces to determine the species affiliated with the Curculionoidea superfamily. Periodic field exits were made weekly (once a week for the specified provinces) from the beginning of march to the end of october, considering the flowering periods of fruit trees. In the research, gardens that could best represent the study area were randomly selected and samples were taken. Sampling was done in 30 gardens in Kahramanmaraş,

Adıyaman, and Gaziantep provinces, as given in Table 1. In addition, care was taken to visit different regions and gardens as much as possible during the sampling. Visual control, impact, and shoot removal methods were applied in species sampling.

Visual inspection method

Depending on the number of trees in the sampling garden, adults visible on the trunk, branches, shoots, leaves, and fruits of the plant were collected by hand or with a mouth aspirator and labeled by walking around each tree for 2-3 minutes. Those in the pre-adult stage were either cut with the plant organ they were located in or taken with forceps, brought to the laboratory with their food, and cultured to observe adult emergence.

Shooting method

Five shoot and branch samples were cut from different directions of the selected trees in the garden, brought to the laboratory in labeled and sealed polyethylene bags, examined with a stereo microscope, and the insects present were recorded.

Impact method

It is a method used to determine harmful and beneficial species on trees and was applied during vegetation. According to this method, the number of trees in the garden where the sampling would be done was taken as a basis. Trees were randomly selected in the garden, and one branch from each of the four directions was hit with a stick (40 cm) with a piece of plastic pipe on its end three times at the same speed, and the insects were allowed to fall into the Japanese umbrella. The samples falling, and then their positions were recorded, and then under the Japanese umbrella was transferred to polyethylene bags, labeled, and their positions recorded. Then, they were placed in an ice box and brought to the laboratory for examination. To reach a general conclusion about the population densities, the insects collected in the umbrella were evaluated according to the scale used by Maçan (1986). According to this scale, the number of samples belonging to a harmful species collected in the Japanese umbrella; if it varies between 1-5, it is considered low (insignificant); if it varies between 6-10, it is considered medium (be maybe necessary) and if it is 11 and above, it is considered high (important significant) (Table 3).

Laboratory studies

Sample bags brought to the laboratory were opened individually, flower samples were checked with a fine-

Table 1. Areas where the study was conducted and the number of sampled orchards in the stone fruit orchards of Kahramanmaraş, Adıyaman, and Gaziantep provinces

Province	Village/Town	Location	Number of gardens sampled
Kahramanmaraş	Sekamer	N37°35'28,975" E37°3'30,066"	1
	Tilkiler	N37°30'36" E37°27'13"	1
	Uzunsöğüt	N37°24'0,6289" E36°47'19,956"	2
		N37°23'35,554"E36°48'59,529"	
	Aşağımülk	N37°26'16,148"E37°30'43,459"	1
	Ulubahçe	N37°30'38,443" E37°22'41,760"	1
	Kozludere	N37°36'51,871 E37°6'27,905"	1
	Gani Dağı	N37°30'44,482"E37°25'10,578"	1
	Kümperli	N37°25'28,976"E36°40'1,6032"	1
	Salmanıpak	N37°25'50,924"E37°12'55,829"	1
	Dereköy	N37°34'46, 189" E37°2'26,443"	1
	KSÜ Kampüs	N37°35'20,914"E36°48'57,960"	2
		N37°35'35"0"E36°49'17.6"	
	Tekerek	N37°35'4,6950"E36°51'50,384"	1
Demrek	N37°37'28,456"E36°38'32,543"	1	
Bulutoğlu	N37°39'54,938"E36°48'37,789"	4	
	N37°38'54,328"E36°46'58,325"		
	N37°38'17,7"E38°46'02,9"		
	N37°38'17,7"E38°46'02,9"		
Gaziantep	Bilek	N37°07'43" E37°33'06"	1
	Yumurtacızade	N37°12'41,635"E37°29'8,4496"	1
	Serintepe	N37°15'59" E37°13'10"	1
	Yalnızbağ	N36°59'31,664"E87°27'41,552	1
	İkizkuyu 2	N36°57'56,529"E37°30'50,309	1
Adıyaman	Yukarçöplü	N37°45'54" E37°43'07"	1
	Şambayat	N37°41'39" E38°01'08"	1
	Şerefli	N37°07'43"E38°03'54"	1
	Kurugöl	N37°37'36N"E37°45'48"	1
	Tepecikli	N37°47'40"E38°04'09"	1
	Çatalağaç	N37°38'03"E37°30'15"	1
Total			30

tipped brush, and counts were performed. Adult individuals obtained from flowers during counts were examined under a stereo microscope and then killed in killing bottles containing potassium cyanide. Adult individuals obtained were pricked with insect needles, labeled, grouped according to order and family levels, placed in appropriate boxes, and made ready for identification.

RESULTS AND DISCUSSION

This study conducted to determine the species belonging to the Curculionoidea superfamily in Kahramanmaraş,

Gaziantep, and Adıyaman provinces, two genera and two species belonging to the Rhynchitinae subfamily of the Rhynchitidae family, eight genera and eleven species belonging to the Curculioninae, Ceutorhynchinae, Lixinae, Entiminae subfamilies of the Curculionidae family were identified (Table 2). In addition, the identified species economically important and unimportant status was determined (Table 3).

Table 2. Species and host plants belonging to the Curculionoidea superfamily identified in Kahramanmaraş, Gaziantep, and Adıyaman provinces in 2022

Superfamily	Family	Subfamily	Species	Host plant
Curculionoidea	Rhynchitidae	Rhynchitinae	<i>Tatianaerhynchites aequatus</i> (Linnaeus, 1767)*, **, ***	<i>Amygdalus communis</i> L.
			<i>Epihynchites smyrnensis</i> (Desbrochers des Loges, 1869) **	<i>Amygdalus communis</i> L.
	Curculionidae	Curculioninae	<i>Anthonomus pyri</i> Boheman, 1843 *	<i>Amygdalus communis</i> L.
			<i>Tychius picirostris</i> (Fabricius, 1787) *	<i>Prunus cerasus</i> L.
				<i>Amygdalus communis</i> L.
			<i>Tychius brevisculus</i> Desbrochers, 1873 *, ***	<i>Prunus domestica</i> L.
				<i>Amygdalus communis</i> L.
				<i>Prunus armeniaca</i> L.
				<i>Prunus avium</i> L.
			<i>Smicronyx jungermanniae</i> Reich, 1797 *, ***	<i>Prunus cerasus</i> L.
		<i>Prunus avium</i> L.		
	Ceutorhynchinae	<i>Ceutorhynchus assimilis</i> Paykull, 1792 *	<i>Amygdalus communis</i> L.	
			<i>Prunus cerasus</i> L.	
		<i>Ceutorhynchus picitarsis</i> Gyllenhal, 1837 *	<i>Amygdalus communis</i> L.	
	Lixinae	<i>Lixus vilis</i> (Rossi, 1790) *, **	<i>Amygdalus communis</i> L.	
Entiminae		<i>Polydrus ponticus</i> Faust, 1888 *	<i>Amygdalus communis</i> L.	
		<i>Sitona macularius</i> (Marsham, 1802)*	<i>Amygdalus communis</i> L.	
		<i>Sitona lineellus</i> (Bonsdorff 1785) *	<i>Amygdalus communis</i> L.	
		<i>Myllocerus damascenus</i> Miller, 1861*	<i>Amygdalus communis</i> L.	

*Kahramanmaraş, ** Adıyaman, ***Gaziantep

Table 3. Damage status of species belonging to the Curculionoidea superfamily detected in Kahramanmaraş, Gaziantep, and Adıyaman provinces in 2022 (1: important, 2: may be important, 3: insignificant)

Superfamily	Family	Species	Kahramanmaraş	Adıyaman	Gaziantep
Curculionoidea	Rhynchitidae	<i>Tatianaerhynchites aequatus</i> (Linnaeus, 1767)	1	1	2
		<i>Epirhynchites smyrnensis</i> (Desbrochers des Loges, 1869)		3	
	Curculionidae	<i>Anthonomus pyri</i> Boheman, 1843	1		
		<i>Tychius picirostris</i> (Fabricius, 1787)	2		
		<i>Tychius brevisculus</i> Desbrochers, 1873	1		3
		<i>Smicronyx jungermanniae</i> Reich, 1797	1		2
		<i>Ceutorhynchus assimilis</i> Paykull, 1792	1		
		<i>Ceutorhynchus picitarsis</i> Gyllenhal, 1837	3		
		<i>Lixus vilis</i> (Rossi, 1790)	3	3	
		<i>Polydrus ponticus</i> Faust, 1888	1		
		<i>Sitona macularius</i> (Marsham, 1802)	3		
		<i>Sitona lineellus</i> (Bonsdorff 1785)	3		
		<i>Mylocerus damascenus</i> Miller, 1861	1		

Family: Rhynchitidae Gistel, 1848

Subfamily: Rhynchitinae Gistel, 1856

Tribus: Rhynchitini Gistel, 1856

Genus: *Tatianaerhynchites* Legalov, 2002

Species: *Tatianaerhynchites aequatus* (L., 1767)

Material examined: Kahramanmaraş/Dulkadiroğlu Sekamer Village, N37°35'28,975" E37°3'30,066", 31.03.2022, *Amygdalus communis* L. (almond), number of samples: 8♀, 7♂; Kahramanmaraş/Pazarçık Tilkiler Village, N37°30'36" E37°27'13", 09.IV.2022, *Amygdalus communis* L. (almond), number of samples: 4♀, 1♂; Adıyaman/Besni Şambayat Village, N37°41'39" E38°01'08", 09.IV.2022, *Amygdalus communis* L. (almond), number of samples: 14♀; Gaziantep/Şehitkamil Bilek Village, N37°07'43" E37°33'06", 10.IV.2022, *Amygdalus communis* L. (almond), number of samples: 2♀, 5♂ (Figure 1).

Distribution in Türkiye: Diyarbakır, Elazığ, Mardin, Siirt, Şanlıurfa, Adıyaman, Manisa, Muğla (Bolu and Özgen 2005, Bolu et al. 2005, Bolu 2006, Bolu and Legalov 2008, Bolu 2016, Tolga and Yoldaş 2020).

Genus: *Epirhynchites* Voss, 1953

Species: *Epirhynchites (Colonnellinius) smyrnensis* (Desbrochers des Loges, 1869)

Material examined: Adıyaman/Gölbaşı Yukarıçöplü Village, N37°45'54" E37°43'07", 09.IV.2022, *Amygdalus communis* L. (almond), number of samples: 3♀ (Figure 2).

Distribution in Türkiye: Ankara, Antalya, Bilecik, Bursa, Balıkesir, Diyarbakır, Edirne, Elazığ, Hatay, İzmir, Kırklareli, Kırşehir, Kapadokya, Kütahya, Malatya, Muğla, Mardin, Sivas, Isparta, Adıyaman, Batman, Gaziantep, Kilis, Siirt, Şanlıurfa, Şırnak, Toros, Tekirdağ and Uşak (Bodemeyer 1900, Lodos 1960, Voss 1969, Tüzün 1975, Maçan 1986, Erol and Önder 1991, Erol 1994, Bolu et al. 2005, Legalov and Friedman 2007, Bolu 2016).

Family: Curculionidae Latreille, 1802

Subfamily: Curculioninae Latreille, 1802

Tribus: Anthonomini Thomson, 1859

Genus: *Anthonomus* Germar, 1817

Species: *Anthonomus (Anthonomus) pyri* Boheman, 1843

Material examined: Kahramanmaraş/Türkoğlu Uzunsöğüt Village, N37°24'0,6289"E36°47'19,956", 27.III.2022, *Amygdalus communis* L. (almond), number of samples: 3♀; Kahramanmaraş/Türkoğlu Uzunsöğüt2 Village, N37°23'35,554"E36°48'59,529", 31.III.2022 *Amygdalus communis* L. (almond), number of samples: 3♀, 4♂; Kahramanmaraş/Pazarcık Ulubahçe Village, N37°30'38,443"E37°22'41,760", 21.IV.2022, *Amygdalus communis* L. (almond), number of samples: 5♀, Kahramanmaraş/Dulkadiroğlu Sekamer, N37°35'28,975"E37°3'30,066", 28.IV.2022, *Amygdalus communis* L. (almond), number of samples: 7♀, 4♂ (Figure 3).

