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Orijinal araştırma (Original article)

Effects of trifloxysulfuron-sodium and fluometuron herbicides on cotton and cocklebur (*Xanthium strumarium* L.)

Trifloxysulfuron-sodium ve fluometuron herbisitlerinin pamuk ve domuz pıtrağı (*Xanthium strumarium* L.) üzerine etkileri

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ABSTRACT

Cotton is an important industrial crop that is the natural raw material for many sectors such as textile, feed and oil industries. One of the factors affecting the yield and quality of cotton is weeds. Among these weeds, cocklebur (*Xanthium strumarium* L.) appears together with cotton and provides strong competition. In this study, field experiments were conducted at two different locations in 2020 to determine the efficacy of herbicides against cocklebur, which is a problem in cotton production, on yield elements in cotton and in cocklebur. The experiments were laid out according to the randomized block trial system with four replications. Different doses of the herbicides trifloxysulfuron sodium (TFS) (10, 15 and 20 g ha^{-1}) and fluometuron (FLM) (2, 2.5 and 3 l ha^{-1}) were used in the field experiments. As a result of the study, the effects of herbicide applications on cotton and cocklebur were evaluated on the 28th day in both sites. It was found that trifloxysulfuron- sodium was more than 90% effective in controlling cocklebur at different doses while fluometuron was found to be less effective at different doses. Regarding the effects of herbicide applications on cotton, trifloxysulfuron-sodium was found to cause no phytotoxicity at different dosages, while fluometuron showed phytotoxicity at different dosages. The highest cotton and fiber yields were obtained when 15 g ha^{-1} of trifloxysulfuron-sodium was applied. It was found that the effects of the applications on cotton fiber fineness, fiber length, and fiber strength were not significant.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.), native to India, is an industrial plant of the mallow family (Malvaceae). In cotton processing, it is the basic material for ginning industry, for textile industry with its fiber, for oil and feed industry with its seeds and for paper industry with its linter. With these aspects, cotton becomes a strategic product in the world. However, with the growth of population and the increase of living standard, the demand for cotton is increasing day by day. For some countries, cotton has become an important economic product due to the high value it generates (Keskinkılıç 2014).

Cotton was grown on an area of 32.01 million hectares worldwide and about 24.6 million tonnes of the cotton lint were obtained in 2023. In countries that meet approximately 83% of the world's cotton production are China, India, Brazil, USA, Pakistan, Australia and Türkiye respectively. Türkiye ranks seventh in the world in cotton production, fifth in consumption and fourth in imports (Anonymous 2024). In addition, Türkiye produces the most efficient and highest quality cotton among countries that do not grow GM (Genetically Modified) cotton (Anonymous 2017). Cotton production in Türkiye is concentrated in the Aegean, Mediterranean, and Southeast Anatolia regions. Approximately 59% of cotton production in Türkiye occurs in the Southeast Anatolia region, 24% in the Aegean region, and 17% in the Mediterranean region (Anonymous 2023).

There are many factors that limit cotton production, and one of the most important factors is weeds (Arslan and Kitiş 2021). Weeds compete with plants for growth factors such as water, nutrients, and light, and as a result of this competition, they cause a decline in crop productivity and quality (Güncan 2016, Sathishkumar et al. 2021). The cotton crop is very sensitive at early growth stages where weed presence during the first 2 months of growth may reduce yield from 10% to 90% (Tariq et al. 2020). To prevent weed-caused yield loss in cotton, weed control generally needs to be carried out between the 1st and 2nd and 7th and 10th weeks after cotton emergence (Tursun et al. 2016). Moreover, weeds compete with cotton at the early stages, hindering its development and significantly reducing its yield if not controlled. In addition to direct yield losses, weeds such as field bindweed (*Convolvulus arvensis* L.), cocklebur (*Xanthium strumarium* L.), black nightshade (*Solanum nigrum* L.), jimsonweed (*Datura stramonium* L.), and green field foxtail (*Setaria viridis* (L.) P. Beauv.), which occur especially after irrigation in the late periods when the cotton plant opens its boll, adhere to the fibers of the cotton plant, making harvesting difficult and reducing quality (Boz and Doğan 2004, Özkil et al. 2019). Therefore, weed control is absolutely necessary for high quality and efficient cotton production (Güncan 2016). Cocklebur (*X. strumarium*), which causes significant yield losses in cotton production, is reported to be an important weed species to be controlled (Özaslan and Bükün 2013, Süer and Tursun 2024).

Cocklebur begins to emerge from the early stages of cotton and may reappear throughout the season after irrigation. Therefore, it is considered one of the most competitive weeds in cotton. It is stated that the presence of one cocklebur in a 9 m cotton row causes a yield loss of about 9% (Coble and Byrd 1992). In the critical period between the 2nd and 10th week after cotton emergence, it causes yield losses of 6-27%. Cocklebur also reduces cotton plant height and boll weight (Byrd and Coble 1991).

It is known that in Türkiye, weeds are hoed by hand or tractor and controlled by chemical means (Pala and Mennan 2018). However, mechanical control with hand or tractor hoe causes difficulties in weed control because of high costs and difficulties in providing labor, so growers prefer chemical control. In chemical control of cotton, herbicides are usually applied pre-emergence or post-emergence. Post-emergence herbicides are generally used to control grass weeds. Herbicides containing the active ingredient trifloxysulfuron sodium are available for broadleaf weed control, but this herbicide is not preferred by growers because it causes phytotoxicity in cotton, and mechanical control is reported to be used because there are not many other herbicide options (Basal et al. 2019). Since there is no hoeing in the late stages of cotton, the need for chemical control of cocklebur is also important (Pala and Mennan 2018).

Cocklebur is reported to be one of the densest and most widespread important weed species in the Southeastern Anatolia region of Türkiye, where cotton production is intensive (Arslan 2018, Özaslan and Bükün 2013, Süer and Tursun 2024). In this study, we aimed to investigate the effect of effective herbicide application against cocklebur on cotton yield and quality.

MATERIALS AND METHODS

Site description and experimental setup

Studies were conducted to evaluate the efficacy of herbicides containing 500 g L^{-1} fluometuron (FLM) and 75% trifloxysulfuron-sodium (TFS) active ingredients against cocklebur, which is a problem in cotton, on yield elements and cocklebur in cotton. In this regard, field experiments were conducted at two different locations during the 2020 cotton production season. The field experiments were established in the experimental field of GAP International Agricultural Research and Training Center Directorate (GAPUTAEM) (37°.56' N, 40°.15' E) on April 22, 2020 and in Elidolu village of Yenişehir, Diyarbakır, Türkiye (37°.58' N, 40°.13' E) on May 5, 2020. Soil samples (0-30 cm) were collected at the beginning of both experiments to determine various physical and chemical properties of the soil. While the soil structure of the experiment laid out in GAPUTAEM is loamy, that of Elidolu is clay loam. It was observed that the organic matter and salt content in the two trial areas where the study was carried out was low, the lime content was at a moderate level, the amount of P_2O_5 was low, the amount of K_2O was sufficient and the pH was alkaline. According to the soil analysis results, it was found that the amount of

Table 1. Soil characteristics of the experimental fields (0–30 cm soil depth)

	GAPUTAEM		Elidolu	
Soil characteristics	**Values**	**Properties**	**Values**	**Properties**
Texture (%)	72.5	Clayey	68.05	Clay-loam
Total salt (%)	0.023	Without salt	0.92	Without salt
pH	8.15	Alkaline	8.2	Alkaline
Lime-$CaCO_3$ (%)	7.31	Medium chalky	11.95	Medium chalky
P_2O_5 (mg kg^{-1})	1.49	Slightly	1.77	Little
K_2O (mg kg^{-1})	94.38	Sufficient	95.60	Sufficient
Organic matter (%)	0.96	Slightly	0.25	Slightly

organic matter in the soil in the GAPUTAEM trial area was higher than in the trial area in Elidolu (Table 1).

Local practices for cotton cultivation were used in preparing the soil for the experiments. The soil was plowed with a chisel, then with a disk harrow, and finally with a harrow to obtain a smooth seedbed. The cotton variety 'Stoneville 468' was sown with a four-row planter with a row spacing of 70 cm, and 8 cm between plant rows. Considering the results of soil analysis, 160 kg ha^{-1} of pure nitrogen (N) and 80 kg ha^{-1} of phosphorus (P) were applied, with half of the nitrogen and all of the phosphorus as 20-20 (N:P) blend fertilizer before sowing. The other half was administered before the first irrigation (approximately 40-45 days after sowing). Fields were irrigated as needed. The experiments in both field experiments were designed as a completely randomized block experiment with four replications. Each experimental plot was 24 m^2 (3 m x 8 m) and had four rows of cotton. To determine the effect (%) of herbicides on cocklebur, two randomly selected gravel plots (1 m^2) were established in each plot. According to the cotton cultivation technique, practices such as hoeing (hand and tractor hoe) and irrigation were uniformly applied in all plots. Cotton pest control was carried out according to the cotton cultivation technique.

Herbicide applications

Herbicide applications were made at GAPUTAEM on June 11, 2020, and at Elidolu on June 19, 2020. Different doses of the herbicides trifloxysulfuron-sodium (75%) and fluometuron (500 g l^{-1}) were applied when cocklebur had two to six leaves and at the six to eight leaf stage of cotton. In addition, the season-long weedy and weed-free control treatments were established in order to compare the efficacy of herbicide doses on cocklebur (Table 2). In weed-free control applications, weeds were periodically hoed with a hand hoe starting from cotton emergence.

Table 2. Herbicides and doses used in the experiment

Treatments	**Application Time**	**Properties**
75% Trifloxysulfuron-sodium	Post emergence	10 g ha^{-1}
75% Trifloxysulfuron-sodium	Post emergence	15 g ha^{-1}
75% Trifloxysulfuron-sodium	Post emergence	20 g ha^{-1}
500 g l^{-1} Fluometuron	Post emergence	2 l ha^{-1}
500 g l^{-1} Fluometuron	Post emergence	2.5 l ha^{-1}
500 g l^{-1} Fluometuron	Post emergence	3 l ha^{-1}

Herbicide was applied with a gasoline backpack sprayer (Oleo-Mac Sp 126 brand) with 6 fan beams (Teejet brand) boom type, 2 m working width, and 3 atmospheres of pressure. 300 l ha^{-1} of water was used for spraying.

Effect of herbicide applications on cotton and cocklebur

The rate of phytotoxicity (%) caused by the application of different doses to cotton was evaluated using the 0-100 scale, by selecting ten cotton plants randomly from the plots, on the 7th, 14th, 21st, and 28th day after herbicide treatment (DAHT).

In determining the effect (%) of herbicide applications on cocklebur, the effect of cocklebur on a fixed area of 1 m^2 and

the cocklebur on which the herbicide application was made were compared with the visual effects on days 7, 14, 21, and 28 DAHT. Herbicide effects (%) were determined visually according to the 0-100 scale (Uygur 1991). In these ratings, 0 = completely healthy, while 100 = dead plant.

Determination of the effects of herbicide applications on the dry weight (g m^{-2}) of cocklebur

Cocklebur plants on the solid plots established in the experimental plots were harvested from the ground 28 DAHT and their dry weight was determined after they were dried in an oven at 105 °C for 24 h (Süer et al. 2024).

Effect of herbicide applications on cotton yield, ginning efficiency and fiber yield

Cotton unseed in the middle two rows of each plot in the applications studied in the experiment was harvested by hand, and cotton yield (kg ha^{-1}), ginning efficiency (GE: %), and cotton fiber yield (CFY: kg ha^{-1}) were calculated.

GE :[Fiber cotton (g)/(Cottonseed (g) + Cotton fiber (g))] x100

Cotton fiber yield (kg ha^{-1}): (Cotton unseed yield x Ginning efficiency) /100

Determination of the effect of herbicide applications on fiber quality characteristics of cotton

Cotton quality analyses were carried out at the GAP International Agricultural Research and Education Center Directorate Cotton Fiber Testing and Analysis Laboratory in Diyarbakır, Türkiye. Analyses were carried out with the HVI 1000 instrument. The effects of different herbicide doses on fiber quality characteristics such as fiber fineness, fiber length, and fiber strength in cotton were determined.

Statistical analysis

In the field experiments, data were evaluated using the IBM SPSS 25 statistical package program in studies to determine the effects of herbicide applications on cocklebur dry weight (g m^{-2}), cotton yield (kg ha^{-1}), ginning yield (%), fiber yield (kg ha^{-1}), and fiber quality traits. Data obtained from the studies were subjected to GLM model One Way (ANOVA) variance analysis, and the difference between the applications was determined by Duncan's multiple comparison test according to the significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Herbicide applications on cotton and cocklebur

In both field experiments conducted in GAPUTAEM and Elidolu villages, it was found that different dose applications of the herbicide containing the active ingredient trifloxysulfuron-sodium (TFS) did not cause phytotoxicity in cotton after the first count on day 7 (Table 3). Similar to our results, Özkil and Üremiş (2021) reported in their study on the control of *Ipomoea triloba* in Mediterranean cotton fields that the application of TFS caused a temporary reduction in growth in cotton, but the symptoms disappeared

Table 3. Phytotoxicity rate (%) caused by herbicides on cotton

GAPUTEAM				
Treatments	**7th day**	**14th day**	**21th day**	**28th day**
TFS (10 g ha^{-1})	0.5±0.5c	0.0±0.0c	0.0±0.0b	0.0±0.0c
TFS (15 g ha^{-1})	4.2±0.4c	2.0±0.8c	0.0±0.0b	0.0±0.0c
TFS (20 g ha^{-1})	5.2±0.4c	3.5±0.6c	0.0±0.0b	0.0±0.0c
FLM (2 l ha^{-1})	18.2±2.8b	14.5±1.7b	6.5±2.8a	1.7±0.8b
FLM (2.5 l ha^{-1})	23.7±3.1a	22.5±2.1a	9.7±3.0a	4.5±0.2a
FLM (3 l ha^{-1})	26.5±1.1a	23.7±1.7a	9.7±4.2a	5.0±0.8a
Elidolu				
Treatments	**7th day**	**14th day**	**21th day**	**28th day**
TFS (10 g ha^{-1})	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0d
TFS (15 g ha^{-1})	0.0±0.0b	0.0±0.0b	0.0±0.0c	0.0±0.0d
TFS (20 g ha^{-1})	0.5±0.5b	0.0±0.0b	0.0±0.0c	0.0±0.0d
FLM (2 l ha^{-1})	10.0±0.0a	8.0±1.4a	4.2±1.2b	3.0±0.8c
FLM (2.5 l ha^{-1})	10.5±0.8a	7.7±1.0a	7.0±0.7a	6.5±0.2b
FLM (3 l ha^{-1})	11.0±1.0a	7.7±1.2a	9.2±1.2a	10.7±0.8a

* TFS: 75% trifloxysulfuron-sodium, FLM: 500 g l^{-1} fluometuron. Each column was evaluated within itself, and the values marked with the same letter are statistically the same ($P > 0.05$)

with time. Richardson et al. (2007a) found that 7 days after post-emergence application of 3.8 g ha^{-1} and 15 g ha^{-1} of the active herbicide TFS to weeds in cotton, phytotoxicity ranged from 19-22%, but at 28 days after application, this rate decreased to 5%-12%. In our study, it was found that no phytotoxicity was observed in cotton plant after 28 days when the herbicide containing the active ingredient TFS was applied at a dose of 15 g ha^{-1}. This difference may be due to the experiments being conducted under different ecological conditions.

It was found that phytotoxicity was observed at all doses of the herbicide containing the active ingredient fluometuron (FLM) at different doses, but decreased after 28 days of application. Similar to our study, Chachalis and Galanis (2007) found that phytotoxicity rate in cotton was less than 4% after 3 weeks when different doses of acetochlor (1.68, 2.10, 2.52 and 2.94 kg ha^{-1}) were applied in combination with FLM dose (1.75 kg ha^{-1}) against weeds in cotton.

It was determined that the effect of TFS herbicide on cocklebur was more than 90% in 28 days in both experimental areas (GAPUTAEM and Elidolu) (Table 4). The study found that the difference between the effects of different TFS doses on cocklebur was insignificant. In studies similar to our results, Richardson et al. (2007a) found that the effects of pre-emergence and post-emergence herbicides applied for weed control in cotton ranged from 96% to 98% of the same herbicide on cocklebur in cotton. In another study, Rezakhanlou et al. (2014) found that the highest efficacy of the applied TFS herbicide in controlling cocklebur in cotton was at doses of 16 and 19 g ha^{-1} and the difference between the two doses was insignificant.

The efficacy of FLM herbicide proved to be quite low compared to TFS. The highest efficacy of FLM on cocklebur was determined after 28 days with 55.5% and 46.0%, respectively. Kaloumenos et al. (2005) evaluated the effects of pre-sowing, pre-emergence, and post-emergence herbicide applications on cotton and weeds in cotton fields in Greece and reported that fluometuron failed to control cocklebur, as occurred in our experiments.

Herbicide applications on the dry weight (g m^{-2}) of cocklebur

The highest dry weight value for herbicide applications was obtained from weedy control plots in GAPUTAEM and Elidolu with 607.0 g m^{-2} and 262.7 g m^{-2}, respectively. The lowest dry weight value was obtained at TFS doses of 10 and 15 g ha^{-1} in both experimental plots. In both field experiments, it was found that herbicide applications of TFS and FLM resulted in a significant decrease in dry weight compared to weedy control plots (Table 5).

In the experiments at both sites (GAPUTAEM and Elidolu), it was determined that the application of 10 and 15 g ha^{-1} of the herbicide TFS had an effect of over 90% on the dry weight of cocklebur, compared to weedy control plots. Rezakhanlou et al. (2014) found that the application of TFS at a dose of 15 g ha^{-1} had an effect of over 90% compared to the weedy control in controlling cocklebur. In addition,

Table 4. Effect of different dosage applications of trifloxysulfuron-sodium (TFS) and fluometuron (FLM) on cocklebur (%)

GAPUTEAM				
Treatments	**7th day**	**14th day**	**21th day**	**28th day**
TFS (10 g ha^{-1})	74.5±6.4a	89.5±2.2a	91.5±1.5a	90.2±4.1a
TFS (15 g ha^{-1})	73.2±4.4a	87.0±1.7a	90.2±1.7a	90.0±0.9a
TFS (20 g ha^{-1})	78.2±2.9a	87.0±1.2a	90.2±1.2a	92.0±1.2a
FLM (2 l ha^{-1})	36.2±5.2b	39.5±4.8b	33.5±3.7c	22.7±5.2c
FLM (2.5 l ha^{-1})	39.0±5.4b	53.2±3.6b	64.0±8.8b	55.5±1.5b
FLM (3 l ha^{-1})	45.7±12.0b	52.0±8.8b	51.7±8.4b	48.7±9.2b
Elidolu				
Treatments	**7th day**	**14th day**	**21th day**	**28th day**
TFS (10 g ha^{-1})	82.7±4.7a	90.7±4.3a	92.0±1.7a	93.0±1.2a
TFS (15 g ha^{-1})	89.7±1.3a	91.0±1.1a	92.5±0.8a	93.5±1.5a
TFS (20 g ha^{-1})	89.7±1.0a	91.2±1.4a	94.2±0.7a	94.2±0.7a
FLM (2 l ha^{-1})	30.7±1.1c	27.5±2.6c	22.7±3.4c	26.2±5.5c
FLM (2.5 l ha^{-1})	29.7±1.8c	31.0±3.2c	27.5±2.7c	30.0±4.0c
FLM (3 l ha^{-1})	42.0±5.6b	41.7±3.8b	42.5±5.4b	46.0±7.6b

* TFS: 75% trifloxysulfuron-sodium, FLM: 500 g l^{-1} fluometuron. Each column was evaluated within itself, and the values marked with the same letter are statistically the same ($P > 0.05$)

Table 5. Herbicide applications on the dry weight (g m^{-2}) of cocklebur

	GAPUTAEM		Elidolu	
Treatments	**Dry weight (g m^{-2})**	**Impact rate (%)**	**Dry weight (g m^{-2})**	**Impact rate (%)**
TFS (10 g ha^{-1})	56.2±11.5c	90.7	15.7±6.3c	94.0
TFS (15 g ha^{-1})	78.0±20.2bc	87.1	17.7±2.6bc	93.2
TFS (20 g ha^{-1})	77.5±14.4bc	87.2	32.2±7.4b	87.7
FLM (2 l ha^{-1})	136.0±16.9b	77.5	30.0±9.2bc	88.5
FLM (2.5 l ha^{-1})	81.0±14.4bc	86.6	30.7±9.3bc	88.3
FLM (3 l ha^{-1})	66.0±22.6bc	89.1	31.7±3.3b	87.9
Weedy control	607.0±105.0a	-	262.7±36.8a	-

* TFS: 75% trifloxysulfuron-sodium, FLM: 500 g l-1 fluometuron. Each column was evaluated within itself, and the values marked with the same letter are statistically the same (P > 0.05)

in the study of Özkil and Üremiş (2021) using different herbicides to control *Ipomoea triloba* in cotton, it was found that the application of TFS at a dose of 15 g ha^{-1} (two applications 2 weeks apart) had an effect on dry weight of *I. triloba* between 92% and 97%. There are similarities between the results obtained and our study.

Herbicide applications on cotton yield, ginning efficiency and fiber yield

In the experiments, the highest seed cotton yield was obtained from season long weed-free control plots in GAPUTAEM and Elidolu with 5022 kg ha^{-1} and 3015 kg ha^{-1}, respectively. In TFS herbicide, the highest yield was obtained with a dose of 15 g ha^{-1} (Table 6). When the yields from the two experiments were compared, it was found that the cotton yield of GAPUTAEM was higher than that of Elidolu. This is thought to be due to the soil structure, organic matter content, and slope of the land where the experiments were conducted.

In experiments, the highest cotton and fiber yields were obtained after weed-free control plots with the 15 g ha^{-1} dose of TFS. Yield at the 20 g ha^{-1} dose is believed to be lower than at the 15 g ha^{-1} dose, possibly due to the herbicide causing phototoxicity in cotton (Table 6). Similar to our results, Rezakhanlou et al. (2014) reported that TFS at a dose of 16 g ha^{-1} gave the highest yield in cotton and that performance at a dose of 19 g ha^{-1} was lower than at a dose of 16 g ha^{-1}, which may be due to phytotoxicity in cotton. The application of FLM herbicides was found to be 3 l ha^{-1} after the yield of the weed-free plot. Similar results were obtained for fiber yield (Table 6). For herbicide application against weeds in cotton, Şahin et al. (2020) reported that the highest cotton yield in cotton was obtained from weed-free control plots and this application followed the application of cycloxydim+triloxsulfuron sodium. In our study, it was found that 2 l ha^{-1} and 3 l ha^{-1} FLM produced higher cotton seed and fiber yield compared to weedy control. Similar to our results, Chachalis and Galanis (2007) found in their study that mixing acetochlor with FLM provided benefits in cotton fiber yield.

Herbicide applications on fiber quality characteristics of cotton

In both field experiments, it was found that the difference between the effects of the treatments on cotton fiber fineness, fiber length, and fiber strength was not statistically significant. The best results in terms of cotton fiber fineness were obtained in GAPUTAEM with the 15 g ha^{-1} application dose of TFS and in the weed-free control plot in Elidolu. The highest fiber length was obtained with a TFS dose of 20 g ha^{-1} in GAPUTAEM and in the weed-free control plot in Elidolu (Table 7).

Table 7.

The quality characteristics of cotton fibers are important in the textile industry. Cotton fibers with the highest fiber strength are preferred in the yarn industry. As a result of the study, it was found that the application of TFS and FLM herbicides had no effect on the quality characteristics of cotton fibers. In parallel with our results, some studies reported that TFS applications have no effect on cotton fiber quality characteristics compared to other applications (Richardson et al. 2003, Richardson et al. 2004, Richardson et al. 2007a, 2007b). In contrast to our study, Şahin et al. (2020) found that cotton fiber quality characteristics were affected by herbicide applications, with the best results obtained from weed-free control plots, and this result was followed by cycloxydim+triloxsulfuron sodium and clethodim+triloxsulfuron sodium applications. The differences between the results we obtained in this study were due to the different ecological conditions of the experimental plots and the differences in the cotton varieties used in the experiments.

Table 6. Herbicide applications on cotton yield, ginning efficiency (GE) and fiber yield (FY)

GAPUTEAM			
Treatments	**Yield (kg ha^{-1})**	**GE (%)**	**FY (kg ha^{-1})**
TFS (10 g ha^{-1})	3930±42.2b	44.0±0.5a	1727±17.9bc
TFS (15 g ha^{-1})	4875±32.1a	44.8±0.4a	2185±14.5ab
TFS (20 g ha^{-1})	3907±12.1b	44.7±0.4a	1748±7.1bc
FLM (2 l ha^{-1})	3017±13.7c	44.7±0.8a	1346±4.9c
FLM (2.5 l ha^{-1})	3870±16.5b	44.3±0.5a	1711±6.4c
FLM (3 l ha^{-1})	4040±37.3b	45.1±0.3a	1822±15.9cb
Weedy control	3187±19.5bc	43.9±0.3a	1622±24.5c
Weed-free control	5022±28.1a	44.4±0.3a	2231±13.2a
Elidolu			
Treatments	**Yield (kg ha^{-1})**	**GE (%)**	**FY (kg ha^{-1})**
TFS (10 g ha^{-1})	2617±8.9bc	44.6±0.4a	1164±7.5ab
TFS (15 g ha^{-1})	2885±11.6ab	44.4±0.5a	1276±4.4ab
TFS (20 g ha^{-1})	2712±14.1abc	44.4±0.2a	1207±5.6ab
FLM (2 l ha^{-1})	2475±18.4c	43.8±0.5a	1086±4.2b
FLM (2.5 l ha^{-1})	2437±9.4c	45.0±0.4a	1130±3.7b
FLM (3 l ha^{-1})	2537±3.5bc	44.5±0.8a	1121±6.0b
Weedy control	1790±6.7d	44.1±0.7a	791±3.7c
Weed-free control	3015±9.2a	44.1±0.2a	1331±10.3a

* TFS: 75% trifloxysulfuron-sodium, FLM: 500 g l-1 fluometuron. Each column was evaluated within itself, and the values marked with the same letter are statistically the same (P > 0.05)

Table 7. Herbicide applications on fiber quality characteristics of cotton

	GAPUTAEM			Elidolu		
Treatments	**Fiber fineness (mic)**	**Fiber length (mm)**	**Fiber Strength (g/tex)**	**Fiber fineness (mic)**	**Fiber length (mm)**	**Fiber Strength (g/tex)**
TFS (10 g ha^{-1})	4.7±0.0a	29.9±0.2a	35.0±0.8a	4.9±0.1a	28.7±0.7a	33.9±0.6a
TFS (15 g ha^{-1})	5.0±0.1a	28.7±0.5a	33.7±0.5a	4.9±0.0a	29.0±0.4a	34.2±0.7a
TFS (20 g ha^{-1})	4.7±0.1a	30.3±0.6a	33.4±0.5a	4.8±0.0a	28.5±0.4a	33.8±0.6a
FLM (2 l ha^{-1})	4.7±0.0a	29.5±0.1a	34.9±0.4a	4.7±0.0a	28.8±0.2a	33.4±0.2a
FLM (2.5 l ha^{-1})	4.7±0.0a	29.3±0.6a	35.6±1.0a	4.9±0.0a	28.9±0.2a	34.7±0.4a
FLM (3 l ha^{-1})	4.7±0.0a	30.0±0.3a	34.9±1.0a	4.7±0.1a	28.6±0.8a	32.9±1.5a
Weedy control	4.6±0.2a	29.8±0.2a	33.3±0.5a	4.9±0.0a	28.9±0.3a	34.5±0.9a
Weed-free control	4.9±0.1a	30.0±0.4a	35.2±1.0a	5.0±0.0a	29.4±0.3a	35.0±0.5a

* TFS: 75% trifloxysulfuron-sodium, FLM: 500 g l-1 fluometuron. Each column was evaluated within itself, and the values marked with the same letter are statistically the same (P > 0.05)

In both experiments, it was determined that trifloxysulfuron-sodium at 10, 15 and 20 g ha^{-1} did not cause any phytotoxicity on cotton and the effect on cocklebur was over 90%. In the field experiments conducted the highest cotton yields, fiber yields, and ginning efficiencies were obtained at an application dose of 15 g ha^{-1} of TFS herbicide. In both field experiments, it was found that the cotton plant became phytotoxic with the application of different doses of the herbicide containing the active ingredient FLM.

Despite the use of pre-sowing and pre-emergence herbicides and cultural and mechanical control of cocklebur in cotton growing areas, adequate control is not possible. Therefore, post-emergence herbicide applications should be combined with these control methods. In post-emergence applications, the active ingredients used to control broadleaf weeds such as cocklebur are limited. In addition, weeds reemerge after irrigation with seed banks present in the soil, making them difficult to control. Therefore, field preparation, irrigation, tillage, hoeing, and chemical control must be timely and appropriate. As a result of the study, it was found that TFS herbicide cocklebur controlled and no phytotoxicity was observed on cotton plant. Therefore, it is important to consider the active ingredient and application dose in the fight against cocklebur. In this way, unnecessary and excessive use of herbicides in cotton growing areas will be prevented and growers will be able to obtain both high quality and productive cotton.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Pamuk işlenmesi bakımından tekstil, yem ve yağ sanayisi gibi birçok sektörün doğal hammaddesi olan önemli bir endüstri bitkisidir. Pamukta verim ve kaliteyi etkileyen faktörlerden biri de yabancı otlardır. Bu yabancı otlardan domuz pıtrağı (*Xanthium strumarium* L.) pamuk ile beraber çıkış yaparak yüksek bir rekabet oluşturur. Bu çalışmada, pamuk üretiminde sorun olan domuz pıtrağına karşı kullanılan herbisitlerin pamukta verim unsurları ve domuz pıtrağına olan etkinliğini belirlemek amacıyla 2020 yılında iki ayrı lokasyonda tarla denemeleri kurulmuştur. Denemeler, tesadüf blokları deneme desenine göre 4 tekerrürlü olacak şekilde yapılmıştır. Tarla denemelerinde trifloxysulfuron-sodium (TFS) (10, 15 ve 20 g ha^{-1}) ve fluometuron (FLM) herbisitlerin (2, 2,5 ve 3 l ha^{-1}) farklı dozları uygulanmıştır. Çalışma sonucunda herbisit uygulamalarının pamuk ve domuz pıtrağına olan etkileri her iki lokasyonda 28. günde değerlendirilmiştir. Trifloxysulfuron-sodium farklı doz uygulamalarında domuz pıtrağı kontrolünde %90'nın üzerinde etkili olduğu, fluometuronun ise farklı doz uygulamalarında etkinliğinin düşük olduğu belirlenmiştir. Herbisit uygulamalarının pamuktaki etkilerine bakıldığında, trifloxysulfuron-sodium'un farklı doz uygulamalarında herhangi bir fitotoksisiteye neden olmadığı, fluometuronun'un ise farklı doz uygulamalarında fitotoksisite gösterdiği görülmüştür. En yüksek pamuk ve lif verimi 15 g ha^{-1} trifloxysulfuron-sodium uygulamasından elde edilmiştir. Uygulamaların pamuk lif inceliği, lif uzunluğu ve lif mukavemeti üzerindeki etkilerinin önemli olmadığı belirlenmiştir.

Anahtar kelimeler: domuz pıtrağı, fitotoksisite, pamuk verimi, lif verimi, lif kalitesi, Türkiye

REFERENCES

Anonymous, 2017. Pamuk sektör raporu. Turkey. http://www.upk.org.tr/User_Files/kitaplik/ulusal-pamuk-konseyi-pamuk-sektor-raporu-2017.pdf (accessed date: 10.06.2024).

Anonymous, 2023. Data portal for statistics. Crop production statistics. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr (accessed date: December 2023).

Anonymous, 2024. Cotton: world markets and trade. https://apps.fas.usda.gov/psdonline/circulars/cotton.pdf (accessed date: 18.07.2024).

Arslan Z.F., 2018. Şanlıurfa ili pamuk tarlalarında sulama sonrası yabancı otlar ile ilgili yaşanan değişimler, sorunlar ve çözüm önerileri. Harran Tarım ve Gıda Bilimleri Dergisi, 22 (1), 109-125. https://doi.org/10.29050/harranziraat.306920

Arslan E., Kitiş Y.E., 2021. Antalya ili pamuk (*Gossypium hirsutum* L.) ekim alanlarında görülen yabancı otlar ve popülasyon durumları. Turkish Journal of Weed Science, 24 (2), 141-149.

Basal H., Karademir E., Goren H.K., Sezener V., Dogan M.N., Gencsoylu I., Erdogan O., 2019. Cotton production in Türkiye and Europe. In: Cotton production. Jabran K., Singh Chauhan B. (Eds.). John Wiley & Sons, Ltd., 297–321 p. https://doi.org/10.1002/9781119385523.ch14

Boz Ö., Doğan M.N., 2004. Aydın ili pamuk ekim alanlarındaki yabancı otlar ve mücadelesi. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 1 (2), 13-16.

Byrd J.D., Coble H.D., 1991. Interference of common cocklebur (*Xanthium strumarium*) and cotton (*Gossypium hirsutum*). Weed Technology, 5 (2), 270-278. https://doi.org/10.1017/S0890037X00028098

Chachalis D., Galanis M., 2007. Weed control and cotton response to combinations of acetochlor with fluometuron. Journal of Food, Agriculture & Environment, 5 (3/4), 198-201.

Coble H.D., Byrd J.D., 1992. Interference of weeds with cotton. In: Weeds of cotton: characterization and control. McWhorter C.G., Abernathy J.R. (Eds.). The Cotton Foundation, Memphis, TN, USA, 73-84 p.

Güncan A., 2016. Yabancı otlar ve mücadele prensipleri. Selcuk Üniversitesi Yayınları, 3. Baskı, Konya, Türkiye, 311 s.

Keskinkılıç K., 2014. Türkiye pamuk durumundaki gelişmeler. İzmir Ticaret Borsası, Raporlar, İzmir, Türkiye, 41 s.

Kaloumenos N.S., Veletza V.G., Papantoniou A.N., Kadis S.G., Eleftherohorinos I.G., 2005. Influence of pyrithiobac application rate and timing on weed control and cotton yield in Greece. Weed Technology, 19 (1), 207-216. doi:10.1614/WT-04-188

Özaslan C., Bükün B., 2013. Determination of weeds in cotton fields in Southeastern Anatolia Region of Turkey. Soil- Water Journal, 2 (2), 1777-1784.

Özkil M., Serim A.T., Torun H., Üremiş İ., 2019. Antalya ili pamuk (*Gossypium hirsutum* L.) tarlalarında bulunan yabancı ot türlerinin, dağılım ve yoğunluklarının saptanması. Turkish Journal of Weed Science, 22 (2), 185-191.

Özkil M., Üremiş İ., 2021. Determination of the control methods of *Ipomoea triloba* L. (three lobe morning glory) in cotton fields. Plant Protection Bulletin, 61 (3), 20-27. https://doi.org/10.16955/bitkorb.880594

Pala F., Mennan H., 2018. Diyarbakır ili pamuk ekim alanlarında sorun olan yabancı otlar ve uygulanan control yöntemlerinin araştırılması. Ege Üniversitesi Ziraat Fakültesi Dergisi, 55 (1), 111-117. doi: 10.20289/zfdergi.330081

Rezakhanlou A., Mirshekari B., Zand E., Farahvash F., Baghestani M.A., 2014. Analyzing the effects of the reduced amounts of the trifloxysulfuron herbicide on controlling the *Xanthium strumarium* weed in cotton. Advances in Environmental Biology, 8 (10), 367-375.

Richardson R.J., Wilson H.P., Armel G.R., Hines T.E., 2003. Mixtures of CGA 362622 and bromoxynil for broadleaf weed control in bromoxynil-resistant cotton (*Gossypium hirsutum*). Weed Technology, 17, 496-502. https://doi.org/10.1614/0890-037X(2003)017[0496:MOCABF]2.0.CO;2

Richardson R.J., Wilson H.P., Armel G.R., Hines T.E., 2004. Influence of adjuvants on cotton (*Gossypium hirsutum*) response to postemergence applications of CGA 362622. Weed Technology, 18 (1), 9-15. https://doi.org/10.1614/WT-02-058

Richardson R.J., Wilson H.P., Armel G.R., Hines T.E., 2007a. Preemergence herbicides followed by trifloxysulfuron postemergence in cotton. Weed Technology, 21 (1), 1-6. https://doi.org/10.1614/WT-05-047.1

Richardson R.J., Wilson H.P., Armel G.R., Hines T.E., 2007b. Growth stage affects cotton (*Gossypium hirsutum*) response to trifloxysulfuron. Weed Technology, 21 (1), 37-40. https://doi.org/10.1614/WT-05-025.1

Sathishkumar A., Srinivasan G., Subramanian E., Rajesh P., 2021. Weed management in cotton: a review. Agricultural Reviews, 43 (1), 1-10.