Distribution in Türkiye: Muğla and Manisa (Tolga and Yoldaş 2020).

Tribus: Tychiini C.G. Thomson, 1859

Genus: *Tychius* Germar, 1817

Species: *Tychius (Tychius) picirostris* (Fabricius, 1787)

Material examined: Kahramanmaraş/Dulkadiroğlu Kozludere Village, N37°36'51,871"E37°6'27,905", 28.IV.2022, *Prunus cerasus* L. (sour cherry) number of samples: 4♀; Kahramanmaraş/Türkoğlu Uzunsöğüt Village, N37°23'35,554"E36°48'59,529", 31.III.2022, *Amygdalus communis* L. (almond), number of samples: 3♀ (Figure 4).

Distribution in Türkiye: Diyarbakır, Mardin, Adana, Osmaniye, Ankara, Antalya, Çankırı, Konya, Sivas, Yozgat (Bolu 2016, Sert 2005).

Species: *Tychius (Tychius) brevisculus* Desbrochers, 1873

Material examined: Kahramanmaraş/Pazarcık Ganıdağı Village, N37°30'44,482" E37°25'10, 578", 07.IV.2022, *Prunus domestica* L. (plum) number of samples: 3♂; Kahramanmaraş/Pazarcık Salmanıpak Village, N37°25'50,924" E37°12'55,829", 21.IV.2022, *Amygdalus communis* L. (almond), number of samples: 5♂; Kahramanmaraş/Dulkadiroğlu Sekamer, N37°35'28,975"E37°3'30,066", 28.IV.2022, *Prunus armeniaca* L. (apricot) number of samples: 1♂, 1♀; Kahramanmaraş/Onikişubat Kümperli Village, N37°25'28,976" E36°40'1,6032", 25.IV.2022, *Prunus avium* L. (cherry) number of samples: 2♂; Gaziantep/Şehitkamil Yumurtacızade Village, N37°12'41,635"E37°29'8,4496", 07.IV.2022, *Amygdalus communis* L. (almond), number of samples: 2♂ (Figure 5).

Distribution in Türkiye: Niğde, Manisa, Burdur, Isparta, Ankara, Eskişehir, Çankırı, Kayseri, Konya, Yozgat, Kırklareli, Uşak (Lodos et al. 1978, Sert and Çağatay 1999a, Tolga and Yoldaş 2020, Sert 2005).

Tribus: Smicronychini Seidlitz, 1891

Genus: *Smicronyx* Schoenherr, 1843

Species: *Smicronyx (Smicronyx) jungermanniae* Reich, 1797

Material examined: Kahramanmaraş/Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'20,914"E36°48'57,960", 31.03.2022, *Prunus domestica* L. (plum) number of samples: 13♀; Gaziantep/Şehitkamil Serintepe Village, N37°15'59"E37°13'10", 28.IV.2022, *Prunus avium* L. (cherry) number of samples: 7♀ (Figure 6).

Distribution in Türkiye: Konya, Nevşehir, Elazığ, Edirne, Muğla and Burdur (Lodos et al. 1978, Lodos et al. 2003, Kaplan and Yücel 2014, Forbicioni et al. 2019, Erbey and Bolu 2021).

Subfamily: Ceutorhynchinae Bedel, 1881

Tribus: Ceutorhynchini Gistel, 1848

Genus: *Ceutorhynchus* Germar, 1824

Species: *Ceutorhynchus assimilis* Paykull, 1792

Material examined: Kahramanmaraş/Onikişubat Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'20,914"E36°48'57,960", 31.03.2022, *Prunus domestica* L. (plum) number of samples: 3♀, 2♂; Kahramanmaraş/Dulkadiroğlu Dereköy Village, N37°34'46,189"E37°2'26,443", 31.III.2022, *Amygdalus communis* L. (almond), number of samples: 3♀, 1♂ (Figure 7).

Distribution in Türkiye: Trakya, Edirne, Erzincan, Erzurum, Tekirdağ, Ankara, İstanbul (Lodos et al. 1978, Sert 2005, Esentürk 2009, Gültekin 2001, Aydın and Hacet 2016).

Species: *Ceutorhynchus picitarsis* Gyllenhal, 1837

Material examined: Kahramanmaraş/Pazarcık Ulubahçe Village, N37°30'38,443"E37°22'41,760", 26.V.2022, *Amygdalus communis* L. (almond), number of samples: 3♀, 1♂ (Figure 8).

Distribution in Türkiye: Artvin, Erzurum, Edirne, Kars, Sivas, İstanbul, İzmir, Çanakkale, İzmir, Tekirdağ, Antalya, Bartın, Bitlis, İçel, Karaman, Kastamonu, Kırıkkale, Konya, Niğde, Ankara, Kayseri, Kırşehir, Sivas, Yozgat, Adana, Antalya, Burdur, Mersin, Niğde, Trabzon, Erzincan (Lodos et al. 1978, Sert 1995, Sert and Çağatay 1999, Lodos et al. 2003, Colonnelli 2004, Sert 2005, Gültekin 2001, Erbey 2010, Aydın 2013, Gürlü 2014, Alaserhat 2019, Hacet and Colonnelli 2019, Gültekin 2020).

Subfamily: Lixinae Schoenherr, 1823

Tribus: Lixini Reitter, 1912

Genus: *Lixus* Fabricius, 1801

Species: *Lixus (Dilixellus) vilis* (Rossi, 1790)

Material examined: Kahramanmaraş/Onikişubat Tekerek District, N37°35'4,6950"E36°51'50,384", 31.III.2022, *Amygdalus communis* L. (almond), number of samples: 4♂; Adiyaman/Merkez Şerefli Village, N37°07'43"E38°03'54", 09.IV.2022, *Amygdalus communis* L. (almond), number of samples: 1♂ (Figure 9).

Distribution in Türkiye: Niğde, Afyon, Aydın, Çanakkale, Edirne, Kırklareli, Manisa, Muğla, Hatay, Aksaray, Ankara, Balıkesir, Bursa, İzmir, Kütahya, Mardin, Isparta (Lodos et al. 1978, Pehlivan et al. 2005, Erbey 2010, Demirözer and Karaca 2011, Tolga and Yoldaş 2020).

Subfamiliya: Entiminae Schoenherr, 1823

Tribus: Polydrosini Champion, 1911

Genus: *Polydrusus* Germar, 1817

Species: *Polydrusus (Eustolus) ponticus* Faust, 1888

Material examined: Kahramanmaraş/Pazarcık Ulubahçe Village, N37°30'38,443" E37°22'41,760", 26.V.2022, *Amygdalus communis* L. (almond), number of samples: 9♀ 3♂; Kahramanmaraş/Dulkadiroğlu Sekamer Village, N37°35'28,975"E37°3'30,066", 25.V.2022, *Amygdalus communis* L. (almond), number of samples: 8♀ 1♂; Kahramanmaraş/Onikişubat Demrek, N37°37'28,456" E36°38'32,543", 06.VI.2022, *Amygdalus communis* L. (almond), number of samples: 2♀, 3♂ (Figure 10).

Distribution in Türkiye: Antalya, Ankara, Amasya, Afyon, Aydın, Aksaray, Adiyaman, Adana, Yozgat, Yalova, Uşak, Siirt, Samsun, Sakarya, Sinop, Samsun, Niğde, Mersin, Mardin, Manisa, Muğla, Malatya, Kütahya, Kocaeli, Kayseri, Kastamonu, Karabük, Karaman Kırklareli, Konya, Kırşehir, Kilis, Konya, Kahramanmaraş, İstanbul, İzmir, Isparta, Hatay, Giresun, Gaziantep, Elazığ, Eskişehir, Edirne, Diyarbakır, Denizli, Çorum, Çanakkale, Çankırı, Bursa, Burdur, Bolu, Bilecik, Bandırma, Bitlis, Balıkesir, Şırnak, Nevşehir, Osmaniye, Erzincan, Gümüşhane, Tekirdağ, Uşak, Zonguldak (Heyden and Faust 1888, Lodos 1960, Voss 1962, Balachowsky and Hoffmann 1963, Tuatay et al. 1972, Tüzün 1975, Lodos 1977, Lodos et al. 1978, Lodos et al. 1987, Çevik 1996, Tamer et al. 1997, Kaya 1999, Lodos et al. 2003, Erbey 2010, Ayaz and Yücel 2010, Kaplan and Yücel 2014, Öztürk and Ulusoy 2014, Tezcan et al. 2014, Yılmaz 2015, Alaserhat 2019, Kapucu 2019, Kaplan 2020, Ayaz 2021, Alaserhat and Bozbek 2021).

Tribus: Sitonini Gistel, 1848

Genus: *Sitona* Germar, 1817

Species: *Sitona macularius* (Marsham, 1802)

Material examined: Kahramanmaraş/Pazarcık Ulubahçe Village, N37°30'38,443" E37°22'41,760", 26.V.2022, *Amygdalus communis* L. (almond), number of samples: 3♀ (Figure 11).

Distribution in Türkiye: Ankara, Adana, Aksaray, Antalya, Adiyaman, Ağrı, Iğdır, Isparta, Ardahan, Afyon, Balıkesir, Bingöl, Bilecik, Bursa, Bolu, Bayburt, Bitlis, Denizli, Yalova, İzmir, Manisa, Muğla, Tekirdağ, Uşak, Elazığ, Erzincan, Erzurum, Edirne, Hakkari, Malatya, Muş, Tunceli, Van, Çankırı, Çorum, Çanakkale, Eskişehir, Gaziantep, Hatay, İçel, Kahramanmaraş, Karabük, Konya, Karaman, Kars, Kastamonu, Kütahya, Kayseri, Kilis, Kırkkale, Kırklareli, Kırşehir, Konya, Nevşehir, Niğde, Osmaniye, Yozgat, Sivas, Diyarbakır, Şanlıurfa (Lodos et al. 1978, Sert and Çağatay 1994, Tamer et al. 1998, Lodos et al. 2003, Coşkuncu and Gencer 2010, Avgın and Colonnelli 2011, Bolu 2016, Erdem 2016, Çekiç 2017, Gözüaçık et al. 2021).

Species: *Sitona lineellus* (Bonsdorff 1785)

Material examined: Kahramanmaraş/Türkoğlu Uzunsöğüt Village, N37°24'0,6289" E36°47'19,956", 27.III.2022, *Amygdalus communis* L. (almond), number of samples: 1♀, 3♂ (Figure 12).

Distribution in Türkiye: Bartın, Kütahya, Edirne, Kars, Artvin, Adiyaman, Zonguldak, Çankırı, Kayseri, Yozgat (Lodos 1977, Lodos et al. 2003, Sert and Kabalak 2013, Delbol and Lempereur 2014).

Tribus: Cyphicerini Lacordaire, 1863

Genus: *Myllocerus* Schoenherr, 1823

Species: *Myllocerus damascenus* Miller, 1861

Material examined: Kahramanmaraş/Onikişubat Bulutoğlu Village, N37°39'54,938"E36°48'37,789", 02.VIII.2022, *Amygdalus communis* L. (almond), number of samples: 13♀, 7♂ (Figure 13).

Distribution in Türkiye: Adiyaman, Batman, Diyarbakır, Gaziantep, Mardin, Malatya Siirt, Şanlıurfa, Şırnak, Mersin, Adana, Antalya, Diyarbakır, Hatay, Kahramanmaraş, Mardin, Niğde, Osmaniye (Osella 1977, Lodos et al. 2003, Öztürk et al. 2004, Bolu and Legalov 2008, Erbey 2010, Avgın and Colonnelli 2011).

While *S. lineellus*, *C. assimilis*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *T. aequatus* species were determined to be the

first record for Kahramanmaraş province, *T. aequatus*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae* were determined to be the first record for Gaziantep province. *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae*, *L. vilis* were determined to be the first record for Adıyaman province.

In addition, *T. picirostris* was first detected in sour cherry, *S. jungermanniae* in cherry, *T. brevisculus* in almond, cherry, plum, and apricot, *A. pyri* and *S. lineellus* in almond, *C. assimilis* in almond and plum.

It was determined that *T. aequatus*, *A. pyri*, *T. brevisculus*, *S. jungermanniae*, *C. assimilis* caused economic losses due to their population density in Kahramanmaraş province. It was observed that they caused damage to stone fruit trees during the flowering period and it was determined that they prevented fruit set as a result of feeding on the flower. *M. damascenus* and *P. ponticus* were found densely in the almond fields of Kahramanmaraş province and it was determined that they feed on the new leaves and young shoots of the almond tree. It was observed that they commonly feed by creating 2-3 cm semicircular holes starting from the leaf edges and then gnawing inwards along the leaf veins. Although it does not cause economic loss, it has been determined that it causes significant damage to the green parts of the almond tree.

As a result, this study has identified species belonging to the Curculionoidea superfamily that cause significant damage to stone fruit orchards in Kahramanmaraş, Adıyaman, and Gaziantep provinces and contributed to the Curculionoidea fauna of Türkiye. In addition, this study has formed the basis for future studies.

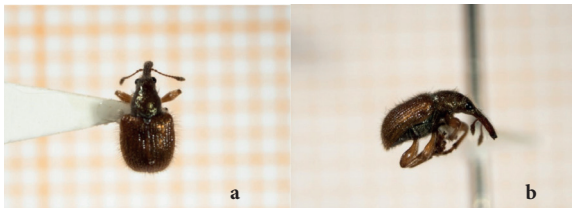


Figure 1. a) dorsal view b) lateral view of *Tatianaerhynchites aequatus* (Linnaeus, 1767)



Figure 2. *Epihynchites (Colonnellini) smyrnensis* (Desbrochers des Loges, 1869)

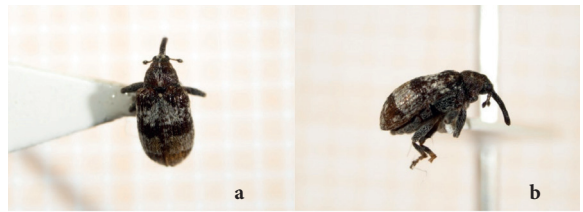


Figure 3. a) dorsal view b) lateral view of *Anthonomus pyri* Boheman, 1843



Figure 4. a) dorsal view b) lateral view of *Tychius picirostris* (Fabricius, 1787)

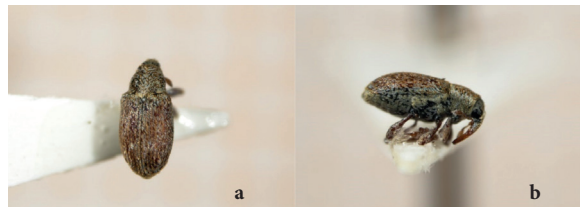


Figure 5. a) dorsal view b) lateral view of *Tychius brevisculus* Desbrochers, 1873

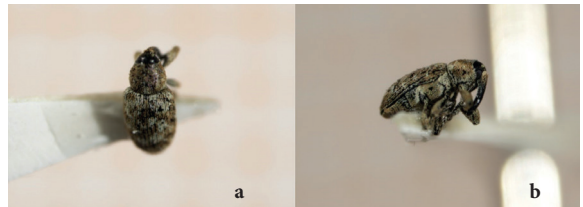


Figure 6. a) dorsal view b) lateral view of *Smicronyx jungermanniae* Reich, 1797

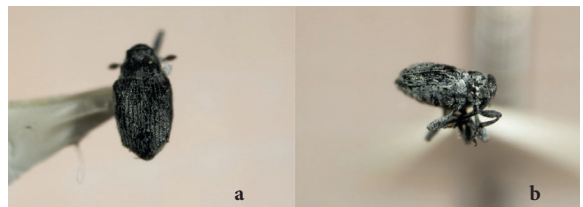


Figure 7. a) dorsal view b) lateral view of *Ceutorhynchus assimilis* Paykull, 1792

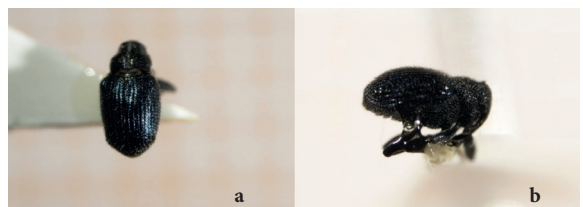


Figure 8. a) dorsal view b) lateral view of *Ceutorhynchus picitarsis* Gyllenhal, 1837

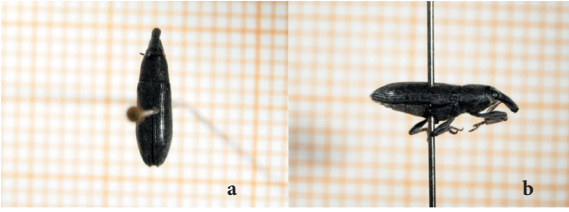


Figure 9. a) dorsal view b) lateral view of *Lixus vilis* (Rossi, 1790)

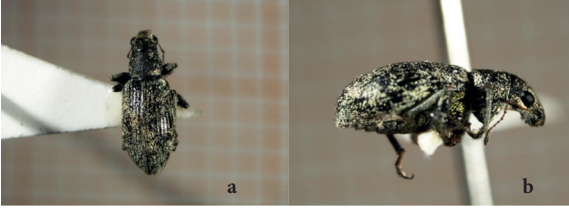


Figure 10. a) dorsal view b) lateral view of *Polydrus ponticus* Faust, 1888

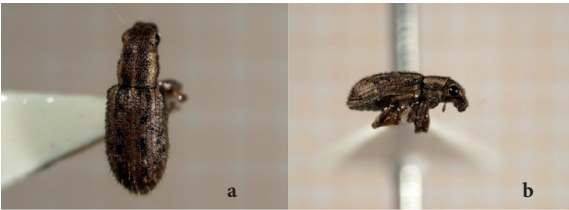


Figure 11. a) dorsal view b) lateral view of *Sitona macularius* (Marsham, 1802)

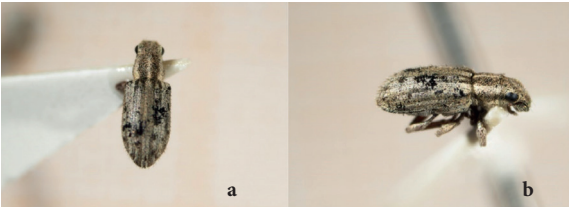


Figure 12. a) dorsal view b) lateral view of *Sitona lineellus* (Bonsdorff 1785)

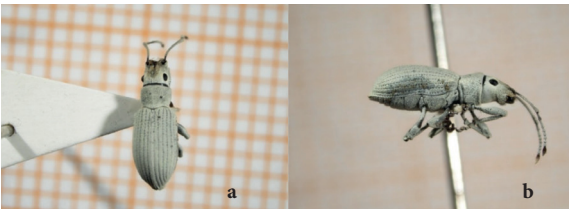


Figure 13. a) dorsal view b) lateral view of *Myllocerus damascenus* Miller, 1861.

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Author's Contributions

The authors have declared no conflict of interest.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Kahramanmaraş, Adıyaman ve Gaziantep illerinde 2022 yılında yapılan bu çalışma ile sert çekirdekli meyve ağaçları badem (*Amygdalus communis* L.), kayısı (*Prunus armenica* L.), kiraz (*Prunus avium* L.), vişne (*Prunus cerasus* L.), şeftali (*Prunus persica* L.), erik (*Prunus domestica* L.) de zarar yapan Curculionoidea (Insecta: Coleoptera) türleri saptanmıştır. Türlerin tespitinde gözle kontrol metodu, sürgün alma metodu ve darbe metodu uygulanmıştır. Sürveyler meyve ağaçlarının çiçeklenme dönemleri göz önünde bulundurularak mart başından - ekim sonuna kadar haftalık (belirtilen iller için haftada 1 kez) periyodik arazi çıkışları yapılmıştır. Çalışma sonucunda; Curculionoidea üst familyasından Rhynchitidae familyasına bağlı 2 tür; *Tatianaerhynchites aequatus* (Linnaeus, 1767), *Epihynchites (Colonnellinius) smyrnensis* (Desbrochers des Loges, 1869) ve Curculionidae familyasına bağlı 11 tür; *Anthonomus (Anthonomus) pyri* Boheman, 1843, *Tychius (Tychius) picirostris* (Fabricius, 1787), *Tychius (Tychius) brevisculus* Desbrochers, 1873, *Smicronyx (Smicronyx) jungermanniae* Reich, 1797, *Ceutorhynchus assimilis* Paykull, 1792, *Ceutorhynchus picitarsis* Gyllenhal, 1837, *Lixus (Dilixellus) vilis* (Rossi, 1790), *Polydrusus (Eustolus) ponticus* Faust, 1888, *Sitona macularius* (Marsham, 1802), *Sitona lineellus* (Bonsdorff 1785), *Myllocerus damascenus* Miller, 1861 olmak üzere toplam 13 tür tespit edilmiştir. Bu türlerden *S. lineellus*, *C. assimilis*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *T. aequatus* türlerinin Kahramanmaraş ili için ilk kayıt, *T. aequatus*, *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae* Gaziantep ili için ilk kayıt ve *A. pyri*, *T. picirostris*, *T. brevisculus*, *S. jungermanniae*, *L. vilis* Adıyaman ili için ilk kayıt olduğu belirlenmiştir.

Anahtar kelimeler: Curculionoidea, Kahramanmaraş, Adıyaman, Gaziantep, sert çekirdekli meyveler

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Original article

Identification of ‘*Candidatus Phytoplasma solani*’ phytoplasma-associated diseases in eggplants exhibiting abnormal flower structure (phyllody and virescence) and witches’ broom symptoms in Şanlıurfa province

Şanlıurfa ilinde anormal çiçek yapısı (phyllody ve virescence) ve cadı süpürgesi simptomları gösteren patlıcan bitkilerinde ‘*Candidatus phytoplasma solani* fitoplazma-ilişkili hastalığın tanımlanması

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ABSTRACT

Phytoplasmas cause infections in numerous plants in agricultural ecosystems, causing significant yield and quality losses in products. In recent years, it has been known that diseases caused by phytoplasmas cause economic losses in eggplant (*Solanum melongena* L.) cultivation. In Turkey, research on infections caused by phytoplasmas in eggplant growing areas is quite limited. This study was carried out to detect phytoplasma infections symptomatologically and molecularly in eggplant production areas in Şanlıurfa province. Fourteen samples were collected from eggplants exhibiting symptoms such as witches' broom, flower abnormalities (virescence, phyllody), elongation of the pedicle, arising of new shoots from flower parts, yellowing and proliferation. Phytoplasma infection was detected in 8 symptomatic samples using 16S rRNA-specific primers, P1/P7 and R16F2n/R16R2, by direct and nested PCR. Sequence information of fragments obtained as a result of molecular studies was extracted and BLAST analyses were performed. According to nucleotide sequence similarity in the 16S rRNA gene region, it was determined that the genetic group of phytoplasma causing infection in eggplant was related to ‘*Candidatus Phytoplasma solani*’ (CaPsoI) belonging to 16SrXII-A subgroup with 98% sequence identity. To our best knowledge, this study suggests comprehensive symptomatic diagnosis of CaPsoI infecting eggplants in Türkiye.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an important crop that can be easily cultivated in tropical and subtropical regions, and it provides nutrition and economic value with a high

yield and a short maturation period. Eggplant, which is commonly grown in China, India, Egypt, France, Italy and Spain (Rao and Kumar 2017), has been largely produced in

Türkiye with 817.591 tons in an area of 166.619 acres (TÜİK 2023). Şanlıurfa province has a significant potential in terms of eggplant production in the Southeastern Region.