Süer İ.E., Eşitmez B., Ateş E., Kahraman Ş., Avşar Ö., 2024. Damla sulama sistemi ile yetiştiriciliği yapılan çeltikte herbisitlerin yabancı otlara karşı etkinliğinin belirlenmesi. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 29 (3), 649-662. https://doi.org/10.37908/mkutbd.1446917

Süer İ.E., Tursun N., 2024. Weeds in the cotton growing areas in the southeastern anatolia region. Harran Tarım ve Gıda Bilimleri Dergisi, 28 (2), 209-221. doi: 10.29050/harranziraat.1406951

Şahin S., Gürbüz R., Çoruh İ., 2020. Iğdır ili pamuk üretim alanlarında görülen yabancı ot türlerinin belirlenmesi ve bazı herbisitlerin yabancı otlanma ile pamuk verimine olan etkilerinin araştırılması,. Journal of Agriculture, 3 (2), 40-48. https://doi.org/10.46876/ja.822131

Tariq M., Abdullah K., Ahmad S., Abbas G., Rahman M.H., Khan M.A., 2020. Weed management in cotton. In: Cotton production and uses. Ahmad, S., Hasanuzzaman, M. (Eds.).. Springer, Singapore, 145-161. https://doi.org/10.1007/978-981-15-1472-2_9

Tursun N., Budak S., Kantarcı Z., 2016. The effects of row spacing on determination of critical period for weed control in cotton (*Gossypium hirsitum* L.). Biotech Studies, 25, 100-105. http://doi.org/10.21566/tarbitderg.281866

Uygur F.N., 1991. Herboloji araştırma yöntemleri. Çukurova Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Yardımcı Ders Notu, Adana, Türkiye, 69 s.

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Orijinal araştırma (Original article)

Investigation of the effects of weeds and different control methods on walnut seedling height and stem diameter in the walnut nursery of Kahramanmaraş province

Kahramanmaraş ili ceviz fidanlığında bulunan yabancı otların ve farklı mücadele yöntemlerinin ceviz fidan boyu ve gövde çapına etkisinin araştırılması

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ABSTRACT

The walnut (*Juglans regia* L.), which plays an important role in human nutrition, is quite significant in terms of omega-3 fatty acids, antioxidants, polyphenols, copper, folic acid, phosphorus, manganese, vitamin B6, and vitamin E. In walnut nurseries, many weeds, diseases, and pests are encountered. One of the main problems in the nursery is the weeds. This study was carried out to determine the problem weed species, families and densities in the walnut (*Juglans regia* L.) nursery of Kahramanmaraş Faculty of Agriculture in the 2022-2023 period. At the same time, the effects of different weed control methods (hand hoe, pendimethalin 455g/l, glyphosate isopropylamine salt and mulch) on walnut sapling height and trunk diameter were investigated. As a result of the study, 66 weed species belonging to 20 different families were identified in the trial area. Of these weed species, 16 are monocotyledonous and 50 are dicotyledonous. According to the density scale, the weed species determined in the walnut experimental area were determined to be very dense with *Elymus repens* (L.) Gould 13.20, *Sorghum halepense* (L.) Pers. 12.69, *Cynodon dactylon* (L.) Pers. 11.58, *Anagallis arvensis* L. 10.70, and *Digitaria sanguinalis* (L.) Scop. 10.30 pieces/m².

In this study, the mulching method reduced weed density by 98.07%, followed by hand hoeing with a reduction of 80.48%. The third method was post-emergence (glyphosate isopropylamin salt) herbicide treatment, which reduced weed density by 76.14%, and the fourth was pre-emergence (pendimethalin) herbicide treatment with a reduction of 74.10%.

Among the weed control methods, the black plastic mulch method increased walnut seedling height by 633.33%, the hand hoeing method by 566.67%, the post-emergence herbicide treatment by 500.00%, and the pre-emergence herbicide treatment by 400.00%.

INTRODUCTION

Walnut (*Juglans regia* L.) belongs to the Juglandaceae family and is one of the economically significant nut crops. In 2023, the People's Republic of China ranked first in the world covering 420.224 ha of walnut plantations, followed by the United States, which has 181.640 ha, securing second place, Iran, with 156.656 ha, ranked third, and Türkiye, in fourth place, with 121.775 ha (FAO 2023). In Türkiye, for walnut production in 2023, Kahramanmaraş ranked first with 13.631 tons, Hakkâri came second with 9.140 tons, while Karaman was third with 8.366 tons (Anonymous 2023).

Walnut is a rich source of nutrients for human nutrition and health. In addition to being consumed directly, walnuts are also used in its dried form, as well as in jam, pestil, and paste. Walnuts are nutritionally significant, being especially rich in omega-3 and omega-6 fatty acids, protein, carbohydrates, potassium, phosphorus, magnesium, and iron. Walnuts contain several key fatty acids, including palmitic, stearic, oleic, linoleic, and linolenic acids. Walnuts are also notably high in antioxidant content. The green outer shell of walnuts is used to prepare walnut liqueur, a traditional product (Akbari et al. 2012, Almeida et al. 2008).

Among the walnut species, *Juglans regia* L. commonly known as Anatolian walnut, Persian walnut, and English walnut, is the most recognized. Its native range spans from the Carpathian Mountains to regions including Türkiye, Iraq, Iran, Afghanistan, Southern Russia, India, Manchuria, and Korea, making it one of the oldest cultivated fruit species in history.

Several diseases, pests, and weed species significantly affect walnut development. For this reason, if weed control is not implemented in walnut orchards, substantial damage can occur. In walnut nurseries and orchards, weeds can cause significant harm by competing for mineral nutrients and water needed by the trees, suppressing root development during the seedling stage with their strong root systems, and by blocking sunlight. Weeds also contribute significantly to the spread of diseases by serving as intermediate hosts for various diseases and pests. Therefore, weed control in walnut nurseries and orchards is crucial.

In the weed survey studies conducted in fruit nurseries in Türkiye, the following species were identified in walnut, citrus, apricot, peach, plum, persimmon, pistachio, pear, apple, loquat, pomegranate, and avocado nurseries in various provinces, including Adana, Antalya, Aydın, Gaziantep, Hatay, İçel, Elazığ, Düzce, Edirne, Karaman, Kırklareli, Malatya, Şanlıurfa, Tekirdağ, and Van: the identified narrow-leaved weed species commonly observed including *Elymus repens* (L.) Gould, *Bromus tectorum* L., *Cyperus rotundus* L., *Lolium temulentum* L., *Sorghum halepense* (L.) Pers., *Cynodon dactylon* (L.) Pers., and *Digitaria sanguinalis* (L.) Scop. Among broad-leaved weed species, *Acroptilon repens* (L.) DC., *Anagallis arvensis* L., *Convolvulus arvensis* L., *Carduus pycnocephalus* L., *Chenopodium album* L., *Cirsium arvense* (L.) Scop., *Conyza canadensis* (L.) Cronquist., *Matricaria chamomilla* L., *Malva neglecta* Wallr., *Papaver rhoeas* L., *Polygonum aviculare* L., *Portulaca oleracea* L., *Senecio vulgaris* L., *Sinapis arvensis* L., *Sonchus asper* (L.) Hill, and *Xanthium strumarium* L. are commonly found (Anonymous 2017, Anonymous 2022a, Kadıoğlu and Uluğ 1993, Karaca and Güncan 2004. Öğüt 2007, Şin et al. 2019, Uludağ and Katkat 1993, Yazlık et al. 2019;).

In weed survey studies conducted in fruit nurseries worldwide, including those in California and North Carolina, the following species have been identified: *Achillea* spp., *Amsinckia* spp., *Alcea rosea*, *Ajuga reptans*, *Anemone* spp., *Arisaema triphyllum*, *Asarum europaeum*, *Astilbe* spp, *Asparagus officinalis*, *Calendula officinalis*, *Campanula latifolia*, *Cardamine hirsuta*, *Digitaria sanguinalis* (L.) Scop., *Ecliptica prostrata*, *Echinochloa colona*, *Echinochloa crus-galli*, *Hordeum leporinum*, *Hyacinthoides hispanicus*, *Hypericum* spp., *Ipomoea* spp., *Mertensia pulmonariode*, *Monarda* spp., *Muscari botryoides*, *Ornithogalum umbellatum*, *Oxalis strica*, *Phalaris canariensis*, *Phyllanthus tenellus*, *Poa annua*, *Salsola tragus*, *Senecio vulgaris*, *Sonchus oleraceus* L., *S. halepense* and *Veronica* spp. (Gina and Neal 2000, Sellmer 2023, Wilen et al. 2022).

Various methods can be applied for weed control in walnut orchards. The first of these methods is soil tillage, which is one of the mechanical control methods. This method not only eliminates weeds but also loosens and ventilates soil particles. Another effective method is hand hoeing. In olive and pomegranate orchards, the hand hoeing method (applied three times) has been found to be 99% successful in controlling fibrous and tap-rooted weeds, while its effectiveness on stoloniferous, rhizomatous, and tuberous-rooted weeds was found to be lower (Üstüner and Avcı 2021, Üstüner and Taylan 2021, Üstüner et al. 2023).

In mulching practices, the soil surface is covered with organic or inorganic materials, preventing moisture loss and blocking sunlight from reaching the soil, thereby inhibiting the germination of many weed seeds. Materials such as black polyethylene covers can be used for this purpose. Black plastic covers not only prevent weed emergence but also increase soil temperature by 3-4 °C. Plastic covers are primarily used in nurseries, as they are not suitable for garden use year-round (File et al. 2000, Lanini and Grant 2000, Kolberg and Wiles 2002, Tarara 2000, Zaragoza 2003). A study conducted in India using various mulch materials

(transparent plastic cover, black plastic cover, wheat straw, rice straw, banana leaves), black plastic cover was reported to be the most effective in weed control, with 92.1% efficacy (Goswami and Saha 2006). Black plastic cover was found to be 98.86-100% successful in all weed control in apple, olive and pomegranate orchards (Ustuner and Ustuner 2011, Üstüner and Avcı 2021, Üstüner and Taylan 2021).

Glyphosate is a highly systemic herbicide that affects annual and biennial weeds by targeting both leaf and root systems (Burt 2018). Postemergence application of corphosate (441 g/l glyphosate isopropylamin salt) successfully controlled weeds in orchards. In North Carolina, 90% (Neal and Adkins 2001), 95.5% (Karaca and Güncan 2004), 90.61% (Ustuner and Ustuner 2011) and 100% efficacy was observed against weeds in nurseries in Türkiye and USA (Brunharo et al. 2020, Üstüner and Avcı 2021, Üstüner and Taylan 2021, Wolter et al. 2023).

When planting in the garden, apple saplings are required to have a diameter of at least 15 mm. While the trunk diameter of Grany Smith saplings is 9.03-11.82 cm, the trunk diameter of saplings is 14.3-20.7 mm in the Mondial Gala / M 9 nursery when different methods are applied. It has been reported that it affects the trunk diameter of saplings in an increasing way when compared to control plots (Atay 2012, Özkan and Yıldız 2009, Robinson 2003).

As a result of this research, the problematic weed species, families, and their density in the walnut nursery in Kahramanmaraş province were identified. Additionally, the effects of different weed control methods applied against these weeds on weed density, walnut sapling height, and stem diameter were investigated. This research highlights the significant weed species in nursery production and the effect of these control methods on sapling height and stem diameter.

MATERIALS AND METHODS

Materials

Materials used in this research include: Maraş 18 walnut saplings, weed species, mulch (black plastic cover), a hand hoe, pre-emergence herbicide Stomp Aqua EC (pendimethalin 455g/l) at a rate of 300 ml/da (Anonymous 2022b), and post-emergence herbicide Korfosat SL (Gglyphosate-isopropylamin salt 480g/l) of at a rate of 300 ml/da (Anonymous 2022c). A backpack sprayer was used to apply the herbicides. A 1m² frame was used for weed counting.

Methods

This research was conducted at the walnut nursery trial area of the Faculty of Agriculture, Kahramanmaraş Sütçü Imam University, during the 2022-2023 period.

Soil analyses for the experimental field were conducted at the Soil Science and Plant Nutrition Laboratory, Faculty of Agriculture, KSU. The soil structure of the trial area is sandy-clay, and its pH is neutral. Soil properties are provided in Table 1.

Table 1. Soil analysis values of the trial area

Ph		6.99
Total Salt	%	0.20
Lime	%	7.15
Organic Matter	%	3.10
Available P	(mg kg-1)	6.7
Available K	(mg kg-1)	226
Available Ca	(mg kg-1)	11300
Available Mg	(mg kg-1)	609
Available Na	(mg kg-1)	32.5
Available Fe	(mg kg-1)	5.40
Available Zn	(mg kg-1)	0.4
Available Cu	(mg kg-1)	0.6
Available Mn	(mg kg-1)	6.50
Available Ni	(mg kg-1)	1.10
Texture		
Sand	%	50.26
Silt	%	25.30
Clay	%	20.52

The experiment was designed using a randomized block design with 5 treatments and 4 replications. The trial plots were arranged to be 5x4 meters in size, with 80 cm between walnut rows and 20 cm between plants within each row.

The experiment design included pre-emergence herbicide, post-emergence herbicide, hand hoeing, mulching (black plastic cover), and control plots. The pre-emergence herbicide was applied before weeds emerged in the walnut nursery. The post-emergence herbicide was applied when the weeds were in the 5-7 leaf stages. A drip irrigation network was installed in the walnut nursery trial area, and irrigation was carried out at regular intervals. After the drip irrigation network was installed, walnut service processes, as well as disease and pest control, were conducted in a timely manner.

In this study, four different weed control methods were applied to the walnut nursery, and the effectiveness of these methods was investigated. Hand hoeing, pre-emergence herbicide, and post-emergence herbicide were each applied three times, while the mulch method was applied once. No weed control methods were applied to the control (non-treated) plots. However, irrigation, fertilization, and disease and pest control were carried out in the control plots at the same time as the other plots.

Determination of weed families and species density in the walnut nursery trial area

Weed counting in the walnut nursery trial plots was conducted three times: on January 30, May 30, and September 30, 2022-2023. The weed count was calculated by dividing the total number of weeds per square meter of each species in a 1 m^2 area by the total surveyed area. Weed density was determined using the following formula (Güncan 2001).

Density= B/n (1)

-B= Total number of individuals in the sample taken, -n= Number of samples taken

A count was made by determining the number of tillers in narrow-leaved weeds and the number of individuals in broad-leaved weeds, and the weed density scale developed by Üstüner and Güncan (2002) was used.

Density scale; (2)

A. Very dense (weed average >10)

B. Dense (weed average between 1-10)

C. Medium dense (weed average between 0.1-1)

D. Low dense (weed average between 0.01-0.1)

E. Rare (weed average less than 0.01)

In weed species identification, Davis (1985-1988) and Serin (2008) were used.

The effect of weed control methods on walnut shoot development (cm) and stem diameter (mm)

To determine the effect of different control methods on the shoot development of walnut saplings in the nursery, sapling height was measured with a measuring tape on the first day of the experiment (January 1, 2022-2023). At the end of the growing season, sapling height measurements were taken again on December 30, 2022-2023. Stem diameter was measured in millimeters using a digital caliper (0.01 mm precision) 15-20 cm above the grafting site, ensuring there was no swelling at the site (Cody et al. 1985). The average of each measurement was calculated by selecting 5 saplings from each plot and then dividing by the number of plots. Shoot development during the growing season was calculated by subtracting the initial measurement values from the final measurement values.

Control methods

Herbicide applications

In the chemical control of weeds in the walnut nursery trial area, pendimethalin EC (455 g/l) pre-emergence herbicide and glyphosate-isopropylamin salt SL (480g/l) post-emergence herbicide were applied using a backpack sprayer on the dates specified in Table 2. The application doses of these herbicides were 300 ml/da.

Table 2. Application times of control methods

Hand hoe	Pendimethalin	Glyphosate isopropylamin salt	Mulch
15.02.2022-2023	01.02.2022-2023	28.02.2022-2023	01.01.2022
25.03.2022-2023	15.03.2022-2023	05.04.2022-2023	
25.05.2022-2023	15.05.2022-2023	10.06.2022-2023	

Hand hoe application

In the walnut nursery trial area, the hand hoeing was performed three times after the weed species reached the 5-6 leaf stage (Table 2). Both narrow and broad-leaved weed species that were problematic in the nursery were targeted with the hand hoe application.

Mulch (black plastic cover) application

Black colored, UV-resistant, 1-meter-wide, 0.30 mm thick PE plastic mulch was used in the trial area. The mulch was laid in the trial area on 01.01.2022, and the drip irrigation pipe was placed under the mulch, for each sapling. The edges of the plastic mulch were covered with soil around the base of the walnut saplings, and the middle parts were secured with staples to complete the installation process. Black plastic covers were laid on both sides of the walnut saplings, extending 50 cm to the right and 50 cm to the left.

Table 3. Scientific name, family, Turkish name and density average of the weed species seen in the walnut nursery trial area for 2022-2023

No	Scientific Name	Family Name	English Name	Weed Density (pcs/m²)
	MONOCOTYLEDONAE (Monocotyledons)			
1	*Aegilops columnaris* Zhuk.	Poaceae	Foxtail	0.50
2	*Aegilops cylindrica* Host.	Poaceae	Beard grass	0.60
3	*Avena sterilis* L.	Poaceae	Wild Oats	3.40
4	*Bromus arvensis* L	Poaceae	Bromine	0.001
5	*Bromus tectorum* L.	Poaceae	Tasseled brome	0.004
7	*Cynodon dactylon* L. Pers.	Poaceae	Bermuda grass	11.58
8	*Dactylis glomerata* L.	Poaceae	Cocksfoot	0.70
9	*Digitaria sanguinalis* (L.) Scop.	Poaceae	Fork grass	10.30
10	*Elymus repens* (L.) Gould	Poaceae	Couch grass	13.20
11	*Lolium temulentum* L.	Poaceae	Darnel ryegrass	2.60
12	*Secale cereale* L.	Poaceae	Wild Rye	0.70
13	*Setaria viridis* (L.) P. Beauv.	Poaceae	Hedgehog millet	0.90
14	*Sorghum halepense* (L.) Pers.	Poaceae	Johnson grass	12.69
15	*Phalaris canariensis* L.	Poaceae	Birdseed grass	6.10
16	*Phragmites australis* (Cav.) Trin.ex.Steud.	Poaceae	Reed	5.30
	DICOTYLEDONAE (Dicotyledons)			
1	*Achillea pseudoaleppica* Huber.-Mor.	Asteraceae	Yarrow	0.01
2	*Acroptilon repens* (L.) DC.	Asteraceae	Achy	0.002
3	*Adonis annua* L.	Ranunculaceae	Blood Drop Herb	0.001
4	*Amaranthus blitoides* S.Watson	Amaranthaceae	Creeping cockscomb	0.001
5	*Anagallis arvensis* L.	Primulaceae	Mouse ear herb	10.70
6	*Anchusa officinalis* L.	Boraginaceae	Beef Tongue	0.21
7	*Anthemis arvensis* L.	Asteraceae	Field Daisy	0.05
8	*Cardaria draba* (L.) Desv.	Brassicaceae	Wild cress	0.75
9	*Carduus nutans* L.	Asteraceae	Donkey Thorn	0.001
10	*Carthamus arborescens* L.	Asteraceae	Yellow thistle	0.001
11	*Chondrilla juncea* L.	Asteraceae	Achillea	0.50
12	*Conyza canadensis* (L.) Cronq.	Asteraceae	Canadian Healing Herb	0.05
13	*Centaurea solstitialis* L.	Asteraceae	Sun thorn	0.80
14	*Chenopodium album* L.	Amaranthaceae	Lambs quarters	0.60
15	*Chenopodium botrys* L.	Amaranthaceae	Redshank	0.04

16	*Cichorium intybus* L.	Asteraceae	Wild Chicory	0.05
17	*Cirsium arvense* L. Scop.	Asteraceae	Creeping thistle	0.80
18	*Convolvulus arvensis* L.	Convolvulaceae	Field ivy	4.50
19	*Euphorbia maculata* L.	Euphorbiaceae	Spurge	0.02
20	*Fumaria officinalis* L.	Papaveraceae	Common fumitory	0.03
21	*Galium aparine* L.	Rubiaceae	Goosegrass	0.24
22	*Lactuca serriola* L.	Asteraceae	Wild Lettuce	2.10
23	*Lamium purpureum* L.	Lamiaceae	Dead nettle	0.01
24	*Lathyrus annuus* L.	Fabaceae	Damson	0.001
25	*Lathyrus sylvestris* L.	Fabaceae	Damson	0.002
26	*Medicago sativa* L.	Fabaceae	Clover	0.010
27	*Oxalis articulata*	Oxalidaceae	Pink sour clover	0.003
28	*Papaver rhoeas* L.	Papaveraceae	Poppy grass	0.006
29	*Plantago lancelota*	Plantaginaceae	Narrow Nerve	1.90
30	*Plantago major* L.	Plantaginaceae	Greater plantain	0.06
31	*Polygonum aviculare* L.	Polygonaceae	Shepherd's crook	0.78
32	*Rumex crispus* L.	Polygonaceae	Curly dock	0.41
33	*Rumex tuberosus* L.	Polygonaceae	Lambskin	0.30
34	*Sinapis arvensis* L.	Brassicaceae	Charlock	0.85
35	*Silene* spp.	Caryophyllaceae	Fly Trap	0.06
36	*Sonchus arvensis* L.	Asteraceae	Donkey lettuce	0.08
37	*Sonchus oleraceus* L.	Asteraceae	Donkey lettuce	0.90
38	*Stellaria media*	Caryophyllaceae	Chickweed	0.06
39	*Taraxacum officinale* Weber ex Wiggers	Asteraceae	Dandelion	0.45
40	*Thlaspi arvense* L.	Brassicaceae	Bird cress	0.001
41	*Tragopogon* spp.	Asteraceae	Goatee	0.35
42	*Tragopogon reticulatus* Boiss.	Asteraceae	Fodder plant	0.02
43	*Trifolium medium*	Fabaceae	Corner clover	0.03
44	*Trifolium repens* L.	Fabaceae	Clover	0.001
45	*Tribulus terrestris* L.	Zygophyllaceae	Iron Thorn	0.001
46	*Vaccaria pyramidata* Medik.	Caryophyllaceae	Arabica broad bean	0.05
47	*Verbascum* spp.	Scrophulariacea	Mullein	3.90
48	*Vicia cracca* L.	Fabaceae	Bird vetch	0.07
49	*Xanthium spinosum* L.	Asteraceae	Thorny cocklebur	0.003
50	*Xanthium strumarium* L.	Asteraceae	Pork Belly	3.50

Control parcels

None of the weed control methods applied in the trial design were used in the control plots. Control plots for both monocotyledonous and dicotyledonous weed species were established. Only general management services such as irrigation, fertilization, insecticide, and fungicide applications were carried out.

Statistical analysis

The obtained data were analyzed using variance analysis with the SAS-JMP 17.0 software, and the means with significant differences were grouped using the LSD multiple comparison test.

RESULTS

Determination of weed families and species density in the walnut nursery experiment

As a result of the two-year study conducted in the walnut sapling production area in Kahramanmaraş province, weed families and species were identified. The two-year survey average revealed a total of 66 weed species from 20 different families in the trial area. These weed species included 1 monocotyledonous family and 19 dicotyledonous families. The distribution of these weed species consists of 16 monocotyledonous species and 50 dicotyledonous species (Table 3).

The two-year average of weed species densities observed in the walnut trial area was calculated. The weed species that were very dense (weed average >10) in the control plots of the walnut trial area were, respectively: *Elymus repens* L. (13.20 pcs/m²), *S. halepense* (12.69 pcs/m²), *C. dactylon* (11.58 pcs/m²), *A. arvensis* (10.70 pcs/m²), and *D. sanguinalis* (L.) Scop. (10.30 pcs/m²). The weed species identified as dense (weed average between 1-10) included: *P. canariensis* (6.10 pcs/m²), *P. australis* (5.30 pcs/m²), *C. arvensis* (4.50 pcs/m²), *Verbascum* spp. (3.90 pcs/m²), *X. strumarium* (3.50 pcs/m²), *A. sterilis* (3.40 pcs/m²), *L. temulentum* (2.60 pcs/m²), *L. serriola* L. (2.10 pcs/m²), and *P. lanceolata* (1.90 pcs/m²) (Table 3).

Effect of weed control methods on walnut shoot development (cm)

In the experimental area, weed species such as *P. australis*, *X. strumarium*, *S. halepense*, *A. sterilis*, *S. arvensis* and *C. arvense* greatly outcompeted walnut saplings in terms of height. As a result, the walnut saplings were at a disadvantage in terms of sunlight acquisition, which suppressed their height development. Additionally, weeds with rhizomes and stolons absorbed more water and nutrients from the soil than the walnut saplings, significantly slowing walnut saplings root and height development. Since walnut saplings were unable to compete with multiple weed species in the control plots, both plant height and root development remained weak. In the control plots, walnut sapling height ranged from 11 to 15 cm, while weeds, especially *X. strumarium*, *S. halepense*, *S. arvensis* and *C. arvense*, grew to 1.30-1.80 meters. *A. sterilis*, *P. canariensis*, *L. serriola* reached heights of 80-90 cm, completely blocking sunlight from the walnut saplings. The root structures of *S. halepense*, *E. repens*, *C. dactylon*, and *C. arvensis* particularly exerted significant pressure on the development of both the roots and aboveground parts of the walnut saplings, leading to an average negative impact of 85% on annual growth.

To determine the effect of the weed control methods used in this trial on walnut sapling height, walnut sapling height measurements were taken in 2022 and 2023 from the mulch, hand hoe, post-emergence herbicide application, pre-emergence herbicide application, and control plots (Table 4).

Table 4. Average shoot length (cm) of walnut saplings in control plots and control methods in the walnut trial area in 2022-2023

Year	Hand hoe (cm)	Pendimethalin (cm)	Glyphosate isopropylamin salt (cm)	Mulch (cm)	Control Parcel (cm)
2022	100.30b*	74.80d	89.10c	110.90a	14.80e
2023	99.70b	75.20d	90.10c	109.10a	15.20e
Average	100.00b	75.00d	90.00c	110.00a	15.00e

* The difference between means shown with different letters in the same row is statistically significant (P≤0.05)

According to the results of this study and the data statistical analysis, the effects of the methods applied in the experimental area on walnut saplings shoot development were evaluated by comparing them to the control plots. The black plastic cover method increased shoot development by 633.33%, hand hoe treatment by 566.67%, post-emergence glyphosate isopropylamin salt treatment by 500.00%, and pre-emergence pendimethalin treatment by 400.00%. All of the weed control methods significantly improved walnut shoot development compared to the control plots.

Effect of weed control methods on walnut sapling stem diameter (mm)

In the experimental area in 2022-2023, when the effect of weed control methods on sapling stem diameter development was compared to the control plots, all control methods showed an increase in sapling trunk diameter (Table 5). The effects of all applied control methods on sapling stem diameter were found to be statistically significant in both 2022 and 2023 ($P \leq 0.05$).

Mulch (black plastic cover) application

In the walnut nursery trial area, the weed density in the plots where mulch was applied was 0.80 pcs/m², while it was 41.50 weeds per square meter in the control plots. This treatment reduced monocotyledonous and dicotyledonous weed density by 98.07%. It was observed that this method was the most effective in reducing weed density, including species with stolon and rhizome root systems.

Table 5. Average stem diameter (mm) of walnut saplings in control parcel and struggle methods in the walnut trial area in 2022-2023

Year	Hand hoe (cm)	Pendimethalin (cm)	Glyphosate isopropylamin salt (cm)	Mulch (cm)	Control Parcel (cm)
2022	13.55bc*	13.30c	13.90b	14.90a	3.95d
2023	13.40c	13.20c	13.70b	15.30a	3.80d
Average	13.47c	13.25c	13.80b	15.10a	3.87d

* The difference between means shown with different letters in the same row is statistically significant (P≤0.05)

Control methods

Pre-emergence herbicide application

In the walnut nursery experimental area, the weed density in the plots where the pre-emergence herbicide (pendimethalin) was applied was 10.75 pcs/m², while it was 41.50 pcs/m² in the control plots. This treatment reduced monocotyledonous and dicotyledonous weed density by 74.10%. The treatment was found to be less effective on species with stolon and rhizome root structures.

Hand hoe application

In the walnut nursery experimental area, the weed density in the plots where hand hoeing was applied was 8.10 weeds per square meter, while it was 41.50 weeds per square meter in the control plots. This treatment reduced monocotyledonous and dicotyledonous weed density by 80.48%. However, the treatment increased the density of species with stolon and rhizome root structures.

Post-emergence herbicide application

In the walnut nursery trial area, the weed density in the plots where the post-emergence herbicide (glyphosate-isopropylamin salt) was applied was 9.90 weeds per square meter, while it was 41.50 pcs/m² in the control plots. This treatment reduced monocotyledonous and dicotyledonous weed density by 76.14%. The treatment effect was observed to be slightly lower in species with stolon and rhizome root structures.

Control plots

None of the weed control methods applied in the control plots that include monocotyledonous and dicotyledonous weed species. In these control plots only general management services such as irrigation, fertilization, insecticide, and fungicide were applied. The two-year average weed density in the control plots was calculated to be 41.50 weeds per square meter.

DISCUSSION

According to the results of this study, a total of 66 weed species belonging to 20 different families were identified in the walnut nursery trial area. These weed species belong to 1 monocotyledonous family and 19 dicotyledonous families. It was determined that 16 of these weed species are monocotyledonous, while the remaining species are dicotyledonous.

Among the weed species identified in the walnut experimental area; *E. repens*, *S. halepense*, *C. dactylon*, *A. arvensis* and *D. sanguinalis* were very dense; *P. canariensis*, *P. australis*, *C. arvensis*, *Verbascum* spp., *X. strumarium*, *A. sterilis*, *L. temulentum*, *L. serriola*, and *P. lancelota* were dense. The weed species identified in this study were found to be similar to those in previous studies conducted in Türkiye. However, weed density varied based on regional climate, soil structure, altitude, and control methods (Karaca and Güncan 2004, Kadıoğlu and Uluğ 1993, Öğüt 2007, Şin et al. 2019, Uludağ and Katkat 1993, Yazlık et al. 2019).

The weed species identified in this study were partially similar to those found in nursery orchards in other countries. This similarity can be attributed to differences in factors such as climate, soil conditions, and altitude, which influence the distribution of weed species (Gina and Neal 2000, Sellmer 2023, Wilen et al. 2022).

In this study, the mulch (black plastic cover) method was the most effective in reducing weed density, followed by hand hoeing, post-emergence (glyphosate-isopropylamin salt) treatment, and pre-emergence (pendimethalin) treatment in that order. Other researchers have reported that black plastic cover, hand hoeing, and both pre-emergence and post-emergence herbicide treatments are effective in controlling weeds in nurseries. Herbicides such as oryzalin, glyphosate, flumioxazin, oxyfluorfen, pendimethalin, isoxaben, and trifluralin are more widely used and have been shown to have potential effects (Abit and Hanson 2013, Altland et al. 2016, Awan et al. 2006, Brunharo et al. 2020, Goswami and Saha 2006, Judge and Neal 2000, Marble et al. 2019, Ramalingam et al. 2013, Richardson and Zandstra 2009, Ustuner and Ustuner 2011, Wilen et al. 2022, Willoughby et al. 2003).

The effectiveness of weed control varies depending on the methods used and the weed species. The findings obtained in this study were similar to those from Türkiye and other countries around the world, but showed numerical variability (Amoroso et al. 2009, Kolberg and Wiles 2002, Lanini and Grant 2000, Tarara 2000, Üstüner and Avcı 2021, Üstüner and Taylan 2021, Üstüner et al. 2023, Wilen et al. 2022).

The reasons for these numerical differences include variations in the active ingredient and dosage applied, differences in cultivar and weed species, and soil and climate characteristics.

According to the results of this study conducted in the walnut nursery, four different weed control methods showed a significant increase in sapling height compared to the control plots. The results obtained in other studies on sapling height increase were found to be numerically different, but all indicated an increase. Weed control methods increased sapling growth of Ilex crenata Thunb. by 60% and that of *Fashion azalea* (Rhododendron × Fashion) by 57% (Amoroso et al. 2009, Berchielli-Robertson et al. 1990, Fretz 1972).

When the effect of the methods applied in this study on sapling stem diameter development was compared to the control plots, a statistically significant increase in sapling stem diameter was observed. The findings obtained by Cody et al. (1985), Robinson (2003), Özkan and Yıldız (2009), and Atay (2012) support the results of this study.

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Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

İnsan beslenmesinde önemli yere sahip olan ceviz (*Juglans regia* L.); omega-3 yağ asitleri, antioksidanlar, polifenoller, bakır, folik asit, fosfor, manganez, B6 ve E vitamini yönünden oldukça önemlidir. Ceviz fidanlığında birçok yabancı ot, hastalık ve zararlı ile karşılaşılmaktadır. Fidanlıkta en önemli sorunlardan bir tanesi de yabancı otlardır. Bu çalışma 2022-2023 yılında Kahramanmaraş Ziraat Fakültesi ceviz (*Juglans regia* L.) fidanlığında sorun olan yabancı ot türleri, familyaları ve yoğunlukları tespit etmek amacıyla yapılmıştır. Aynı zamanda bu yabancı otlar ile farklı mücadele yöntemlerinin (el çapası, pendimethalin 455 g/l, glyphosate isopropylamin tuzu ve malç) ceviz fidan boyu ve gövde çapına etkisi araştırılmıştır. Çalışma sonucunda deneme alanında 20 farklı familyaya ait 66 adet yabancı ot türü tespit edilmiştir. Bu yabancı ot türlerinden 16 tanesi tek çenekli ve 50 tanesi ise çift çeneklidir. Yoğunluk skalasına göre ceviz deneme alanında belirlenen yabancı ot türlerinin; *Elymus repens* (L.) Gould 13.20, *Sorghum halepense* (L.) Pers. 12.69, *Cynodon dactylon* (L.) Pers. 11.58, *Anagallis arvensis* L. 10.70 ve *Digitaria sanguinalis* (L.) Scop. 10.30 adet/m^2 çok yoğun olduğu tespit edilmiştir.

Bu araştırmada yabancı ot yoğunluğunu, malç yöntemi %98.07 oranında azaltırken, bunu el çapa uygulaması %80.48 oranıyla ikinci sırada izlemiştir. Üçüncü sırada çıkış sonrası (glyphosate isopropylamin tuzu) aktif maddeli herbisit uygulaması %76.14 ve dördüncü sırada çıkış öncesi (pendimethalin) aktif maddeli herbisit uygulaması %74.10 oranıyla izlemiştir.

Yabancı ot mücadele yöntemlerinden siyah plastik örtü yöntemi ceviz fidan boyu gelişimini %633.33, el çapa uygulaması yöntemi %566.67, çıkış sonrası herbisit uygulaması %500.00 ve çıkış öncesi herbisit uygulaması %400.00 oranında artırmıştır.

Anahtar kelimeler: ceviz (*Juglans regia* L.), fidanlık, yabancı ot, mücadele yöntemleri, fidan boyu

REFERENCES

Abit M.J.M., Hanson B.D., 2013. Evaluation of preemergence and post-directed herbicides on rootstock safety in field-grown almond nursery stock. HortTechnology, 23, 462–467. doi: 10.21273/HORTTECH.23.4.462

Altland J.E., Boldt J.K., Krause C.C., 2016. Rice hull mulch affects germination of bittercress and creeping woodsorrel in container plant culture. American Journal of Plant Sciences, 7, 2359–2375. doi: 10.4236/ajps.2016.716207

Akbari V., Jamei R., Heidari R., Esfahlan J., 2012. Antiradical activity of different parts of walnut (*Juglans regia* L.) fruit as a function of genotype. Food Chemistry, 135, 2404-2410. https://doi.org/ 10.1016/j. foodchem.2012.07.030

Almeida I.F., Fernandes E., Lima J.L.F., Costa P.C., 2008. Walnut (*Juglans regia* L.) leaf extracts are strong scavengers of pro-oxidant reactive species. Food Chemistry, 106 (3), 1014-1020. https:// doi.org/10.1016 /j.foodchem.2007.07.017

Amoroso G., Fini A., Piatti R., Frangi P., 2009. Mulching as alternative to chemical weed control in nursery containerized crops. Advances in Horticultural Science, 23, 276. doi: 10.1400/121245

Anonymous 2017. Ceviz Entegre Mücadele Teknik Talimatı. Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü. https://www.tarimorman.gov.tr/TAGEM/Belgeler/Entegre/ceviz%20entegre.pdf (accessed date: 12.03.2021).