Among these biotic stress factors, phytoplasmas, which have no direct control method, are of particular importance. Phytoplasmas are bacterial plant pathogens, a group of microorganisms that lack a cell wall and are genetically related to a Gram-positive ancestor (Weisburg et al. 1989). They are restricted to the phloem tissue in plants and are transmitted by sap-sucking insect vectors such as Cicadellidae, Fulgoromorpha, and Psyllidae (Weintraub and Beanland 2006). Phytoplasmas, whose molecular differences were defined using the 16S ribosomal gene as the basic standard (Duduk et al. 2010), have been classified into 48 tentative species and more than 150 subgroups so far, and 27 complete genomes have been sequenced (Wang et al. 2024). Among these genetic groups, 'Candidatus Phytoplasma solani' (CaPsoI) (subgroup 16SrXII-A), which is particularly widespread in the vineyards of the Euro-Mediterranean basin and poses a potential threat to viticulture worldwide and causes losses in members of the Solanaceae, has become remarkable in agroecosystems (Navrátil et al. 2009, Quaglino et al. 2019). Infections caused by CaPsoI have been reported from different geographical regions of Türkiye and various agricultural ecosystems where perennial or annual plants such as tobacco, pepper, tomato and grapevine are grown (Erilmez et al. 2022, Usta et al. 2022, Zelyüt 2023, Zelyüt et al. 2022). Studies on phytoplasma-associated infections in eggplant cultivation areas of our country are quite limited and have only been reported from the Eastern Mediterranean and Eastern Anatolia regions of Turkey (Sertkaya et al. 2007, Usta et al. 2022). Moreover, it has been reported from different countries of the world that phytoplasma-induced infections in eggplant plants are associated with six different ribosomal groups (16SrI, 16SrII, 16SrIII, 16SrVI, 16SrIX, 16SrXII) (Rao and Kumar 2017). More specifically, phytoplasma-associated diseases have been reported to cause losses of up to 40% in eggplant plants (Mitra et al. 1993, Rao et al. 2011).

Phytoplasmas were identified in more than 1000 plant species with diseases and different symptoms exhibited in field crops, horticultural and ornamental plants and weeds (Bertaccini 2022, Bertaccini et al. 2022) and exhibit quite diverse symptoms from the behaviour and physiology of normal plants. However, eggplants infected with phytoplasma-associated diseases show widespread symptoms including dwarfism, witches' broom, little leaf formation, phyllody (Li et al. 2019, Gawande et al. 2022), hypertrophy of the calyces (Usta et al. 2022) and abnormal

development of floral parts into leafy structures (Arocha et al. 2007, Asudi et al. 2021, Bertaccini et al. 2014, Karthikeyan et al. 2024, Šafářová et al. 2016).

Phytoplasmas have a wide host range, infecting plants and replicating within the bodies of insect vectors. Beyond their transmission by phloem-feeding insect vectors such as leafhoppers, planthoppers, and psyllids (Asudi et al. 2021, Gonella et al. 2008, Huang et al. 2021), they can also spread through alternative mechanisms. These include parasitic plants like dodder that connect to the vascular tissues of host plants (Akhtar et al. 2009, Montano et al. 2001), vegetative propagation materials such as grafts, cuttings, storage roots, rhizomes, bulbs (Omar and Foissac 2012), and stem segments (Bertaccini 2007, Tedeschi et al. 2006, Wang et al. 2024), as well as, in some cases, seeds (Kirdat et al. 2023, Randa-Zelyüt et al. 2022, Wang et al. 2024).

In phytoplasma-infected plants, specific symptoms can sometimes help diagnose the disease. However, in certain cases, infected plants may remain asymptomatic or display symptoms that are hard to differentiate from those caused by viral infections or physiological disorders (Wang et al. 2024). Thus, identification relies on techniques like electron microscopy, histochemical staining, serological assays, and molecular diagnostic methods. In additions, with the technological advances in recent years, the use of the *rp* (ribosomal protein) operon, *tuf*, *secY*, *secA*, *groEL* (*cpn60*) and *rpoB* marker genes (Botti and Bertaccini 2003, Lee et al. 2006, Lee et al. 2010, Marcone et al. 2000, Martini et al. 2002, Martini et al. 2007, Mitrovic et al. 2011, 2015, Hodgetts et al. 2008, Valiunas et al. 2013) has been developed to distinguish phytoplasmas and various genes encoding surface proteins such as *vmp1* (Cimerman et al. 2009, Fialová et al. 2009), *imp* (Danet et al. 2011), *amp* (Kakizawa et al. 2006), *stamp* (Fabre et al. 2011), and *hflB* (Schneider and Seemüller 2009) which appear to be more determinant at the strain level, has become widespread.

Almost half of the phytoplasma diseases observed in vegetables belong to *Solanaceae* family diseases and in eggplants. Distinct phytoplasma groups and subgroups have been reported in different countries, including the 16SrI group in Bangladesh (Kelly et al. 2009) and India (Kumar et al. 2012); 16SrI-B subgroup in Japan (Lee et al. 1998, Okuda et al. 1997); 16SrII-D subgroup in India (Kumar 2015, Yadav et al. 2016), Iran (Siampour et al. 2013), Oman (Al-Subhi et al. 2011) and Egypt (Omar and Foissac 2012); 16SrIII-J and -U subgroup in Brazil (Amaral Mello et al. 2007, Barros et al. 1998); 16SrVI-A subgroup in Türkiye (Sertkaya et al. 2007); 16SrVI-D subgroup in India (Azadvar and Branwal 2012, Kumar 2015); Bangladesh (Siddique et al. 2001, Wei

et al. 2008); 16SrIX-C subgroup in Iran (Tohidi et al. 2015) 16SrXII-A subgroup in Russia (Ember et al. 2011) and Türkiye (Usta et al. 2018).

This study was conducted to identify and characterize the presence of phytoplasma in eggplant plants, which exhibit suspicious phytoplasma symptoms in fields where eggplant is cultivated in Şanlıurfa province. PCR-based techniques were employed for this purpose.

MATERIALS AND METHODS

Sample collection

A total of 14 samples exhibiting phytoplasma symptoms such as phyllody, virescence, little leaf formation, witches' broom and yellowing were collected from eggplant cultivation fields in July-August 2023 in Eyyübiye, Haliliye, Karaköprü and Siverek districts of Şanlıurfa province. All plant samples were transported to Niğde Ömer Halisdemir University-Plant Production and Technologies Laboratory and stored at -20 °C prior to molecular analysis.

DNA extraction and PCR analysis

Total DNA was extracted from 0.5 g of plant tissue from young shoots, midribs, and flowers belonging to symptomatic eggplants using the cetyl trimethyl ammonium bromide (CTAB) technique (Doyle and Doyle 1990). To amplify the 16S rRNA gene region, the extracted DNA was amplified with the P1 (5'-AAGAGTTTGATCCTGGCTCAGGATT-3') / P7 (5'-CGTCCTTCATCGGCTCTT-3') (Deng and Hiruki 1991, Schneider et al. 1994) primer pair for direct PCR and with the R16F2n (5'-GAAACGACTGCTAAGACTGG-3') / R16R2 (5'-TGACGGGCGGTGTGTACAAACCCCG-3') (Gundersen and Lee 1996) primer pair for nested PCR in two-stage (direct and nested) PCR studies. Amplicons obtained from direct PCR products were diluted at a 1/50 ratio and used as template DNA in nested PCR. Thermocycling conditions were regulated to direct and nested PCR. For direct PCR: 3 min at 94 °C for the first denaturation, followed by 35 cycles of 1 min at 94 °C, 2 min at 50 °C and 3 min at 72 °C, finally 10 min at 72 °C. For nested 5 min at 94 °C for the first denaturation, followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 2 min at 72 °C, finally 10 min at 72 °C. The 25 µl of PCR reaction mixes consisted of 1 µl of DNA as a template, 2.5 µl of 10x PCR buffer, 1.5 µl of MgCl₂ (25 mM), 1 µl of dNTP (10 mM), 1 µl of reverse and forward primer (10 µM), 0.2 µl of Taq DNA polymerase (2 unite) (Thermo-Fisher Scientific). A 'Ca. P. mali' (AP) isolate, used as a positive control, was kindly provided by Dr. B. Schneider (Germany). To visualize the products amplified by nested PCR, they were subjected to electrophoresis on a 1% agarose gel in 1xTAE (Tris Acetic EDTA) buffer at 120 V for 40 minutes. The gel was then

stained with Ethidium Bromide and visualized with a UV transilluminator (Biorad).

DNA analysis

Amplified nested PCR products were sequenced using the amplification primers from both sides with Applied Biosystems®3500 by MedSanTek (İstanbul/Türkiye). Geneious Prime software was used to check the quality of the sequences and to expand the entire sequence of the fragments by merging the overlaps. The obtained sequence was blasted in the NCBI database (www.ncbi.nlm.nih.gov) and for further analysis similar sequences were retrieved. The aligned sequences were deposited in the GenBank database and an accession numbers were obtained.

The Neighbor-Joining (NJ) method inferred the phylogenetic tree (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980). Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021).

RESULTS

Symptomatology

The most dramatic symptoms related to phytoplasma agents in diseased eggplants were observed as flower organ abnormalities (phyllody, virescence). Additionally, yellowing of the entire plant, little leaf formation, new shoots from the flower parts, elongation of the flower stalk, and witches' broom were commonly observed symptoms (Figure 1).

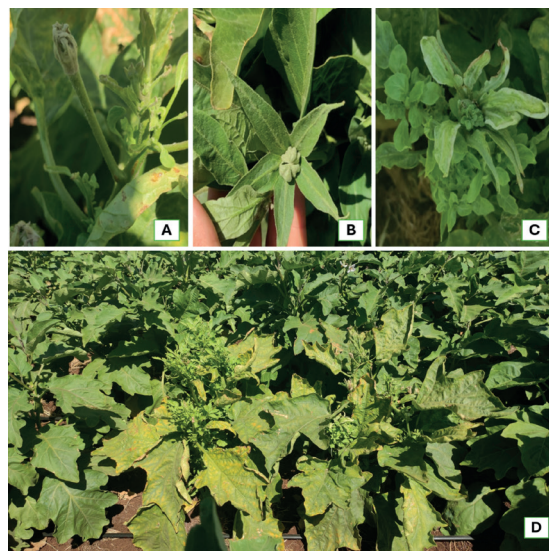


Figure 1. Typical symptoms observed in phytoplasma-infected plants: A. elongation of the flower stalk, B. Phyllody, C. Virescence and formation of new plants from flower parts, D. yellowing, witches' broom and proliferation

Detection and identification of phytoplasma agents

Fourteen symptomatic plants were tested against phytoplasma using 16S rRNA-specific primers. Amplifications of approximately 1200 bp in length expected for primer pairs R16F2n / R16R2 were obtained in 8 of all samples. The trimmed 991 nt nucleotide sequence of the PH50 isolate showed 98.85% similarity to the member of the 16SrXII-A 'Ca. P. solani' sequence, an eggplant isolate from Turkey deposited in NCBI with the accession number KT595210. The phylogenetic tree constructed with CaPsol strains detected in Türkiye from different hosts was shown in Figure 2. The eggplant isolate showed 98.85% identity with all CaPsol strains from Türkiye.

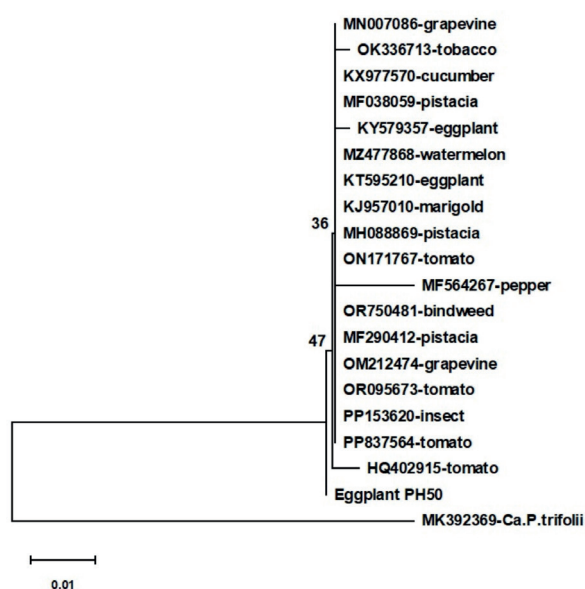


Figure 2. The phylogenetic tree constructed using the CaPsol strains detected in Türkiye from different hosts. 'Ca. Phytoplasma trifolii' used as outgroup (Bootstrap is 1000)

DISCUSSION

Phylogenetic analyses of 16S rRNA sequences of phytoplasmas worldwide revealed that phytoplasma-associated diseases in eggplants belong to different groups and subgroups of the pathogen. In Brazil, Amaral-Mello et al. (2011) identified that 16SrIII-B subgroups were associated with eggplant giant calix disease exhibiting symptoms such as leaf chlorosis, proliferation, shortened internodes, dwarfism, enlarging of calyces, small flowers and reduction of fruit size. In a study, carried out on eggplant in India, Venkataravanappa et al. (2018) detected mixed infections with both phytoplasma and begomovirus in showing little leaf formation and mosaic symptoms, and identified the

phytoplasma-associated disease as Clover proliferation belonging to the 16SrVI group. Li et al. (2019) detected that the phyllody phytoplasma (eggplant phyllody phytoplasma – EPP) strain was associated to the 16SrII-D group in phylogenetic analyses based on 16S rRNA and *secA* gene sequences of eggplant plants showing phyllody, little leaf and witches' broom symptoms. Omar et al. (2020) identified peanut witches' broom phytoplasma belonging to 16SrII-X subgroup in infected eggplants displaying symptoms including phyllody, little leaf formation and witches' broom. Usta et al. (2022) detected CaPsol belonging to 16SrXII-A in eggplants exhibiting symptoms such as fruit deformation, hypertrophy of the calyces and yellowing. Gawande et al. (2022) found that 'Candidatus Phytoplasma trifolii' was associated with Brinjal little leaf (BLL) disease in eggplants showing symptoms including little leaf formation, phyllody and witches' broom. Darabakula et al. (2024) detected phytoplasma associated diseases in infected eggplants showing brinjal little leaf symptoms; 16SrI, -II, -V, -VI, and -XII phytoplasma groups identified in the first-generation seedling produced from these infected eggplants whereas only 16SrI and 16SrXII groups identified in the second-generation seedlings. Karthikeyan et al. (2024) identified 'Ca. P. trifolii' belonging to 16SrVI Clover proliferation group in eggplants exhibiting symptoms such as little leaf formation, excessive growth of axillary shoots, virescence, phyllody, stunted growth, leaf chlorosis and witches' broom symptoms, and insect vectors.

In this study, CaPsol belonging to the 16SrXII-A phytoplasma group was identified based on the 16S rRNA conserved region in eggplant plants. CaPsol, the causative agent of stolbur disease, has a wide host range that includes both cultivated and wild plants (CABI 2024). In the 4 samples where phytoplasma could not be detected, it is likely that the phytoplasma density was at an undetectable density. CaPsol affects various members of the same species such as Solanaceae plants, grapes, lavender, strawberry, sugarcane, bindweed and common morning glory (Danet et al. 2003, Fos et al. 1992, Garnier 2000, Langer and Maixner 2004, Quaglino et al. 2013). In particular, the period when eggplant is grown at the same time as other crops raises concerns about the natural spread of phytoplasmas among different plant species. Therefore, effective control methods should be implemented in an integrated manner by choosing resistant plant species and taking into account regional conditions.

In this study, both symptomatic detection and molecular detection based on the 16S rRNA conserved region were performed in eggplant which is a significant host

of phytoplasma-associated diseases. Symptoms such as witches' broom, flower abnormalities (virescence, phyllody), elongation of the peduncle, production of new shoots from flower parts, yellowing and proliferation were observed in infected eggplants. Pathogen was identified as 16SrXII-A subgroup belonging to CaPSol with bioinformatics' analysis. To detect other possible hosts belonging to this phytoplasma species and insect vectors, advanced studies are necessary. Further studies are needed to identify other possible hosts and vector insects of this phytoplasma species.

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Author's Contributions

The authors have declared no conflict of interest.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Fitoplazmalar tarımsal ekosistemlerdeki çok sayıda bitkide enfeksiyonlara neden olarak ürünlerde önemli verim ve kalite kayıplarına yol açmaktadır. Son yıllarda patlıcan (*Solanum melongena* L.) yetiştiriciliğinde fitoplazmalardan kaynaklanan hastalıkların ekonomik kayıplara neden olduğu bilinmektedir. Türkiye'de patlıcan yetiştirilen alanlarda fitoplazmaların neden olduğu enfeksiyonlara ilişkin araştırmalar oldukça sınırlıdır. Bu çalışma, Şanlıurfa ilinde patlıcan üretim alanlarında görülen fitoplazma enfeksiyonlarının semptomatolojik ve moleküler olarak tespiti amacıyla yürütülmüştür. Cadı süpürgesi hastalığı, çiçek anormallikleri (viresens, fillodi), çiçek sapının uzaması, çiçek kısımlarından yeni sürgünlerin çıkması, sararma ve çoğalma gibi belirtiler gösteren patlıcanlardan 14 örnek toplanmıştır. 8 semptomatik örnekte 16S rRNA-spesifik primerler, P1/P7 ve R16F2n/R16R2 kullanılarak direkt ve nested PCR ile fitoplazma enfeksiyonu tespit edilmiştir. Moleküler çalışmalar sonucunda elde edilen fragmentlerin sekans bilgileri çıkarılmış ve BLAST analizleri yapılmıştır. 16S rRNA gen bölgesindeki nükleotid dizi benzerliğine göre, patlıcanda enfeksiyona neden olan fitoplazmanın genetik grubunun %98 dizi benzerliği ile 16SrXII-A alt grubuna ait '*Candidatus* Phytoplasma solani' (CaPSol) ile ilişkili olduğu belirlenmiştir. Bilgilerimize göre, bu çalışma Türkiye'de patlıcanları enfekte eden CaPSol'ün kapsamlı semptomatik teşhisini önermektedir.

Anahtar kelimeler: patlıcan, Şanlıurfa, CaPSol, nested-PCR, moleküler karakterizasyon

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Original article

Effects of CO₂ and temperature levels on glyphosate activity and growth of seven weed species

CO₂ ve sıcaklık seviyelerinin glifosat aktivitesi ve yedi yabancı ot türünün büyümesi üzerindeki etkileri

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ABSTRACT

Changes in environmental conditions have a major impact on weed growth and their susceptibility to applied herbicides. We studied the effects of CO₂ and temperature levels on the glyphosate (480 g/l Glyphosate Isopropylamin salt) activity and growth of seven weed species. Three temperature levels (control or normal temperature 26/16 °C (14/10 day/night), normal temperature + 3°C i.e., 29/19 °C and normal temperature + 6 °C i.e., 32/22 °C), and four CO₂ levels (control i.e., 400 ppm, 600 ppm, 800 ppm, and 1000 ppm) were tested. Six doses of glyphosate were: i) ¼, ii) ½, iii) full dose (1440 g a.i./ha), iv) 2-times, v) 4-times, and vi) 8-times of the recommended dose, at 4-6 leaf stage. Generally, the increase in CO₂ and temperature improved weed growth. For most weed species, the most favourable temperature and CO₂ levels were 29 °C and 800 ppm to 1000 ppm. The ED₅₀ (effective dose 50) value for *Echinochloa colonum* (L.) Link., *Amaranthus retroflexus* L., *Amaranthus palmeri* S. Watson, *Portulacaca oleracea* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers. and *Physalis angulata* L. showed that some weeds will likely become tolerant to glyphosate with climate change. With increasing temperature and CO₂ concentration, ED value increases, meaning higher herbicide doses are required to control these weeds. As a result, using more herbicides in agricultural areas in the coming years will cause producers to experience more costs and the herbicide resistance problem in weeds will increase to much higher levels.

INTRODUCTION

Rising carbon dioxide (CO₂) levels in the atmosphere, droughts, global warming, floods and uneven rainfall are important components of climate change. Global warming results from the earth's temperature rising due to a large

increase in population and the burning of fossil fuels, which has added a huge amount of CO₂ and other toxic gases to the atmosphere. It has been observed that the most advanced form of climate change is the enhancement of CO₂

in the atmosphere (IPCC 2020). The current atmospheric concentration of CO₂ is almost 400 ppm, down from below 300 ppm before the global industrial revolution, and it is expected to rise to 700 ppm by the end of this century (IPCC 2020).

These climatic changes can affect different physiological processes in a crop, such as stomatal opening and closing, photosynthetic rate and growth rate (DaMatta et al. 2010, Pernicová et al. 2024). Climate change sometimes negatively impacts crop productivity, reducing crop potential and yields (Asseng et al. 2014, Yang et al. 2024). Some of these climatic changes not only affect the plants but also significantly impact the pest populations of these crops (Elad and Pertot 2014, Jabran et al. 2020, Olesen and Bindi 2002). Elevated CO₂ concentrations can affect various physiological processes in plants and produce a mixed response (both positive and negative) (Misra et al. 2019). C₃ and C₄ species also respond differently to this increase in CO₂ concentration (Hamim 2011). C₃ species respond more quickly and positively than C₄ species because C₄ species are sensitive to CO₂ accumulation (Mooney et al. 1999).

Several invasive weed species have been found to benefit (directly or indirectly) from climate change (Blumenthal and Kray 2014). These weed species develop physiological and morphological adaptations to climate change, allowing them to grow easily and reproduce efficiently as compared to the native plants of the area (Ziska and McConnell 2016). In addition to affecting physiological processes, it also affects weed management programs. The current climate change scenario may favour weeds due to their wide range of environmental tolerances, large dispersal rates, and rapid colonisation (Bajwa et al. 2018), although this prediction may not apply to all weeds (Roger et al. 2015). Weed infestation is one of the most damaging biotic factors. Crop losses due to weeds are more than 30%, which is a higher percentage than crop losses due to diseases and insect pests (Oerke 2006).

Glyphosate is a non-selective broad-spectrum herbicide used to control weeds in cultivated and uncultivated areas. Due to climate change, i.e., increases in temperature and CO₂ concentrations, the effectiveness of glyphosate is largely disturbed. There may be some reasons for this, including increased leaf thickness under elevated CO₂ conditions, which reduces stomata conductance and thus limits leaf uptake of herbicides. This reduction in stomata conductance then reduces the rate of transpiration, which ultimately decreases the herbicide uptake from the soil. Due to the decrease in the absorption rate of herbicide,

the effect of herbicide on plant function decreases causing the ineffectiveness of applied herbicide. However, some literature suggests that the changing climate conditions (warming and rising atmospheric CO₂) will have a neutral effect on the efficacy of herbicides (glyphosate) (Jabran and Doğan 2018). Under current circumstances, it is necessary to determine the effects of applied herbicides on the growth of weeds exposed to changing climate scenarios. Extensive research has been conducted on the impact of climate change on the growth of various crops and weed species as well as on the effectiveness of herbicides against weeds. However, the weeds included in this study have not been previously studied for the impacts of climate change on their growth and the efficacy of herbicides applied to control these weeds. The current study was therefore designed to determine the ultimate effect of climate change on glyphosate activity and weed growth. The effect of temperature and CO₂ on the morphological parameters of C₃ and C₄ weeds was determined. The susceptibility of weeds to glyphosate at high temperatures and CO₂ concentrations was also determined.

MATERIALS AND METHODS

Study site

The study was conducted at the Faculty of Agriculture, Malatya Turgut Ozal University, Malatya, Türkiye. The faculty has a facility of automated growth rooms (5 m × 5 m) where desired CO₂, temperature and humidity levels can be maintained.

Selection of weed species

Seven weed species were selected for this research work (Table 1). Many crops in Türkiye and around the world suffer from these weeds. In addition, they are considered invasive and harmful in agricultural areas (Balah and Balah 2022, Costea et al. 2003, Edmonds and Chweya 1997, Holm et al. 1977, Matzrafi et al. 2023, Rao 2021).

Determining the effect of temperature and CO₂ levels on weed growth

The IPCC (2002) predictions were used to decide the temperature levels in this study. This study had three temperature levels including (i) Control or normal temperature 26/16 °C (14/10 day/night), (ii) normal temperature + 3 °C i.e., 29/19 °C (14/10 day/night), and (iii) normal temperature + 6 °C i.e., 32/22 °C (14/10 day/night). The second and third temperature treatments were considered as medium and high warming.

Four CO₂ levels were tested in this study. (i) Control i.e., 400 ppm, (ii) 600 ppm, (iii) 800 ppm, and (iv) 1000 ppm. The

Table 1. Scientific names, growth habits and families of the weed species used in the experiment.