Anonymous 2023. İllere göre ceviz yetiştiriciliği. https://data.tuik.gov.tr/Kategori/GetKategori?p=Tarim-111 (accessed date: 02.03.2022).

Anonymous 2022a. Ceviz Entegre Mücadele Teknik Talimatı, Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı. https://www.tarimorman.gov.tr/TAGEM/Belgeler/Entegre/Ceviz%20Entegre%20M%C3%BCcadele%20Teknik%20Talimat%C4%B1.pdf (accessed date: 14.02.2022).

Anonymous 2022b. Stomp Aqua (455 g/l Pendimetalin) https://www.agro.basf.com.tr/tr/%C3%9Cr%C3%BCnler/%C3%9Cr%C3%BCn-Bilgileri/Herbisit/Stomp-Aqua.html (accessed date: 04.01.2022).

Anonymous 2022c. Korfosat (480 g/L glyphosate isopropylamin tuzu). https://korumatarim.com/ilac/korfosat-48-sl/ (accessed date: 04.01.2022).

Atay E., 2012. Dallı fidan eldesinde kullanılan farklı yöntemlerin elmalarda fidan kalitesi ve fizyolojisi üzerine etkileri. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 112 s., Isparta, Türkiye.

Awan T., Safdar M., Manzoor Z., Ashraf M., 2006. Screening of herbicides as post-emergence application for effective weed control without affecting growth and yield of direct seeded rice plant. The Journal of Animal and Plant Sciences, 16, 60–64.

Berchielli-Robertson D.L., Gilliam C.H., Fare D.C., 1990. Competitive effects of weeds on the growth of container-grown plants. HortScience, 25, 77–79. doi:10.21273/HORTSCI.25.1.77

Brunharo C.A., Watkins S., Hanson B.D., 2020. Season-long weed control with sequential herbicide programs in California tree nut crops. Weed Technology, 34 (6), 834–842. https://doi.org/10.1017/wet.2020.70

Burt J., 2018. Pomegranates in Western Australia, https://agric.wa.gov.au/n/2078 (accessed date: 03.07.2022).

Cody C.A., Larsen F.E., Fritts R.Jr., 1985. Stimulation of lateral branch development in tree fruit nursery stock with GA4+7+BA. HortScience, 20 (4), 758-759.

Davis P.H., 1985-1988. Flora of Turkey and The East Aegean Islands. Vol. I-X Edinburgh University Press.

FAO 2023. Walnut production. https://www.fao.org/faostat/en/#home (accessed date: 02.01.2023).

Fretz T.A., 1972. Weed competition in container grown Japanese holly. HortScience, 7, 485 486.

File S.L., Knight P., Gilliam C., Reynolds D., Altland J., 2000. Evaluation of alternative weed control options for ornamentals grown in large containers. In: Proceedings of Southern Nursery Association (SNA) Research Conference. Bryson, L. J., (Ed.). USA, 45, 397-402.

Gina M.P., Neal J.C., 2000. Weed scouting in container nurseries. SNA Research Conference, 45, 387-390.

Güncan A., 2001. Yabancı otlar ve mücadelesi, Selçuk Üniversitesi, Ziraat Fakültesi, Ders kitabı, Basımevi yayını, Konya, 311 s.

Goswami S.B., Saha S., 2006. Effect of organic and inorganic mulches on soil-moisture conservation, weed suppression and yield of elephant-foot yam (*Amorphophallus paeoniifolius*). Indian Society of Agronomy. 51 (2), 154-156. https://doi.org/10.59797/ija.v51i2.4996

Judge A.C., Neal J.C., 2000. Susceptibility of common nursery weeds to preemergence herbicides. In: Proceedings of Southern Nursery Association (SNA) Research Conference. McDaniel, G., (Ed.). 45, 370-374.

Kadıoğlu İ., Uluğ E., 1993. Akdeniz bölgesi meyve fidanlıklarında yabancı otların belirlenmesi üzerine araştırmalar. Türkiye I. Herboloji Kongresi, 3-5 Şubat 1993, Adana,163-174.

Karaca M., Güncan A., 2004. Karaman ili genç elma bahçelerinde sorun olan yabancı otların mücadelesinde en etkili yöntemin belirlenmesi üzerine bir araştırma. Türkiye I. Bitki Koruma Kongresi, Bildiri Özetleri, 8-10 Eylül 2004, Samsun, 223.

Kolberg R.L., Wiles L.J., 2002. Effect of steam application on cropland weeds. Weed Technology, 16 (1), 43–49. https://doi.org/10.1614/0890037X(2002)016[0043:EOSAOC]2.0.CO;2

Lanini T.W., Grant J.A., 2000. Organic weed management in walnut orchards. Page: 5. Cooperative Extension Weed Ecologist, Cooperative Extension Farm Advisor, University of California.

Marble S.C., Steed S.T., Saha D., Khamare Y., 2019. On-farm evaluations of wood derived, waste paper, and plastic mulch materials for weed control in Florida container nurseries. HortTechnology. 29, 866–873. doi: 10.21273/HORTTECH04437-19

Neal J.C., Adkins C.R., 2001. Comparison of glyphosate and clopyralid for mugwort (*Artemisia vulgaris*) control in field-grown nursery crops. In: Proceedings of the Southern Nursery Association (SNA) Research Conference. McDaniel, G., (Ed.). USA, 46, 420-421.

Öğüt D., 2007. Aydın ili fidanlıklarında sorun olan yabancı otların saptanması ve bazı uygulamaların incir fidanlığındaki yabancı otlara etkinliğinin belirlenmesi. Aydın Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 88 s., Aydın.

Özkan Y., Yıldız K., 2009. Vegetative and generative characteristics in Granny Smith apple variety Budded/Grafted on M 26 and MM 106 rootstocks. Tarım Bilimleri Araştırma Dergisi, 2009 (2), 133-135.

Ramalingam S.P., Chinnagounder C., Perumal M., Palanisamy M.A., 2013. Evaluation of new formulation of oxyfluorfen (23.5% EC) for weed control efficacy and bulb yield in onion. American Journal of Plant Sciences, 4, 890–895. doi:10.4236/ajps.2013.4410

Richardson R.J., Zandstra B.H., 2009. Weed control in Christmas trees with flumioxazin and other residual herbicides applied alone or in tank mixtures. HortTechnology. 19, 181–186. doi: 10.21273/HORTSCI.19.1.181

Robinson T., 2003. Apple orchard planting systems. In: Apples: Botany, Production and Uses. Ferree, D.C., Warrington, I.J. (Eds.). Cambridge, USA, 345-407 p.

Sellmer J., 2023. Landscaping and gardening around walnuts and other juglone producing plants. Pennstate University Extension.

Serin Y., 2008. Türkiye'nin çayır mera bitkileri. T.C. Tarım ve Köy işleri Bakanlığı, Tarımsal Üretim ve Geliştirme Genel Müdürlüğü, Ankara, 476 s.

Şin B., Öztürk L., Sivri N., Avcı G.G., Kadıoğlu İ., 2019. Weed flora of cherry, walnut, apple, almond and pear orchards in Northwestern Marmara region of Turkey. Turkish Journal of Agriculture-Food Science and Technology, 7 (12), 2252–2258. https://doi.org/10.24925/turjaf.v7i12.2252-2258.3017

Tarara J.M., 2000. Microclimate modification with plastic mulch. HortScience, 35 (2), 169-180. https://doi.org/10.21273/HORTSCI.35.2.169

Uludağ A., Katkat M., 1993. Güneydoğu Anadolu bölgesi meyve fidanlıklarında bulunan yabancı otlar ve yoğunluklarının belirlenmesi üzerine çalışmalar. Türkiye I. Herboloji Kongresi, 3-5 Şubat 1993, Adana, 175-178.

Üstüner T., Güncan A., 2002. Niğde ve yöresi patates tarlalarında sorun olan yabancı otların yoğunluğu ve önemi ile topluluk oluşturmaları üzerine araştırmalar. Türkiye Herboloji Dergisi, 5 (2), 30-42.

Ustuner T., Ustuner M., 2011. Investigation on different mulch materials and chemical control for controlling weeds in apple orchard in Turkey. Scientific Research and Essays. 6 (19), 3979–3985. http://www.academicjournals.org/SRE

Üstüner T., Taylan G.S., 2021.Investigation of different control methods against weeds causing problems in pomegranate (*Punica granatum*) garden. Çukurova 7th International Scientific Researches Conference, 7-8 September, 2021, Adana, Türkiye, 579-595.

Üstüner T., Avcı A., 2021. Weed species causing problems in olive (*Olea europaea* L.) gardens, their density, fighting methods, and effects on yield. Çukurova 7th International Scientific Researches Conference, 7-8 September, 2021, Adana, Türkiye, 596-611.

Üstüner T., Sakran M.A., Üstüner M., 2023. Effects of some control methods on Johnson grass and yield components in tomato fields. Turkish Journal of Agriculture and Forestry, 47 (3), 308-318. https://doi.org/10.55730/1300-011X.3088

Yazlık A., Çöpoğlu E., Özçelik A., Tembelo B., Yiğit M., Albayrak B., Baykuş M.A., Aydınlı V., 2019. Yabancı ot türleri ve etkileri: Düzce'de meyve fidanlık alanı örneği. Tekirdağ Ziraat Fakültesi Dergisi, 16 (3), 389-401. https://doi.org/10.33462/jotaf.578999

Wilen C.A., Koike S.T., Ploeg A.T., Tjosvold S.A., Bethke J.A., Mathews D.M., Stapleton J.J., 2022. Revised continuously. UC IPM Pest Management Guidelines: Floriculture and Ornamental Nurseries. UC ANR Publication 3392. https://ipm.ucanr.edu/agriculture/floriculture-and-ornamental-nurseries/authors-and credits/#gsc.tab=0

Willoughby I., Clay D., Dixon F., 2003. The effect of pre-emergent herbicides on germination and early growth of broadleaved species used for direct seeding. Forestry, 76, 83-94. doi: 10.1093/forestry/76.1.83

Wolter D.A., Kyser G.B., Hanson B.D., 2023. Herbicide management of threespike goosegrass in California orchards. Hortechnology, 33 (2), 176-180. https://doi.org/10.21273/HORTTECH05159-22

Zaragoza C., 2003. Weed management in vegetables. In: weed management for developing countries, Labrada, R., (Ed.). Food and Agriculture Organization of the United Nations, Addendum, 1, 277 p.

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Orijinal araştırma (Original article)

Synergistic effects of endophytic bacteria and silicon on controlling common bacterial blight disease in beans

Fasulye bakteriyel adi yanıklık hastalığının kontrolünde endofitik bakteri ve silisyumun sinerjistik etkileri

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ABSTRACT

Enhancing the effectiveness of environmentally friendly and sustainable practices in plant disease management is crucial for promoting their wider adoption and use. In this context, the combined use of bacterial biocontrol agents and silicon applications holds significant potential. This study aimed to evaluate the effects of individual and combined applications of endophytic bacteria (EB) and silicon on controlling common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) in beans. Additionally, the effects of these treatments on plant biomass and chlorophyll content were investigated. Bean plants (*Phaseolus vulgaris* cv. Gina) were grown in a peat/perlite medium under soilless conditions in a climate chamber. Silicon dioxide (SiO_2) (30 mM) and endophytic bacteria were applied to the root collar using the drenching method. The pathogen Xap was inoculated by spraying the leaves, and disease severity was assessed using a 1–5 scale. Plant growth parameters were also recorded. Among the tested EB isolates, *Pseudomonas caspiana* V30G2 was the most effective in suppressing disease severity. Disease severity was reduced by 31% with V30G2 and by 21% with SiO_2 when applied individually. Notably, the combined application of both agents exhibited a synergistic effect, reducing disease severity by 55%. Although some improvements were observed in specific parameters, such as leaf number, neither the individual nor the combined treatments significantly influenced overall plant biomass or chlorophyll content. Nevertheless, the results suggest that the combined application of silicon and endophytic bacteria, when appropriately selected, has significant potential for environmentally friendly and sustainable disease management, enhancing the disease suppression efficacy of each treatment.

INTRODUCTION

In today's intensive agricultural production systems, disease control is essential; however, traditional methods have significant risks to both the environment and human health. While biological control presents promising results in sustainable agricultural practices, its practical application faces several challenges. The inability of biocontrol agents to consistently replicate their laboratory success under field conditions, their susceptibility to environmental

fluctuations, and the instability of their activity are critical issues that need to be addressed. In this context, enhancing the effectiveness of biocontrol agents is as crucial as their development.

Plant growth-promoting bacteria (PGPBs) represent one of the most widely studied and applied eco-friendly strategies for plant disease control, though their field efficacy remains limited and requires enhancement. In this regard, silicon has significant potential, both as a direct treatment and in combination with other application methods, to enhance the stability and efficiency of biocontrol agents (Guerriero et al. 2016, Guével et al. 2007, Sahebi et al. 2015).

PGPBs can colonize plants as epiphytes, residing on the plant surface, or as endophytes, inhabiting internal plant tissues. Endophytic bacteria (EB) are defined as microorganisms that can spread throughout the plant without causing harm and spend at least part of their life cycle within plant tissues (Hallmann 1997, Hardoim et al. 2008). EB can promote plant growth and development through both direct and indirect mechanisms (Hardoim et al. 2008, İmriz et al. 2014).

EB contribute directly to plant growth by facilitating nitrogen fixation, enhancing phosphorus solubilization, promoting the uptake of iron and other nutrients, and producing phytohormones (Grobelak et al. 2015). Additionally, they play an indirect role in plant protection by activating mechanisms such as antibiosis, competition, hyperparasitism, and plant-induced resistance or tolerance against harmful organisms (Santoyo 2016).

Compared to epiphytic bacteria, endophytic bacteria offer several advantages. Because they reside within plant tissues, their metabolites can interact directly with and be readily absorbed by the plant (Akköprü et al. 2021, Romano et al. 2020). Furthermore, endophytic bacteria can reach and influence all plant tissues through the plant's vascular system, thereby exerting more widespread effects (Hardoim et al. 2008, Mercado-Blanco and Lugtenberg 2014, Romano et al. 2020).

Silicon (Si) is second only to oxygen in abundance in the Earth's crust (Kim et al. 2002). Vermiculite, smectite, kaolin, orthoclase, feldspars, plagioclase (silicates in the form of crystal), amorphous silica, and quartz are the main Si components in most soils structures (Luyckx et al. 2017, Sahebi et al. 2015). The major soluble forms of Si in the soil are poly- and monosilicic acids. It converts silicon into forms that the plant can use by decomposing silicate minerals (Sahebi et al. 2015). Some plants such as rice accumulate silicon at rates above 1–5% and are considered as accumulator plants, while others such as tomato, cucumber, maize, barley and soybean accumulate at rates lower than 1% (Sahebi et al. 2015).

Though Si addition via silicate slags or solutions to soils or nutrient solutions is common, interest in foliar applications remains high due to their ease of use (Guével et al. 2007). Studies indicated that many plant disease caused by bacteria and fungi, are less severe when silicon is made available resulting in slower disease progress and less disease severity (Fortunato et al. 2015, Guerriero et al. 2016, Luyckx et al. 2017). Rodrigues et al. (2015) indicated that although foliar-applied Si is effective in reducing some foliar diseases, applying silicon to the roots is more effective. Many studies conducted in recent years have shown that silicon has direct or indirect positive effects on the development and functions of plants (Guerriero et al. 2016, Luyckx et al. 2017, Savant et al. 1997). This shows that Si can be included in environmentally friendly sustainable practices in disease control.

In this context, bean, which we have chosen as a model plant, is an important crop and we can test our hypothesis by using EB and Si together in controlling bacterial blight disease, which causes great losses.

Bean (*Phaseolus vulgaris*) is one of the most produced legumes due to their high nutritional content and their significant contributions to soil (Duman and Soylu2019). Türkiye ranks in the top ten producers of beans producers (FAO 2023). The beans are negatively impacted by many bacterial, fungal, and viral pathogens. One of the most important bean bacterial diseases in worldwide and Türkiye that causes significant economic losses is the "common leaf blight" caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Bozkurt 2009, Karavina et al. 2011). The presence of this pathogen has been documented in various studies conducted in countries with commercial bean production (Osdaghi 2014).

The Xap is prevalent in temperate and tropical climates, leading to substantial yield losses in bean cultivation regions (Gilbertson and Maxwell 1992, Saettler 1989). The bacteria enter the plant through natural openings and wounds (Ertekin et al. 2016). While the pathogen triggers infections in all above-ground parts of the plant, the symptoms are more severe in leaves and pods. Leaf symptoms appearance of a typical narrow, lemon-yellow halo around it (Rudolph 1993, Vidaver 1993). Beyond leaf symptoms, the pathogen also causes issues in fruits, reducing seed germination in severe cases (Ertekin et al. 2016)

For disease control, cultural measures are typically implemented. Additionally, chemical applications like foliar and seed spraying with copper compounds are recommended (Bozkurt 2009, Schwartz et al. 2007). However, chemical

spraying alone is inadequate in preventing fruit infections and may not yield satisfactory results (Opio et al. 1996, Park et al. 1999). The use of antibiotics has been restricted in several countries, and the pathogen's rapid development of resistance to antibiotics and copper preparations has been observed (İmriz et al. 2014).

In this context, within the framework of an environmentally friendly and sustainable agricultural approach, the use of appropriate combinations can enhance the effectiveness of biological control microorganisms or applications holds significant potential. This study aimed to evaluate the potential of single or combined applications of endophytic bacteria (EB) and silicon (Si) in controlling "common leaf blight" disease caused by *Xanthomonas axonopodis* pv. phaseoli in beans.

MATERIALS AND METHODS

Plant material and plant growth medium

Bean plants (*Phaseolus vulgaris* cv. Gina) were cultivated under soilless conditions using a peat/perlite (1:1, v/v) substrate. Seeds were sown into 250 ml plastic pots filled with the growth medium and maintained in a climate-controlled chamber set at 25 ± 2 °C, with a photoperiod of 14 hours and approximately 50% relative humidity (Figure 1a). To ensure adequate nutrition, a nutrient solution formulated according to the composition outlined in Table 1 was supplied regularly throughout the experiment (Hoagland and Arnon 1950). The study was carried out in the climate chambers of Van Yüzüncü Yıl University, Faculty of Agriculture, Plant Protection Department in 2020.

Endophytic bacteria and its application

Endophytic bacteria (EB) were selected from isolates whose some properties were determined by previous studies (Babier and Akköprü 2020, Olur 2019) (Table 2). *Pseudomonas fluorescens WCS365* isolate, have some PGP traits and it is trigger plant immunity (Bolwerk et al. 2003, Kamilova et al. 2005), was used as reference isolate obtained from Dr. Kamilova. EB were cultivated in King's-B medium (KB) (peptone 20 g/l, K_2HPO_4 1.5 g/l, $MgSO_4 7H_2O$ 1.5 g/l, glycerol 10 ml/l, agar 15 g/l) and incubated at 27 °C for 24 hours. Bacterial suspensions were prepared from the colonies, adjusted to an OD_{600} of 0.1 (approximately 10^8

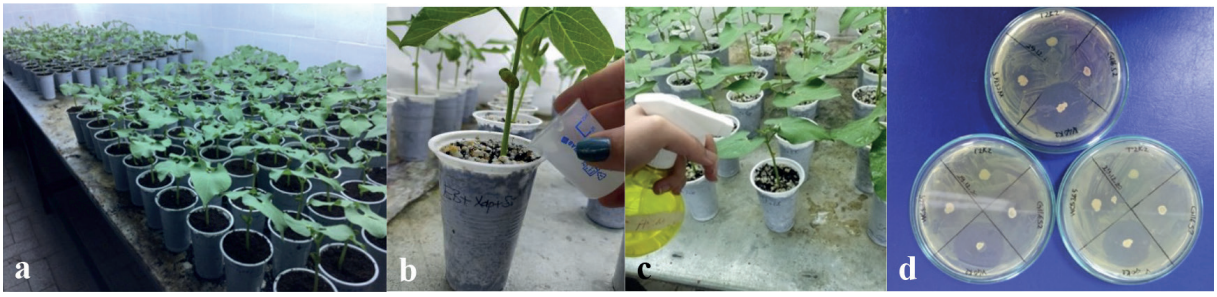

Figure 1. Bean seedlings prepared for applications (a), application of SiO_2 and Endophytic bacteria to the root collar by drenching method (b), patogen *Xanthomonas axonopodis* pv. *phaseoli* application to the seedlings by pulverization (c), *in vitro* antagonistic test (d).

Table 1. Composition of nutrient solution used to meet the nutritional needs of beans seedlings according to recipt of Hoagland and Arnon (1950)

A nutrient solution (%)		**B nutrient solution (%)***	
Total Nitrogen (N)	10.3	Total Nitrogen (N)	2.1
Ammonium (NH4)	1.6	Nitrate (NO3)	2.1
Nitrate (NO_3)	8.7	Potassium oxide (K2O)	11.6
Potassium oxide (K_2O)	7.5	Phosphorus pentoxide (P2O5)	6.4
Calcium (Ca)	8.6	Magnesium (Mg)	1.6
Iron DTPA (Fe)	0.3	Manganese (Mn)	0.1
		Zinc (Zn)	0.01
		Boron (B)	0.03
		Copper (Cu)	0.003
		Molybdenum (Mo)	0.004

CFU/ml) using a spectrophotometer. The prepared the EB suspensions were applied to each seedling by drenching from the root collar of the plants as 20 ml (Figure 1b). EB application was done twice; the first application was carried out 8 days after planting, and the second application was carried out 10 days later. The groups that did not receive EB application were given water in the same amount and method.

Determination of disease severity

Disease symptoms observed 21 days after the inoculation with the pathogen Xap were evaluated using a 1–5 disease severity scale. The scale was defined as follows: 1 = no symptoms; 2 = necrosis or isolated spots affecting 1–5% of the leaf area; 3 = symptoms and necrosis affecting 6–25% of the leaf; 4 = symptoms and necrosis affecting 26–50% of the leaf; and 5 = symptoms, necrosis, or complete leaf death affecting more than 50% of the leaf surface (Akköprü 2020). The recorded scale values were converted into disease severity percentages using the Townsend Heuberger formula (1) (Townsend and Heuberger 1943). The percentage of disease reduction was calculated as the relative difference in disease severity between the treatment and the control groups.

$$DS\ (\%) = \Sigma\ ((S \times L) / (M \times Smax)) \times 100. \quad (1)$$

where in DS: disease severity, S = scale value, L = the number of plant leaves evaluated in each scale, M = the total number of plant leaves, and Smax = the highest scale value.

Table 2. The endophytic bacteria, (EB) and their plant growth promoting traits, isolated and identification by Kamilova et al. (2005), Olur (2019), Babier and Akköprü (2020)

EB isolates	NCBI Acs. Num.	ACC-d	Sid (mm)	P (mm)	IAA (ppm)
Pseudomonas caspiana V30G2	(MN128080)	-	4.50	+	-
Pantoea sp. V31Y4	(MT249279)	3	2.25	-	25.03
Pseudomonas fluorescens WCS365	-	ND	6	+	8
Bacillus velezensis V40K2	MN186863	3	7.00	-	1.38
(ND) G116S2	-	-	1.25	+	ND

*EB: Endophytic bacteria, ACC-d: 1-aminocyclopropane-1-carboxylate deaminase, Sid: Siderophore activity, P: Phosphate solubilizing activity, IAA: Indole-3-acetic acid, NCBI Acs. Num.: NCBI Genbank Accession number, ND: Undetermined

Silicon and its application

The Silicon dioxide (SiO_2) (Si) form of silicon was used in the study. Si was applied to the plant once using the drenching method (Çelik 2021, Çelik and Akköprü 2025). Si suspensions were prepared at 30 mM concentrations with the help of sterile pure water. The 15 ml of Si suspensions per plant was applied into the root collar by the drenching method (Figure 1b). Si was applied 9 days after seed sowing, when the dual leaves were fully open and the triple leaves were forming.

Pathogen and its application

In the study, *Xanthomonas axonopodis* pv. *phaseoli* (Xap), whose virulence was determined and used in previous studies, was used (Akköprü 2020). A 48-hour Xap culture grown in KB medium was adjusted to 10^8 CFU/ml using a spectrophotometer, with 0.01% Tween added. The suspension was uniformly applied to leaves via hand sprayer 48 hours post-Si and 24 hours post-EB application (Figure 1c). Only sterile water was sprayed on the leaves of plants that did not receive pathogen application. After the application, the plants were covered with a transparent polyethylene cover for 48 hours and exposed to high relative humidity and kept in a lightless environment for the first 24 hours. Then, the plants were left to develop in the climate chamber under normal conditions (with a photoperiod of 14 hours and approximately 50% relative humidity and at 25±2 °C).

Determination of plant growth parameters and total chlorophyll content

Leaf number was recorded on day 21 at the end of the experiment. Bean plants were uprooted and cleaned of the growing medium residues by washing. The shoot fresh weights were determined by cutting them from the root collar and weighing them. After the roots were dried with the help of blotting papers, they were weighed and their fresh weights were determined. The plant roots and shoots, whose fresh weights were determined, were placed in aluminum foil containers and dried in an oven at 65 °C for 48 hours, and then weighed and their dry weights were determined.

A chlorophyll meter (Minolta brand SPAD) device was used to determine the total chlorophyll content in the leaves of bean seedlings treated with Xap, SiO_2, and EB. For

this purpose, readings were taken from three leaves of the same age in each seedling, and the chlorophyll values were determined by taking the average of the three readings.

In vitro antagonistic effect

In this study, the in vitro antagonistic effects of SiO_2 on EB and Xap, as well as the effect of EB on Xap, were investigated. For this purpose, 100 µl of a Xap suspension with a concentration of 10^6 CFU/ml was evenly spread onto King's B (KB) agar medium and allowed to dry. Similarly, 100 µl of an EB suspension, adjusted to an optical density (OD) of 0.05 using a spectrophotometer, was spread on a separate KB medium plate and dried. Following the drying process, four sterile paper disks were placed equidistantly on the surface of each prepared Petri dish. Subsequently, 10 µl of either SiO_2 or EB suspension was applied to each disk. After a two-day incubation period, inhibition zones or Xap growth around the disks were examined (Figure 1d).

Data analysis

Experiments were repeated twice. The obtained data were subjected to variance analysis with the help of SPSS (SPSS, Inc. 2007) software package, and the means were evaluated with Duncan's multiple comparison test. Each treatment group had three replications, and at least 15 seedlings were used in each replication. The study was conducted according to Table 3.

Table 3. Treatment groups designed according to randomized plots experimental design

1) NC	5) PC
2) EB	6) EB+*Xap*
3) SiO_2	7) SiO_2+*Xap*
4) EB+SiO_2	8) EB+SiO_2+*Xap*

*SiO_2: Silicon dioxide, EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, PC: positive control, NC: Negative Control

RESULTS

In vitro antagonistic effect of Si and EB

Certain EB isolates exhibited antagonistic activity against *Xanthomonas axonopodis* pv. *phaseoli* (Xap). Among these, isolates V40K2 and V30G2 demonstrated the highest levels of inhibition, with inhibition zone diameters of 13.5 mm and 10 mm, respectively (Figure 1d). In contrast, silicon (Si) at a concentration of 30 mM showed no observable antagonistic effect on either Xap or the EB isolates.

EB selection

The most successful EB isolate was selected according to its level of disease suppression and contribution to shoot development. It was observed that all EB isolates used in the studies suppressed the disease caused by Xap between 38 and 51% (Figure 2). Among these isolates, V30G2 with disease severity values of 10.87 and G116S2 with 10. respectively, V30G2 and G116S2 stood out (Figure 2).

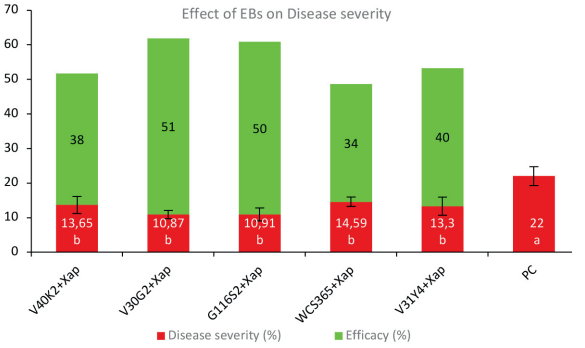

Figure 2. Effect of endophytic bacteria (EB) on disease severity (red parts of columns) of common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) on bean seedlings and efficacy percentage (green parts of columns) of EB treatment on disease severity

* Values with the same letter in the column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

On the other hand, none of the isolates used made significant contributions to the number of leaves (LN), shoot fresh (SFW) and dry (SDW) weight (Table 4). In fact, it was observed that the G116S2 isolate reduced LN and SFW. Similar effects were observed under disease pressure (Figure 2).

In light of these data, EB V30G2 isolate was selected to be used in the next stages of the study. This selected isolate was used with 30 mM SiO2 to investigate its potential to control the disease.

Effect of SiO_2 and EB V30G2 isolate on disease severity and plant development

In this stage of the study, the effects of SiO_2 and EB V30G2 single and combined applications on disease caused by Xap and plant development were investigated. All application groups showed an effect between 21 and 55% in suppressing disease severity. Single applications of SiO_2 and EB V30G2 reduced disease severity. However, the decrease obtained from V30G2+Xap application was found to be statistically significant. In addition, the combined use of SiO_2 and EB V30G2 reduced disease severity by 55%, more than the individual effect of both applications (Figure 3).

Table 4. Effects of endophytic bacteria (EB) application on leaf number, shoot fresh weight and shoot dry weight of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Treatments	LN	SFW (g)	SDW (g)
NC	17.87±0.99 a	6.38±0.44 ab	0.69±0.10 a
EB V40K2	14.80±1.54 cde	5.28±0.88 def	0.50±0.10 de
EB V30G2	14.30±1.25 de	5.33±1.15 def	0.50±0.13 de
EB G116S2	14.10±1.66 e	4.96±1.39 ef	0.49±0.11 de
EB WCS365	16.40±1.71 abcd	5.74±0.80 bcdef	0.58±0.05 bcde
EB V31Y4	15.30±2.21 bcde	4.73±1.03 f	0.46±0.13 e
PC	16.00±2.00 bcde	6.69±1.20 a	0.66±0.14 bc
EB V40K2+*Xap*	15.40±3.09 abcde	5.74±0.90 bcdef	0.52±0.10 de
EB V30G2+*Xap*	14.30±2.94 de	5.55±1.34 cdef	0.46±0.16 e
EB G116S2+*Xap*	14.90±1.85 bcde	4.95±0.90 ef	0.46±0.09 e
EB WCS365+*Xap*	16.66±1.11 abc	5.74±0.73 bcdef	0.54±0.09 cde
V31Y4+*Xap*	17.10±3.17 ab	5.99±1.41 bcde	0.55±0.15 cde

*EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, SFW: Shoot fresh weight, SDW: Shoot dry weight, LN: Leaf number, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

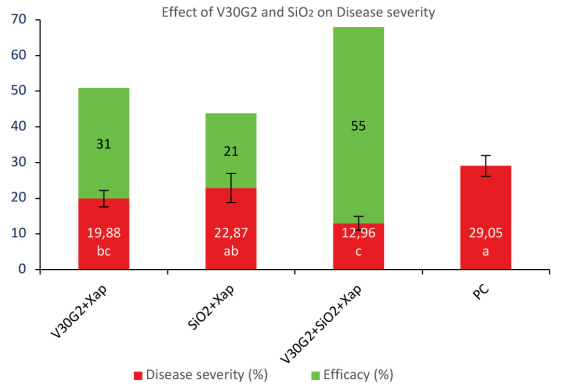

Figure 3. Effect of endophytic bacteria (EB) and silicon (red parts of columns) treatment on severity of common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) on bean seedlings and efficacy percentage (green parts of columns) of this treatment on disease severity

* Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

SiO_2 and EB V30G2 isolate applied individually and in combination did not show a significant effect on shoot fresh, shoot dry and root dry weight. However, single and combined applications of the agents significantly increased root fresh weight (Table 5).

Under disease pressure, single or combined applications of SiO_2 and EB V30G2 did not show a significant effect on root and shoot fresh/dry weights (Table 5). It was determined that SiO_2 and EB V30G2 isolate applied individually and in combination did not have a significant effect on leaf number and chlorophyll content. However, it was determined that leaf number increased significantly in the group where SiO_2 and EB V30G2 were used together under disease pressure (V30G2+SiO_2+Xap) compared to the group where only pathogen was applied (PC).

In the groups where there was no disease pressure, the applications did not cause an increase in total chlorophyll content. On the other hand, SiO_2 application under disease pressure significantly increased total chlorophyll content compared to PC (Table 6).

DISCUSSION

Biological control agents (BCAs) used in the management of plant diseases may exhibit limitations in their biocontrol efficacy due to inherent biological characteristics. For instance, their performance can be markedly influenced by environmental fluctuations, and their interactions with various host plants may vary, potentially restricting their effectiveness under field conditions. These challenges can undermine the reliability and broader adoption of biocontrol strategies based on BCAs. Therefore, enhancing the efficacy of BCAs remains a critical objective (Spadaro and Gullino 2005). In this regard, supplementing BCA applications with supportive agents may offer promising results. Among these, silicon (SiO_2) has attracted considerable attention due to its potential role in enhancing plant resistance and microbial efficacy. In the present study, the potential of silicon (SiO_2) to enhance the biocontrol effectiveness of endophytic

Table 5. Effects of endophytic bacteria (EB) V30G2 and SiO2 application on leaf number, shoot and root fresh/dry weight of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Treatments	SFW (g)	SDW (g)	RFW (g)	RDW (g)
NC	0.68±0.09 a	6.66±0.76 a	0.52±0.30 c	0.08±0.01 a
V30G2	0.55±0.22 ab	5.86±1.56 ab	1.21±0.67 ab	0.06±0.02 ab
SiO_2	0.58±0.16 ab	5.72±1.73 ab	1.62±0.49 a	0.08±0.02 a
V30G2+SiO_2	0.66±0.19 ab	6.49±1.45 a	1.64±0.59 a	0.07±0.04 ab
PC (Xap)	0.51±0.12 b	4.57±0.97 b	1.06±0.15 b	0.05±0.01 b
V30G2+*Xap*	0.55±0.14 ab	4.82±1.25 b	1.11±0.63 b	0.06±0.02 ab
SiO2+*Xap*	0.63±0.15 ab	5.52±1.34 ab	0.97±0.19 b	0.06±0.01 ab
V30G2+SiO_2+*Xap*	0.65±0.10 ab	5.80±1.10 ab	1.18±0.28 b	0.07±0.02 ab

*EB: Endophytic bacteria, SiO_2: 30 mM silicon dioxide, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

Table 6. Effects of endophytic bacteria (EB) V30G2 and SiO_2 application on leaf number and total chlorophyll content of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Gruplar	LN	Chlorophyll
NK	18.80±2.09 ab	39.29±3.66 a
EB V30G2	19.30±4.02 ab	36.85±6.12 ab
SiO_2	17.40±3.33 ab	37.36±4.10 ab
EB V30G2+SiO_2	17.40±4.57 ab	39.21±5.51 a
PK	16.00±2.74 b	34.60±3.06 b
EB V30G2+*Xap*	18.44±2.87 ab	36.36±2.23 ab
SiO2+Xap	19.11±3.25 ab	40.53±3.27 a
EB V30G2+SiO_2+*Xap*	19.90±1.52 a	37.98±2.19 ab

*EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, LN: Leaf number, Chlo: total chlorophyll content, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

bacteria against common bacterial blight disease, caused by *Xanthomonas axonopodis* pv. *phaseoli* in bean plants, was investigated.

Many studies have been conducted to control common leaf blight disease in beans with the help of PGPRs or EB and successful results have been obtained (Belete et al. 2021, Corrêa et al. 2017, Sallam and Aldayel 2025). Bozkurt (2009) showed that antagonistic bacteria isolated from bean plants inhibited the disease by 42-72% in capsule tests, 32-67% in pot tests and 30-55% in field tests. It was observed that the five EB isolates we used in our study suppressed the disease severity between 34 and 51% (Figure 2). Among these isolates, *Pseudomonas caspiana* V30G2, the most successful isolate in disease suppression, was selected to be used together with Si. The disease suppression ability of the V30G2 isolate may be due to its antagonistic effect. This is because *in vitro* tests have determined that the V30G2 isolate has an antagonistic effect against Xap (Figure 1d). However, it may have used other biocontrol mechanisms and/or triggered induced plant resistance. There are many studies on the abilities of *Pseudomonas* sp. in this direction (Alattas et al. 2024).