	Weed species	Growth habit	Family	C ₃ or C ₄
1.	<i>Amaranthus retroflexus</i> L.	Annual, broad-leaved	Amaranthaceae	C ₄
2.	<i>Amaranthus palmeri</i> S. Watson	Annual, broad-leaved	Amaranthaceae	C ₄
3.	<i>Portulaca oleracea</i> L.	Annual, broad-leaved	Portulacaceae	C ₄
4.	<i>Solanum nigrum</i> L.	Annual, broad-leaved	Solanaceae	C ₃
5.	<i>Physalis angulata</i> L.	Annual, broad-leaved	Solanaceae	C ₃
6.	<i>Echinochloa colonum</i> (L.) Link.	Annual, narrow-leaved	Poaceae	C ₄
7.	<i>Sorghum halepense</i> (L.) Pers.	Perennial, narrow-leaved	Poaceae	C ₄

first CO₂ level represents the current CO₂ concentrations on our planet. The other three levels represent the future CO₂ levels as predicted by IPCC (2007).

Experimental materials and setup

Seeds of weed species (Table 1) were collected from the agricultural fields around the Faculty of Agriculture, Malatya Turgut Ozal University, Malatya. The plastic pots used in the study had dimensions of 18 × 18 × 15 cm and a volume of 3.8 liters. These pots were filled with a mixture of compost, sand, and perlite (1:1:1). For each weed species, ten seeds were sown in a pot, and later, a single plant was maintained. Four replications were performed in this experiment using a completely randomized design.

Effect of temperature and CO₂ levels on morphological parameters of weeds

All the weed plants were exposed to the above temperature and CO₂ levels starting from germination until the 8th week of growth. The weeds were then harvested, and growth data were recorded. The data included shoot fresh weight (g), plant height (PH) (cm), root fresh weight (RFW) (g), root length (RL) (cm), plant dry weight (PDW) (g) and root dry weight (RDW) (g). Plant and root dry weights were recorded after drying the relevant parts of the plant in an oven at 65 °C for 48 hours.

Effect of temperature and CO₂ levels on weed susceptibility to glyphosate

Herbicide application is a popular method for controlling weeds, so in this study, the effect of glyphosate on weeds (Table 1) was studied under the established temperature and CO₂ levels. Therefore, weeds grown in greenhouses with different environmental conditions were treated with various doses of glyphosate (480 g/l Glyphosate Isopropylamin salt) herbicide at the 4-6 leaf stage. Six doses of herbicide were applied, including: i) ¼, ii) ½, iii) full dose, iv) 2-times, v) 4-times, and vi) 8-times of the recommended dose. The recommended dose of glyphosate

(480 g/l Glyphosate Isopropylamin salt) was 1440 g a.i./ha. Herbicide applications were carried out with an automatic laboratory sprayer fitted with an 11002 flat-fan nozzle with a spray volume of a pressure of 200 l/ha and a spray pressure of 304 kPa pressure and 5 km/h. First, a stock solution was prepared and diluted to the required concentrations for the herbicide doses. Each herbicide treatment was replicated three times. Herbicide-treated plants were closely monitored, and their dry weight was measured four weeks after glyphosate application.

Dose-response curves were constructed based on the weed biomass obtained after herbicide application on plants grown at each temperature and CO₂ level. Effective doses (ED₅₀ and ED₉₀) were calculated along with the dose-response curves. ED₅₀ (50% control) and ED₉₀ (90% control) values were determined as a measure of the level of control of weeds by the herbicide.

DM reduction (%) data were subjected to a nonlinear regression analysis over herbicide dose using the four-parameter log-logistic model (Knezevic et al. 2007, Ulloa et al. 2011), with the lower asymptote (C) fixed at 0 and the upper asymptote (D) fixed at 100:

$$Y = C + \frac{(D - C)}{\{1 + \exp[B(\log X - \log E)]\}} \quad (1)$$

In this equation, Y is the response (e.g., the percentage reduction in DM), C is the lower limit, D is the upper limit, X is the dose of glyphosate, E is the dose that produces in a 50% and 90% response between the lower and upper limits (also known as the inflection point, I50 or ED₅₀; I90 or ED₉₀) and B is the degree of slope of the slope line.

For the other data, statistical analyses were performed using General Linear Model (GLM) and analysis of variance (ANOVA) using Statistix 8.1 software. Duncan's multiple range test was used to determine the differences among treatments.

Table 2. Effect of temperature levels and CO₂ concentration on morphological parameters of *Amaranthus palmeri* S. Watson and *Amaranthus retroflexus* L.

Variation levels	<i>Amaranthus palmeri</i> S. Watson						<i>Amaranthus retroflexus</i> L.					
	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	35.2 B	12.9 B	4.7 B	0.54 B	1.08 C	0.15 B	28.1 C	23.1 C	4.5 B	0.76 B	1.15 B	0.23 C
29°C	81.3 A	32.9 A	10.9 A	1.42 A	2.95 A	0.56 A	59.9 A	44.4 A	15.8 A	2.84 A	3.34 A	1.05 A
32°C	80.0 A	29.8 A	10.2 A	1.72 A	2.43 B	0.53 A	37.4 B	30.9 B	7.5 B	1.49 B	1.78 B	0.51 B
CO ₂ levels												
400 ppm	61.0 BC	21.1 A	6.6 B	1.01 A	1.80 B	0.36 A	43.9 A	27.7 A	10.1 A	1.54 A	2.45 A	0.56 A
600 ppm	68.4 AB	25.5 A	9.9 A	1.49 A	2.39 AB	0.48 A	40.6 A	35.8 A	7.7 A	1.48 A	1.91 AB	0.52 A
800 ppm	75.1 A	27.9 A	10.9 A	1.36 A	2.51 A	0.43 A	42.6 A	36.7 A	10.3 A	2.01 A	2.43 A	0.69 A
1000 ppm	57.5 C	26.3 A	7.2 B	1.07 A	1.89 B	0.38 A	40.3 A	30.9 A	8.9 A	1.75 A	1.58 B	0.61 A
Temperature × CO ₂												
26°C × 400 ppm	32.5 c	13.9 cde	3.9 d	0.54 a	1.19 de	0.18 b	33.7 de	13.5 d	5.4 b	0.79 bc	1.45 bc	0.26 c
26°C × 600 ppm	31.8 c	9.0 e	4.8 cd	0.51 a	0.92 e	0.09 b	29.7 de	24.8 abcd	4.4 b	0.91 bc	1.15 c	0.27 c
26°C × 800 ppm	38.8 c	16.4 bcde	6.4 bcd	0.64 a	1.25 de	0.15 b	22.9 e	31.6 abcd	4.9 b	0.93 bc	1.09 c	0.26 c
26°C × 1000 ppm	37.7 c	12.3 de	3.7 d	0.49 a	0.97 e	0.17 b	26.2 de	22.3 cd	3.30 b	0.42 c	0.91 c	0.15 c
29°C × 400 ppm	99.1 ab	25.9 abcde	9.7 abc	1.22 a	2.76 abc	0.54 ab	63.4 a	46.3 a	16.5 a	2.47 abc	3.99 a	0.91 abc
29°C × 600 ppm	92.0 ab	37.9 a	14.5 a	2.15 a	3.94 a	0.86 a	54.9 abc	44.1 abc	12.8 ab	2.20 abc	3.28 ab	0.89 abc
29°C × 800 ppm	80.8 b	35.3 ab	12.5 a	1.49 a	3.14 ab	0.49 ab	61.9 ab	44.5 ab	17.2 a	3.50 a	4.15 a	1.28 a
29°C × 1000 ppm	53.1 c	32.5 abcde	7.2 bcd	0.86 a	1.96 bcde	0.34 ab	59.8 ab	42.6 abc	16.8 a	3.19 ab	1.96 bc	1.11 ab
32°C × 400 ppm	51.5 c	23.6 abcde	6.2 bcd	1.28 a	1.47 cde	0.37 ab	34.5 de	23.3 bcd	8.9 ab	1.36 abc	1.89 bc	0.52 abc
32°C × 600 ppm	81.3 b	29.5 abcde	10.3 ab	1.80 a	2.34 bcd	0.49 ab	37.3 cde	38.5 abc	5.9 b	1.34 abc	1.30 c	0.41 bc
32°C × 800 ppm	105.8 a	32.1 abcd	13.7 a	1.93 a	3.16 ab	0.65 ab	43.1 bcd	34.1 abcd	8.8 ab	1.60 abc	2.04 bc	0.53 abc
32°C × 1000 ppm	81.6 b	34.0 abc	10.7 ab	1.85 a	2.76 abc	0.62 ab	34.9 de	27.9 abcd	6.7 b	1.65 abc	1.88 bc	0.57 abc

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 3. Effect of temperature levels and CO₂ concentration on morphological parameters of *Echinochloa colonomum* (L.) Link. and *Sorghum halepense* (L.) Pers.

Variation levels	<i>Echinochloa colonomum</i> (L.) Link.						<i>Sorghum halepense</i> (L.) Pers.					
	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	56.7 C	22.5 B	7.6 A	0.96 B	1.51 A	0.26 B	69.2 C	34.9 B	4.7 B	1.51 B	0.88 B	0.37 C
29°C	72.6 B	34.9 A	9.6 A	1.56 AB	1.93 A	0.74 AB	102.1 B	57.2 A	15.5 A	5.6 A	4.1 A	2.23 A
32°C	93.2 A	36.9 A	10.5 A	3.52 A	2.38 A	1.08 A	133.9 A	60.7 A	12.2 A	5.2 A	3.05 A	1.22 B
CO ₂ levels												
400 ppm	78.3 A	28.7 A	11.8 A	1.71 A	2.57 A	0.74 A	94.0 A	60.7 A	8.1 B	2.18 B	2.06 B	0.91 B
600 ppm	67.7 A	31.1 A	8.3 A	1.73 A	1.78 A	0.59 A	97.3 A	47.5 A	9.7 AB	4.1 AB	2.20 B	1.00 B
800 ppm	74.5 A	31.7 A	8.2 A	1.59 A	1.64 A	0.58 A	116.6 A	45.3 A	15.0 A	6.0 A	3.94 A	2.17 A
1000 ppm	76.3 A	34.3 A	8.6 A	3.01 A	1.78 A	0.83 A	99.1 A	50.3 A	10.5 AB	4.2 AB	2.53 AB	0.99 B
Temperature × CO ₂												
26°C × 400 ppm	65.7 bcde	22.6 b	10.8 a	1.16 b	2.30 a	0.35 b	56.5 d	29.2 b	2.04 b	0.64 b	0.32 b	0.13 b
26°C × 600 ppm	50.4 de	18.7 b	8.7 a	0.75 b	1.79 a	0.19 b	54.0 d	25.3 b	3.3 b	1.2 b	0.41 b	0.18 b
26°C × 800 ppm	45.8 e	30.2 b	4.7 a	1.22 b	0.59 a	0.23 b	85.0 bcd	40.8 ab	5.4 b	1.5 b	0.83 b	0.29 b
26°C × 1000 ppm	65.0 bcde	18.5 b	6.1 a	0.73 b	1.38 a	0.25 b	81.6 cd	44.3 ab	8.0 b	2.6 b	1.96 b	0.85 b
29°C × 400 ppm	80.2 abcd	34.7 ab	9.9 a	1.41 b	2.20 a	0.85 ab	97.0 abcd	81.7 a	12.8 b	3.1 b	3.46 b	1.66 b
29°C × 600 ppm	59.5 cde	39.4 ab	8.0 a	1.41 b	1.78 a	0.82 ab	96.9 abcd	49.1 ab	11.9 b	4.3 b	2.86 b	1.41 b
29°C × 800 ppm	84.0 abc	36.1 ab	14.3 a	2.43 ab	2.94 a	1.01 ab	131.0 abc	51.0 ab	28.3 a	11.8 a	7.7 a	4.89 a
29°C × 1000 ppm	66.7 abcde	29.5 b	5.8 a	0.98 b	0.79 a	0.28 b	83.4 bcd	47.0 ab	9.1 b	3.2 b	2.37 b	0.94 b
32°C × 400 ppm	89.1 abc	28.8 b	14.5 a	2.56 ab	3.19 a	1.04 ab	128.5 abc	71.1 ab	9.3 b	2.8 b	2.41 b	0.95 b
32°C × 600 ppm	93.1 ab	35.1 ab	8.0 a	3.02 ab	1.78 a	0.79 ab	141.0 a	68.0 ab	13.7 b	6.7 ab	3.35 b	1.41 b
32°C × 800 ppm	93.7 ab	28.9 b	5.8 a	1.14 b	1.38 a	0.51 ab	133.8 ab	44.2 ab	11.3 b	4.7 b	3.21 b	1.34 b
32°C × 1000 ppm	97.0 a	54.7 a	13.8 a	7.3 a	3.16 a	1.97 a	132.4 abc	59.5 ab	14.5 b	6.8 ab	3.25 b	1.17 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 4. Effect of temperature levels and CO₂ concentration on morphological parameters of *Solanum nigrum* L. and *Physalis angulata* L.