Silicon protects the plant against biotic stresses such as plant diseases and insect pests as well as abiotic stress and helps its development (Ma 2004, Sahebi et al. 2015, Sistani et al. 1997, Rajput et al. 2021). Although the effectiveness of silicon varies depending on its form, application method and dose, there are many publications indicating that it suppresses many plant diseases (Luyckx et al. 2017, Sahebi et al. 2015). Previous studies have reported that silicon application significantly suppresses diseases caused by *Meloidogyne incognita*, *Pectobacterium betavasculorum*, *Rhizoctonia solani*, *Xanthomonas axonopodis* pv. *phaseoli*, and *Pseudomonas syringae* pv. *tomato* (Andrade et al. 2013,

Çelik and Akköprü 2025, Khan et al. 2020, Shetty et al. 2011, Siddiqui et al. 2020). Consistent with these findings, our study also demonstrated a 34% reduction in disease severity following silicon treatment.

Si has been reported to accumulate in plant cell walls, where it contributes to stress tolerance by forming a physical barrier against various biotic and abiotic stress factors (Luyckx et al. 2017, Sahebi et al. 2015). Additionally, Si enhances plant defense mechanisms by stimulating the activity of defense-related enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and lipoxygenase, thereby restricting pathogen penetration (Cai et al. 2008, Chérif et al. 1994, Fauteux et al. 2005; Polanco et al. 2012, Shetty et al. 2011). Furthermore, Si is also reported to modulate the content and activity of plant hormones, influencing physiological responses to stress (Luyckx et al. 2017).

Despite these well-documented roles of Si in enhancing plant resistance, our study did not reveal a significant effect of Si on plant growth parameters. Similarly, previous studies have reported that Si applications can increase resistance to biotic stress without necessarily promoting overall plant development (Çelik and Akköprü 2025, Guével et al. 2007).

The aim of combining various control methods in control plant diseases is to obtain a synergistic effect rather than just an additive. In this context, the use of Si, which is safe for human health and the environment, together with BCAs has great potential (Etesami 2024, Sahebi et al. 2015). One of the important factors here is to be able to create appropriate combinations.

Some studies have shown that the combined use of PGPR and Si can protect the plant under abiotic stress and support its development. Mahmood et al. (2016) the combined application of the *Bacillus drentensis* with Si resulted in the greatest enhancement of mung bean physiology, growth, and yield under the salinity stress (Mahmood et al. 2016). Also, Kubi et al. (2021) reported that the application of *Pseudomonas psychrotolerans* CS51 + Si combinations was the most successful application by significantly increasing maize biomass and chlorophyll content under salinity stress.

However, neither our literature review nor that conducted review by Etesami (2024) revealed any studies investigating the combined use of silicon (Si) and biological control agents (BCAs) against plant aboveground diseases. The findings demonstrated that the combined application of the endophytic bacterial isolate V30G2 and silicon dioxide (SiO_2) shown the synergistic effect and significantly enhanced disease suppression compared to individual treatments. While individual applications of the EB V30G2 and SiO_2 resulted in 31% and 21% disease suppression, respectively, their combined application achieved a suppression rate of 55% (Figure 3).

This synergistic effect may have resulted from the activation of plant-induced resistance mechanisms. PGPR have long been recognized for their capacity to induce plant resistance. In recent decades, however, growing evidence has demonstrated that Si also plays a crucial role in enhancing plant defense responses. Silicon contributes to plant resistance against pathogenic infections by upregulating defense-related gene expression; increasing the synthesis of phenolic compounds, callose, phytoalexins, and lignin; and boosting levels of polyphenols, antimicrobial flavonoids, and anthocyanins (Verma et al. 2022). Notably, Kubi et al. (2021) reported that the combined application of the biological control agent *Pseudomonas psychrotolerans* CS51 and silicon significantly reduced abscisic acid (ABA) levels by 1.5-fold and jasmonic acid (JA) content by 14.89%. Furthermore, this co-application enhanced the antioxidant system by increasing flavonoid content by 97% and polyphenol content by 19.64%.

On the other hand, microbial biocontrol agents can facilitate the uptake of Si, which is difficult to absorb and has low solubility in soil. Bacteria belonging to genera such as *Enterobacter*, *Pseudomonas*, *Proteus*, *Bacillus*, *Rhizobia* and *Burkholderia* are known to increase the uptake of Si in soils (Etesami and Jeong 2022, Raturi et al. 2021). This may contribute to plant protection. Sahebi et al. (2015) stated that increasing the Si content in the plant body was related to the decrease in disease occurrence.

The *Pseudomonas caspianhalide* V30G2 isolate used in our study has the ability to solubilize phosphate (Table 2) (Babier and Akköprü 2020). Also, it is known that phosphate-solubilizing bacteria affected the availability of Si and increase the uptake of this element by plants (Etesami et al. 2021). Different bacterial species are known to degrade various types of silicates using mechanisms such as acid production, exopolysaccharide secretion, and chelate-forming metabolites (Etesami 2024, Rezakhani et al. 2022). Phosphate-solubilizing bacteria (PSB) facilitate P uptake by plants through organic P mineralization and solubilization of insoluble mineral phosphates (Sharma et al. 2013). PSBs do this by reducing soil pH and producing organic acids and phosphatases. Similarly, silicon-solubilizing bacteria promote silicate dissolution by creating acidic conditions and producing various organic acids involved in this process (Etesami 2024, Etesami et al. 2021). Rezakhani et al. (2022) observed that the application of silicon (Si) in combination with PSB isolates, such as *Pseudomonas* sp. FA1 and *Bacillus simplex* UT1, enhanced the availability of Si forms that can be absorbed by plants. Furthermore, the study revealed that

the combined application of Si and PSB strains significantly increased Si accumulation in plant shoots compared to both the control group and the individual applications of Si or PSB.

In addition, as is known, the rhizosphere is one of the most effective elements in maintaining soil properties and plant health. In this context, silicate-solubilizing bacteria in the soil can convert insoluble silicates into soluble Si (Rajput et al. 2021). This can contribute to the plant by changing the soil microbiota. In fact, it has been determined that Si applications can increase microbial biomass in soils and plants' access to Si (Rajput et al. 2021).

The combined application of EB and Si did not cause a significant increase in other growth parameters except for the number of leaves. Guével et al. (2007) and Çelik and Akköprü (2025) also reached similar findings.

In conclusion, it is well-documented that Si and plant PGPRs individually enhance plant resistance to various biotic and abiotic stress factors or mitigate their adverse effects (Etesami 2024, Rajput et al. 2021). However, studies investigating the combined application of Si and PGPRs against biotic diseases remain scarce or non-existent. In this context, the findings of the present study are considered original. Our results demonstrate that the combined application of Si and EB provides greater protection against common bacterial blight of bean caused by *Xanthomonas axonopodis* pv. *phaseoli* compared to their individual use. Nonetheless, this combined treatment did not yield a significant improvement in overall plant growth. These findings suggest that the strategic combination of an appropriate Si dose and form with effective EB or PGPR isolates holds considerable promise for environmentally friendly and sustainable agricultural practices.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of intere

ÖZET

Bitki hastalıklarının yönetiminde çevre dostu ve sürdürülebilir uygulamaların etkinliğini artırmak, onların daha geniş çapta benimsenmesi ve kullanımını teşvik etmek için çok önemlidir. Bu bağlamda, bakteriyel biyokontrol ajanları ile silisyumun (Si) birlikte kullanılması önemli bir potansiyel taşımaktadır. Bu çalışma, fasulyelerde *Xanthomonas axonopodis* pv. *phaseoli* (Xap)'nin neden oluğu adi yaprak yanıklığı hastalığının kontrolünde Endofitik Bakteriler (EB) ve silisyumun teksel ve birlikte uygulamalarının etkilerini belirlemeyi amaçlamıştır. Ek olarak, bu uygulamaların bitki biyokütlesi ve klorofil içeriği üzerindeki etkileri araştırılmıştır. Fasulye (*Phaseolus vulgaris* cv. Gina) fideleri, iklim odasında topraksız tarım sisteminde torf ve perlitten oluşan yetiştirme ortamında geliştirilmiştir. Silisyum dioksit (SiO_2) (30 mM) ve EB, içirme yöntemi kullanılarak kök boğazına uygulanmıştır. Patojen Xap, yapraklara püskürtülerek uygulanmış ve hastalık şiddeti 1-5 skalası kullanılarak değerlendirilmiştir. Test edilen EB arasında, *Pseudomonas caspiana* V30G2 hastalık şiddetini baskılamada en etkili izolat olmuştur. Teksel uygulamalarda hastalığın şiddeti V30G2 ile %31, SiO_2 ile %21 düzeyinde azaltılmıştır. Ancak, her iki etkenin birlikte uygulanması sinerjistik bir etki göstererek hastalık şiddetini %55 oranında azaltmıştır. Yaprak sayısı gibi belirli parametrelerde bazı pozitif etkiler gözlemlenmiş fakat ne tek başına ne de birlikte yapılan uygulamalar genel bitki biyokütlesini veya klorofil içeriğini önemli ölçüde etkilememiştir. Sonuç olarak, uygun şekilde seçilmiş silikon ve endofitik bakterilerin birlikte uygulanmasının çevre dostu ve sürdürülebilir hastalık yönetimi için önemli bir potansiyele sahip olduğunu ve her bir uygulamanın hastalık baskılama etkinliğini artırdığını göstermektedir.

Anahtar kelimeler: biyokontrol, *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas caspiana*, silisyum dioksit

REFERENCES

Akköprü A., 2020. Potential using of transgenerational resistance against common bacterial blight in *Phaseolus vulgaris*. Crop Protection, 127, 104967. https://doi.org/10.1016/j.cropro.2019.104967

Akköprü A., Akat Ş., Özaktan H., Gül A., Akbaba M., 2021. The long-term colonization dynamics of endophytic bacteria in cucumber plants, and their effects on yield, fruit quality and angular leaf spot disease. Scientia Horticulturae, 282, 110005. doi:10.1016/j.scienta.2021.110005

Alattas H., Glick B.R., Murphy D.V., Scott C., 2024. Harnessing *Pseudomonas* spp. for sustainable plant crop protection. Frontiers in Microbiology, 15:1485197. https://doi.org/10.3389/fmicb.2024.1485197

Andrade C.C.L., Resende R.S., Rodrigues F.A., Ferraz H.G.M., Moreira W. R., Oliveira J.R., Marian R.L.R., 2013. Silicon reduces bacterial speck development on tomato. Tropical Plant Pathology, 38 (5), 436–442. https://doi.org/10.1590/S1982-56762013005000021

Babier Y., Akköprü A., 2020. Çeşitli kültür bitkilerinden izole edilen endofitik bakterilerin karakterizasyonu ve bitki patojeni bakterilere karşı antagonistik etkilerinin belirlenmesi. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 30 (3), 521–534. https://doi.org/10.29133/yyutbd.727138

Belete T., Bastas K.K., Francesconi S., Balestra G.M., 2021. Biological effectiveness of *Bacillus subtilis* on common bean bacterial blight. Journal of Plant Pathology, 103 (1), 249–258. https://doi.org/10.1007/s42161-020-00727-8

Bolwerk A., Lagopodi A.L., Wijfjes A.H., Lamers G.E., Chin-A-Woeng T.F., Lugtenberg B.J., Bloemberg G.V., 2003. Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Molecular Plant-Microbe Interactions, 16 (11), 983–993. https://doi.org/10.1094/MPMI.2003.16.11.983

Bozkurt I.A., 2009. Fasulye bakteriyel yanıklık hastalığına (*Xanthomonas axonopodis* pv. *phaseoli*) karşı antagonistik bakterilerle mücadele olanakları. Ege Üniversitesi Fen Bilimleri Enstitüsü, Basılmamış Doktora Tezi, 171 s., İzmir.

Cai K., Gao D., Luo S., Zeng R., Yang J., Zhu X., 2008. Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. Physiologia Plantarum, 134 (2), 324–333. https://doi.org/10.1111/j.1399-3054.2008.01140.x

Chérif M., Asselin A., Bélanger R.R., 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Phytopathology, 84, 236–242. https://doi.org/10.1094/Phyto-84-236

Corrêa B.O., Soares V.N., Sangiogo M., de Oliveira J.R., Moura A.B., 2017. Interaction between bacterial biocontrol-agents and strains of *Xanthomonas axonopodis* pv. *phaseoli* effects on biocontrol efficacy of common blight in beans. African Journal of Microbiology Research, 11 (32), 1294–1302. https://doi.org/10.5897/AJMR2017.8565

Çelik R., 2021. Endofit bakteri ve silisyum dioksitin fasulyede adi yaprak yanıklığı (*Xanthomonas axonopodis* pv. *phaseoli*) üzerine etkileri. Van Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Basılmamış Yüksek Lisans Tezi, 92 s., Van.

Çelik R., Akköprü A., 2025. Evaluating the control potential of silicon dioxide against *Xanthomonas axonopodis* pv. *phaseoli* in beans. Turkish Journal of Agriculture - Food Science and Technology. In print.

Duman K., Soylu S., 2019. Characterization of plant growth-promoting traits and antagonistic potentials of endophytic bacteria from bean plants against *Pseudomonas syringae* pv. *phaseolicola*. Bitki Koruma Bülteni, 59 (3), 59–69. https://doi.org/10.16955/bitkorb.597214

Ertekin D.Ç., Çalış Ö., Yanar Y., 2016. Orta Karadeniz Bölgesi'nde *Pseudomonas savastanoi* pv. *phaseolicola* ve *Xanthomonas axonopodis* pv. *phaseoli*'nin izolasyonu ve tanılanması. Mediterranean Agricultural Sciences, 34 (1), 25–32. https://doi.org/10.29136/mediterranean.776787

Etesami H., 2024. Enhancing crop disease management through integrating biocontrol bacteria and silicon fertilizers: challenges and opportunities. Journal of Environmental Management, 371, 123102. doi: 10.1016/j.jenvman.2024.123102

Etesami H., Jeong B.R., 2022. Biodissolution of silica by rhizospheric silicate-solubilizing bacteria-chapter 19. In: silicon and nano-silicon in environmental stress management and crop quality improvement. Etesami H., Al Saeedi A.H., El-Ramady H., Fujita M., Pessarakli M., Hossain M.A. (Eds.). Academic Press, London, 265–276 p.

Etesami H., Jeong B.R., Glick B.R., 2021. Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant. Frontiers in Plant Science, 12:699618. https://doi.org/10.3389/fpls.2021.699618

FAO, 2023. Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC (accessed date: 01.04.2025).

Fauteux F., Remus-Borel W., Menzies J.G., Bélanger R.R., 2005. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiology Letters, 249 (1), 1–6. https://doi.org/10.1016/j.femsle.2005.06.022

Fortunato A.A., Rodrigues F.A., Datnoff L.E., 2015. Silicon control of soil-borne and seed-borne diseases. In: Silicon and Plant Diseases. Rodrigues F.A., Datnoff L.E. (Eds.). Springer, Cham, 39–59. https://doi.org/10.1007/978-3-319-22930-0_3

Gilbertson R.L., Maxwell D.P., 1992. Common blight of bean. In: Diseases of international importance. Vol 2. Chaube, H.S., Kumar, J.,, Mukhopadyay, A.N., Singh, U.S. (Eds.). Prentice Hall, Inglewood Cliffs, New Jersey. 18–39.

Grobelak A., Napora A., Kacprzak M., 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecological Engineering, 84, 22–28. https://doi.org/10.1016/j.ecoleng.2015.07.019

Guerriero G., Hausman J-F., Legay S., 2016. Silicon and the plant extracellular matrix. Frontiers in Plant Science, 7: 463. doi:10.3389/fpls.2016.00463

Guével M.H., Menzies J.G., Bélanger R.R., 2007. Effect of root and foliar applications of soluble silicon on powdery mildew control and growth of wheat plants. European Journal of Plant Pathology, 119, 429–436. https://doi.org/10.1007/s10658-007-9181-1

Hallmann J., Quadt-Hallmann A., Mahaffee W.F., Kloepper J.W., 1997. Bacterial endophytes in agricultural crops. Journal of Microbiology, 43 (10), 895–914. https://doi.org/10.1139/m97-131

Hardoim P.R., Van Overbeek L.S., Van Elsas D.J., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends in Microbiology, 16 (10), 463–471. doi: 10.1016/j.tim.2008.07.008

Hoagland D.R., Arnon D.I., 1950. The water-culture method for growing plants without soil. California College Agricultural Experiment Station Circular, 347, Berkeley.

İmriz G., Özdemir F., Topal İ., Ercan B., Taş M.N., Yakışır E., Okur O., 2014. Bitkisel üretimde bitki gelişimini teşvik eden rizobakteri (PGPR)'ler ve etki mekanizmaları. Elektronik Mikrobiyoloji Dergisi, 12 (2), 1–19.

Kamilova F., Validov S., Azarova T., Mulders I., Lugtenberg B., 2005. Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environmental Microbiology, 7 (11), 1809–1817. https://doi.org/10.1111/j.1462-2920.2005.00889.x

Karavina C., Mandumbu R., Parwada C., Tibugari H., 2011. A review of the occurrence, biology and management of common bacterial blight. Journal of Agricultural Technology, 7(6), 1459–1474.

Khan M.R., Siddiqui Z.A., 2020. Use of silicon dioxide nanoparticles for the management of *Meloidogyne incognita*, *Pectobacterium betavasculorum* and *Rhizoctonia solani* disease complex of beetroot (*Beta vulgaris* L.). Scientia Horticulturae, 265, 109211. https://doi.org/10.1016/j.scienta.2020.109211

Kim S.G., Kim K.W., Park E.W., Choi D., 2002. Silicon-induced cell wall fortification of rice leaves: a possible cellular mechanism of enhanced host resistance to blast. Phytopathology, 92 (10), 1095–1103. doi: 10.1094/PHYTO.2002.92.10.1095

Kubi H.A.A., Khan M.A., Adhikari A., Imran M., Kang S.-M., Hamayun M., Lee I.-J., 2021. Silicon and plant growth-promoting rhizobacteria *Pseudomonas psychrotolerans* CS51 mitigates salt stress in *Zea mays* L. Agriculture, 11 (3), 272. https://doi.org/10.3390/agriculture11030272

Luyckx M., Hausman J-F., Lutts S., Guerriero G., 2017. Silicon and plants: current knowledge and technological perspectives. Frontiers in Plant Science, 8, 411. https://doi.org/10.3389/fpls.2017.00411

Ma J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Science and Plant Nutrition, 50, 11-18. https://doi.org/10.1080/00380768.2004.10408447

Mahmood S., Daur I., Al-Solaimani S.G., Ahmad S., Madkour M.H., Yasir M., Hirt H., Ali S., Ali Z., 2016. Plant growth promoting rhizobacteria and silicon synergistically enhance salinity tolerance of mung bean. Frontiers in Plant Science, 7, 876. https://doi.org/10.3389/fpls.2016.00876

Mercado-Blanco J., Lugtenberg B.J., 2014. Biotechnological applications of bacterial endophytes. Current Biotechnology, 3 (1), 60–75.

Olur Ü., 2019. Tuzlu ortamda gelişen bitkilerden izole edilen endofit bakterilerin hıyar bitkisinde köşeli yaprak leke hastalığı (*Pseudomonas syringae* pv. *lachrymans*), tuz stresi ve bitki gelişimine etkileri. Van Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 87 s., Van.

Opio A.F., Allen D.J., Teri J.M., 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. Plant Pathology, 45 (6), 1126–1133.

Osdaghi E., 2014. Occurrence of common bacterial blight on mung bean (*Vigna radiata*) in Iran caused by *Xanthomonas axonopodis* pv. *phaseoli*. New Disease Reports, 30 (1), 9.

https://doi.org/10.5197/j.2044-0588.2014.030.009

Park S.J., Rupert T., Anderson T.R., 1999. White mold: germplasm screening under various field conditions in Ontario. Annual Report of the Bean Improvement Cooperative, 42, 51–52.

Polanco L.R., Rodrigues F.A., Nascimento K.J.T., Shulman P., Silva L.C., Neves F.W., Vale F.X.R., 2012. Biochemical aspects of bean resistance to anthracnose mediated by silicon. Annals of Applied Biology, 161 (2), 140–150. https://doi.org/10.1111/j.1744-7348.2012.00558.x

Rajput V.D., Minkina T., Feizi M., Kumari A., Khan M., Mandzhieva S., Sushkova S., El-Ramady H., Verma K.K., Singh A., van Hullebusch E.D., Singh R.K., Jatav H.S., Choudhary R., 2021. Effects of silicon and silicon-based nanoparticles on rhizosphere microbiome, plant stress and growth. Biology, 10 (8), 791. https://doi.org/10.3390/biology10080791

Raturi G., Sharma Y., Rana V., Thakral V., Myaka B., Salvi P., Singh M., Dhar H., Deshmukh R., 2021. Exploration of silicate solubilizing bacteria for sustainable agriculture and silicon biogeochemical cycle. Plant Physiology and Biochemistry, 166, 827–838. doi: 10.1016/j.plaphy.2021.06.039

Rezakhani L., Motesharezadeh B., Tehrani M.M., Etesami H., Hosseini H.M., 2022. The effect of silicon fertilization and phosphate-solubilizing bacteria on chemical forms of silicon and phosphorus uptake by wheat plant in a calcareous soil. Plant and Soil, 477, 259–280. https://doi.org/10.1007/s11104-021-05274-4

Rodrigues F., Dallagnol L.J., Duarte H.S.S., Datnoff L.E., 2015. Silicon control of foliar diseases in monocots and dicots. In: Silicon and plant diseases. Rodrigues, F., Datnoff, L. (Eds.). Springer, Cham. https://doi.org/10.1007/978-3-319-22930-0_4

Romano I., Ventorino V., Pepe O., 2020. Effectiveness of plant beneficial microbes: overview of the methodological approaches for the assessment of root colonization and persistence. Frontiers in Plant Science, 11, 6. https://doi.org/10.3389/fpls.2020.00006

Rudolph K., 1993. Infection of the plant by *Xanthomonas*. In: *Xanthomonas*. Swings, J.G., Civerolo, E.L. (Eds.). Chapman and Hall, London, 193–264.

Saettler A.W., 1989. The need for detection assay, detection of bacteria in seed and other planting material. In: Detection of bacteria in seed and other planting material. Saettler, A.W., Schaad, N.W., Roth, D.A. (Eds.). APS Press, 122 p.

Sahebi M., Hanafi M.M., Siti Nor Akmar A., Rafii M.Y., Azizi P., Tengoua F.F., Nurul Mayzaitul Azwa J., Shabanimofrad M., 2015. Importance of silicon and mechanisms of biosilica formation in plants. Biomed Research International, 2015, 396010. https://doi.org/10.1155/2015/396010

Sallam N.M.A., Aldayel M.F., 2025. Synergistic effects of *Rahnella aquatilis* and *Trichoderma orientale* in biocontrol of common bacterial blight in bean. Egyptian Journal of Biological Pest Control, 35, 9. https://doi.org/10.1186/s41938-025-00847-2

Santoyo G., Moreno-Hagelsieb G., del Carmen Orozco-Mosqueda M., Glick B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiological Research, 183, 92–99. https://doi.org/10.1016/j.micres.2015.11.008

Savant N.K., Snyder G.H., Datnoff L.E., 1997. Silicon management and sustainable rice production. In: Advances in agronomy. Sparks, D.L. (Ed.). Academic Press, San Diego, CA, USA, 58, 151–199. https://doi.org/10.1016/S0065-2113(08)60255-2

Schwartz H.F., Gent D.H., Franc G.D., Harveson R.M., 2007. Dry bean, disease, common bacterial blight. High Plains IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University, and Montana State University.

Sharma S.B., Sayyed R.Z., Trivedi M.H., Gobi T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus, 2, 587. doi: 10.1186/2193-1801-2-587

Shetty R., Frette X., Jensen B., Shetty N.P., Jensen J.D., Jørgensen H.J.L., NewmanM.A., Christensen L.P., 2011. Silicon-induced changes in antifungal phenolic acids, flavonoids, and key phenylpropanoid pathway genes during the interaction between miniature roses and the biotrophic pathogen *Podosphaera pannosa*. Plant Physiology, 157 (4), 2194–2295. https://doi.org/10.1104/pp.111.185215

Siddiqui Z.A., Hashmi A., Khan M.R., Parveen A., 2020. Management of bacteria *Pectobacterium carotovorum*, *Xanthomonas campestris* pv. *carotae*, and fungi *Rhizoctonia solani, Fusarium solani* and *Alternaria dauci* with silicon dioxide nanoparticles on carrot. International Journal of Vegetable Science, 26 (6), 547–557. https://doi.org/10.1080/19315260.2019.1675843

Sistani K.R., Savant N.K., Reddy K.C., 1997. Effect of rice hull ash silicon on rice seedling growth. Journal of Plant Nutrition, 20 (1), 195–201. https://doi.org/10.1080/01904169709365242

Spadaro D., Gullino M.L., 2005. Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Protection, 24 (7), 601–613. https://doi.org/10.1016/j.cropro.2004.11.003

Townsend G.R., Heuberger J.W., 1943. Methods for estimating losses caused by diseases in fungicide experiments. The Plant Disease Reporter, 27, 340-343.

Verma K.K., Song X.P., Li D.M., Singh M., Wu J.M., Singh R.K., Sharma A., Zhang B.Q., Li Y.R., 2022. Silicon and soil microorganisms improve rhizospheric soil health with bacterial community, plant growth, performance, and yield. Plant Signaling & Behavior, 17 (1), 2104004. doi: 10.1080/15592324.2022.2104004

Vidaver A., 1993. *Xanthomonas campestris* pv. *phaseoli:* cause of common bacterial blight of bean. In: *Xanthomonas*. Swings, J.G., Civerolo, E.L. (Eds.). London, UK: Chapman & Hall, 40-44.

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Orijinal araştırma (Original article)

Effect of temperature on the migration of the Sunn pest *Eurygaster maura* L. 1758 (Hemiptera: Scutelleridae) from overwintering area

Süne, *Eurygaster maura* L. 1758 (Hemiptera: Scutelleridae)'nın kışlaktan göç seyrine sıcaklığın etkisi

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ABSTRACT

Sunn pest (*Eurygaster* spp.) is one of the most important pests adversely affecting the yield and quality of wheat, which is one of the main nutritional products for humans. In controlling the sunn pest, determining the migration pattern of the pest from its overwintering sites to wheat fields is of critical importance. The ability to predict the onset and end of this migration event forms the basis of forecasting and warning systems for controlling the sunn pest. In this study, conducted over four life cycles from 2014 to 2018 in two wintering sites, the predictability of the onset and end of the sunn pest's migration from wintering sites to wheat fields using temperature data was investigated. The obtained data were evaluated using pure temperature values, the day-degree model using effective temperature sums, and machine learning (decision tree) methods using cumulative temperature values. The study revealed that the migration pattern of the sunn pest cannot be explained solely by temperature data.

INTRODUCTION

Sunn pest (*Eurygaster* spp.) is one of the pests causing economic losses in wheat in the Middle East, Eastern and Northeastern Europe, and Africa (Critchley 1998). Both adult and nymph stages of the sunn pest pierce and suck wheat grains during various phenological stages, resulting in significant losses in germination capacity as well as bread and pasta-making qualities. Failure to control the pest in places and years with high infestation can lead to up to 100% loss in terms of both quality and quantity (Anonymous 2008, Özkan et al. 2017).

The pest, which reproduces once a year, has an active and passive phase in its life cycle. It spends approximately 8-9 months in a passive phase called overwintered site in the mountains. Pests that have hibernated spend the spring flying from the mountains to wheat fields in the plains. The passive period ends and the active period begin with the start of flights to the plains. Upon reaching the plains, overwintered adults feed, mate, and lay their eggs for 1.5-2 months. At the end of this period, adults die. The eggs hatch within 2-3 weeks depending on climatic conditions, and

nymphs emerge. Nymphs that go through 5 instars within an average of 1 month become the new generation adults (NGA). The new generation of adults feed voraciously, store the necessary energy, and retreat to their overwintering areas with the harvest. The passive phase consists of two periods: the post-harvest period, from July (after harvest) until October-November, known as the summering period; and the period from October-November to March-April of the following year, known as the overwintering period.

Sunn pest control, carried out within the framework of integrated control principles, is mainly based on chemical control. The annual pest control season begins with the spring wintering survey, in which the population density of overwintered adults (OA) that can land from wintering places to grain areas is determined. Following the spring wintering survey, the first movements, that is the first descents, are detected to determine the migration of overwintered adults from the wintering sites to the plains. This event marks the first critical point in pest control and is referred to as the onset of descents. The second critical point is the commencement of the survey to determine the density and distribution of OA pests in wheat cultivation areas. For this purpose, the completion of the descent of 95% of OA pest populations from overwintered sites to the plains is determined (termination of descents). As is evident, in pest control, determining two critical biological points – the onset and termination of descent – is crucial. In predictive warning studies conducted by various researchers on combating pests, these two critical points, which are important to determine, have generally been attempted to be predicted by associating them only with temperature values (Agacino 1972, Aleksandrov 1949, Amir-Maafi et al. 2007, Arkhangel'skii 1939, Barbulescu 1967, 1971, Duman 2015, Gözüaçık et al. 2016, Grigorov and Gospodinov 1964, Ionescu and Mustatea 1975, İleri 1957, İslamoğlu 2010, Karaca et al. 2009, Kılıç and Karslıoğlu 1961, Kiliç 1978, Lodos 1961, Lazarov et al. 1969, Memişoğlu 1985, Mozaffari and Azizian 2011, Nakova and Urukov 1976, Öncüer and Kıvan 1995, Paulian and Popov 1980, Peredelskii et al. 1951, Popov and Barbulescu 1978, Silvestri 1934, Smolyannikov 1955, Tafaghodinia and Majdabadi 2006, Vojdani 1954, Yüksel and Bayseç 1964). In this study, the explicability of the migration pattern from the overwintering sites, which is critical in combating pests, was investigated solely based on temperature values.

MATERIALS AND METHODS

The studies were conducted in the wintering sites of Kırşehir-Hasanpaşa hill (wintering plants: hedgehog-vetch, hedgehog herb, thyme, oregano) and Aksaray-Ekecik mountain (wintering plant: oak) from 2014 to 2018, characterized by high population density of pests and different wintering plants. In both overwintering sites, the sunn pest population was overwhelmingly dominated by the species *Eurygaster maura*, accounting for 99.5% of the total. Koçak and Babaroğlu (2005) also reported that *E. maura* comprised 99.5% of the population in the aforementioned overwintering sites. To obtain temperature data, a meteorological station (IMetos) was installed at each wintering site.

To determine the main critical points for control, the life cycle critical periods of the pest were considered and divided into three phases: Phase 1: It covers approximately an 8-9 month period, starting with the movement of the new generation of adults to the overwintering sites following the completion of wheat harvest and ending with the first movements of overwintered adult sunn pests in spring at the overwintering grounds. This period, which is the sum of the summer dormancy (estivation) and winter dormancy (hibernation) periods, is also referred to as the passive period. Phase 2: It includes the period from the initial movement of overwintered adult sunn pests in spring to the completion of the descent of 95% of the adult population in the wintering site. Phase 3: This phase, also known as the active period, begins with the completion of the descent of 95% of the overwintered adult population in the wintering site and ends with the time spent in the field until the new generation of adult's moves to the wintering site after the completion of wheat harvesting. In Phase 1, covering the passive period, just before the initial movement of the sunn pests in the wintering site, the sunn pest densities in both wintering sites were determined (spring wintering site census).

Following the spring wintering site survey, the wintering sites were checked every 2-3 days to determine the first movements of the sunn pests. Counting continued until 95% of the sunn pest population in the wintering site had completed its descent after the detection of the initial movement of the sunn pests.

Counts in the wintering sites were conducted according to the type of vegetation cover. If the vegetation cover consisted of plants such as oak or pine, 32 counts were conducted using 1/16 m^2 (25x25 cm) frames to determine the number of individuals under fallen leaves. If the vegetation cover consists of plants such as coneflower, milkvetch, and thyme, counts were conducted taking into account the plant numbers in Table 1 (Dörtbudak et al. 1991).

The predictability of the start and completion times of the migration of overwintered adults from wintering sites to wheat fields based on temperature values has been

Table 1. Basis plant sizes and corresponding plant numbers per m² for small, medium, and large categories in wintering area plants

Plant grub	Vegetation					
	Coneflower		Coneflower-Milkvetch		Thyme	
	Diameter (cm) (narrowest X largest)	Number of plants in m²	Diameter (cm) (narrowest X largest)	Number of plants in m²	Diameter (cm) (narrowest X largest)	Number of plants in m²
Small	20x30	17	20x30	26	20x27	26
Medium	31x40	9	31x50	8	28x35	14
Big	41<	3	51<	8	36x60	8

investigated using three different methods, which are crucial points in the execution of pest control.

1- The daily average, minimum, and maximum temperatures from January 1st until the start and end of the migration were evaluated without undergoing any processing. The logistic regression method was utilized for the evaluations.

2- Forecast warning models based on the calculation of the total daily average temperatures above the development threshold (degree-days) were investigated to determine the start and end times of descents from the wintering sites. In degree-day calculations, two different starting points were used, including the beginning of Phase 1, and the average calculation method [Effective temperature sum = (maximum temperature + minimum temperature)/2) - development threshold] was used. Various researchers used different development threshold temperatures (0-20 °C) in the calculations of the effective temperature sum method, which is utilized in the forecast warning studies for pests. In this study, commonly used development threshold values of >0 °C and >10 °C were adopted for the calculations related to effective temperature sums (Amir-Maafi et al. 2007, Ionescu and Mustatea 1975, Paulian and Barbulescu 1970, Popov and Barbulescu 1978). The nominal logistic regression method was used in the evaluations.

3- Artificial intelligence, machine learning, and data mining techniques have been utilized to predict the start and end times of descent from wintering sites with minimal error, based on the relationships between temperature values and the onset of the sunn pest biological events. Decision tree models have been developed to predict pest migration onset and completion times from wintering sites.

For this purpose, measurement and label data were collected during the studies conducted between 2014 and 2018. Measurement data consisted of temperature values (average, minimum, maximum), including date and location information. Accumulative measurements of values above 0 °C throughout each life cycle of the pest were calculated from the values directly measured periodically by the sensors. Label data, primarily collected through field studies under natural conditions, mainly consists of phase information for all days.

During the study, models were developed using a dataset consisting of 2925 daily records for 2 regions and 4 life cycles each. Since there was no separate dataset available for testing the models, they were evaluated using a 10-fold cross-validation method.

Statistical Analyses were performed using SPSS 21 statistical software (IBM Corp. 2021). For phase estimation, decision tree algorithms integrated into the WEKA software package were utilized (Bouckaert et al. 2016).

RESULTS AND DISCUSSION

Pure temperature values

According to researchers, climatic conditions, especially temperature, play a significant role in the initiation and continuation of descent from wintering sites, with various threshold values (10-25 °C). Therefore, the effect of temperature on the initial movement and descent pattern of the pests in the wintering sites was investigated during the four life cycles studied (2014-2018). The first critical point for pest control, the start of sunn pest descents from wintering sites, occurred on different dates from year to year during the study period, but it consistently took place in April in both wintering sites (Table 2). In both wintering sites, descents typically begin when the maximum temperature in the wintering site rises above 10 °C (ranging from 9.2 °C to 20 °C). However, it was determined that, despite occasional rises in temperature in the days leading up to the onset of migration, there was no movement observed in the overwintered adults.