Variation levels	<i>Solanum nigrum</i> L.						<i>Physalis angulata</i> L.					
	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels												
26°C	29.4 B	32.4 B	5.8 C	1.85 C	1.48 B	0.49 C	20.8 B	9.7 C	3.9 C	0.39 C	0.34 C	0.05 C
29°C	41.3 A	40.4 A	13.9 A	4.4 B	2.70 A	1.26 A	47.7 A	52.9 A	24.7 A	2.92 B	2.95 A	0.95 A
32°C	32.9 B	46.7 A	8.0 B	5.6 A	1.49 B	0.73 B	50.0 A	36.3 B	20.9 B	6.2 A	2.49 B	0.82 B
CO ₂ levels												
400 ppm	31.9 BC	35.2 BC	9.3 A	3.43 B	1.69 A	0.86 A	34.8 B	25.8 C	10.7 C	1.41 B	1.33 C	0.33 D
600 ppm	36.2 AB	48.5 A	9.5 A	3.96 AB	2.02 A	0.84 A	41.4 AB	40.5 A	17.9 AB	3.79 A	2.09 AB	0.67 B
800 ppm	38.9 A	42.9 AB	9.5 A	4.8 A	1.99 A	0.75 A	44.1 A	34.8 AB	22.5 A	3.80 A	2.64 A	0.89 A
1000 ppm	31.2 C	32.8 C	8.6 A	3.8 AB	1.87 A	0.85 A	37.7 AB	30.6 BC	14.9 BC	3.74 A	1.64 BC	0.52 C
Temperature × CO ₂												
26°C × 400 ppm	27.7 cd	33.8 abcd	6.9 cd	2.86 cde	1.68 bcd	0.75 bcd	27.0 def	6.3 e	4.9 f	0.36 ef	0.39 fg	0.05 d
26°C × 600 ppm	34.0 abcd	37.6 abcd	7.7 bcd	2.19 cde	1.84 bcd	0.48 cd	27.8 def	20.3 de	7.5 ef	0.95 def	0.72 efg	0.12 d
26°C × 800 ppm	31.2 bcd	35.8 abcd	4.7 d	1.62 de	1.35 cd	0.44 cd	11.5 f	3.5 e	0.92 f	0.12 f	0.06 g	0.01 d
26°C × 1000 ppm	24.9 d	22.3 d	3.9 d	0.75 e	1.03 d	0.26 d	17.0 ef	8.7 e	2.28 f	0.16 ef	0.20 fg	0.02 d
29°C × 400 ppm	38.3 abc	38.9 abcd	13.3 ab	3.22 cde	2.13 abcd	1.01 abc	35.8 cde	37.8 c	8.3 def	1.04 def	1.34 def	0.46 c
29°C × 600 ppm	41.9 ab	55.1 a	13.2 ab	3.41 cd	2.83 ab	1.24 ab	51.0 abc	57.0 ab	28.6 b	3.08 cd	3.54 b	1.04 b
29°C × 800 ppm	42.1 a	38.1 abcd	12.5 abc	4.6 bc	2.40 abc	1.28 ab	64.0 a	70.0 a	44.4 a	5.2 bc	5.1 a	1.83 a
29°C × 1000 ppm	42.8 a	29.5 cd	16.7 a	6.5 ab	3.43 a	1.50 a	40.0 bcd	47.0 bc	17.6 cde	2.36 def	1.81 cde	0.46 c
32°C × 400 ppm	29.7 cd	32.8 bcd	7.7 bcd	4.2 bcd	1.26 cd	0.83 bcd	41.8 bcd	33.5 cd	18.9 bc	2.81 cde	2.26 cd	0.48 c
32°C × 600 ppm	32.7 abcd	52.6 ab	7.5 bcd	6.3 ab	1.38 cd	0.79 bcd	45.5 abcd	44.4 bc	17.8 cd	7.4 ab	2.02 cd	0.86 b
32°C × 800 ppm	43.3 a	55.0 a	11.6 abc	8.1 a	2.20 abcd	0.51 cd	56.8 ab	31.1 cd	22.0 bc	6.1 ab	2.78 bc	0.84 b
32°C × 1000 ppm	25.8 d	46.5 abc	5.3 d	4.0 bcd	1.13 cd	0.79 bcd	56.0 ab	36.3 cd	24.9 bc	8.70 a	2.78 bc	1.10 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

Table 5. Effect of temperature levels and CO₂ concentration on morphological parameters of *Portulaca oleracea* L.

<i>Portulaca oleracea</i> L.						
Variation levels	Plant height (cm)	Root length (cm)	Shoot fresh weight(g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Temperature levels						
26°C	25.1 B	7.7 B	8.6 B	0.28 B	0.78 B	0.04 B
29°C	34.9 A	15.2 A	28.2 A	0.53 A	2.08 A	0.34 A
32°C	37.4 A	10.4 B	10.9 B	0.20 B	0.73 B	0.05 B
CO ₂ levels						
400 ppm	32.1 A	8.6 B	15.1 A	0.28 B	1.14 AB	0.07 A
600 ppm	34.3 A	11.0 AB	18.5 A	0.43 A	1.45 A	0.09 A
800 ppm	30.9 A	10.1 B	14.5 A	0.28 B	1.04 B	0.06 A
1000 ppm	32.6 A	14.7 A	15.4 A	0.35 AB	1.17 AB	0.35 A
Temperature × CO ₂						
26°C × 400 ppm	29.3 bc	10.2 b	13.4 c	0.43 abc	1.32 bcd	0.06 b
26°C × 600 ppm	27.4 c	8.2 b	10.2 cd	0.38 bcd	1.08 cde	0.06 b
26°C × 800 ppm	17.1 d	5.8 b	4.2 d	0.09 d	0.19 e	0.01 b
26°C × 1000 ppm	26.5 c	6.7 b	6.4 cd	0.21 cd	0.54 de	0.03 b
29°C × 400 ppm	34.3 abc	9.8 b	24.3 b	0.29 bcd	1.57 bc	0.09 b
29°C × 600 ppm	36.7 ab	14.8 ab	34.4 a	0.69 a	2.57 a	0.20 ab
29°C × 800 ppm	34.9 abc	13.9 ab	27.2 ab	0.56 ab	2.18 ab	0.14 b
29°C × 1000 ppm	34.1 abc	22.4 a	26.8 ab	0.56 ab	2.01 ab	0.94 a
32°C × 400 ppm	32.8 abc	6.0 b	7.6 cd	0.12 d	0.52 de	0.05 b
32°C × 600 ppm	38.8 a	10.0 b	10.9 cd	0.22 cd	0.69 cde	0.04 b
32°C × 800 ppm	40.6 a	10.8 b	12.1 cd	0.19 cd	0.74 cde	0.04 b
32°C × 1000 ppm	37.1 ab	15.0 ab	13.0 cd	0.28 bcd	0.96 cde	0.07 b

Means not sharing a letter (capital letter for main effects and small letters for interactive effects) in common differ significantly at 5% probability level.

RESULTS

Temperature levels, CO₂ concentrations and their interactions had a significant effect on plant height, shoot fresh weight, and shoot dry weight of *A. palmeri* (Table 2). The root length and root dry weight of *A. palmeri* were affected by CO₂ levels and temperature × CO₂, while CO₂ concentration had no significant effect on root length. The fresh root weight of *A. palmeri* was only affected by CO₂ concentration, while temperature × CO₂ had a non-significant effect. The growth parameters of *A. retroflexus* were only significantly affected by temperature levels and temperature × CO₂ concentration. Shoot dry weight was an exception and was also affected by CO₂ concentration.

At different CO₂ levels, *A. retroflexus* plant height, shoot fresh weight, root fresh weight, root length, and root dry

weight were similar, but these parameters were significantly affected by the temperature levels (Table 2). A temperature of 29 °C was more favourable for the *A. retroflexus* than the other two temperature levels in the study (26 °C and 32 °C). At 29 °C, the weed had the highest plant height, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight compared to the other temperature levels. Moreover, an interaction of 29 °C with different levels of CO₂ was the most favourable for this weed. An interaction of 29 °C × 400 ppm provided the highest plant height and root length of *A. retroflexus*, while 29 °C × 800 ppm provided the highest root fresh weight and root dry weight.

The growth parameters of *E. colonum* were not affected by the CO₂ levels, but the highest temperature level (32 °C) had increased the plant height, root length, root fresh weight and root dry weight of this weed (Table 3). The interaction of the

highest levels of temperature and CO₂ (32 °C × 1000 ppm) provided the highest plant height, root length, fresh root weight and dry root weight.

Temperature significantly affected the growth parameters of *S. halepense* (Table 3). The highest plant height was recorded at 32 °C, while the highest root dry weight was recorded at 29 °C. The *S. halepense* plants grown at 29 °C and 32 °C had a statistically similar (and higher than the plants at 26 °C) root length, shoot fresh weight, root fresh weight and shoot dry weight. Furthermore, the highest shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of *S. halepense* were recorded when these were grown under 800 ppm CO₂. An interaction of 32 °C × 600 ppm CO₂ produced the *S. halepense* plants with the greatest plant height, while 29 °C × 400 ppm produced the greatest root length. Moreover, 29 °C × 800 ppm CO₂ interaction produced the *S. halepense* plants with the highest shoot fresh weight, root fresh weight, shoot dry weight and root dry weight compared to other interaction treatments in the experiment.

A temperature of 29 °C was the most favourable for the growth of *S. nigrum* followed by 32 °C, while 26 °C decreased the weed growth compared to other temperature levels (Table 4). The highest plant height, shoot fresh weight, shoot dry weight, and root dry weight of *S. nigrum* were recorded at 29 °C, while the highest root length and root fresh weight were recorded at 32 °C. CO₂ levels did not affect shoot fresh weight, shoot dry weight and root dry weight of *S. nigrum*, but plant height, root length and root fresh weight of the weed were improved by 600-800 ppm CO₂. The interaction between CO₂ and temperature also had a significant effect, and the temperature level of 29 °C + higher CO₂ levels considerably impacted all growth parameters.

For *P. angulata* weed growth, the temperature of 29 °C was the most favourable followed by 32 °C, while 26 °C decreased weed growth compared to other temperatures (Table 4). While the highest root length, shoot fresh weight, shoot dry weight, and root dry weight were recorded at 29 °C, the highest plant height and root fresh weight were observed at 32 °C. Plant height and root fresh weight were not significantly affected by CO₂ levels, but the shoot fresh weight, the shoot dry weight, and the root dry weight were significantly affected by 600-800 ppm CO₂. The interactive effect of 29 °C × 800 ppm CO₂ produces a higher plant height, shoot fresh weight, shoot dry weight and root dry weight.

The growth of *P. oleracea* was significantly influenced by 29 °C temperature (Table 5). Root length, root fresh weight, shoot fresh weight, shoot dry weight, and root dry weight

were increased at 29 °C, while plant height was increased at 32 °C. CO₂ levels did not affect plant height, shoot fresh weight and root dry weight, while root length, root fresh weight and shoot dry weight were significantly influenced at 600 ppm CO₂. When comparing the interactions, the maximum shoot fresh weight, root fresh weight and root dry weight were obtained by the 29 °C × 600 ppm treatment, while the highest plant height was obtained at the 32 °C temperature and 600 ppm and 800 ppm CO₂ treatments, respectively.