The effect of daily average, minimum, and maximum temperatures on the onset of migration and the descent pattern of the pest in the wintering area is provided (Table 3). The general trend indicates that both average and

Table 2. Descent pattern of overwintered adult sunn pest populations from the Ekecik mountain (Aksaray) and Hasanpaşa hill (Kırşehir) wintering sites from 2014 to 2018

Wintering site	Stage	Phase cycle	Status	Life cycle			
				Cycle 1	Cycle 2	Cycle 3	Cycle 4
				2014-2015	2015-2016	2016-2017	2017-2018
Kırşehir Hasanpaşa hill	Passive period	$Phase_1$	Beginning	21.7.2014	30.7.2015	26.7.2016	27.7.2017
			Ending	28.4.2015	8.4.2016	20.4.2017	5.4.2018
			Time	274 day	258 day	270 day	254 day
		$Phase_2$	Beginning	29.4.2015	9.4.2016	21.4.2017	9.4.2018
			Ending	8.5.2015	25.4.2016	4.5.2017	19.4.2018
			Time	10 day	17 day	14 day	10 day
	Active period	$Phase_3$	Beginning	9.5.2015	26.4.2016	5.5.2017	20.4.2018
			Ending	29.7.2015	25.7.2016	26.7.2017	
			Time	81 day	90 day	81 day	
Aksaray Ekecik mountain	Passive period	$Phase_1$	Beginning	21.7.2014	30.7.2015	16.7.2016	19.7.2017
			Ending	28.4.2015	6.4.2016	20.4.2017	5.4.2018
			Time	273 day	263 day	277 day	259 day
		$Phase_2$	Beginning	29.4.2015	7.4.2016	21.4.2017	9.4.2018
			Ending	8.5.2015	22.4.2016	2.5.2017	19.4.2018
			Time	10 day	18 day	12 day	10 day
	Active period	$Phase_3$	Beginning	9.5.2015	23.4.2016	3.5.2017	20.4.2018
			Ending	29.7.2015	15.7.2016	18.7.2017	
			Time	82 day	84 day	76 day	

Table 3. The relationship between the migration pattern of the sunn pest and temperature

Descriptive variables	B	Std. Error	Wald	df	Sig.	Exp(B)
Temperature (Mean)	,559	,084	43,846	1	,000	1,749
Temperature (Minimum)	-,175	,063	7,754	1	,005	,839
Temperature (Maximum)	,034	,033	1,015	1	,314	1,034
Constant	-5,543	,425	169,852	1	,000	,004
Number of observations	1204	Missing cases		11		
Log-Likelihood value	521,294					
Cox&Snell R^2	0,271					
Nagelkerke R^2	0,512					
Hosmer and Lemeshow Test	X^2=8,675; df=8; p=0,370					
Classification Table		Predicted				
Observed		$Phase_1$	$Phase_2$	Percentage correct		
$Phase_1$		1009	35	96.6%		
$Phase_2$		84	65	43.6%		
Overall Percentage		92.3%	65.0%	90.0%		

minimum temperatures affect the onset of movement for the pest in the wintering site and its migration pattern to the fields (Wald statistic sign. value). It can be observed that with an increase in temperature, both movement onset and migration accelerate (Exp(B)). However, upon examining the Classification Matrix, it was found that the prediction accuracy was low, particularly with a significant amount of error in predicting both the onset of movement and the migration pattern (56.40%). The Cox-Snell and Nagelkerke values also confirm our findings regarding the explanatory power of average and minimum temperatures on the migration pattern. This situation demonstrates that temperature may not be the main factor in initiating migration from the wintering sites. This indicates that the onset of migration from the wintering sites and the course of migration, which are the first critical points in forecasting-warning, cannot be determined solely based on temperature values.

When other studies are examined; it will be observed that temperatures at the wintering sites vary significantly (10-25 °C) depending on factors such as studies, years, countries, and regions within the same country when migration from the wintering sites begins. The dominant sunn pest species in the study area is *E. maura*. Although the findings were obtained from *E. maura*, they show considerable consistency with the results derived from other sunn pest species, as demonstrated in the studies presented below.

Silvestri (1934) found that sunn pests become active and migrate when the temperature rises above 10 °C during spring term in Italy. According to Arkhangel'skii (1939), migration from the wintering sites in the North Caucasus occurs when the temperature rises above 21 °C in the second half of April. On the other hand, Peredelskii et al. (1951) report that migration takes place when the average temperature in the first week of April is 16 °C. Similarly, Smolyannikov (1955) reports that overwintered adults migrate from wintering sites to grain fields when the temperature reaches 20-22 °C at the end of April in the same region.

In Iran's significant cereal production region of Varamine, studies on the migration of sunn pest (*E. integriceps*) from wintering sites to fields indicated that migration started when temperatures reached 20-22 °C according to Aleksandrov (1949), 18-20 °C according to Vojdani (1954), 20 °C according to Agacino (1972), and 13.5-14.3 °C according to Radjabi (2000). Tafaghodinia and Majdabadi (2006) reported that in their studies conducted in five different regions in Iran, the landing dates of *Eurygaster integriceps* from wintering sites to wheat fields and the temperatures on these dates varied from year to year. They mentioned that during the descents from the wintering sites, the long-term average temperatures were 12 °C in the Shazand, Arak, and Tafresh regions, and 14 °C in the Saveh region.

In studies conducted in Türkiye (specifically in Diyarbakır-Karacadağ wintering sites), sunn pests (*E. integriceps*) migrate from the wintering sites to the plains when the daily average air temperature in spring reaches 17 °C according to Lodos (1961), and when it exceeds 13 °C according to Karaca et al. (2009). In the same region, Kılıç (1978) reports that Sunn pests migrate from the wintering sites to the plains when temperatures in the wintering sites reach 18 °C in the second half of March to the beginning of April. Memişoğlu (1985) reported that the migration of sunn pests (*E. maura*) from wintering sites in Ankara province began in May with daily average temperatures of 14.8 °C, 15.1 °C, and 20.0 °C in the years 1981, 1982, and 1983, respectively.

Furthermore, in other studies conducted in Türkiye, according to İleri (1957), migration from the wintering sites occurs in the second half of March when temperatures reach 15-17 °C. Kılıç and Karslıoğlu (1961) state that migration occurs when the average temperature in the wintering sites ranges from 14-22 °C. Yüksel and Bayseç (1964) suggest a temperature threshold of 12 °C for migration, while Öncüer and Kıvan (1995) indicate that sunn pest (*E. integriceps*)'s migration occurs when temperatures in the wintering sites reach 14-16 °C.

According to studies conducted in Bulgaria, Grigorov and Gospodinov (1964) report that the temperature in the wintering sites reaches 17-18 °C in spring when adult sunn pests (*E. integriceps*) migrate from the wintering grounds to wheat fields. Similarly, Lazarov et al. (1969) state that migration occurs when temperatures reach 14-15 °C, while Nakova and Urukov (1976) indicate that migration begins when temperatures reach 18.6 °C.

In Romania, according to Barbulescu (1967, 1971), migration from wintering sites to wheat fields occurs when temperatures reach 12 °C. Similarly, Ionescu and Mustatea (1975) suggest that migration happens when temperatures are above 10 °C and, according to Paulian and Popov (1980), sunn pest (*E. integriceps*)'s migration occurs when temperatures reach 12-13 °C.

While there are numerous other studies similar to those mentioned above, it was deemed sufficient to omit them for the sake of discussion, given the literature provided. When the results obtained from our study are evaluated together with the studies conducted so far, it is observed that predictions based on daily temperature values may lead to erroneous conclusions.

Therefore, the applicability of forecast warning models

based on the accumulation of temperatures above the developmental threshold (degree-days) for each life stage of insects, which are part of decision support systems aimed at assisting in determining survey and spraying times, has also been investigated in predicting the migration pattern of the sunn pest from wintering sites.

Total effective temperature

As a result of the studies, it has been determined that the effective temperature sums obtained for critical points in sunn pest control (onset and end of migration from wintering sites) show a high degree of variation (Table 4-5). The totals of effective temperatures for overwintered adults (two starting times (January 1, July) and threshold temperature (>0 °C, >10 °C) until they start and finish their descent vary according to years (min-max values) in the same location and locations in the same year. For instance, at the Kırşehir Hasanpaşa hill transmitting station wintering sites, calculations based on data collected from January 1st and using a threshold temperature above 10 °C indicate that the effective temperature sum for the start of descent of overwintered adults' averages 13.49 degree-days. However, it has been determined that this value varies significantly between years, ranging from 3.19 to 29.94 degree-days (Table 4). A similar situation is observed when considering a threshold temperature of 0 °C. The same situation was also observed as a result of studies conducted using data from the Ekecik mountain wintering site in Aksaray. It has been determined that the cumulative effective temperature sums until the completion of migration from the wintering sites also exhibit variation between years at the same location and between locations in the same year, similar to the variation seen at the start of descent (Table 4).

As the starting point for the day-degree calculation, the date of return of the new generation adults to the wintering sites for summer was taken as July. It has been determined that there was variation between years at the same location and between locations in the same year for both threshold temperatures, from the start to the completion of descent from the wintering sites (Table 5).

Table 5.

The analysis results for predicting Phase 2, which is the descent process of the sunn pest from the wintering sites, using cumulative effective temperature sums are provided in Tables 6-9. When examining the tables, it can be seen that while there was a high success rate in predicting the phases, the correct prediction rate of Phase 2 remained at a low level.

According to the analysis conducted for predicting the

Table 4. Total effective temperatures (degree-days) until the start of descent of overwintered adults from the wintering sites to the fields (1 January)

Location	Total effective temperatures (degree-days) until the start of descent of overwintered adults (January 1st)				Total effective temperatures (degree-days) until the completion of descent of overwintered adults (January 1st)			
	Kırşehir- Hasanpaşa hill		Aksaray-Ekecik mountain		Kırşehir- Hasanpaşa hill		Aksaray-Ekecik mountain	
Descriptive Statistics	Threshold temperatures		Threshold temperatures		Threshold temperatures		Threshold temperatures	
	>0 °C	>10 °C	>0 °C	>10 °C	>0 °C	>10 °C	>0 °C	>10 °C
Mean	342.64	13.49	290.47	6.67	492.84	38.27	419.19	24.86
Std. error	21.60	4.65	21.10	2.15	44.79	7.55	36.84	4.69
Minimum	273.07	3.19	218.54	1.43	394.06	20.76	320.27	13.22
Maximum	387.09	29.94	342.61	11.42	624.80	63.54	525.50	39.93
Std. deviation	48.29	10.39	47.18	4.82	100.15	16.89	82.38	10.48
Confidence interval 95%	282.68	0.58	231.89	0.69	368.49	17.30	317.62	11.85
	402.61	26.39	349.05	12.65	617.19	59.24	522.20	37.88

Table 5. Total effective temperatures (degree-days) until the completion of descent of overwintered adults from the wintering sites to the fields (July)

Location	Total effective temperatures (degree-days) until the start of descent of overwintered adults (July)				Total effective temperatures (degree-days) until the completion of descent of overwintered adults (July)			
	Kırşehir-Hasanpaşa hill		Aksaray-Ekecik mountain		Kırşehir-Hasanpaşa hill		Aksaray-Ekecik mountain	
Descriptive Statistics	Threshold temperatures		Threshold temperatures		Threshold temperatures		Threshold temperatures	
	>0 °C	>10 °C	>0 °C	>10 °C	>0 °C	>10 °C	>0 °C	>10 °C
Mean	2109.54	651.62	2027.48	613.91	2285.95	678.44	2190.85	636.68
Std. error	57.77	27.45	71.79	64.44	83.10	28.93	50.27	62.59
Minimum	1936.73	587.44	1813.69	422.60	2057.71	610.93	2052.81	453.20
Maximum	2175.26	715.83	2122.36	701.72	2415.11	752.07	2279.46	726.21
Std. deviation	115.54	54.90	143.58	128.89	166.20	57.87	100.54	125.18
Confidence interval 95%	1925.69	564.27	1799.02	408.82	2021.48	586.36	2030.87	437.49
	2293.38	738.97	2255.95	819.00	2550.42	770.52	2350.82	835.86

phases using cumulative temperatures above 0 °C from January 1st until the start of descent of overwintered adults, a success rate of 93.2% has been achieved, but the correct prediction rate for Phase 2 remains at 54.8% (Table 6). This indicates a high error rate of 45.2% in predicting the start and completion of the descent of overwintered adults from the wintering sites. When the developmental threshold was taken as 10 °C, the analysis results show that the correct prediction rate for Phase 2 remains similarly low at 49.6% (Table 7).

When the start of estivation (Phase 1) was taken as the starting point for degree-day calculations, it was determined that the success rates for predicting the start and end times of the sunn pest migration were very low for both threshold temperatures (Table 8-9).

As can be seen from the results obtained, meaningful conclusions cannot be drawn using effective temperature sums. It is clear that predictions made using these data will be insufficient to explain the course of the pest's migration.

When examining the studies conducted on the subject so far, it will be observed that there are significant differences in the developmental threshold values used in calculations related to effective temperature sums. Additionally, significant differences will be observed in the cumulative effective temperatures until the start and completion of descent of overwintered adults obtained as a result of calculations.

According to the study conducted by Ionescu and Mustatea (1975) in Romania over four years (1971-1973) at six different locations, they reported that the cumulative effective temperatures were between 13.8-19.4 degree-days when descent began and between 33.4-112.2 degree-days when descent ended (with a developmental threshold of 10 °C). The researchers stated that the cumulative effective temperature varies depending on the conditions during the descent. They indicate that under favorable conditions for sunn pest descent, the cumulative effective temperatures were low (33.40 degree-days) with average temperatures above 12 °C until May and low precipitation. However, under unfavorable conditions, such as average temperatures below 12 °C until May with precipitation, the cumulative effective temperatures were high (99.00 degree-days).

Popov and Barbulescu (1978) report that in the wintering sites of the Fundelea region of Romania (from 1965 to 1976), when the migration of sunn pests from the wintering sites to the grain fields began, the cumulative effective temperatures above 10 °C varied between 22.5 and 84.5 degree-days. They

Table 6. Total effective temperatures (degree-days) until the start of descent of overwintered adults from the wintering sites to the fields (1 January)

Descriptive variables	B	Std. Error	Wald	df	Sig.	Exp(B)
Total effective temperature >0 °C (gd)	-,015	,001	106,044	1	,000	,985
Number of observations	1580	Missing cases		0		
Likelihood Ratio Tests	X^2=2440,553; df=2; p=0,000					
Cox&Snell R^2	0,787					
Nagelkerke R^2	0,953					
Goodness-of-Fit (Deviance)	X^2=470,764; df=2564; p=1,00					
Classification Table			Predicted			
Observed	$Phase_1$	$Phase_2$	$Phase_3$	Percentage correct		
$Phase_1$	780	14	0	98.2%		
$Phase_2$	23	74	38	54.8%		
$Phase_3$	0	33	618	94.9%		
Overall Percentage	50.8%	7.7%	41.5%	93.2%		

Table 7. Total effective temperatures (degree-days) until the start of descent of overwintered adults from the wintering sites to the fields (1 January)

Descriptive variables	B	Std. Error	Wald	df	Sig.	Exp(B)
Total effective temperature >10 °C (gd)	-,084	,008	100,832	1	,000	,919
Number of observations	1580	Missing cases		0		
Likelihood Ratio Tests	X^2=2333,682; df=2; p=0,000					
Cox&Snell R^2	0,772					
Nagelkerke R^2	0,917					
Goodness-of-Fit (Deviance)	X^2=499,611; df=1318; p=1,00					
Classification Table			Predicted			
Observed	$Phase_1$	$Phase_2$	$Phase_3$	Percentage correct		
$Phase_1$	790	4	0	99.5%		
$Phase_2$	39	67	29	49.6%		
$Phase_3$	0	33	618	94.9%		
Overall Percentage	52.5%	6.6%	40.9%	93.2%		

also mention that the cumulative effective temperatures varied from year to year over the 12 years.

Radjabi (2000) reports that there is no correlation between the onset of sunn pest migration from the wintering sites to the grain fields and the effective temperature totals (degree-days).

Amir-Maafi et al. (2007) examined the relationship between cumulative effective temperature sums during the period from January 1st to the beginning of the first sunn pest descent to grain fields using 7 different threshold temperatures (0-2-4-6-8-10 and 12 °C) in Iran from 1992 to 2002. They found high variations from year to year and between locations, suggesting that cumulative effective temperature totals cannot be used to predict the initial migration of sunn pests from wintering sites.

Mozaffari and Azizian (2011) reported that the annual effective temperature sum varies according to the climatic conditions of the year, with the development threshold temperature taken as 6 °C ranging from 413.8 to 1132.7 degree-days. They indicate that along with the increase in the annual effective temperature sum, there will also be increases in the population density of sunn pests.

Table 8. Total effective temperatures (degree-days) until the start of descent of overwintered adults from the wintering sites to the fields (July)

Descriptive variables	B	Std. Error	Wald	df	Sig.	Exp(B)
Total effective temperature >0 °C (gd)	-,011	,001	295,667	1	,000	,989
Number of observations	2925	Missing cases		0		
Likelihood Ratio Tests	X^2=3356,69; df=2; p=0,000					
Cox&Snell R^2	0,682					
Nagelkerke R^2	0,903					
Goodness-of-Fit (Deviance)	X^2=757,70; df=4812; p=1,00					
Classification Table			Predicted			
Observed	$Phase_1$	$Phase_2$	$Phase_3$	Percentage correct		
$Phase_1$	2125	15	0	99.3%		
$Phase_2$	45	41	49	30.4%		
$Phase_3$	13	19	619	95.1%		
Overall Percentage	74.6%	2.6%	22.8%	95.2%		

Table 9. Total effective temperatures (degree-days) until the start of descent of overwintered adults from the wintering sites to the fields (July)

Descriptive variables	B	Std. Error	Wald	df	Sig.	Exp(B)
Total effective temperature >10 °C (gd)	-,018	,001	190,633	1	,000	,982
Number of observations	2925	Missing cases		0		
Likelihood Ratio Tests	X^2=1497,21; df=2; p=0,000					
Cox&Snell R^2	0,401					
Nagelkerke R^2	0,530					
Goodness-of-Fit (Deviance)	X^2=757,70; df=4812; p=1,00					
Classification Table			Predicted			
Observed	$Phase_1$	$Phase_2$	$Phase_3$	Percentage correct		
$Phase_1$	2140	0	0	100.0%		
$Phase_2$	122	0	13	0.0%		
$Phase_3$	192	0	459	70.5%		
Overall Percentage	83.9%	0.0%	16.1%	88.8%		

Decision tree models

A third method was used to predict the first of the field-based surveys, which is the first of the primary survey times for control purposes. This method relies on the starting times of the biological events of the sunn pest, explaining the relationship between temperature values and the beginnings of these biological events by using machine-learning techniques. The results obtained from this method are presented in Table 10. The predictions made using only temperature data obtained from meteorological stations in the wintering sites resulted in an accuracy rate of over 90% (ranging from 82.94% to 96.44%). However, as can be seen upon examining in the error matrix, more than 100 (ranging from 104 to 499) incorrect predictions were made. Especially, the accuracy rate in predicting the duration of Phase 2, where the valuation survey is determined, remains very low (ranging from 0.00 to 38.00).

Conclusions

Studies investigating the applicability of forecast warning models created solely with temperature data in the context of sunn pest control have shown that temperature values alone (minimum, average, maximum) cannot explain the migration pattern of the pest from overwintering sites, which is a critical aspect in pest management.

Table 10. Prediction results for the onset of assessment surveys using one of temperature data

Measured data		The accurate prediction rate (%)	Number of prediction		Total	Error Matrix				The accurate prediction rate (%)	Location	Number of loops
			Incorrect	Correct								
Air temperature	Max.	96.44	104	2821	2925	Phases	P_1	P_2	P_3		2	4
						P_1	2129	10	0	99.53		
						P_2	48	48	32	38.00		
						P_3	6	6	644	97.87		
	Avg.	93.95	177	2748	2925	Phases	P_1	P_2	P_3		2	4
						P_1	2123	0	16	99.25		
						P_2	77	1	50	0.78		
						P_3	28	6	624	94.83		
	Min.	82.94	499	2426	2925	Phases	P_1	P_2	P_3		2	4
						P_1	2138	0	1	99.95		
						P_2	128	0	0	0		
						P_3	370	0	288	43.77		

Error type	Measured data		
	Minimum	Average	Maximum
Mean Absolute Error	0,0047	0,0058	0,0045
Root Mean Square Error	0,0642	0,0733	0,0653
Relative Absolute Error	1,7086%	2,0946%	1,5959%
Root Relative Squared Error	17,2271%	19,6748%	17,4761%

It is evident that insect bioecology, which involves multiple relationships, cannot be explained by a single factor. We believe that forecast warning models created using artificial intelligence techniques and utilizing all meteorological data will explain migration patterns, which are influenced by multiple meteorological factors, with higher accuracy rates.

Based on our research specifically focused on the sunn pest, we conclude that forecast warning models created for pest control, incorporating all meteorological data and utilizing commonly used artificial intelligence techniques, will yield higher accuracy in predictions.

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Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

İnsan beslenmesinin vazgeçilmez ürünlerinin başında gelen buğdayın, verim ve kalitesini olumsuz yönde etkileyen önemli zararlıların başında Süne (Eurygaster spp.) gelmektedir. Süne ile mücadelede, zararlının kışlaklardan buğday alanlarına göç seyrinin belirlenmesi kritik öneme sahiptir. Bu göç olayının başlangıç ve son bulmasının tahmin edilebilmesi süne mücadelesi tahmin uyarı çalışmalarının temelini oluşturmaktadır. Sünenin kışlaktan ovaya inişinin başlama ve bitişini sıcaklık verileri ile tahmin edilebilirliğinin araştırıldığı bu çalışma; 2014-2018 yıllarında 2 kışlakta 4 yaşam döngüsü süresince yürütülmüştür. Elde edilen veriler 3 yöntem; herhangi bir işlem yapılmadan salt sıcaklık değerleriyle, etkili sıcaklık toplamlarının kullanıldığı gün derece modeli ve birikimli sıcaklık değerlerinin kullanıldığı makine öğrenmesi (karar ağacı) yöntemleri ile değerlendirilmiştir. Yapılan çalışmalar sonucunda; sünenin ovaya göç seyrinin sadece sıcaklık verileri ile açıklanamayacağı belirlenmiştir.

Anahtar kelimeler: süne, buğday, tahmin-uyarı

REFERENCES

Agacino M.E., 1972. Informe de la F.A.O. al gobierno del Iran sobre ecologia de la chinche de los cereales y la Lucia contra este parasito. Bulletin Informativo de Plages (1972), 91, 25-56.

Alexandrov N., 1947-1949. *Eurygaster integriceps* Put. a Varamine et ses parasites [In Persian with French Summary]. Entomologie et Phytopathologi Apliques, 5, 11-14, 29-41; 6-7, 8-17, 28-47; 8, 13-20, 16-52.

Amir-Maafi M., Majdabadi M., Aghdasi F., Parker B.L., Parsi F., 2007. Degree days for forecasting migration of sunn pest. 139-145 pp. In: sunn pest management: a decade of progress 1994-2004. Parker B.L., Skinner M., El Bouhssini, M., Kumari S.G., (Eds.). Published by the Arab Society for Plant Protection, Beirut, Lebanon, 432 pp.

Anonymous, 2008. Zirai Mücadele Teknik Talimatları. Cilt 1. Tarım ve Köyişleri Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü. Başak Matbaacılık ve Tan. Hiz. Ltd. Şti. Ankara. ISBN:978-975-407-262-4. 283 s. (in Turkish).

Arkhangel'skii N.N., 1939. A study upon on the injurious corn bug *Eurygaster integriceps* Put. in order to elaborate control measures against it. Summary of the scientific research work of the institute of plant protection for the year 1939. Med. 8 vo, pp. 26-35. (CAB abstracts 1941).

Barbulescu A.L., 1967. Some biological and ecological aspects of the cereal bugs. Analele Institutului de Cercetari pentru Cereale si Plante Tehnice-Fundulea, 3, 169-176.

Barbulescu A.L., 1971. Evolutia aparitiei ploşnitelor cerealelor (*Eurygaster* sp.) in perioda 965-1969 Fundulea. [Evolution of cereal bug (*Eurygaster* sp.) appearance during 1965-1969 at Fundelea]. Analele Institutului de Cercetari pentru Cereale si Plante Tehnice-Fundulea, 1971, 7, 149-158.

Bouckaert R.R., Frank E., Hall M., Kirkby R., Reutemann P., Seewald A., Scuse D., 2016. WEKA manual for version 3-9-1. University of Waikato, Hamilton, New Zealand, 1-341.

Critchley B.R., 1988. Literature review of sunn pest, *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Crop Protection, 17 (4), 271-287. https://doi.org/10.1016/S0261-2194(98)00022-2

Dörtbudak Y., Memişoğlu H., Özkan M., Melan K., Kiliç A.U. 1991. Orta Anadolu Bölgesinde (*Aelia rostrata* Boh.)'ın popülasyon yoğunluğunu etkileyen faktörle, neden olduğu ürün kayıpları ve kimyasal mücadelesi üzerinde araştırmalar. Proje KKGA-B-U3,U/A Nihai Raporu, 93 s. (in Turkish).

Duman M., 2015. Güneydoğu Anadolu Bölgesi'nde süne (*Eurygaster integriceps* Put.,Hem:Scutelleridae)'nin hasat sonrasında göç davranışları, ergin parazitoidlerinin bazı biyoekolojik özellikleri ve moleküler karekterizasyonu. TAGEM-BS-12/12-01/01-01 No'lu Doktora Projesi Sonuç Raporu, 145 s.

Grigorov S., Gospodinov G., 1964. The noxius pentatomid *Eurygaster integriceps* Put. Rastifelna Zashchita, 12 (2), 3-10. (CAB abstracts 1966)

Gözüaçık C., Yiğit A., Şimşek Z., 2016. Predicting the development of critical biological of sunn pest, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), by using sum of degree-days for timing its chemical control in wheat. Turkish Journal of Agriculture and Forestry, 40 (4), 577-582. https://doi.org/10.3906/tar-1511-64

IBM Corp. 2021. IBM SPSS Statistics for Windows (Version 21). SPSS Inc., Chicago, IL, USA

Ionescu C., Mustatea D., 1975. Contrubutii la cunaaşterea unor aspect biologiei si ecologiei plaşnitelor optime de combetera din Romonia. (Contributions to the knowledge of some aspects of the biology and ecology of cereal bugs for forecasting optimum control period in Romania). Analele Institutului de Cercetari pentru Cereale si Plante Tehnice-Fundulea, 11, 119-131.

İleri M., 1957. Türkiye'de süne (*Eurygaster integriceps* Put) ve kımıl (*Aelia rostrata* Boh.) durumu. Tomurcuk, 6 (66), 13-18. (in Turkish).

İslamoğlu M., 2010. Süne (*Eurygaster integriceps* Put.) (Heteroptera: Scutelleridae)'nin bazı kışlama özelliklerinin belirlenmesi ve yumurta parazitoitleri *Trissolcus* spp. (Hymenoptera: Scelionidae)'nin kitle üretiminde kışlayan ergin Süne'nin kullanım olanaklarının araştırılması. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, 179 s., Adana.

Karaca V., Mutlu Ç., Gözüaçik C., Duman M., Şimşek Z., 2009. Güneydoğu Anadolu Bölgesi'nde süne, *Eurygaster integriceps* Put. (Het.: Scutelleridae)'nin kışlak popülasyonu ve bunun mücadele yapılan alanlara olan etkisi. Türkiye III. Bitki Koruma Kongresi Özetleri, 15-18 Temmuz 2009, Van. 73 s. (in Turkish).

Kılıç A.U., Karslıoğlu S., 1961. FAO'nun Rusya'da süne mevzuunda tertiplemiş olduğu iki aylık kursa ait teknik rapor. 23 s. (in Turkish).

Kiliç A.U., 1978. Sunn pest, *Eurygaster integriceps*, in Southeastern Turkey. Wheat pests. Symposium held in Ankara, June 1-3, 1976, at the University of Ankara. CENTO Scientific Programme 1978. Report No: 22, 13-20.

Koçak E., Babaroğlu N., 2005. Orta Anadolu Bölgesi kışlaklarındaki *Eurygaster* (Heteroptera: Scutelleridae) türleri. Türkiye Entomoloji Dergisi, 29, (4), 301-307. (in Turkish).

Lazarov A., Grivanov S., Arabadjiev D., Kontev H., Kaitazov A., Popov V., Gospodinov G., Bogdanov V., Furtunov D., Ducevski V., 1969. Jitnite dirveniti v Bulgaria i borbata s teah. Sofia: Bulgarian Academy of Sciences Press, 141 pp.

Lodos N., 1961. Türkiye, Irak, İran ve Suriye'de süne (*Eurygaster integriceps* Put.) problemi üzerinde araştırmalar (yayılışı, zararları, biyolojisi, parazitleri ve savaşı). Ege Üniversitesi Ziraat Fakültesi Yayınları. No: 51, 115 s. (in Turkish).

Memişoğlu H., 1985. Ankara ilinde süne türlerinin (*Eurygaster* spp.) (Hemiptera: Pentatomidae) yayılışı ve *E. maura* L.'nin biyo-ekolojisi ile savaş yöntemleri üzerinde araştırmalar. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, (Basılmamış) Doktora Tezi,194 s., Ankara. (in Turkish).

Mozaffari Gh.A., Azizian M.S., 2001. A study about wheat sunn pest diffusion according temperature characteristics in Kordestan province. Physical Geography Research Quateryl, 76, 121-135.

Nakova N., Urukov G., 1976. The control of the *Eurygaster integriceps* in the Pleven districts. Rastitelna Zashchita, 24 (2), 17-19. (CAB Abstracts 1976-1978).

Öncüer C., Kivan M., 1995. Tekirdağ ve çevresinde *Eurygaster* Lap. (Heteroptera: Scutelleridae) türleri, tanınmaları, yayılışları ve bunlardan *Eurygaster integriceps* Put.'in biyolojisi ve doğal düşmanları üzerinde araştırmalar. Türk Tarım ve Ormancılık Dergisi, 19 (4), 223-230. (in Turkish).

Özkan M., Babaroğlu N.E., Gökdoğan A., Kan M., Koçak E., 2017. Orta Anadolu Bölgesi'nde buğdayda Avrupa sünesi (*Eurygaster maura* L. Hemiptera: Scutelleridae)'nin neden olduğu ürün kayıpları ve ekonomik zarar eşiğinin belirlenmesi. (abstract in English). Bitki Koruma Bülteni, 57 (2), 137-203. https://doi.org/10.16955/bitkorb.298560

Paulian F., Barbulescu A.L., 1970. Ploşnitele cerealelor biologie pagube şi masuri de combatere. Redactia Revistelor Agricola, Bucureşti, 40 p.

Paulian F., Popov C., 1980. Sunn pest of cereal bug. Wheat Technical Monograph. Giba-Geigy Ltd. Basel, Switzerland, 69-75 p.

Peredelskii A.A., Bucharova O.M., Starovoitova M.R., 1951. Characteristics of the migration of the noxious little tortoise (*Eurygaster integriceps* Put.) from its hibernation sites in the department of Krasnodar. Doklady Akademii Naouk, 77 (3), 499-502.

Popov C., Barbulescu A.L., 1978. Studies on the spring migration of *Eurygaster integriceps* Put. in the period 1965-1976 at Fundulea. Probleme de Protectia Plantelor, 6 (2), 81-104.

Radjabi G., 2000. Ecology of cereals' sunn-pests in Iran. Tehran, Iran, Ministry of Jihad and Agriculture, Agricultural Research, Education and Organization Publication.

Silvestri F., 1934. *Eurygaster maurus* (L.). Compendio di Entomologia Applicata (Agraria- Forestale-Medica-Veterinaria), Vol: 1, 241-242.

Smolyannikov V.V., 1955. Matériaux pour l'écologie de la punaise des cereals *Eurygaster integriceps* [Hemiptera-Heteroptera, Pentatomidae] dans le Pré-Caucase. Entomologichescoe Obozrenie, 34, 88-92 (CAB Abstracts 1957).

Tafaghodinia B., Majdabadi M., 2006. Temperature based model to forecasting attack time of the sunn pest, *Eurygaster integriceps* Put., in wheat fields of İran. Proceedings of the 2006 WSEAS, International Conference on Mathematical, Biology and Ecology. Miami, Florida USA, January 18-20, 2006, 1-3 pp.

Vojdani S., 1954. Contribution a l'etude des punaises cereales et en particulier d'*Eurygaster integriceps* Put. (Heteroptera, Pentatomidae, Scutelleridae). [A contribution to the study of the Hemiptera of cereals and especially of *Eurygaster integriceps* Put.]. Annals des Epiphyties, 2, 105-160.

Yüksel M., Bayseç B., 1964. Mayıs-Haziran-Temmuz aylarında Moskova-Krasnodar ve Leningrad'da yapılan beynelmilel süne seminerine ait Tarım Bakanlığına verilen rapor (5.10.1964 tarih ve 1932 sayılı rapor) (in Turkish).

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Orijinal araştırma (Original article)

Potential of a *Bacillus* spp. consortium as a biological control agent of purple spot (*Alternaria porri* (Ellis) Cif.) and enhancement of shallot growth and production

Bacillus spp. konsorsiyumunun mor leke (*Alternaria porri* (Ellis) Cif.) hastalığının biyolojik kontrol ajanı olarak potansiyeli ve arpacık soğanı büyümesi ve üretiminin artırılması

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ABSTRACT

Purple spot disease caused by *Alternaria porri* poses a significant threat to shallot plants, potentially leading to yield reductions of up to 40%. Addressing this issue is crucial for maintaining agricultural productivity and ensuring food security. One promising approach to controlling purple spot disease is the utilization of *Bacillus* spp. consortium, which offers a cost-effective and environmentally friendly solution. In a recent study, researchers aimed to identify the most effective *Bacillus* spp. consortium for controlling *A. porri* while also enhancing the growth and yield of shallot plants. The study was employed a completely randomized design (CRD) with seven treatments and five replicates. These treatments included various combinations of *Bacillus* strains along with positive and negative controls and a fungicide containing mancozeb 80%. The results of the study demonstrated that the consortium treatment consisting of *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 was the most effective in reducing purple spot disease development. This treatment exhibited a disease incidence of 17.00% and disease severity of 13.33%. Moreover, the consortium treatment significantly promoted the growth and production of shallot plants. Specifically, plants treated with this consortium exhibited a notable increase in plant height, leaf number, and both fresh and dry bulb weights. The enhanced growth parameters included a plant height of 49.83 cm, a number of leaves of 53.33 strands, and fresh and dry bulb weights of 127.08 g and 96.65 g, respectively.

INTRODUCTION

Shallots (*Allium ascalonicum* L.) hold significant importance as a horticultural commodity within Indonesia, being utilized diversely in various sectors such as health (Aryanta 2019), the food industry (Ibrahim and Elihami 2020), and export markets. Over recent years, Indonesia has witnessed a notable increase in shallot productivity, with yields reaching 13.16 tons/ha in 2019, 15.12 tons/ha in 2020, and 18.15 tons/ha in 2021. Similarly, in West Sumatra, productivity surged from 12.02 tons/ha in 2019 to 19.07 tons/ha in 2021 (Anonymous 2021). Despite these advancements, shallot

productivity continues to fall short of the optimal level of 20 tons/ha (Susanti et al. 2018).

The relatively low productivity of shallots can be attributed, in part, to various plant pathogens (Rachmatunnisa et al. 2017). Among the pathogens affecting shallot plants are *Peronospora destructor*, causing feather dew disease; *Colletotrichum gleosporoides*, responsible for anthracnose disease; *Fusarium oxysporum* f. sp. *cepae*, leading to Fusarium wilt disease (Supyani et al. 2021, Yağmur et al. 2024); *Erwinia caratovora* pv. *caratovora*, causing tuber rot disease; *Pantoea ananatis*, inducing bacterial leaf blight disease (Yanti et al. 2021); *Cercospora duddiae*, resulting in leaf spot disease; the Onion yellow dwarf virus, causing onion mosaic disease; and *Alternaria porri*, responsible for purple spot disease (Aldo and Putra 2020).

Purple spot disease poses a significant threat to onion plants, often reducing bulb production (Kim et al. 2022). Uncontrolled infections of *A. porri* in shallots can lead to yield losses of up to 40% (Sutariati et al. 2020). Typical symptoms of an *A. porri* infestation include the development of white spots that evolve into purplish lesions, expanding with a surrounding yellow halo (Hersanti et al. 2019). Control measures against the *A. porri* pathogen have been predominantly cultural and technical, involving environmental management through appropriate cultivation practices (Agastya et al. 2017), mechanical methods such as the removal of infected plant parts (Sumartini 2012), as well as the utilization of resistant cultivars and synthetic fungicides containing propineb (Ruswandari et al. 2020). However, the excessive and continuous use of synthetic fungicides poses environmental and health risks (Bansal 2020), necessitating the exploration of alternative, cost-effective, and eco-friendly control methods such as biological agents (Kim et al. 2022). *Bacillus* spp. have been investigated as potential biological agents for controlling plant pathogens, as they can suppress pathogens and enhance plant growth quality directly or indirectly (Miljaković et al. 2020).