The ED₉₀ value for *E. colonum* was 1816.89 at 26 °C + 800 ppm CO₂, for *A. retroflexus* it was 754.784 at 26 °C + 1000 ppm CO₂, for *A. palmeri* it was 1245.794 at 26 °C + 600 ppm CO₂, for *P. oleracea* it was 1161.96 at 26 °C + 800 ppm CO₂, for *S. nigrum* it was 1307.002 at 26 °C + 600 ppm CO₂, for *S. halepense* it was 370.067 at 32 °C + 400 ppm CO₂ and, for *P. angulata* it was 490.528 at 29 °C + 400 ppm CO₂. Data for ED₅₀ value showed that some of the weeds are likely to become tolerant to glyphosate with climate change. With increasing temperature and CO₂ concentration, the ED₅₀ value increased, which means a higher dose is required to control these weeds (Table 6). The ED₅₀ value (g a.i./ha) for *E. colonum* was 103.55 at 26 °C + 800 ppm CO₂, for *A. retroflexus* it was 47.35 at 29 °C + 800 ppm CO₂, for *A. palmeri* it was 79.64 at 26 °C + 1000 ppm CO₂, for *P. oleracea* it was 109.43 at 29 °C + 1000 ppm CO₂, for *S. nigrum* it was 87.12 at 29 °C + 1000 ppm CO₂, for *S. halepense* it was 37.86 at 32 °C + 1000 ppm CO₂ and, for *P. angulata* it was 18.61 at 29 °C + 800 ppm CO₂.

DISCUSSION

The study showed that weeds were significantly influenced by climate change and developed some tolerance to herbicide use. In the case of *A. retroflexus*, germination was increased by increasing temperature. The higher ED value shows that the efficacy rate of a specific dose of glyphosate decreases, leading to increased tolerance. The previous studies show that maximum germination of *A. retroflexus* was observed at high temperatures (25-35 °C), indicating that the increase in temperature favours its germination and growth (Guo and Al-Khatib 2003, Safavi et al. 2023). Another recent study also showed that the germination rate of *Amaranthus retroflexus* was high at higher temperatures (Khan et al. 2023). Increases in temperature and CO₂ have a positive effect on growth rate. The interactive effect of increased CO₂ and other resources significantly impacts plant height, leaf area, and total biomass of *Amaranthus retroflexus* (Valerio et al. 2011). Herbicide efficacy decreased as CO₂ concentration increased (Ziska et al. 2004) due to

Table 6. Effect of temperature levels and CO₂ concentration on ED₅₀ and ED₉₀ values of weeds

Temperature (°C)	400 ppm			600 ppm			800 ppm			1000 ppm		
	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀	b	ED ₉₀	ED ₅₀
26°C												
<i>Echinochloa colonom</i>	-0.87692	715.46	58.4	-0.79957	963.257	61.701	-0.76697	1816.89	103.55	-1.22955	268.625	44.984
<i>Solanum nigrum</i>	-1.01705	448.155	51.664	-0.62635	1307.002	39.154	-0.67672	933.201	36.298	-0.69589	1016.269	43.227
<i>Amaranthus retroflexus</i>	-1.00584	361.731	40.709	-0.76815	665.312	38.086	-0.74314	678.206	35.262	-0.70468	754.784	33.395
<i>Amaranthus palmeri</i>	-0.91083	1226.93	109.94	-0.75727	1245.794	68.445	-0.72572	1203.331	58.277	-1.60012	314.438	79.649
<i>Portulaca oleracea</i>	-0.37005	113.857	0.30036	-0.84442	200.278	14.845	-0.68187	1161.96	46.316	-0.83516	1041.35	74.99
29°C												
<i>Echinochloa colonom</i>	-0.60356	197.169	5.174	-0.95102	151.732	15.055	-0.9628	226.743	23.143	-3.60138	118.484	64.371
<i>Solanum nigrum</i>	-0.56473	197.4333	4.0336	-1.19545	540.63	86.035	-1.07415	561.8	72.647	-1.26985	491.57	87.12
<i>Amaranthus retroflexus</i>	-1.59867	193.33	48.911	-1.4189	194.552	41.355	-1.5636	193.05	47.357	-1.32548	240.823	45.896
<i>Amaranthus palmeri</i>	-1.0916	588.869	78.678	-1.01745	491.323	56.687	-0.72902	650.087	31.918	-0.61168	218.1201	6.0071
<i>Portulaca oleracea</i>	-1.47499	187.475	42.266	-1.9562	173.712	56.496	-0.96084	338.061	34.345	-2.15839	302.87	109.43
<i>Sorghum halepense</i>	-1.55041	226.454	54.891	-1.15288	235.764	35.057	-1.26954	177.13	31.38	-1.2467	147.688	25.347
32°C												
<i>Echinochloa colonom</i>	-1.08357	166.178	21.874	-1.208	267.75	43.427	-1.01457	503.464	57.734	-2.7578	189.519	85.435
<i>Solanum nigrum</i>	-3.1001	140.6	69.21	-1.13218	375.413	53.911	-1.05765	436.903	54.721	-0.69278	487.579	20.447
<i>Amaranthus retroflexus</i>	-1.2162	201.543	33.096	-0.83672	228.033	16.502	-0.80268	266.147	17.231	-0.64306	341.742	11.215
<i>Amaranthus palmeri</i>	-0.85799	363.358	28.064	-1.0207	310.584	36.082	-1.17656	377.379	58.308	-1.17889	372.331	57.742
<i>Portulaca oleracea</i>	-0.7005	422.005	18.326	-1.9155	139.87	44.418	-0.56705	317.9733	6.6005	-0.54045	230.1461	3.9478
<i>Physalis angulata</i>	-1.00533	199.29	22.403	-0.97986	149.86	15.916	-1.16609	114.72	17.43	-0.74403	135.5574	7.0727
<i>Sorghum halepense</i>	-0.62503	370.067	11.005	-0.70496	328.23	14.54	-0.60005	246.8763	6.3416	-1.46408	169.839	37.868

anatomical changes that affect herbicide uptake rate (Manea et al. 2011). The growth of *A. palmeri* also increased with increasing temperature and CO₂ concentration. Norsworthy et al. (2008) observed that the LD₅₀ for the glyphosate resistant biotype was 2,820 g/ha, which was 79-115 times higher than the LD₅₀ for the glyphosate sensitive biotypes. Furthermore, this amount of glyphosate was more than three times the normal application rate of 840 g/ha. The work of Mohseni-Moghadam et al. (2013) showed that a glyphosate resistant *A. palmeri* had an LD₅₀ of 458 g/ha, which is approximately three times lower than the amount of glyphosate applied in this study to achieve a 50% reduction in weed biomass. Based on the shikimate accumulation and the dose response studies, Brazilian populations of the weed were found to be highly glyphosate-resistant; the herbicide quantity required to reduce the weed growth by 50% was about 4 kg/ha, more than twice the usual application rates (Küpper et al. 2017). For example, *A. palmeri*, an introduced weed in Brazil, is highly resistant to glyphosate, and the dose required to control 80% of the population of this resistant weed is more than 4.5 kg a.i./ha, and this application rate is not economically viable (Carvalho et al. 2015). Sosnoskie et al. (2011) also confirmed multiple resistance in *A. palmeri* to glyphosate and pyriithiobac in Georgia. They observed that 12 and 14-fold higher doses of glyphosate were required to obtain 50% control of *A. palmeri* biotype as compared to the susceptible biotype. Data related to shikimic acid showed glyphosate-susceptible biotypes of *A. palmeri* had shikimate in their leaf tissues after the application of glyphosate, but shikimate was not present in the resistant biotypes (Culpepper et al. 2006). The presence of shikimate in the plants treated with glyphosate indicates that EPSP activity is affected by the herbicide application (Mueller et al. 2003).

In the case of *E. colonum*, a wide range of temperatures exists for germination. It has been observed that *E. colonum* seeds can germinate at 20-34 °C, and 25/15 to 35/25 °C (Peerzada et al. 2016). Recent studies have evaluated some suspected glyphosate resistant *E. colonum* populations in Australia (Werth et al. 2012) and northern California (Alarcón-Reverte et al. 2015). In *S. halepense*, both an increase in temperature and CO₂ levels support the growth parameters. Vila-Aiub et al. (2013) observed that the appearance of glyphosate resistance is strongly temperature-dependent, but a biochemical basis for this dependence is still unknown. Another study shows that high CO₂ levels can flatten and prolong the growth rate of *S. halepense* (Carter and Peterson 1983).

For *P. oleracea*, there was no significant effect of increasing temperature on germination. These results are supported

by Chauhan and Johnson (2009), who found that under laboratory conditions, germination was not influenced by the different temperature levels (35/25 °C, 30/20 °C, and 25/15 °C). Germination of *S. nigrum* was also affected by increasing temperature but started to decrease at higher temperatures. Dong et al. (2020) observed that the germination rate of *S. nigrum* was maximum at 30 °C and started to decrease above 35 °C. *Physalis angulata* germination remained constant at the temperature levels in the study. The response of *P. angulata* showed that at 25 °C and 30 °C temperatures, the germination rate was maximum, while at 40 °C, it started to reduce (Bell and Oliver 1979). Data from different studies show that climate change induces morpho-physiological changes in plants, due to which the uptake and translocation rate of herbicides decreases (Jabran and Dogan 2022, Manea et al. 2011, Ziska et al. 2004). This ultimately causes an increase in the E₅₀ value, which enhances the weeds' ability to tolerate applied herbicides. Consequently, a higher dose of herbicide will be necessary to control these specific weeds. As a result, the increasing use of herbicides in agricultural areas will lead to greater economic costs for producers and a significant rise in weed resistance levels in the coming years.

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Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Çevresel koşullardaki değişikliklerin yabancı otların büyümesi ve uygulanan herbisitlere duyarlılıkları üzerinde büyüktür. Bu çalışmada, CO₂ ve sıcaklık seviyelerinin glifosat (480 g/l Glyphosate Isopropylamin Tuzu) aktivitesi ile yedi yabancı ot türünün büyümesi üzerindeki etkileri incelenmiştir. Üç sıcaklık seviyesi (kontrol veya normal sıcaklık 26/16 °C (14/10 gün/gece), normal sıcaklık +3 °C, 29/19 °C ve normal sıcaklık +6 °C, 32/22 °C) ve dört CO₂ seviyesi (kontrol 400 ppm, 600 ppm, 800 ppm ve 1000 ppm) test edilmiştir. Altı doz glifosat: i) önerilen dozun ¼'ü, ii) ½'si, iii) tam doz (1440 g a.i./ha), iv) önerilen dozun 2 katı, v) 4 katı ve vi) 8 katı, 4-6 yaprak aşamasında uygulanmıştır. Genel olarak, artan CO₂ ve sıcaklık seviyelerinde, yabancı otların büyümesini artırmıştır. Yabancı ot türlerinin çoğu için en uygun sıcaklık ve CO₂ seviyeleri sırasıyla 29 °C ve 800 ppm ile 1000 ppm olarak tespit edilmiştir. *Echinochloa*

colinum (L.) Link., *Amaranthus retroflexus* L., *Amaranthus palmeri* S. Watson, *Portulaca oleracea* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers. ve *Physalis angulata* L. için ED₅₀ (etkili doz 50) değeri, iklim değişikliğiyle birlikte bazı yabancı otların glifosata karşı tolerans geliştirme olasılığının yüksek olduğunu göstermiştir. Sıcaklık ve CO₂ konsantrasyonundaki artışla birlikte ED değeri de artmakta olup, bu durum yabancı otların kontrolü için daha yüksek herbisit dozlarına ihtiyaç duyulacağına işaret etmektedir. Sonuçta ileriki yıllarda tarımsal alanlarda daha fazla herbisit kullanımı üreticilerin ekonomik olarak daha fazla masraf yaşamasına ve yabancı otlarda dayanıklılık sorununun çok daha yüksek seviyelere çıkacağına sebebiyet verebilecektir.

Anahtar kelimeler: patlıcan, Şanlıurfa, CaPsol, nested-PCR, moleküler karakterizasyon

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