The utilization of Bacillus spp. for controlling plant diseases is typically conducted individually (Miljaković et al. 2020). However, to enhance its effectiveness, Bacillus can be applied in combination by utilizing two or more isolates, a practice known as a consortium (Aiman et al. 2017). A consortium refers to a blend of two or more species of microorganisms that collaborate synergistically to provide various more efficient control mechanisms (Yanti et al. 2021). The objective of the study was to identify the most effective *Bacillus* spp. consortium for the control of *Alternaria porri* while simultaneously promoting shallot growth and increasing production.

MATERIALS AND METHODS

The study was conducted using a completely randomized design (CRD) consisting of seven treatments and five replicates. The bacterial isolates used in this study were obtained from previous exploration research conducted by Yanti et al. (2019). These are individual bacteria that were isolated and characterized in that study, not commercial products. The combinations of Bacillus species used in this study were based on previous research by Yanti et al. (2021). According to Yanti et al. (2021), the combinations were determined through compatibility testing between the bacterial isolates. The compatibility tests enabled the identification of bacterial combinations that could effectively work together without inhibiting each other's growth or antagonistic effects. The specific combinations that passed these compatibility tests are shown in Table 1.

The research was conducted in the microbiology laboratory of the Department of Andalas University and the experimental garden of Andalas University from July 2023 to January 2024.

Table 1. Treatment and consorsium *Bacillus* spp.

Treatment	Accepted
A	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3
B	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain SNE 2.2
C	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain TLE 1.1
D	*Bacillus pseudomycoides* strain EPL 1.1.4 + *Bacillus cereus* strain TLE 2.3 + *Bacillus cereus* strain TLE 1.1 + *Bacillus cereus* strain SNE 2.2
E	Positive control: without *Alternaria porri* inoculation and without *Bacillus* spp. consortium treatment.
F	Negative control: inoculated with *Alternaria porri* and without *Bacillus* spp. consortium treatment.
G	Fungicide: active ingredient mancozeb 80%

Preparation of Bacillus spp.

Bacillus spp. samples were gathered in microtubes, revitalized using the scratch technique on Tryptic Soy Agar (TSA) medium, and cultivated for two consecutive periods of 24 hours each. Following this incubation, they were examined for morphological traits of colony growth. To ensure purity, *Bacillus* spp. isolates underwent verification through Gram staining and hypersensitivity reaction testing.

Isolation and identification of Alternaria porri

The fungal inoculum of *A. porri* was obtained from symptomatic shallot leaves through isolation using the moist chamber method. When symptomatic plants were discovered in the field, they were promptly transported to the laboratory. Subsequently, surface sterilization was performed by immersing the symptomatic plant parts, cut into 1x1 cm^2 pieces, in sterile distilled water and alcohol for 1 minute. These pieces were then placed onto Petri dishes containing sterile Potato Dextrose Agar (PDA) media and incubated for seven days (Wiyatiningsih 2009). Identification of the pathogenic fungi was conducted through both macroscopic and microscopic observations. Macroscopic observation of *A. porri* morphology focused on colony growth, shape, color, and texture, while microscopic examination involved assessing the shape of hyphae and conidia, as well as the color and shape of conidiophores, using a binocular microscope with a magnification of 40x10 (Rachmatunnisa et al. 2017).

Pathogenicity test of Alternaria porri

A Petri dish containing a pure culture of A. porri fungus was filled with 10 ml of sterile distilled water. Subsequently, the fungus was gently crushed using a small sterile brush to release the hyphae and conidia. The resulting suspension, which primarily contained conidia, was transferred to a test tube and homogenized using a vortex. Serial dilutions were performed up to 10^{-3}. The fungal suspension was examined using a Haemocytometer Neubauer Improved, and the conidia density was calculated under a microscope. The required conidia density for the test was 10^7 conidia/ml (Hersanti et al. 2019). The homogenized conidial suspension was then transferred into a sprayer. The pathogenicity test of *A. porri* was conducted on Birma variety shallot plants two weeks after planting. The fungus was inoculated by spraying 5 ml per plant onto shallot leaves that had been wounded with a sterile needle. Observations were subsequently made to determine whether the leaves exhibited symptoms of purple spot disease.

Bacillus spp. complex propagation

Bacillus spp. that were revitalized on TSA media underwent a multiplication process in liquid culture, which comprised two stages—in the initial stage, the pre-culture phase, a single colony of *Bacillus* spp. pure culture was transferred into 25 ml of TSB medium within a culture bottle. Subsequently, this mixture was incubated on a rotary shaker at 150 rpm for 24 hours. Following this, the second stage, referred to as the main culture phase, involved the creation of the Bacillus consortium by combining two compatible Bacillus species. For this stage, 1 ml of liquid culture from each Bacillus species obtained from the pre-culture phase was transferred into 198 ml of sterile coconut water within a culture bottle. The mixture was then incubated for 48 hours on a rotary shaker at 150 rpm, maintaining room temperature. After the incubation period, the concentration of the bacterial suspension used in the experiment was determined by comparing the turbidity of the suspension with a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/ml.

Preparation of planting media

The shallot planting substrate consisted of soil, which was thoroughly cleaned and sifted to remove any impurities, combined with manure sourced from the Animal Husbandry UPT of Universitas Andalas at a ratio of 2 parts soil to 1 part manure (Yanti et al. 2019). This mixture was then filled into transparent, heat-resistant plastic containers with a capacity of 5 kg. The planting medium underwent sterilization in a pot for one hour, after which the soil was allowed to cool for a day. Subsequently, the planting substrate was transferred into polybags.

Bacillus spp. consortium introduction and planting

The introduction of the consortium involved cutting the top one-third portion of the shallot bulb. Subsequently, the cut portion was immersed in the *Bacillus* spp. consortium solution corresponding to the designated treatment for 15 minutes. Following this, two treated bulbs were planted by submerging them into a single polybag with a capacity of 10 kg, filled with soil mixed with manure (Hersanti et al. 2019).

Inoculation of Alternaria porri

The inoculation of the *A. porri* fungus occurs when the shallot plants reach the age of two weeks. The fungus is inoculated by spraying 5 ml of *A. porri* conidia suspension (10^7 conidia/ml) onto the shallot leaves that have been previously wounded with a sterile needle. Subsequently, the wounded leaves are covered with clear plastic for three days (Hersanti et al. 2019). The variables under scrutiny encompassed disease development, which involved assessing the incubation period, disease incidence, and disease severity.

Incubation period (days after planting)

The incubation period was observed after inoculation with *A. porri* on the shallot plants, noting the first appearance of purple spot disease symptoms on the plants.

Disease incidence (%)

Disease incidence was monitored weekly after inoculation until the plants were 8 weeks old. The disease incidence was calculated using the following formula:

$$DI\% = \frac{n}{N} X100\% \quad \text{..... Formula 1}$$

where:

DI%: Percentage of disease incidence

n: Number of leaves infected by A. porri

N: Total number of leaves

Disease severity (%)

Disease severity was assessed weekly after inoculation until the plants were 8 weeks old. The severity of the disease was calculated using the following formula:

$$S = \frac{\Sigma(ni \times vi)}{N \times V} X100\% \quad \text{..... Formula 2}$$

where:

S: Disease severity

ni: Number of plants with the same disease score

vi: Disease score for each plant

N: Total number of sample plants

V: Maximum value of the damage category

Table 2. Scale and degree of infection by *Alternaria porri* (modified from Hersanti et al. 2019)

Scale	Symptoms
0	No symptoms
1	Leaves infected 0 < X < 12%
2	Leaves infected 13% < X < 25%
3	Leaves infected 26% < X < 50%
4	Leaves infected 51% < X < 75%
5	Leaves infected 76% < X < 100%

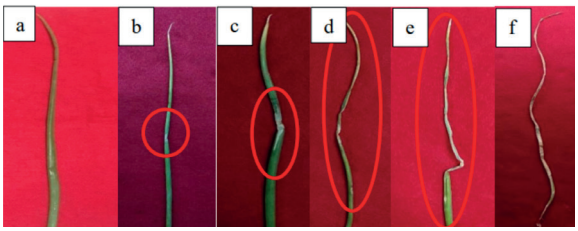

Figure 1. Scale of purple spot disease symptoms on shallots: (a) scale 0, (b) scale 1, (c) scale 2, (d) scale 3, (e) scale 4, (f) scale 5

Shallot plant growth

Number of leaves (count): Counting was performed once a week from 1 week after planting until 6 weeks after planting.

Plant height (cm): Measurement was done once a week from 1 week after planting until 6 weeks after planting. Observations were made from the soil surface to the tip of the highest leaf on the sample plants.

Fresh bulb weight (g): The weight was measured by weighing the shallot bulbs per cluster in each treatment within the polybag. The bulbs were cleaned of any remaining soil, then the leaves at the top of the bulbs were trimmed before weighing.

Dry bulb weight (g): The dry weight was measured after the shallot bulbs from each cluster were air-dried for 14 days, then the bulbs were weighed.

Data analysis

Data were analyzed using analysis of variance (ANOVA). If significant differences were found, further analysis was conducted using Duncan's New Multiple Range Test (DNMRT) at a 5% significance level.

RESULTS

Disease progression

Incubation period (hsi)

Shallot plants treated with a *Bacillus* spp. consortium showed a notable difference in the incubation period of purple spot disease compared to the negative control. Specifically, Consortium D demonstrated a significant difference when compared to the treatment with Consortium B. In contrast, while the treatments with Consortiums C and A did not show significant differences from each other, they were significantly different from both the fungicide (active ingredient: mancozeb 80%) and the negative control (Table 3).

Disease incidence (%)

Disease incidence was monitored weekly after inoculation until the plants were 8 weeks old. The percentage of disease incidence was calculated using the following Formula 1. Shallot plants treated with the *Bacillus* spp. consortium exhibited a notably distinct impact on the incidence of purple spot disease compared to the control group. The negative control exhibited a notable variation between Consortia C and A, while no significant differences was found between the two consortia themselves. Conversely, Consortium B displayed significant distinctions from both the fungicide treatment and Consortium D. Notably, no significant difference was observed between the fungicide treatment of 80% mancozeb and Consortium D (Table 4).

Table 3. The incubation period of purple spot disease in shallot plants introduced with the *Bacillus* spp. consortium

Treatment	Incubation period (the day after inoculation) ± SD
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	9.67 ± 0.57 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	9.00 ± 0.00 b
(C) *B. pseudomycoides* EPL 1.1.4 + B. cereus TLE 2.3 + *B. cereus* TLE 1.1	8.67 ± 0.57 bc
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	8.33 ± 0.57 bc
(G) Fungicide: active ingredient mancozeb 80%	8.00 ± 0.00 cd
(F) Negative control	5.00 ± 0.00 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 4. The incidence of purple spot disease in shallot plants following their exposure to the *Bacillus* spp. consortium

Treatment	Disease incidence (%) ± SD
(F) Negative control	54.73 ± 4.33 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	39.77 ± 1.16 b
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	36.90 ± 1.15 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	28.17 ± 2.28 c
(G) Fungicide: active ingredient mancozeb 80%	20.23 ± 0.63 d
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	17.00 ± 2.64 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level

Disease severity (%)

Disease severity was assessed weekly after inoculation until the plants were 8 weeks old. The severity of the disease was calculated using the following Formula 2. Shallot plants treated with the *Bacillus* spp. consortium exhibited a significantly varied impact on the severity of purple spot disease compared to the control group. Notably, the negative control group exhibited a significant difference when compared to the treatments with Consortiums C and A; however, no significant difference was found between the two consortia. Consortium B showed significant differences when compared to the fungicide treatment and Consortium D. Interestingly, there was no significant difference observed between the fungicide treatment and Consortium D (Table 5).

A comparison of the severity of purple spot disease is shown in Figure 2. The initial symptoms caused by the *A. porri* fungus appear as minor white spots on the leaf surface. Subsequently, with continued infection, the color transitions to a purplish hue, and shallot leaves exhibit curling, accompanied by yellow circles forming along the leaf edges. Severe infections may lead to complete leaf necrosis, thereby delaying bulb maturation.

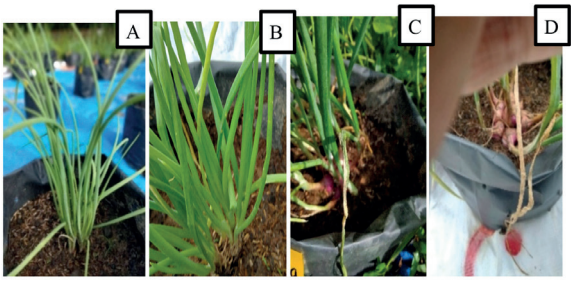

Figure 2. Severity of purple spot disease: (a) shallot leaves without symptoms of purple spot disease, (b) early symptoms of purple spot disease, (c) purple spot disease begin to spread, (d) purple spot disease begins to spread on all parts of the affected leaves, and leaves die

Table 5. The severity of purple spot disease in shallot plants following their exposure to the *Bacillus* spp. consortium

Treatment	Disease Severity (%) ± SD
(F) Negative control	53.20 ± 3.55 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	35.93 ± 2.06 b
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	33.10 ± 1.22 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	26.70 ± 3.11 c
(G) Fungicide: active ingredient mancozeb 80%	17.57 ± 2.41 d
(D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	13.33 ± 0.90 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level

Onion plant growth

Number of leaves (blade)

Onion plants that were introduced to a consortium of *Bacillus* spp. demonstrated a significantly different effect on the number of leaves compared to the control group. Specifically, Consortia D and B showed significant differences when compared to Consortia A and C. However, there were no significant differences among the treatments within the same consortium. Additionally, both Consortia A and C exhibited significant differences when compared to the fungicide and the control group, but again, there were no significant differences among the treatments within the consortia (see Table 6).

Conversely, no significant difference was observed between the fungicide treatment and the control across the treatments (Table 7).

Fresh weight of tuber (g)

Shallot plants introduced with the Bacillus spp. consortium exhibited a notably distinct impact on bulb fresh weight compared to the control group. Notably, Consortium D demonstrated a significantly different effect compared to Consortium A treatment. Furthermore, Consortium A treatment displayed significant differences from the treatments of both Consortium B and C. However, no significant difference was observed between Consortium B and C treatments, although they were significantly different

Table 6. Number of leaves of shallot plants inoculated with *Bacillus* spp. complex

Treatment	Number of leaves (Leaves) ± SD (%)
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	55.33 ± 3.01 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	54.66 ± 3.07 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	51.33 ± 1.36 b
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	51.33 ± 1.36 b
(G) Fungicide: active ingredient mancozeb 80%	35.00 ± 2.09 c
(E) Positive control	33.16 ± 2.31 c

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Plant height (cm)

The consortium exhibited a notably distinct impact on plant height compared to the control group. Interestingly, all *Bacillus* spp. consortia did not demonstrate significant differences amongst themselves. However, they significantly differed from the fungicide treatment and the control. from both the fungicide treatment and the positive control (Table 8).

Tuber dry weight (g)

Shallot plants treated with the Bacillus spp. consortium exhibited a notably distinct impact on the dry weight

Table 7. The plant height measurements of shallots following their exposure to the *Bacillus* spp. consortium

Treatment	Plant Height (cm) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	49.83 ± 4.87 a
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	48.83 ± 2.73 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	48.33 ± 4.95 a
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	47.33 ± 3.98 a
(G) Fungicide: active ingredient mancozeb 80%	38.50 ± 1.37 b
(E) Positive control	35.50 ± 1.87 b

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 8. The weight for shallot bulbs following their exposure to the *Bacillus* spp. consortium

Treatment	Fresh Weight (g) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	127.08 ± 5.91 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	87.52 ± 2.03 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	67.95 ± 1.59 c
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	64.90 ± 1.58 c
(G) Fungicide: active ingredient mancozeb 80%	51.33 ± 1.54 d
(E) Positive control	49.43 ± 1.45 d

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

Table 9. The dry weight measurements for shallot bulbs following their exposure to the *Bacillus* spp. consortium

Treatment	Dry Weight (g) ± SD
D) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2	96.65 ± 0.80 a
(A) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3	81.27 ± 1.37 b
(B) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* SNE 2.2	47.07 ± 1.44 c
(C) *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1	46.43 ± 0.83 c
(G) Fungicide: active ingredient mancozeb 80%	38.43 ± 1.27 d
(E) Positive control	35.45 ± 1.98 e

Numbers followed by identical lowercase letters within the same column do not display significant differences, as determined by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

of the bulbs compared to the control group. Notably, Consortium D demonstrated a significantly different effect compared to Consortium A treatment. Furthermore, the Consortium A treatment displayed significant differences from the treatments of both Consortium B and C. However, Consortium B and C treatments were significantly different from the fungicide treatment (Table 9).

DISCUSSION

The introduction of a *Bacillus* spp. consortium in shallot plants has been found to extend the incubation period, reduce disease incidence, and mitigate disease severity compared to both the negative control and fungicide treatments. Among the various treatments, the consortium consisting of *B. pseudomycoides* EPL 1.1.4, *B. cereus* TLE 2.3,

B. cereus TLE 1.1, and *B. cereus* SNE 2.2 proved to be the most effective. This can be attributed to the utilization of multiple isolates in the consortium, which yields superior outcomes in suppressing the development of purple spot disease. The efficacy of *Bacillus* spp. consortium in acting as a biocontrol agent against the *A. porri* pathogen in shallot plants is underscored by its collaborative action in inhibiting pathogen growth. This notion is supported by Hadi et al. (2021), who suggest that bacterial consortia exhibit enhanced efficacy in plant protection due to their interactive mechanisms that control pathogen intrusion and exert physiological influences.

Utilizing the consortium consisting of *B. pseudomycoides* EPL 1.1.4, *B. cereus* TLE 2.3, *B. cereus* TLE 1.1, and *B. cereus* SNE 2.2 confers an advantage in suppressing plant diseases compared to alternative treatments. This Bacillus consortium is known to produce salicylic acid, which plays a pivotal role in inducing plant resistance (Lugtenberg and Kamilova 2009). Each Bacillus strain harbors unique advantages when incorporated into the consortium, as it facilitates various mechanisms and synergistic effects that effectively suppress plant diseases. Including *B. cereus* and *B. pseudomycoides* in the consortium significantly enhances the production of chitinase enzymes, which help decompose chitin substances and hinder the growth of plant pathogens. This finding is supported by research conducted by Win et al. (2021), demonstrating that a combination of *B. subtilis*, *B. velezensia*, and *Penicillium* sp. resulted in a substantial reduction (60-63%) in disease severity caused by *Fusarium* sp. and *Alternaria* sp. on banana plants. Additionally, Krestini et al. (2020) reported promising outcomes in reducing the intensity of Fusarium wilt disease in garlic plants by employing a consortium comprising *B. subtilis*, *T. harzianum*, *A. chroococcum*, and *P. cepacian*.

Bacillus spp. possess the capability to produce antibiotic compounds and enzymes that serve as signaling molecules, prompting the attacked plant to activate its self-defense mechanisms. Among these enzymes, chitinase, lipoxygenase, and glucanase are notable examples found in plants as part of their self-defense mechanisms against pathogens. This statement is supported by research conducted by Butarbutar et al. (2018), which highlighted that *Bacillus* sp. has the ability to produce chitinase enzymes, fix nitrogen, and solubilize phosphate. These capabilities allow *Bacillus* sp. to outcompete white root fungi for nutrients in plants, thereby suppressing the growth of the fungi. Additionally, Mageshwaran et al. (2022) reported the antagonistic activity of *B. subtilis* against wilt disease in chickpea plants caused by various soil-borne pathogens. Furthermore, studies by El-Kareem et al. (2021) demonstrated that *B. pumilus* effectively mitigated the severity of black rot disease in strawberry plants induced by pathogens such as *F. solani*, *R. solani*, and *Pythium* sp. by 65.3% to 67.3%. Moreover, Djaenuddin et al. (2017) found that *B. subtilis* significantly suppressed the development of the soil-borne pathogenic fungus *R. solani* in corn plants by 63.4%. Additionally, Saputri et al. (2020) reported that *Bacillus* spp. they inhibited dauqan midrib rot disease caused by the pathogen *R. solani* by 56.93% and 51.52% in corn plants.

The introduction of a *Bacillus* spp. consortium to shallot plants has been shown to enhance plant growth and productivity. Among the Bacillus consortia tested, the combination of *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 emerged as the most effective in promoting plant height, leaf count, and bulb weight, with plant height reaching 49.83 cm, leaf count at 55.33 leaflets, fresh bulb weight of 127.08 g, and dry bulb weight of 96.65 g. This enhancement in growth is attributed to the production of Indole Acetic Acid (IAA) by Bacillus, known as a plant growth promoter. Additionally, *Bacillus* spp. contribute to plant growth through the synthesis of phytohormones and siderophores. Rabbe et al. (2019) highlighted that Bacillus spp. can produce siderophores, bacteriocins, and other volatile compounds that stimulate plant growth. This finding is corroborated by Gau et al. (2021), who reported that *B. subtilis* application in shallots increases plant height, leaf count, and fresh bulb weight. Furthermore, Ernita et al. (2016) demonstrated through their research that *B. pumilis* can augment plant height, leaf count, and shallot yield, reaching 15.2 tons/ha.

The application of the consortium comprising *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 exhibited superior efficacy in mitigating the incidence and severity of purple spot disease, resulting in a disease incidence of 17.00% and disease severity of 13.33%. Additionally, this treatment demonstrated notable enhancements in shallot plant growth and production, evidenced by a plant height of 49.83 cm, a total of 53.33 leaves, a fresh bulb weight of 127.08 g, and a dry bulb weight of 96.65 g. In our study, the antagonistic potential of the isolate appeared to be suppressed in consortium D compared to consortium C. This could be attributed to several factors. The increased complexity of consortium D, which includes *Bacillus pseudomycoides* EPL 1.1.4, *Bacillus cereus* TLE 2.3, *Bacillus cereus* TLE 1.1, and *Bacillus cereus* SNE 2.2, might lead to competitive interactions among the strains for resources such as nutrients and space. This competition could reduce the overall antagonistic activity of each strain. Additionally, certain strains may produce antimicrobial compounds or engage in quorum sensing that affects the

antagonistic capabilities of other strains. In consortium D, the inclusion of *Bacillus cereus* SNE 2.2 might have altered the production or effectiveness of these compounds, leading to a diminished antagonistic effect. Furthermore, while consortium D effectively reduced disease incidence and severity, the interactions among the four strains may not have been synergistic, impacting the overall effectiveness. These findings suggest that optimizing the composition of microbial consortia to balance strain interactions could enhance their disease-suppressing capabilities. The use of the consortium *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2 has advantages in suppressing the development of plant diseases compared to other treatments. This is because the Bacillus consortium produces salicylic acid which functions to induce plant resistance (Lugtenberg and Kamilova 2009). Each of the Bacillus used has advantages when introduced as a consortium because the consortium has various mechanisms and synergistic effects that can suppress plant diseases. The use of *B. cereus* and *B. pseudomycoides* produces the chitinase enzyme where the enzyme can decompose chitin, so that the chitin produced by Bacillus is able to inhibit the growth of plant pathogens. This is supported by research by Win et al. (2021) reported that the combination of *B. subtilis*, *B. velezensis* and *Penicillium* sp. was able to reduce the severity of disease from various types of pathogenic fungi in banana plants caused by *Fusarium* sp. and *Alternaria* sp. pathogens by up to 60-63%.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Alternaria porri'nin neden olduğu mor leke hastalığı, arpacık soğanı bitkileri için önemli bir tehdit oluşturmakta ve potansiyel olarak %40'a varan verim kayıplarına yol açmaktadır. Bu sorunun ele alınması, tarımsal verimliliğin sürdürülmesi ve gıda güvenliğinin sağlanması açısından hayati önem taşımaktadır. Mor leke hastalığının kontrolüne yönelik umut vadeden bir yaklaşım, uygun maliyetli ve çevre dostu bir çözüm sunan *Bacillus* spp. konsorsiyumunun kullanılmasıdır. Yakın zamanda yapılan bir çalışmada, araştırmacılar *A. porri*'yi kontrol altına alırken aynı zamanda arpacık soğanı bitkilerinin büyümesini ve verimini de artıran en etkili *Bacillus* spp. konsorsiyumunu belirlemeyi amaçlamıştır. Çalışmada, yedi uygulama ve beş tekerrürden oluşan tamamen tesadüfi tasarım (CRD) kullanılmıştır. Bu uygulamalar, pozitif ve negatif kontrollerin yanı sıra çeşitli *Bacillus* strainlerinin kombinasyonlarını ve %80 oranında mancozeb içeren bir fungisiti içermektedir. Çalışmanın sonuçları, *B. pseudomycoides* EPL 1.1.4 + *B. cereus* TLE 2.3 + *B. cereus* TLE 1.1 + *B. cereus* SNE 2.2'den oluşan konsorsiyum uygulamasının mor nokta hastalığının gelişimini azaltmada en etkili olduğunu göstermiştir. Bu uygulama %17.00 hastalık insidansı ve %13.33 hastalık şiddeti göstermiştir. Dahası, konsorsiyum uygulaması arpacık soğanı bitkilerinin büyümesini ve üretimini önemli ölçüde teşvik etmiştir. Özellikle, bu konsorsiyum ile tedavi edilen bitkiler bitki boyu, yaprak sayısı ve hem taze hem de kuru soğan ağırlıklarında dikkate değer bir artış göstermiştir. Geliştirilmiş büyüme parametreleri arasında 49.83 cm bitki boyu, 53.33 şerit yaprak sayısı ve sırasıyla 127.08 g ve 96.65 g taze ve kuru soğan ağırlığı yer almıştır.

Anahtar kelimeler: *Alternaria porri*, arpacık soğanı, mor leke, konsorsiyum

REFERENCES

Agastya I.M.I., Julianto R.P.D., Amir H., 2017. Teknik pengendalian penyakit antraknosa (patek) di sentra tanaman cabai (*Capsicum annuum* L.) menggunakan pendekatan PHT. Jurnal Akses Pengabdian Indonesia, 1 (2), 28-31.

Aiman U., Tantriati, Sriwijaya B., 2017. Pemberian macam konsorsium bakteri hasil isolasi tumbuhan pantai pada kangkung (*Ipomoea reptans* Poirs.). (abstract in English). Planta Tropika: Jurnal Agrosains (Journal of Agro Science), 5 (1), 1-6. https://doi.org/10.18196/pt.2017.065.1-6

Aldo D., Putra S.E., 2020. Expert system for diagnosis of pests and diseases of shallots using the dempster shafer method. Komputika: Journal of Computer Systems, 9 (2), 85-93. https://doi.org/10.34010/komputika.v9i2.2884

Anonymous, 2021. Agricultural data and information center. Ministry of Agriculture, Ministry of Agriculture of the Republic of Indonesia, Central Bureau of Agricultural Statistics, Jakarta Central Java Agriculture.

Aryanta I.W.R., 2019. Bawang merah dan manfaatnya bagi kesehatan (abstract in English). Jurnal Widya Kesehatan, 1 (1), 29-35. https://doi.org/10.32795/widyakesehatan.v1i1.280

Butarbutar R., Marwan H., Mulyati S., 2018. Eksplorasi *Bacillus* spp. dari rizosfer tanaman karet (*Hevea brasilliensis*)

dan potensinya sebagai agens hayati jamur akar putih (*Rigidoporus* sp.). Jurnal Agroecotania, 1 (2), 31-41.

Djaenuddin N., Nonci N., Muis A., 2017. Efektivitas formula *Bacillus subtilis* TM4 untuk pengendalian penyakit pada tanaman jagung. (abstract in English). Jurnal Fitopatologi Indonesia, 13 (4), 113-118. https://doi.org/10.14692/jfi.13.4.113

Bansal O.P., 2020; Impact of pesticides on human and environment: A review. Egyptian Scientific Journal of Pesticides, 6 (1), 1–7.

El-Kareem F.A, Elshahawy I.E., Abd-elgawad M.M.M., 2021 Application of *Bacillus pumilus* isolates for management of black rot disease in strawberry. Egyptian Journal of Biological Pest Control, 31, 25. https://doi.org/10.1186/s41938-021-00371-z

Ernita M., Zahanis, Jamilah., 2016. Aplikasi rizobakteri dalam meningkatkan pertumbuhan, hasil dan ketahanan pada tanaman bawang merah. (abstract in English). Jurnal Pengabdian Kepada Masyarakat, 22 (3),131-134.

Gau A.D.T., Syam'um E., Ulfa F., 2021. Application of *Bacillus subtilis* on red onion (*Allium ascalonicum*). IOP Conference Series: Earth and Environmental Science, 921: 012078. doi: 10.1088/1755-1315/921/1/012078

Hadi A.E., Khalisha A., Pambudi A., Effendi Y., 2021. Potential of bacteria consortium as growth controller of pathogenic fungi *Fusarium oxysporum* f.sp. *cubense* (Foc). IOP Conference Series: Earth and Environmental Science, 637 (1), 1-11.

Hersanti H., Sudarjat S., Damayanti A., 2019. Kemampuan *Bacillus subtilis* dan *Lysinibacillus* sp. dalam silika nano dan serat karbon untuk menginduksi ketahanan bawang merah terhadappenyakit bercak ungu (*Alternaria porri* (Ell.) Cif). (abstract in English). Jurnal Agrikultura, 30 (1), 8-16. https://doi.org/10.24198/agrikultura.v30i1.22698

Ibrahim I., Elihami E., 2020. Pembuatan bawang goreng raja di kabupaten enrekang. (abstract in English). Maspul Journal of Community Empowerment, 2 (2), 6-17. e-ISSN: 2716-4225. https://ummaspul.e-journal.id/pengabdian/article/view/766

Krestini E.H., Rusmawati U., Susilawati A., 2020. Effectiveness of microbial consortium on growth, yield, and intensity of withered disease (F*usarium oxysporum* Schelecht) on garlic plants. ICWEB 2019, BIO Web of Conferences, 20, 4 p. https://doi.org/10.1051/bioconf/20202003009

Lugtenberg B., Kamilova F., 2009. Plant growth promoting rhizobacteria: bacteria that cause indirect plant growth promotion or biological control. Annual Review of Microbiology, 63, 541-56. doi: 10.1146/annurev.micro.62.081307.162918

Mageshwaran V., Gupta R., Singh S., Sahu P.K., Singh U.B., Chakdar H., 2022. Endophytic *Bacillus subtilis* antagonizes soil-borne fungal pathogens and suppresses wilt complex disease in chickpea plants (*Cicer arietinum* L.). Frontiers in Microbiology, 13:994847. doi: 10.3389/fmicb.2022.994847

Miljaković D., Marinković J., Balešević-Tubić S., 2020. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms, 8 (7), 1037. https://doi.org/10.3390/microorganisms8071037

Kim M.Y., Han J.W., Dang Q.L., Kim J-C., Kim H., Choi G.J., 2022. Characterization of *Alternaria porri* causing onion purple blotch and its antifungal compound magnolol identified from *Caryodaphnopsis baviensis*. PLoS ONE, 17 (1): e0262836. https://doi.org/10.1371/journal.pone.0262836

Rabbe M.F., Ali M.D.S., Choi J., Hwang B.S., Jeong S.C., Baek K.H., 2019. *Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes. Molecules, 24 (6), 1046 https://doi.org/10.3390/molecules24061046.

Rachmatunnisa R., Rukmi I., Pujiyanto S., 2017. Aktivitas antagonistik kapang endofit duwet (*Syzygium cumini* (L.) skeels) terhadap *Alternaria porri* penyebab bercak ungu pada bawang merah (*Allium ascalonicum* L.) secara *in-vitro*. (abstract in English). Jurnal Akademika Biologi, 6 (1), 71-78.

Ruswandari V.R., Syauqi A., Rahayu T., 2020. Uji antagonis jamur *Trichoderma viride* dalam menghambat pertumbuhan jamur patogen *Alternaria porri* penyebab penyakit bercak ungu pada tanaman bawang merah (*Allium ascalonicum* L.): Fungi antagonism test of *Trichoderma viride* in inhibiting growth pathogenic fungi of *Alternaria porri* that causes of the purple spot on shallots (*Allium ascalonicum* L.). Jurnal Ilmiah Biosaintropis (Bioscience-Tropic), 5 (2), 84–90. https://doi.org/10.33474/e-jbst.v5i2.255

Saputri A., Soesanto L.E., Mugiastuti A., Umayah, Sarjito A., 2020. Eksplorasi dan uji virulensi bakteri *Bacillus* sp. endofit jagung terhadap penyakit busuk pelepah jagung. (abstract in English). Jurnal Ilmu-Ilmu Pertanian Indonesia, 22 (2), 70-78. https://doi.org/10.31186/jipi.22.2.70-78

Sumartini. (2012). Penyakit tular tanah (*Sclerotium rolfsii* dan *Rhizoctonia solani*) pada tanaman kacang-kacangan dan umbi-umbian serta cara pengendaliannya. (abstract in English). Jurnal Litbang Pertanian, 31 (1), 27-34.

Supyani S.H.P., Supriyadi F.I.P., Putri D.H., Putri D.T., Hadiwiyono, 2021. Disease intensity of moler and yield

losses of shallot cv. Bima caused by *Fusarium oxysporum* f.sp. cepae in Brebes Central Java. IOP Conference Series: Earth and Environmental Science, 905 (1), 11-16. https://doi.org/10.1088/1755-1315/905/1/012049

Susanti H., Budiraharjo K., Handayani M., 2018. Analisis pengaruh faktor-faktor produksi terhadap produksi usahatani bawang merah di kecamatan Wanasari kabupaten Brebes. (abstract in English). Agrisocionomics: Jurnal Sosial Ekonomi dan Kebijakan Pertenian, 2 (1), 23-30. https://doi.org/10.14710/agrisocionomics.v2i1.2673

Sutariati G.A.K., Khaeruni A., Madiki A., 2020. Bakteri asal Wakatobi menghambat pertumbuhan koloni *Alternaria porri* dan *Fusarium oxyporum* penyebab penyakit pada bawang merah secara *in vitro*. (abstract in English). Jurnal Fitopatologi Indonesia, 16 (3), 105-111). https://doi.org/10.14692/jfi.16.3.105-111

Win T.T., Bo B., Malec P., Fu P., 2021. The effect of a consortium of *Penicillium* sp. and *Bacillus* spp. in suppressing banana fungal diseases caused by *Fusarium* sp. and *Alternaria* sp. Journal of Applied Microbiology, 131 (4), 1890-1908. doi: 10.1111/jam.15067

Wiyatiningsih S., 2009. Etiologi penyakit moler pada bawang merah. UPN University Press. Surabaya.

Yağmur A., Demir S., Canpolat S., Danesh Y.R., Farda B., Djebaili R., Pace L., Pellegrini M., 2024. Onion Fusarium basal rot disease control by arbuscular mycorhizal fungi and *Trichoderma harzianum*. Plants, 13, 386. https://doi.org/10.3390/plants1303038.

Yanti Y., Arneti N.M., 2019. Characterization of biocontrol ability of indigenous endophytic bacteria for the control of *Ralstonia syzygii* subsp. *indonesiensis* in chili. National Seminar in the framework of the 43rd Anniversary of UNS 2019, 3 (1).

Yanti Y., Hamid H.R., 2021. Development of the PGPR and cyanobacteria consortium for growth promotion and control of *Ralstonia syzigii* subsp. *indonesiensis* of tomato. IOP Conference Series: Earth and Environmental Science, 709, 1-11. doi:10.1088/1755-1315/709/1/012085

Yanti Y., Hamid H., Yaherwandi R., 2021. Biological control of *Sclerotium rolfsii* on tomato seedlings using *Bacillus* spp. consortium. Earth and Environmental Science, 741 (1), 1-5. doi: 10.1088/1755-1315/741/1/012063

Yanti Y., Hamid H., Yaherwandi R., Warnita, Habazar T., 2020. The ability of indigenous *Bacillus* spp. consortia to control the anthracnose disease (*Colletrotricum capsici*) and increase the growth of chili plants. Biodiversity, 21 (1), 179-186. doi: 10.13057/biodiv/d210123

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Orijinal araştırma (Original article)

Management of Egyptian broomrape [*Phelipanche aegyptiaca* (Pers.) Pomel] using biofungicide (*Trichoderma* spp.) in tomato

Domateste biyofungisit (*Trichoderma* spp.) kullanılarak Mısırlı canavar otu [*Phelipanche aegyptiaca* (Pers.) Pomel]'nun mücadelesi

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ABSTRACT

Phelipanche aegyptiaca (Pers.) Pomel, commonly known as Egyptian broomrape, is a root parasitic plant that causes significant yield losses in tomato production. This study aimed to evaluate the efficacy of a commercial bioproduct containing *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain ICC080, against *P. aegyptiaca* in tomato. The experiment was conducted under controlled greenhouse conditions using three concentrations of the bioproduct (N: recommended dose, N/2, and 3N/2) in a randomized block design with four replications. Applications were made in two programs: Program A (one week before and one day after planting) and Program B (an additional application 15 days after planting). Results showed that the biological control agent (BCA) applications significantly increased disease severity in *P. aegyptiaca* shoots, with the values ranging from 69.5% to 79.6%, compared to 40% in the control. The number of shoots exhibiting the highest scale value on necrotic area increased significantly from three in the control to 17-19.5 in BCA-treated pots. Additionally, the average number of dead tubercles on tomato roots was 32 in BCA-treated pots, compared to 16 in untreated controls. The fresh weight of BCA-treated broomrape shoots was significantly different from the control, while there was no significant difference in the dry weight of the shoots among treatments. BCA did not significantly alter the weights of aerial parts and the roots of tomato compared to the control. These results suggest that *Trichoderma* species can control broomrape through multiple mechanisms. Further field trials are recommended to validate these findings under natural conditions.

INTRODUCTION

Broomrape species (*Phelipanche* spp. and *Orobanche* spp.) are obligate parasitic plants that establish direct connections with the vascular systems of their host plants, causing significant economic losses in various crops (Dörr and Kollmann 1995). Among these, *Phelipanche aegyptiaca* (Pers.) Pomel (Egyptian broomrape) is particularly damaging to Solanaceous crops, including tomato, with yield losses ranging from 5% to 100% depending on

infestation density and environmental conditions (Joel et al. 2013, Parker 2009). In Türkiye, where tomato production accounts for approximately 7% of global output, ranking it as the world's third-largest producer after China and India (FAOSTAT 2022), broomrape-induced yield losses have been reported at 24% (Aksoy and Uygur 2008).

The life cycle of broomrape enters the parasitic phase when the plant establishes a connection to the host's vascular system via the haustorium, enabling it to extract water and nutrients. This parasitic relationship severely compromises the host plant's growth and productivity, leading to significant yield losses (Yoshida et al. 2016). Following attachment, the broomrape develops a tubercle, which serves as the foundation for the emergence of shoots. These shoots eventually break through the soil surface, flower, and produce seeds, completing the life cycle (Rispail et al. 2007).

Conventional control methods are often ineffective or economically unfeasible, prompting the exploration of biological control strategies. *Trichoderma* spp. are widely recognized as effective biocontrol agents, playing a significant role in suppressing plant pathogens (Shoresh et al. 2010). *Trichoderma* spp. exhibit a broad spectrum of biocontrol activity, effectively managing a wide range of foliar (Elad 2000), root (Amira et al. 2017), and fruit pathogens (Li et al. 2025), as well as invertebrates such as nematodes (Poveda et al. 2020). They are known for their ability to antagonize plant pathogens, induce systemic resistance, and enhance plant growth and development, making them effective biological control agents (Harman et al. 2004, Howell 2003, Vinale et al. 2008). Additionally, *Trichoderma* species have been shown to mitigate a wide range of abiotic stresses, such as drought (Shukla et al. 2012), salinity (Rawat et al. 2011), extreme temperatures (Montero-Barrientos et al. 2010), and cold stress (Afrouz et al. 2023). They are widely used in vegetable crops and the most useful strains exhibit the ability to colonize plant roots, a feature known as 'rhizosphere competence' (Harman et al. 2004). For instance, *Trichoderma gamsii* has been shown to enhance systemic resistance in crops, such as maize, against fungal pathogens like *Fusarium verticillioides* (Galletti et al. 2020), while long-term field studies have demonstrated the efficacy of *T. asperellum* and *T. gamsii* in protecting perennial crops (Di Marco et al. 2022). Additionally, *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 strains have been reported to up-regulate key defense-related genes, such as *pr1*, *sod*, *pgip2*, and *pal1*, in wheat, enhancing resistance against *Fusarium* pathogens (Cesarini et al. 2025).

Recent studies have also highlighted the potential of *Trichoderma* species in controlling parasitic weeds. *Trichoderma harzianum* significantly inhibits *Striga hermonthica* seed germination and haustorium initiation, while also enhancing host plant growth, highlighting its potential as a biocontrol agent against parasitic weeds (Azarig et al. 2020). Studies have shown that *Trichoderma viride* can significantly influence *S. hermonthica* seed germination, with higher concentrations (75%) completely inhibiting germination, while also enhancing the growth and vigor of millet varieties, such as increased shoot length, root length, and dry weight (Hassan et al. 2013). For example, *T. asperellum* significantly reduces the germination of *Orobanche cumana* seeds in sunflower (Maširević et al. 2014), while *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) significantly enhance growth parameters in faba bean plants, such as shoot length, shoot fresh weight, shoot dry weight, and leaf number, even in the absence of *Orobanche crenata* infection, demonstrating their potential as effective bio-control agents (El-Dabaa and Abd-El-Khair 2020). Recent studies have also demonstrated that *T. harzianum* suppresses *Phelipanche ramosa* infestation in tomatoes by inhibiting tubercle formation and enhancing antioxidant defenses (Fidan and Tepe 2024). Furthermore, different application methods of *Trichoderma* culture filtrates, such as foliar sprays and soil drenches, have been shown to significantly reduce *P. aegyptiaca* infection in tomatoes. For instance, foliar application of *T. virens* reduced the number of aboveground stalks and underground juveniles by 83% and 66%, respectively, while increasing tomato fruit fresh and dry weights by 86% and 90%. Similarly, soil drench application of *T. brevicompactum* reduced the fresh and dry weights of *P. aegyptiaca* stalks and juveniles by 77%, 52%, 75%, and 49%, respectively (Jalali et al. 2024). Despite the demonstrated efficacy of *Trichoderma* species against various parasitic weeds, the potential of *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080, formulated in a commercial product, to control *P. aegyptiaca* in tomato has not been previously investigated.

The aim of this study is to evaluate the efficacy of *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080, present in a commercial formulation, against *P. aegyptiaca* in tomato under *in vivo* conditions.

MATERIALS AND METHODS

Plant material

The experiment was conducted using the commonly grown commercial tomato (*Solanum lycopersicum* cv. Bizimköy F1) variety. The seeds of *P. aegyptiaca* used in the experiments were obtained from a prior study (Cignitas and Kitis 2022).

Biological Control Agent (BCA)

The commercial biological fungicide was used *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain

ICC080 at a concentration of 10^8 CFU/g, presented in a wettable powder (WP) formulation. It is registered for use at a rate of 300 g/da for root rot fungal pathogens (*Fusarium oxysporum*, *Macrophomina phaseolina*, *Pythium* spp., *Rhizoctonia solani*) in strawberry, and 250 g/da for root rot fungal pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium* spp.) in pepper, and for root rot fungal pathogens (*Pythium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Fusarium* spp.) in tomato.

Preparation of the BCA inoculum

The BCA suspensions were prepared at three different concentrations (N: recommended dose, N/2, 3N/2). An amount of 250 g (registered dose for pepper and tomato) and 300 g (registered dose for strawberry) of the BCA formulation was dissolved in 3 liters of sterile distilled water for N dose. The other doses, N/2 and 3N/2, were adjusted by calculating according to recommended dose. To stimulate the sporulation, the suspensions were placed on a circular shaker for 24 hours at room temperature (25 ± 1°C) before applying the treatments.

Greenhouse experiment

The experiment was conducted in a greenhouse at Batı Akdeniz Agricultural Research Institute (BATEM), Antalya province in Türkiye, during the fall season in 2023. The *P. aegyptiaca* seeds (20 mg/kg soil) were blended with sterilized soil mixture of silt: peat: perlite (1: 1: 1) in 3-liter plastic pots. Thirty-day-old tomato seedlings were used for the host plant of the broomrape. The BCA applications were applied at 100 ml per seedling and performed in two programs: A, B. Program A included two different application times: the first one is seven days before planting the seedlings, and second is one day after planting. Program B included a third one in addition to these two applications, which is 15 days after planting the seedlings. As a negative control, the pots left without BCA, and as a non-infested control, the pots contained neither BCA nor broomrape seeds. The experiment was set up with four replications, with each replication consisting of one plant per pot, under 14 h natural light at 23 ± 1 °C. The study was conducted as a randomized complete block design. The experimental design was shown in Table 1.

Assessment of the experiment

Ninety days after planting the seedlings, the shoots and tubercles of the broomrapes, as well as the roots and aerial parts of the tomato plants, were carefully harvested to evaluate efficacy parameters (Figure 1). Re-isolation of the BCA was performed from the infected parts of the broomrape, fulfilling Koch's postulates.

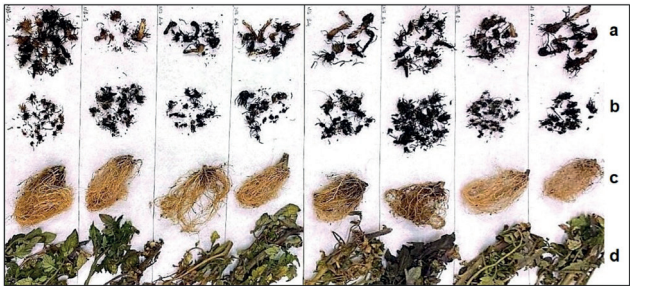

Figure 1. Harvested parts of the Egyptian broomrape and tomato plants. Shoots (a) and tubercles (b) of the *Phelipanche aegyptiaca*, roots (c) and aerial parts (d) of the tomato

Assessment of the effect of the BCA on broomrape

To evaluate the effect of the BCA on the shoots, the necrotic area was scored from 0 to 4 (Figure 2). Based on the scales, percentages of disease severity (DS) were calculated for each treatment according to Townsend-Heuberger formula:

$$DS\ (\%)=\sum((n\times v))/((Z\times N))\times 100$$

Table 1. The experimental design of the applications

BLOCKS			
I	II	III	IV
3N/2 A	Negative control	N A	N/2 B
N A	N/2 A	Non-infested control	Negative control
N/2 A	3N/2 A	N/2 B	N B
Non-infested control	N/2 B	3N/2 B	N/2 A
3N/2 B	Non-infested control	3N/2 A	N A
N B	3N/2 B	N/2 A	3N/2 A
N/2 B	N A	Negative control	Non-infested control
Negative control	N B	N B	3N/2 B

where *n* is the number of shoots in the disease scale, *v* is a numerical value of the disease score, *Z* is the highest score value, and *N* is the total number of shoots (Townsend-Heuberger 1943).

Figure 2. Scale used for evaluation of necrotic area of *Phelipanche aegyptiaca* shoots. 0=Healthy shoot, 1=1-25% necrosis of shoot, 2=26-50% necrosis of shoot, 3=51-75% necrosis of shoot, 4=76-100% necrosis of shoot, dead

To evaluate the effect of the BCA on the tubercles, the average number of healthy and dead tubercles was calculated in per pot both with and without BCA treatments. Tubercles that showed blackening caused by necrosis were considered as dead.

Assessment of the effect of the BCA on biomass

Fresh and dry weights of the parts of broomrape and tomato plant were measured to evaluate the effect of the BCA treatments on biomass. After evaluating regarding necrosis parameters, the shoots and tubercles were weighed, subsequently incubated at 65 °C for 48 h, and then weighed again. The process was also performed for the roots and aerial parts of tomato plants.

Statistical analysis

The data were subjected to ANOVA analysis using the SAS statistical software (SAS Institute, Cary, NC, USA). Differences between treatments were determined using Tukey's test at $P < 0.05$.

RESULTS

Re-isolation of the BCA

As a result of BCA re-isolation, morphological and microscopic observations were confirmed that the causal agent was *Trichoderma* spp. (Figure 3).

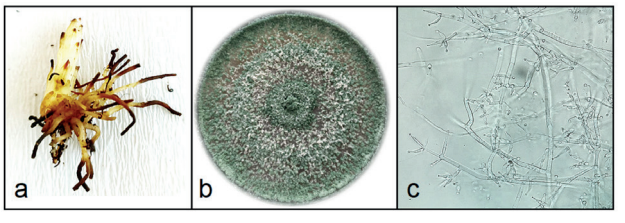

Figure 3. Necrotic roots of the *Phelipanche aegyptiaca* shoots (a), fourteen-day-old *Trichoderma* spp. culture on PDA (b), mycelial structures of the *Trichoderma* spp. at 20x magnification (c)

Effects of the BCA on broomrape

According to the evaluation of the shoots, DS (%) values in the pots treated with BCA were significantly different from the control. The DS values ranged from 69.5% to 79.6% across treatments (Figure 4). There was no statistical difference between the doses and the programs.

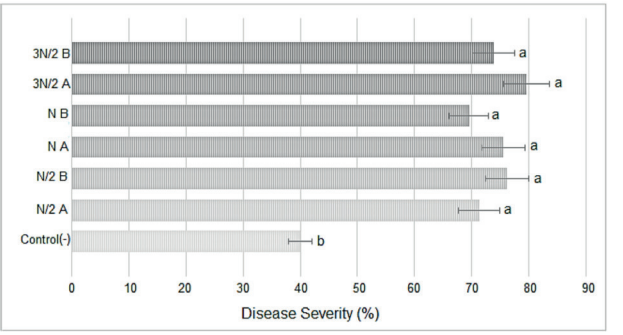

Figure 4. Disease severity (%) values of the treatments

The number of the shoots scored as 0 was 12 in the control, while none of the BCA-treated shoots were scored with 0. As expected, the number of the shoots treated with BCA in N, N/2 and 3N/2 doses peaked at scale 4, while for the control, this scale value was lowest, with the values of 19.5, 17, 18.5 and 3 shoots per pot, respectively (Figure 5).

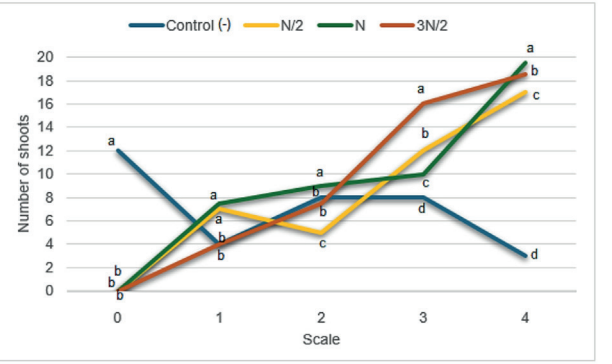

Figure 5. Number of the shoots per pot in treatments on a scale of 0-4

There was a significant difference both with and without BCA in terms of healthy and dead tubercles. Average number of healthy and dead tubercles in the non-treated and BCA-treated BCA pots was 2, 16 and 1.3, 32.1 per pot, respectively (Figure 6).

Effect of the BCA on biomass of broomrape and tomato plants

Fresh weight of the shoots treated with BCA was significantly different from the control, ranging from 3.80 g to 5.71 g. Dry weight of the shoots treated with BCA ranged from 0.56 g to 0.73 g, and there was no difference between any treatments. On the other hand, mean fresh weight of the tubercles

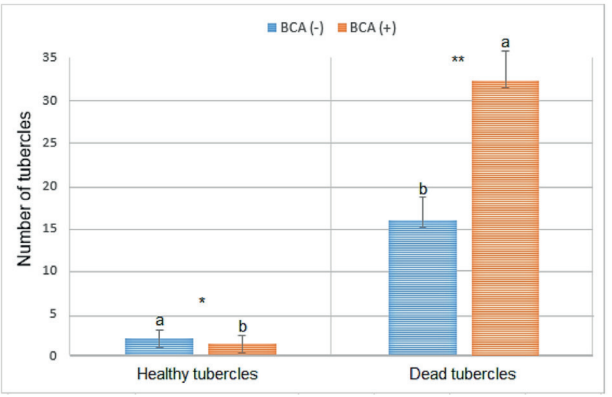

Figure 6. Average number of healthy and dead tubercles per pot in the treatments

ranged from 1.98 g to 4.30 g, and dry weight of the tubercles ranged from 0.29 g to 0.54 g in pots treated with BCA (Table 2).

Fresh weight of the roots of BCA-treated plants ranged from 2.91 g to 4.53 g, with no significant difference from the negative control. Dry weight of the roots of these treatments ranged from 0.54 g to 0.59 g. On the other hand, fresh weight of the aerial parts of BCA-treated plants ranged from 12.7 g to 16.6 g, and dry weight ranged from 1.89 g to 3.32 g. There was no significant difference between the BCA-treated and negative control pots. Non-infested control treatment had the highest value in terms of all the parameters (Table 3).

DISCUSSION

The results showed that applying the biological control agent (BCA) containing *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 significantly increased disease severity (DS) in *Phelipanche aegyptiaca* shoots on tomato plants, with DS values ranging from 69.5% to 79.6% compared to 40% in the control. All treatments nearly doubled the disease severity in *P. aegyptiaca* shoots compared to the control. Furthermore, while the number of shoots naturally exhibiting the highest disease severity (scale 4) averaged 3 in the control, this number significantly increased to 17-19.5 in pots treated with the BCA. These findings indicate that BCA application not only enhances disease severity but also substantially increases the proportion of severely affected

Table 2. Effect of the BCA on biomass of shoot and tubercle of *Phelipanche aegyptiaca*

Treatment	Shoots		Tubercles	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Negative control	7.38 a ± 0.97	0.94 a ± 0.09	1.54 a ± 1.45	0.18 b ± 0.13
N/2 A	5.55 ab ± 2.17	0.73 a ± 0.39	2.73 a ± 2.31	0.34 ab ± 0.20
N/2 B	5.71 ab ± 1.19	0.71 a ± 0.08	2.59 a ± 1.12	0.42 ab ± 0.10
N A	4.31 b ± 1.04	0.56 a ± 0.14	3.50 a ± 2.61	0.38 ab ± 0.17
N B	3.80 b ± 2.64	0.64 a ± 0.49	1.98 a ± 1.10	0.29 ab ± 0.16
3N/2 A	4.82 b ± 1.23	0.67 a ± 0.27	2.09 a ± 1.47	0.34 ab ± 0.16
3N/2 B	4.49 b ± 0.86	0.60 a ± 0.13	4.30 a ± 2.90	0.54 a ± 0.26

Mean ± standard deviation (n = 4).
Means with the same letter are not significantly different from each other (Tukey's test, $P < 0.05$).

Table 3. Effect of the BCA on biomass of root and aerial parts of tomato

Treatment	Shoots		Tubercles	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Non-infested control	6.56 a ± 0.43	0.68 a ± 0.11	25.6 a ± 0.62	4.56 a ± 0.11
Negative control	4.13 b ± 1.31	0.60 a ± 0.16	11.7 b ± 0.99	1.93 b ± 0.24
N/2 A	3.34 b ± 1.26	0.57 a ± 0.09	12.7 b ± 1.67	1.89 b ± 0.51
N/2 B	4.13 b ± 0.49	0.59 a ± 0.13	15.1 b ± 5.81	1.89 b ± 0.63
N A	4.53 b ± 0.12	0.55 a ± 0.04	16.6 b ± 3.47	2.66 b ± 0.31
N B	2.91 b ± 0.40	0.57 a ± 0.19	13.3 b ± 3.50	3.32 b ± 0.33
3N/2 A	4.17 b ± 0.32	0.54 a ± 0.05	14.1 b ± 1.30	2.13 b ± 0.40
3N/2 B	3.48 b ± 0.67	0.57 a ± 0.02	13.2 b ± 1.31	2.25 b ± 0.14

Mean ± standard deviation (n = 4).
Means with the same letter are not significantly different from each other (Tukey's test, $P < 0.05$).

shoots and inhibits tubercle formation. When the tubercles formed on the host root were examined, the average number of dead tubercles was 32 in pots with BCA application, compared to 16 in those without BCA. This suggests that after *P. aegyptiaca* attaches to the host root, *Trichoderma* species likely trigger the plant's defense responses at an early stage, leading to necrosis in the tubercles and preventing successful parasitism. These results highlight the potential of BCA in disrupting both the shoot development and tubercle formation of *P. aegyptiaca*, contributing to its overall suppression. In support of these findings, Azarig et al. (2020) investigated the effects of *T. harzianum* on the early developmental stages (seed germination) and incidence of *S. hermonthica*. They reported that *T. harzianum* reduced *S. hermonthica* seed germination and incidence by approximately 50% compared to the control. These findings align with previous studies showing that *Trichoderma* species can effectively reduce parasitic weed infestation by inhibiting tubercle formation and enhancing host plant resistance (Fidan and Tepe 2024, Maširević et al. 2014).

The observed effects may be attributed to the BCA's ability to induce systemic resistance in tomato plants, as evidenced by the up-regulation of defense-related genes such as *pr1*, *sod*, *pgip2*, and *pal1* (Cesarini et al. 2025). This mechanism is consistent with previous findings that *Trichoderma* species can enhance plant defense mechanisms against various pathogens and parasitic weeds (Galletti et al. 2020, Howell 2003). Additionally, the ability of *Trichoderma* strains to colonize plant roots and exhibit rhizosphere competence (Harman et al. 2004) likely contributed to their effectiveness in reducing *P. aegyptiaca* infestation.

On the other hand, the results indicated that the BCA did not significantly alter the weights of tomato shoots and roots compared to the control in the 90-day pot experiment. However, under field conditions, it is likely that the BCA applications will reduce the number of tubercles and shoots through enhanced parasitism, thereby improving the growth parameters of tomato plants. Biomass of reproductive tissues, which is one of the most important growth parameters for crops, is negatively affected by the presence of *P. aegyptiaca* (Fernández-Aparicio et al. 2016). Furthermore, other studies have also demonstrated that *Trichoderma* species can improve plant growth and development, even under parasitic weed pressure (El-Dabaa and Abd-El-Khair 2020, Hassan et al. 2013). For instance, *T. viride* has been shown to enhance the growth and vigour of millet varieties by increasing shoot length, root length, and dry weight (Hassan et al. 2013), while *T. harzianum* has been reported to suppress *P. ramosa* infestation in tomatoes by inhibiting tubercle formation and enhancing antioxidant defenses (Fidan and Tepe 2024).

Despite these promising results, it is important to note that this study was conducted under controlled conditions, which may not fully replicate field environments. The lack of significant differences in some growth parameters between BCA-treated and control plants suggests that further optimization of application methods and dosages may be necessary. Future studies should investigate the long-term effects of BCA applications under field conditions and explore the potential synergistic effects of combining BCA with other biocontrol agents, such as mycorrhiza or plant growth-promoting rhizobacteria. Additionally, the impact of environmental factors, such as soil type and climate, on the efficacy of *Trichoderma* strains should be evaluated.

In conclusion, the findings demonstrated that BCA formulation containing *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 has potential as an effective bioproduct against *P. aegyptiaca*, offering a sustainable solution for managing parasitic weeds in tomato production. The ability of these strains to reduce parasitic weed infestation underscores their value in integrated pest management systems. Further research is needed to fully exploit their potential and develop practical applications for sustainable agriculture.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Mısırlı canavar otu olarak bilinen *Phelipanche aegyptiaca* (Pers.) Pomel, domates üretiminde önemli verim kayıplarına neden olan kök paraziti bir bitkidir. Bu çalışmada, *Trichoderma asperellum* ICC012 ve *Trichoderma gamsii* ICC080 suşlarını içeren ticari bir biyolojik ürünün domateste canavar otu üzerindeki etkinliği değerlendirilmiştir. Deneme, ürünün üç farklı konsantrasyonunda (N: tavsiye dozu, N/2 ve 3N/2) uygulanmasıyla, tesadüf blokları deneme desenine göre dört tekerrürlü olarak kurulmuş ve kontrollü sera koşullarında yürütülmüştür. Uygulamalar iki programda gerçekleştirilmiştir: Program A (fide dikimden bir hafta önce ve bir gün sonra) ve Program B (fide dikiminden 15 gün sonra ek bir uygulama). Sonuçlar, biyolojik kontrol ajanı (BCA) uygulamalarının *P. aegyptiaca* sürgünlerinde hastalık şiddetini önemli ölçüde artırdığını, bu değerlerin kontroldeki %40'a kıyasla %69.5 ile %79.6 arasında değiştiğini göstermiştir. Nekrotik alandaki en yüksek skala değerini alan sürgün sayısı, kontrolde ortalama üç iken BCA uygulaması yapılan saksılarda ortalama 17-19.5 ile önemli ölçüde artmıştır. Ayrıca, BCA uygulanan saksılarda ölü tüberküllerin ortalama sayısı 32 iken,

uygulama yapılmayan saksılarda 16 olarak belirlenmiştir. BCA uygulanan canavar otu sürgünlerinin yaş ağırlığı kontrole göre önemli bulunurken, kuru sürgün ağırlığında hiçbir uygulama arasında önemli bir fark bulunmamıştır. Domates bitkisinin büyüme parametrelerinde kontrole kıyasla önemli bir fark gözlemlenmemiştir. Bu sonuçlar, *Trichoderma* türlerinin çoklu mekanizmalarla canavar otunu kontrol edebileceğini düşündürmektedir. Bu bulguların doğal koşullarda doğrulanması için saha denemelerinin yapılması gerekmektedir.

Anahtar kelimeler: domates, *Phelipanche aegyptiaca*, *Trichoderma* spp., biyolojik kontrol ajanı

REFERENCES

Afrouz M., Sayyed R.Z., Fazeli-Nasab B., Piri R., Almalki W., Fitriatin B.N., 2023. Seed bio-priming with beneficial *Trichoderma harzianum* alleviates cold stress in maize. Peer J, 11, e15644.

Aksoy E., Uygur F.N., 2008. Effect of broomrapes on tomato and faba bean crops. Türkiye Herboloji Dergisi, 11 (1), 1-7.

Amira M.B., Lopez D., Mohamed A.T., Khouaja A., Chaar H., Fumanal B., Gousset-Dupont A., Bonhomme L., Label P., Goupil P., 2017. Beneficial effect of *Trichoderma harzianum* strain Ths97 in biocontrolling *Fusarium solani* causal agent of root rot disease in olive trees. Biological Control, 110, 70-78.

Azarig M.A., Hassan M.M., Rugheim A.M., Ahmed O.M.M., Abakeer R.A., Abusin R., Abdelgani M.E., 2020. Impact of *Trichoderma harzianum* and bacterial strains against *Striga hermonthica* in sorghum, 9 (10), 4049-4059.

Cesarini M., Petrucci A., Hotaj E., Venturini G., Liguori R., Sarrocco S., 2025. Use in a controlled environment of *Trichoderma asperellum* ICC012 and *Trichoderma gamsii* ICC080 to manage FHB on common wheat. Microbiological Research, 290, 127941. https://doi.org/10.1016/j.micres.2024.127941

Cignitas E., Kitis Y.E., 2022. Molecular identification of *Phelipanche* species from the western Mediterranean region of Türkiye. p. 198. In: Book of Abstracts: 19th European Weed Research Society Symposium, Athens, Greece.

Di Marco S., Metruccio E.G., Moretti S., Nocentini M., Carella G., Pacetti A., Battiston E., Osti F., Mugnai L., 2022. Activity of *Trichoderma asperellum* strain ICC 012 and *Trichoderma gamsii* strain ICC 080 toward diseases of esca complex and associated pathogens. Frontiers in Microbiology, 12, 813410. doi: 10.3389/fmicb.2021.813410

Dörr I., Kollmann R., 1995. Symplasmic sieve element continuity between orobanche and its host. Botanica Acta, 108 (1), 47-55. https://doi.org/10.1111/j.1438-8677.1995.tb00830.x

El-Dabaa M.A.T., Abd-El-Khair H., 2020. Applications of plant growth promoting bacteria and *Trichoderma* spp. for controlling *Orobanche* crenata in faba bean. Bulletin of the National Research Centre, 44 (4), 1-10. https://doi.org/10.1186/s42269-019-0263-y

Elad Y., 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection, 19 (8-10), 709-714. https://doi.org/10.1016/S0261-2194(00)00094-6

FAOSTAT. 2022. Crops and livestock products, (accessed date: 10.03.2024). https://www.fao.org/faostat/en/#data/QCL.

Fernández-Aparicio M., Reboud X., Gibot-Leclerc S., 2016. Broomrape weeds. Underground mechanisms of parasitism and associated strategies for their control: a review. Frontiers in Plant Science, 7, 135. doi: 10.3389/fpls.2016.00135

Fidan E., Tepe I., 2024. Physiological effects of arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPRs), and *Trichoderma harzianum* on tomato (*Solanum lycopersicum* L.) infected with branched broomrape [*Phelipanche ramosa* (L.) Pomel]. 03 April 2024, Preprint (Version 1). https://doi.org/10.21203/rs.3.rs-4186595/v1

Galletti S., Paris R., Cianchetta S., 2020. Selected isolates of *Trichoderma gamsii* induce different pathways of systemic resistance in maize upon *Fusarium verticillioides* challenge. Microbiological Research, 233, 126406. https://doi.org/10.1016/j.micres.2019.126406

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M., 2004. Trichoderma species—opportunistic, avirulent plant symbionts. Nature Reviews, Microbiology, 2 (1), 43-56. doi:10.1038/nrmicro797

Hassan M.M., Daffalla H.M., Modwi H.I., Osman M.G., Ahmed I.I., Gani M.E.A., Abdel El Gabar E., 2013. Effects of fungal strains on seeds germination of millet and *Striga hermonthica*. Universal Journal of Agricultural Research, 2 (2), 83-88. doi:10.13189/ujar.2014.020208

Howell C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Disease, 87 (1), 4-10.

Jalali F., Abbasi S., Salari H., 2024. The activity of *Trichoderma* spp. culture filtrate to control *Phelipanche aegyptiaca* infection in tomato. Journal of Plant Protection Research, 64 (2), 189-199. https://doi.org/10.24425/jppr.2024.150252

Joel D.M., Gressel J., Musselman L.J., 2013. Parasitic mechanisms and control strategies. In: Parasitic Orobanchaceae. Joel, D.M., Gressel, J., Musselman, L.J., (Eds.). Springer Berlin, Heidelberg, XVII, 513 p. https://doi.org/10.1007/978-3-642-38146-1

Li X., Liao Q., Zeng S., Wang Y., Liu J., 2025. The use of *Trichoderma* species for the biocontrol of postharvest fungal decay in fruits and vegetables: challenges and opportunities. Postharvest Biology and Technology, 219, 113236. https://doi.org/10.1016/j.postharvbio.2024.113236

Maširević S., Medić-Pap S., Škorić D., Terzić A., 2014. Effect of roots of different sunflower hybrids and bio agent based on *Trichoderma asperellum* on broomrape germination. 89-94 p. In: Proceedings of Third International Symposium on Broomrape (*Orobanche* spp.) in Sunflower. Córdoba, Spain. Int. Sunflower Assoc., Paris, France.

Montero-Barrientos M., Hermosa R., Cardoza R.E., Gutiérrez S., Nicolás C., Monte E., 2010. Transgenic expression of the *Trichoderma harzianum* hsp70 gene increases *Arabidopsis* resistance to heat and other abiotic stresses. Journal of Plant Physiology, 167 (8), 659-665. doi: 10.1016/j.jplph.2009.11.012

Parker C., 2009. Observations on the current status of orobanche and striga problems worldwide. Pest Management Science: formerly Pesticide Science, 65 (5), 453-459. https://doi.org/10.1002/ps.1713

Poveda J., Abril-Urias P., Escobar C., 2020. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. Frontiers in Microbiology, 11, 992. doi: 10.3389/fmicb.2020.00992

Rawat L., Singh Y., Shukla N., Kumar J., 2011. Alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum* L.) by seed biopriming with salinity tolerant isolates of *Trichoderma harzianum*. Plant and Soil, 347 (1), 387-400. doi:10.1007/s11104-011-0858-z

Rispail N., Dita M.A., González-Verdejo C., Pérez-de-Luque A., Castillejo M.A., Prats E., Román B., Jorrín J., Rubiales D., 2007. Plant resistance to parasitic plants: molecular approaches to an old foe. New Phytologist, 173 (4), 703-712. https://doi.org/10.1111/j.1469-8137.2007.01980.x

Shoresh M., Harman G.E., Mastouri F., 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review Of Phytopathology, 48 (1), 21-43. doi: 10.1146/annurev-phyto-073009-114450

Shukla N., Awasthi R., Rawat L., Kumar J., 2012. Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. Plant Physiology and Biochemistry, 54, 78-88. doi:10.1016/j.plaphy.2012.02.001

Townsend G.R., Heuberger J.W., 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter, 27, 340-343.

Vinale F., Sivasithamparam K., Ghisalberti E.L., Marra R., Woo S.L., Lorito M., 2008. *Trichoderma*–plant–pathogen interactions. Soil Biology and Biochemistry, 40 (1), 1-10. https://doi.org/10.1016/j.soilbio.2007.07.002

Yoshida S., Cui S., Ichihashi Y., Shirasu K., 2016. The haustorium, a specialized invasive organ in parasitic plants. Annual Review Of Plant Biology, 67 (1), 643-667.

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Orijinal araştırma (Original article)

Otospermum glabrum extracts: antioxidant properties and bioactivity against fungal pathogens and *Aphis fabae*

Otospermum glabrum özleri: fungal patojenlere ve *Aphis fabae*'ye karşı antioksidan özellikler ve biyoaktivite

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ABSTRACT

The adoption of plant-based biopesticides as sustainable alternatives to synthetic chemicals in agricultural pest management has gained increasing attention due to their safety and ecological benefits. Many of these natural compounds demonstrate potent antifungal activity, mitigating foodborne fungal contamination and associated mycotoxins. This study evaluated the antioxidant, antifungal, and aphicidal potential of *Otospermum glabrum* (Asteraceae) extracts derived from its aerial parts. Three extracts −ethyl acetate, methanolic, and aqueous− were assessed for total polyphenolic and flavonoid contents, antioxidant activity (DPPH radical scavenging, β-carotene bleaching, and total antioxidant capacity), antifungal effects against six post-harvest and crop fungal pathogens, and aphicidal activity against the black bean aphid (*Aphis fabae* Scop.). The ethyl acetate extract (EaE) exhibited the highest polyphenols (57.60 ± 0.17 µg GAE/mg) and flavonoids (49.46 ± 0.66 µg QE/mg), while the methanolic extract (ME) demonstrated the strongest DPPH scavenging activity (IC_{50} = 56.05 ± 0.03 µg/ml) and β-carotene bleaching inhibition, comparable to BHT. The aqueous and ethyl acetate extracts showed the highest total antioxidant capacity (252.60 ± 0.20 and 249.10 ± 0.81 µg AAE/mg, respectively). Antifungal assays revealed that ethyl acetate and methanolic extracts were the most effective, with inhibition percentages (IP) exceeding 65% against all tested fungi. Additionally, the methanolic extract at 30% concentration induced 100% mortality in A. fabae after 72 h and exhibited significant repellency (48.98 ± 8.76%). These findings highlight *O. glabrum* as a promising source of natural biopesticides for integrated pest management.

INTRODUCTION

Ensuring food security is essential for sustaining the growing populations of both humans and animals. Food stability can be significantly impacted by unexpected challenges in food production caused by various organisms, including bacteria, viruses, fungi, and insects (Hendel et al. 2021, Mwangi et al. 2023).

Fungal pathogens are responsible for 70–80% of microbial-related agricultural losses, with approximately 8.000 species causing nearly 100.000 plant diseases, while recent studies report over 19.000 fungal species as phytopathogens (Deresa and Diriba 2023). Highly destructive genera such as *Aspergillus*, *Penicillium*, and *Fusarium* induce critical crop diseases; including corn ear rot (*Fusarium* spp.), cotton boll rot (*Aspergillus* spp.), grape blue mold (*Penicillium* spp.), and wheat Fusarium crown rot (*Fusarium* spp.), resulting in severe pre- and postharvest yield reductions (Alananbeh et al. 2024, Ghuffar et al. 2021, Zakaria 2024). At the same time, insects and pests significantly impact global food production across all stages of crop growth, harvest, and storage, leading to an estimated annual reduction of 18–20% of agricultural yield, equivalent to over US$ 470 billion (Souto et al. 2021). The black bean aphid (*Aphis fabae* Scopoli), a polyphagous pest infesting over 200 plant species, is particularly destructive to fava beans, with Algerian outbreaks causing up to 50% yield loss through direct damage (sap depletion, honeydew excretion, tissue deformation/gall formation) and indirect harm as a vector for more than 30 plant viruses (Benbelkhir et al. 2024). These impacts are compounded by rising costs of chemical control measures.

Conventional management of black bean aphids and fungal diseases depends on synthetic pesticides (neonicotinoids, pyrethroids, azoles, and strobilurins) for their broad-spectrum efficacy (Almogdad and Semaškienė 2021, Pandey and Rathore 2023, Pintye et al. 2024). However, their overuse endangers human health, ecosystems, and accelerates pesticide resistance (Hernández-Ceja et al. 2021, Li et al. 2024). Plant-derived biopesticides offer a sustainable alternative, combining low environmental persistence, minimal mammalian toxicity, and complex phytochemical profiles that limit resistance evolution. These attributes, alongside proven efficacy against diverse pests and pathogens, position botanical extracts as environmentally compatible tools for integrated pest management (Ahmed et al. 2020, Noureldeen et al. 2022).

Approximately 2.500 plant species from 235 botanical families show efficacy against pathogens and insects through their secondary metabolites (Ngegba et al. 2022). Notably, polyphenols serve dual protective functions, exhibiting both potent antioxidant capacity and enhanced defense mechanisms against biotic and abiotic stressors, thereby significantly improving plant stress tolerance (Hbika et al. 2022, Šamec et al., 2021).

Otospermum glabrum (Lag.) Willk. (Asteraceae) is an annual glabrous herb characterized by erect or ascending stems, 1-3 pinnatipartite leaves, and small corymbose inflorescences. The flowers feature white, tridentate ligules and black-bordered linear-obtuse bracts (Quezel and Santa 1962). As a member of the *Anthemideae* tribe, which includes bioactive genera like *Achillea*, *Artemisia*, and *Matricaria*, it likely shares their characteristic phytochemicals (phenolics, flavonoids, terpenoids, alkaloids, and coumarins) responsible for demonstrated antioxidant, antimicrobial, and insecticidal properties (El Mihyaoui et al. 2022, Kaczorová et al. 2021, Kursa et al. 2022, Silva-Beltran et al. 2023, Yang et al. 2024). This phytochemical similarity suggests that *O. glabrum* may possess comparable biological activities worthy of investigation.

To date, there is no existing documentation in the current literature regarding *O. glabrum*, aside from limited botanical and taxonomical reports. Therefore, this study aims to assess the total polyphenol and flavonoid content, explore the antioxidant and antifungal activities of *O. glabrum* extracts, and evaluate their aphicidal effects against *A. fabae*, known as the black bean aphid.

Despite the well-documented bioactivity of *Anthemideae* tribe members, *O. glabrum* remains pharmacologically unexplored, with only limited taxonomic descriptions available. This study therefore investigates the total polyphenol and flavonoid content, antioxidant and antifungal potential of its extracts, and aphicidal activity against *A. fabae*, a destructive pest of leguminous crops.

MATERIALS AND METHODS

Plant material

Aerial parts of *O. glabrum* were collected during the flowering period in April 2022 from the northern region of M'sila (35.8498° N, 4.5426° E), Algeria. The plant material was authenticated by Dr. Djamel Sarri, with a voucher specimen (OG2865QS28N) deposited in the herbarium of the Laboratory of Biology: Applications in Health and Environment at M'sila University. Following collection, the plant material was shade-dried at ambient temperature (25 ± 2 °C) and stored in paper bags until further use.

Plant extract preparation

The aqueous extract (AE) was prepared using a standardized decoction method (Ljubuncic et al. 2005). Briefly, plant

powder (50 g) was soaked in distilled water (500 ml) and heated to 90 °C for 15 min with constant stirring. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated at 40 °C using a drying oven. The final extract was stored at 4 °C until further use.

The ethyl acetate (EaA) and methanolic (ME) extracts were prepared by maceration (Mbarga et al. 2022). Plant powder (50 g) was soaked in 450 ml either methanol (Merck 99.9%) or ethyl acetate (Merck 99.8%) with continuous stirring for 24 hours, then filtered through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure at 40 °C and stored at 4 °C until further use.

Total phenolic content (TPC)

Total phenolic content (TPC) in *O. glabrum* extracts was determined using a modified Folin-Ciocalteu reagent (FCR) method (Elbouzidi et al. 2023). Extract aliquots (100 µl) were mixed with 500 µl of 10% FCR, followed by addition of 400 µl of 7.5% Na_2CO_3 after 4 minutes. After 2 hours of incubation at room temperature, absorbance was measured at 765 nm against a blank, using a UV-Visible Spectrophotometer (CHIMADZU UV-1280 Multipurpose). A gallic acid standard curve was used to calculate results, expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg).

Total flavonoid content (TFC)

Total flavonoid content (TFC) in *O. glabrum* extracts was determined using an aluminum chloride colorimetric assay modified from Madjitoloum Betoloum et al. (2018). Each extract aliquot (1 ml) was mixed with 1 ml of 2% $AlCl_3$ solution. After 10 minutes of incubation at room temperature, absorbance was measured at 415 nm against a blank. Results were calculated from a quercetin standard curve and expressed as micrograms of quercetin equivalents per milligram of extract (µg QE/mg).

Evaluation of the antioxidant activity

Total antioxidant capacity (TAC) assay

Total antioxidant capacity was determined using a modified phosphomolybdenum assay (Aazza et al. 2024) with ascorbic acid as standard. The reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate) was prepared, and reaction mixtures containing 0.1 ml extract and 0.9 ml reagent solution were incubated at 95 °C for 90 min. After cooling at room temperature, absorbance was measured at 695 nm against a blank. Results were calculated from an ascorbic acid (AA) standard curve and expressed as micrograms of AA equivalents per milligram extract (µg AAE/mg).

Free radical scavenging assay

Free radical scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay following Mokhtari et al. (2023) with some modification. Methanolic solutions of test extracts (1 ml) were mixed with 1 ml of 0.004% DPPH in methanol and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm against a DPPH solution control, with butylated hydroxytoluene (BHT) as positive control. Radical scavenging activity was calculated as:

$$I\ (\%) = [(A_c - A_t)/ A_c] \times 100$$

where A_c is the control absorbance and A_t is the test sample absorbance. The IC_{50} value (concentration yielding 50% inhibition) was determined from dose-response curves of inhibition percentage versus extract concentration.

β-carotene bleaching test

Antioxidant activity was evaluated using a β-carotene/ linoleic acid bleaching assay modified from Tabet Zatla et al. (2023). The β-carotene stock solution was prepared by dissolving 1 mg β-carotene in 5 ml chloroform with 25 µl linoleic acid and 200 mg Tween 40. After chloroform evaporation at 40 °C under vacuum, the residue was emulsified in 100 ml oxygen-saturated distilled water by vigorous shaking. For testing, 0.5 ml of methanolic extract solutions were mixed with 2 ml emulsion, with initial absorbance (t=0) measured immediately at 470 nm. Samples were then incubated at 50 °C for 120 min, followed by final absorbance measurement. Butylated hydroxytoluene (BHT) served as positive control. β-carotene bleaching inhibition was calculated as:

$$I\% = [(A_t - C_t / C_0 - C_t)] \times 100$$

where A_t is sample absorbance at 120 min, C_t is control absorbance at 120 min, and C_0 is control absorbance at t=0 min. The IC_{50} was determined from dose-response curves of inhibition percentage versus extract concentration.

Antifungal activity test

The fungal strains used in this study; *Aspergillus flavus*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium culmorum*, *Penicillium expansum*, and *Penicillium italicum*, were obtained from the department of microbiology & biochemistry's laboratory of microbiology, M'sila University, Algeria. These molds were previously isolated and identified by Hendel et al. (2024). They were cultivated on potato dextrose agar (PDA) slants and kept at 4 °C for storage until required.

Antifungal activity was evaluated on potato dextrose agar (PDA) using a modified agar incorporation method (Fatehi et al. 2021). Extracts (AE, ME, EaA) were dissolved in DMSO and incorporated into molten PDA at 10 mg/ml. Aliquots (20 ml) were dispensed into 90 mm Petri dishes and allowed to solidify. A 6 mm mycelial disc from the growing margin of 7-day-old fungal cultures was aseptically transferred to each plate center. Control plates contained DMSO without extract. After 7 days of incubation at 25 °C, fungal growth inhibition was calculated as:

$$I\% = [(D_C - D_T) / D_C] \times 100$$

where D_C is the control colony diameter and DT is the treated colony diameter.

Evaluation of the aphicidal activity

Adult black bean aphids and Vicia faba leaves used in this study were collected in April 2024 from an infested fava bean field in the Bordj Bou Arréridj area (36.0391° N, 4.8847° E), Algeria. Aphid-infested plant sections were carefully transferred to ventilated rearing cages and transported to the laboratory under controlled conditions to preserve colony integrity. To standardize bioassays, wingless adult aphids of uniform size were selectively isolated using a fine bristle brush to minimize physical stress. The selected individuals were subsequently transferred to Petri dishes for immediate bioassay procedures. Taxonomic identification was confirmed as *A. fabae* following Martin's (1983) morphological key, with voucher specimens deposited in the laboratory archive.

Toxicity test

Aphid bioassays were conducted in 90 mm Petri dishes under controlled laboratory conditions (25 ± 2 °C, 60 ± 20% RH, 16:8 L:D photoperiod). Four concentrations (5%, 10%, 20%, and 30% w/v) of AE and ME were prepared by dissolving *O. glabrum* extracts in distilled water. Following Salari et al. (2010), twenty-seven Petri dishes were prepared with three replicates per concentration. Fresh *Vicia faba* leaves were immersed in extract solutions for 10 seconds, air-dried, and placed on moist filter paper in each dish. Fifteen wingless adult aphids were then introduced per dish. Control treatments received distilled water only. Mortality was assessed at 24, 48, and 72 hours post-treatment, with aphids considered dead when showing no movement of legs or antennae.

Repellency test

Repellent activity of AE and ME was evaluated using a modified choice test (Moawad and Al-Barty 2011). Twenty-four Petri dishes (90 mm) were prepared with three replicates per concentration. Each dish contained filter paper divided into two equal sections. Pairs of surface-sterilized *V. faba* leaves were prepared - one leaf immersed in extract solution (5 min) and air-dried, while the other (untreated) served as control. Fifteen wingless adult aphids were released at the center of each dish. After 24 hours at 25 ± 2 °C, aphid distribution was recorded. Repellency percentage (RP) was calculated as:

$$RP\% = [(N_C - N_T) / (N_C + N_T)] \times 100$$

where N_C = number of aphids on control leaf and N_T = number on treated leaf.

Statistical analyses

For antioxidant and antifungal assays, all samples were analyzed in triplicate. Results are expressed as mean ± SD (n = 3). Data were analyzed by two-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism 6.01 (GraphPad Software). Statistical significance was set at $p < 0.05$.

Corrected mortality (CM%) data were analyzed using generalized linear models (GLMs), followed by probit analysis to determine lethal concentration (LC_{50}) values with their confidence intervals. A multivariate analysis of variance (MANOVA) was performed to assess aphid mortality, with extract type, concentration, and exposure time as fixed factors and mortality as the response variable. Tukey's post hoc test was used for mean comparisons at a 5% significance level. All statistical analyses were conducted using R Studio (version 1.2.5019) with R software (version 3.6.1).

RESULTS

Extraction yield

In this study, three extraction methods were employed: aqueous extraction by decoction yielded 23.91 ± 1.43% (w/w), while methanolic and ethyl acetate extractions by maceration yielded 17.89 ± 1.82% and 6.53 ± 0.76% (w/w), respectively.

Polyphenolics and antioxidant activity

Table 1 presents the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity profiles of the plant extracts. Spectrophotometric analysis revealed significant differences in phytochemical composition, with ethyl acetate extract (EaE) showing the highest TPC (57.60 ± 0.17 µg GAE/mg) and TFC (49.46 ± 0.66 µg QE/mg), followed by methanolic extract (ME) and aqueous extract (AE) ($p < 0.05$).

The antioxidant properties of *O. glabrum* extracts, evaluated by TAC, DPPH radical scavenging, and β-carotene/linoleic acid assays, are summarized in Table 1. The AE exhibited the highest TAC value (252.60 ± 0.20 µg AAE/mg), followed closely by the EaE (249.10 ± 0.81 µg AAE/mg), while the ME recorded the lowest TAC (154.92 ± 1.06 µg AAE/mg). DPPH radical scavenging assays revealed ME as the most potent (IC_{50} = 56.05 ± 0.03 µg/ml), though three-fold less active than the BHT standard (21.69 ± 0.04 µg/ml). Both AE and EaE showed minimal DPPH scavenging (IC_{50} > 200 µg/ml). In the β-carotene/linoleic acid system, all three extracts demonstrated significant inhibition of linoleic acid oxidation at 500 µg/ml. The ME achieved complete inhibition (100%, IC_{50} = 7.27 ± 0.95 µg/ml), comparable to BHT, with no significant difference ($p < 0.05$) compared to the standard, while the AE and EaE exhibited inhibition rates of 93.42% and 89.26%, with corresponding IC50 values of 63.67 ± 3.92 µg/ml and 26.66 ± 2.70 µg/ml, respectively.

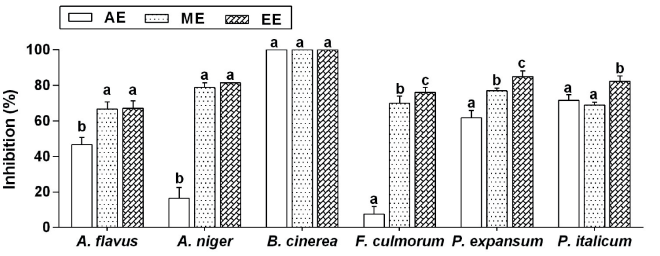

Figure 1. Inhibitory effect of the AE, ME, and EE of *Otospermum glabrum* on the tested molds. No significant differences are indicated by the same letters on each mold column (Tukey's multiple comparisons test, $p<0.05$; data are means (n=3) ± SD)

Evaluation of the aphicidal activity

Toxicity assays against *A. fabae* revealed significant concentration- and time-dependent mortality effects for *O. glabrum* extracts (Table 2). Both extract concentration ($F(3.32) = 15.601$, $p = 0.000^{***}$) and exposure duration ($F(3.32) = 71.390$, $p = 0.0000^{***}$) significantly influenced aphid mortality. The methanolic extract (ME) demonstrated superior efficacy, achieving 100% mortality at 30% concentration (72-h LC_{50} = 51.02%), while the aqueous extract (AE) showed 86.24% mortality (72-h LC_{50} = 46.77%). Lower concentrations (5-20%) exhibited progressive mortality increases: ME (31.11-72.49%) and AE (33.33-78.09%) across 24-72 h exposure periods.

Table 1. Polyphenolic and flavonoid contents and antioxidant activity of *Otospermum glabrum* extracts as measured by multiple assays

Extract / Standard	Test				
	Polyphenols (µg GAE/mg)	**Flavonoids (µg QE/mg)**	**TAC (µg AAE/mg)**	**DPPH (IC_{50}, µg/ml)**	**β-carotene (IC_{50}, µg/ml)**
ME	42.80 ± 0.56	17.61 ± 0.10	154.92 ± 1.06	56.05 ± 0.03	7.27 ± 0.95[a*]
EaE	57.60 ± 0.17	49.46 ± 0.66	249.10 ± 0.81	241.23 ± 0.06	63.67 ± 3.92
AE	19.39 ± 0.16	7.23 ± 0.12	252.60 ± 0.20	267.69 ± 0.47	26.66 ± 2.70
BHT	-	-	-	21.69 ± 0.04	4.73 ± 0.40[a]

*Values with the same superscript letter are not statistically different according to the two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, $p<0.05$. Values are means (n = 3) ± SD

Evaluation of the antifungal activity

The antifungal activity of *O. glabrum* extracts (10 mg/ml) was evaluated by measuring fungal growth inhibition. All extracts demonstrated significant antifungal effects against the tested strains (Figure 1), with complete inhibition of *B. cinerea* growth observed for all treatments. Ethyl acetate extract (EaE) showed the strongest overall inhibition, followed by methanolic extract (ME), with both maintaining >65% inhibition across all fungi. Notably, EaE and ME exhibited statistically similar efficacy ($P < 0.05$) against *A. flavus* and *A. niger*. In contrast, aqueous extract (AE) displayed the weakest activity (7.56-71.56% inhibition), except against *P. italicum*, where its performance slightly exceeded ME ($p < 0.05$).

Repellency test

Repellent activity of *O. glabrum* extracts against *A. fabae* showed concentration-dependent effects (Table 3). Both extract type ($F(1.16) = 4.5$, $p = 0.048^{*}$) and concentration ($F(3.16) = 5.19$, $p = 0.01071$) significantly influenced repellency. The methanolic extract (ME) exhibited stronger

Table 2. Lethal concentrations of *Otospermum glabrum* extract against Aphis fabae aphid population following 72 h exposure

Extract	Exposure time	Concentration (%)	Corrected mortality (%)*	LC_{50} (%)	95% Confidence Interval	Slope ± SE	χ^2
AE	24 h	5	33.33±0.00^{lk}	49.73±0.29	46.75–55.46	1.4 ± 0.2	2.1
		10	51.11±2.22^{jihg}				
		20	53.33l±3.84^{ihgf}				
		30	55.56±4.44^{lihgfe}				
	48 h	5	41.75±3.21^{lkj}	46.94±2.40	35.45–57.53	1.5 ± 0.3	2.4
		10	53.49±2.06^{lihgf}				
		20	58.10±4.22^{lihgfe}				
		30	65.08±0.79^{hgfed}				
	72 h	5	61.31±7.92^{lihgfe}	46.77±6.03	45.77–76.83	1.2 ± 0.3	2.6
		10	69.93f±3.85^{edc}				
		20	78.09±3.48^{dcb}				
		30	86.24±3.13^{b}				
ME	24 h	5	31.11±0.00^{l}	49.74±0.25	45.53–73.24	1.3 ± 0.2	2.3
		10	31.11±2.22^{l}				
		20	42.22±5.87^{lkj}				
		30	68.89±2.22^{gfedc}				
	48 h	5	44.29±2.97^{lkji}	48.37±2.06	37.67–59.77	1.4 ± 0.3	2.1
		10	48.73±3.09^{kjih}				
		20	48.57±5.71^{kjih}				
		30	83.65±2.55cb				
	72 h	5	59.21±5.01^{lihgfe}	51.02±0.01	49.37–69.03	1.1 ± 0.2	2.5
		10	64.80±2.29^{hgfed}				
		20	72.49±6.27^{edcb}				
		30	100±0.00^{a}				
F-(1.12)=0.231. P=.63		F-(2.12)=124.61 P=.0.00000		F- (3.12)=71.30 P=.0.00000			

*Values are presented as mean ± SE (n=3). significant differences were determined at P ≤ 0.05; different superscript letters within a column indicate statistically significant differences at P < 0.05

Table 3. Repellent activity of *Otospermum glabrum* extract against *Aphis fabae* populations

Plant extracts	Concentration (%)	RP (%)*	Class
AE	5	12.50±00.00^{b}	I
	10	12.50±00.00^{b}	I
	20	31.94±10.84^{ab}	II
	30	31.94±10.84^{ab}	II
ME	5	26.38±06.94^{ab}	II
	10	19.44±06.94^{ab}	I
	20	38.88±05.55^{ab}	II
	30	48.98±08.76^{a}	III
F-value (1. 16) =4.5. P=.048		F-value (3. 16)=5.19.P=.01071	

*RP - Repellency percentage (mean ± SE, n = 3). Different superscript letters within columns indicate statistically significant differences (p < 0.05)

repellent effects (26.38-48.98%) compared to the aqueous extract (AE; 12.50-31.94%), with efficacy increasing proportionally to concentration.

DISCUSSION

Extraction yields followed the polarity-dependent trend: ethyl acetate extract (EaE) < methanolic extract (ME) < aqueous extract (AE), consistent with the higher efficiency of polar solvents for phytochemical extraction. This pattern reflects the differential solubility of plant metabolites, where solvent polarity critically influences extraction efficiency (Molole et al. 2022). The superior yield of AE likely results from both the high polarity of water and the elevated temperature during decoction, which enhances solubility of polar compounds including flavonoids, alkaloids, and polysaccharides (Kumar et al. 2023). Similar polarity-yield relationships have been documented in related Anthemideae genera; *Achillea* (Toplan et al. 2022) and *Artemisia* (Trifan et al. 2022), confirming our observations.

Total phenolic (TPC) and flavonoid (TFC) contents exhibited a consistent solvent polarity-dependent trend: aqueous < methanolic < ethyl acetate extracts. This pattern reflects the preferential solubility of polyphenolic compounds - including phenolic acids, terpenes, and methoxylated flavonoid aglycones - in less polar solvents (Palaiogiannis et al. 2023). The aqueous extract's lower yields may result from both the limited solubility of non-polar phenolics in water and potential thermal degradation during decoction (Lezoul et al. 2020). These findings align with previous reports on Algerian *Matricaria chamomilla* (Khennouf et al. 2013) and *Achillea* species (Kaczorová et al. 2021), where chloroform and ethyl acetate consistently outperformed polar solvents in extracting polyphenols, confirming that medium-polarity solvents optimally recover these bioactive compounds. Comparative analysis reveals superior polyphenolic content in *O. glabrum* relative to related species. Our TPC and TFC values significantly exceeded those reported for *Achillea* species (TPC: 16.34-27.48 µg GAE/mg; TFC: 11.31-27.13 µg QE/mg) (Mehmood et al. 2022) and approached the highest levels documented in *Artemisia* species (methanol extracts: 106.34 µg GAE/g in *A. vulgaris*; 47.74 µg RE/mg in *A. annua*) (Trifan et al. 2022). Notably, our extracts consistently outperformed the lowest-yielding chloroform extracts of *A. absinthium* (TPC: 5.78 µg GAE/g; TFC: 0.37 µg RE/mg). These findings position *O. glabrum* as a particularly rich source of bioactive polyphenols, with significant potential for biologically protective applications.

The antioxidant results revealed an inverse relationship between *O. glabrum*'s total phenolic/flavonoid content (TPC/TFC) and total antioxidant capacity (TAC), with the aqueous extract (AE) showing the highest TAC despite lower TPC/TFC levels. This apparent discordance results from three key factors: the presence of non-phenolic antioxidants (e.g., carotenoids, tocopherols) not detected by TPC assays, structural efficacy of specific compounds that disproportionately contribute to antioxidant activity, and synergistic interactions between phytochemicals that enhance overall activity. These findings align with reports in *Artemisia* species (Guenane et al. 2024, Trifan et al. 2022), confirming that antioxidant potential cannot be predicted by polyphenolic content alone. Notably, *O. glabrum*'s TAC (up to 252.60 µg AAE/mg) exceeded values reported for related species like *Achillea coarctata* (226.82 µg AAE/mg) and *Matricaria chamomilla* (100–124 µg AAE/mg), highlighting its exceptional antioxidant profile (Albayrak and Silahtarlıoğlu, 2019).

The DPPH radical scavenging results revealed that the EaE of *O. glabrum*, despite containing the highest levels of TPC and TFC, exhibited significantly lower antioxidant activity compared to the ME. This apparent contradiction stems from the structural specificity of DPPH radicals, which preferentially react with flavonoids containing hydroxyl groups on the β-ring and polyhydroxy aromatic acids (Mammeri et al. 2022), rather than responding to overall phenolic concentration. The superior activity of the ME can be attributed to its greater polarity, which enhances extraction of hydroxyl-rich phenolic compounds that are particularly effective in radical neutralization (Kaczorová et al. 2021). These findings are consistent with studies on *Achillea* species (Kaczorová et al. 2021, Şabanoğlu et al. 2019) and *Artemisia* species (IC_{50}: 107.2-227 µg/ml) (Ranjbar et al. 2020), confirming that antioxidant efficacy depends more on molecular structure than total polyphenol content. Notably, *O. glabrum*'s ME (IC_{50} = 56.05 µg/ml) showed stronger radical scavenging activity than most reported values for related Anthemideae species, including various populations of *Matricaria chamomilla* (IC_{50}: 19.23-73.35 µg/ml) (Hassanpour et al. 2020), highlighting its exceptional antioxidant potential despite having only moderate TPC levels. This pattern underscores the importance of considering both chemical composition and specific antioxidant mechanisms when evaluating plant extracts for their radical scavenging capacity.

The β-carotene bleaching assay revealed that *O. glabrum*'s EaE extract, despite having the highest TPC/TFC levels, showed the weakest antioxidant activity, underscoring that polyphenols play only a limited role in preventing lipid peroxidation (Faraone et al. 2018). This paradox reflects the system's dependence on non-phenolic antioxidants (carotenoids, tocopherols) that more effectively quench lipid-derived radicals (Othman et al. 2014), as previously

observed in *Achillea* species (Agar et al. 2015, Gharibi et al. 2013). Notably, *O. glabrum* extracts demonstrated superior inhibition of linoleic acid oxidation compared to related Anthemideae species - exceeding the 30-90% range reported for *Achillea* (Gharibi et al. 2015) and matching or surpassing *Artemisia* species (*A. campestris*: 88.03%; *A. herba-alba*: 67.56%; *A. absinthium*: 48.7%) (Bouguerra et al. 2020, Mourad et al. 2018). These results position *O. glabrum* among the most effective antioxidant species in the tribe, while confirming that lipid peroxidation inhibition depends on specific antioxidant profiles rather than total phenolic content.

The antifungal activity of *O. glabrum* extracts showed a strong positive correlation with their total phenolic and flavonoid content, consistent with the established antimicrobial properties of polyphenolic compounds (Lagnika et al. 2016). This relationship explains the observed variability in efficacy across extracts, where ethyl acetate extracts with higher TPC/TFC demonstrated superior antifungal activity compared to aqueous extracts. The differential sensitivity of fungal species to specific secondary metabolites (Salem et al. 2019) and the polarity-dependent extraction efficiency of bioactive compounds further account for these variations. Our findings align with current literature demonstrating that organic extracts typically outperform aqueous extracts in antimicrobial activity (Rahim et al. 2023), and moderately polar solvents optimally extract antimicrobial phytochemicals (Lagnika et al. 2016).

The aqueous extract (AE) showed unexpectedly high activity against *Penicillium italicum*, potentially due to the fungus' sensitivity to water-soluble compounds, and synergistic effects of non-phenolic phytochemicals (alkaloids, glycosides, terpenoids) with demonstrated antifungal properties (Chioma et al. 2021, Onanuga and Oloyede 2022). This species-specific response likely reflects differences in fungal cell wall composition and metabolic pathways that influence susceptibility to particular phytochemical structures (Silva-Beltran et al. 2023).

Otospermum glabrum extracts exhibited superior antifungal activity compared to related Anthemideae species. While *Matricaria aurea* showed moderate inhibition (31.48-53.44%) against *Aspergillus* spp. (Rizwana et al. 2016), and *Artemisia campestris* methanol extract achieved 51.96-56.47% inhibition against *Botrytis cinerea* and *P. expansum* (Hendel et al. 2021), *O. glabrum* demonstrated consistently stronger efficacy. The variable performance of *Achillea* and *Artemisia* species (18-63.82% inhibition) (Andreu et al. 2018, Kursa et al. 2022, Salem et al. 2019) further highlights *O. glabrum*'s potential as a promising candidate for the development of new biofungicides, particularly given the economic importance of the target pathogens.

The aphicidal activity of *O. glabrum* extracts arises from its rich range of secondary metabolites, including phenolics, flavonoids, terpenoids, and alkaloids (Li et al. 2024, Noureldeen et al. 2022). These bioactive compounds disrupt key physiological processes in aphids; deterring feeding, impairing development, and reducing reproductive capacity (Lebbal et al. 2023). Our findings align with documented efficacy of plant extracts against multiple aphid species (*Aphis fabae*, *Macrosiphum rosae*, *Brevicoryne brassicae*, *Aphis craccivora*) (Abdel-Rahman et al. 2019, Ahmed et al. 2020, Thakshila et al. 2022).

Otospermum glabrum demonstrated particularly strong insecticidal effects against *A. fabae*, with methanolic extract (ME) achieving 100% mortality at 72 h (highest concentration) versus 80% for aqueous extract (AE). ME also showed superior repellency (48.98% maximum) compared to AE (31.94% maximum). This differential bioactivity reflects variations in extract composition, where ME's lower polarity likely enhanced extraction of non-polar bioactive compounds with greater aphid toxicity.

Comparative analysis reveals differential aphicidal efficacy among Anthemideae species. While *Artemisia judaica* ethanolic extract achieved 100% *Aphis fabae* mortality within 2 hours at 12.5 mg/ml (Acheuk et al. 2017), O. glabrum required higher concentrations and longer exposure (72 h). Conversely, *Matricaria chamomilla* aqueous extracts showed only 35% mortality after 120 h (Biniaś and Gospodarek 2017), demonstrating *O. glabrum*'s intermediate potency. Against other aphid species, *Achillea millefolium* essential oil exhibited superior contact toxicity (LC_{50} 0.34%) and repellency (58.1%) to *Myzus persicae* (Czerniewicz et al. 2018), while *Artemisia* spp. ethyl acetate extracts showed complete *Macrosiphoniella sanborni* control within 7 days (Yang et al. 2024). *O. glabrum* displayed faster toxicity but weaker repellency than these counterparts, suggesting distinct mode-of-action profiles. Beyond aphids, *Artemisia absinthium* methanol extract caused 100% *Sitophilus oryzae* mortality within 24 h (Dane et al. 2016), whereas O. glabrum required 72 h for complete *A. fabae* control. However, *O. glabrum* achieved comparable efficacy at lower concentrations than *A. absinthium* against *Tribolium castaneum* (93.3% mortality at 95 mg/ml; Naimi et al. 2025). These variations likely reflect species-specific susceptibility and differential phytochemical bioavailability.

While this study provides the first evidence of *O. glabrum*'s bioactivity, its laboratory-scale design using crude extracts

limits field relevance and mechanistic understanding. Future work should employ bioassay-guided fractionation (HPLC/LC-MS) to isolate active compounds, validate efficacy in field trials, and expand testing to diverse pests/pathogens. Investigating synergies with existing biocontrol agents would further support its integration into sustainable pest management systems.

This study provides the first comprehensive evaluation of *Otospermum glabrum* as a source of bioprotective agents, demonstrating significant antioxidant, antifungal, and aphicidal activities. The ethyl acetate extract showed superior phenolic and flavonoid contents, correlating with enhanced bioactivity, while the methanolic extract exhibited the strongest aphid toxicity. These findings highlight the influence of extraction solvents on phytochemical recovery and biological efficacy. As a novel botanical resource, *O. glabrum* shows exceptional potential for developing multi-target, eco-friendly biopesticides. Future research should focus on isolating active compounds through bioassay-guided fractionation and validating field efficacy to facilitate its integration into sustainable crop protection strategies that address both pest management and food security challenges.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Tarımsal zararlı yönetiminde sentetik kimyasallar yerine bitki bazlı biyopestisitlerin benimsenmesi, güvenlikleri ve ekolojik faydaları nedeniyle giderek artan bir ilgi çekmektedir. Bu doğal bileşiklerin birçoğu güçlü antifungal aktivite göstererek gıda kaynaklı mantar kontaminasyonunu ve ilişkili mikotoksinleri azaltmaktadır. Bu çalışmada, *Otospermum glabrum*'un (Asteraceae) toprak üstü kısımlarından elde edilen ekstraktların antioksidan, antifungal ve afisidal potansiyeli değerlendirilmiştir. Üç ekstrakt –etil asetat, metanolik ve sulu– toplam polifenolik ve flavonoid içerikleri, antioksidan aktiviteleri (DPPH radikal süpürme, β-karoten ağartma ve toplam antioksidan kapasitesi), altı hasat sonrası ve bitki fungal patojenine karşı antifungal etkileri ve bakla yaprakbitine (*Aphis fabae* Scop.) karşı afisidal aktiviteleri açısından değerlendirilmiştir. Etil asetat özütü (EaE), test edilen özütler arasında en yüksek polifenol (57.60 ± 0.17 µg GAE/mg) ve flavinoid (49.46 ± 0.66 µg QE/mg) seviyesine sahipken; metanolik özüt (ME), en yüksek DPPH temizleme aktivitesi (IC50= 56.05±0.03 µg/ml) ve β-karoten ağartmasını önlemede en yüksek etkinliğini göstererek BHT'ninkine eşdeğer bir inhibitör aktivitesine ulaşmıştır. Sulu özüt ve etil asetat özütü, en yüksek toplam antioksidan kapasitesini göstermiştir (sırasıyla 252.60±0.20 µg AAE/mg ve 249.10±0.81 AAE/mg). Antifungal testlerine göre etil asetat özütü ve metanolik özütü tüm test edilen funguslara karşı %65'i geçen inhibisyon yüzdeleri (IP) ile en belirgin etkiyi ortaya koymuştur. Ek olarak, %30 konsantrasyonundaki metanolik ekstrakt, 72 saat sonra *A. fabae*'de %100 ölüme neden olmuş ve önemli bir kovucu etki (%48.98 ± 8.76) göstermiştir. Bu bulgular, *O. glabrum*'un entegre zararlı yönetimi için umut verici doğal bir biyopestisit kaynağı olduğunu göstermektedir.

Anahtar kelimeler: *Otospermum glabrum*, polifenoller, antioksidan aktivite, antifungal aktivite, afisidal aktivite

REFERENCES

Aazza S., El-Guendouz S., Miguel M.G., 2024. Antioxidant and α-amylase inhibition activities of six plants used in the management of diabetes in Morocco. Letters in Applied NanoBioScience, 13 (1), 17. https://doi.org/10.33263/LIANBS131.017

Abdel-Rahman R.S., Ismail I.A., Mohamed T.A., Hegazy M.E.F., Abdelshafeek K.A., 2019. Laboratory and field evaluation of certain wild plant extracts against *Aphis fabae* Scop. (Homoptera: Aphididae) and its predators. Bulletin of the National Research Centre, 43, 44. https://doi.org/10.1186/s42269-019-0084-z

Acheuk F., Lakhdari W., Abdellaoui K., Belaid M., Allouane R., Halouane F., 2017. Phytochemical study and bioinsecticidal effect of the crude ethanolic extract of the Algerian plant *Artemisia judaica* L. (*Asteraceae*) against the black bean aphid, *Aphis fabae* Scop. The Journal Agriculture and Forestry, 63 (1), 95-104. https://doi.org/10.17707/AgricultForest.63.1.11

Albayrak S., Silahtarlıoğlu N., 2019. Determination of biological activities of essential oil and extract obtained from *Achillea coarctata* Poir. Oriental Pharmacy and Experimental Medicine, 19, 135-146. https://doi.org/10.1007/s13596-019-00378-w.

Agar O.T., Dikmen M., Ozturk N., Yilmaz M.A., Temel H., Turkmenoglu F.P., 2015. Comparative studies on phenolic composition, antioxidant, wound healing and cytotoxic activities of selected *Achillea* L. species growing in Turkey. Molecules, 20 (10), 17976–18000. https://doi.org/10.3390/molecules201017976

Ahmed M., Peiwen Q., Gu Z., Liu Y., Sikandar A., Hussain D., Javeed A., Shafi J., Iqbal M.F., An R., Guo H., Du Y., Wang W., Zhang Y., Ji M., 2020. Insecticidal activity and biochemical composition of *Citrullus colocynthis*, *Cannabis indica*, and *Artemisia argyi* extracts against cabbage aphid (*Brevicoryne brassicae* L.). Scientific Reports, 10, 522. https://doi.org/10.1038/s41598-019-57092-5

Alananbeh K.M., Al-Abdallat A., Al-Hiary H., 2024. First report of *Fusarium culmorum* causing crown rot on wheat in Jordan. Plant Disease, 108 (3), 799. https://doi.org/10.1094/PDIS-08-23-1714-PDN

Almogdad M., Semaškienė R., 2021. The occurrence and control of black bean aphid (*Aphis fabae* Scop.) in broad bean. Zemdirbyste-Agriculture 108 (2), 165-172. https://doi.org/10.13080/z-a.2021.108.022

Andreu V., Levert A., Amiot A., Cousin A., Aveline N., Bertrand C., 2018. Chemical composition and antifungal activity of plant extracts traditionally used in organic and biodynamic farming. Environmental Science and Pollution Research International, 25 (30), 16987–17000. https://doi.org/10.1007/s11356-018-1320-z

Benbelkhir F.Z., Allali K., Benadjila A., Goudjal Y., Medjekal S., Zamoum M., 2024. Development of bioinsecticide based on *Streptomyces griseoflavus* PAL114 for control of black bean aphids *Aphis fabae*. Biocontrol Science and Technology, 34 (8), 736–753. https://doi.org/10.1080/09583157.2024.2373477

Biniaś B., Gospodarek J., 2017. Effect of water extract from chamomile on black bean aphid and Colorado potato beetle. Journal of Ecological Engineering, 18 (3), 118–124. https://doi.org/10.12911/22998993/69363

Bouguerra A., Djebili S., Zouaoui N., Barkat M., 2020. Evaluation of phenolic contents and antioxidant activities of some medicinal plants growing in Algerian Aurès Mountains. Acta Scientifica Naturalis, 7 (2), 15–30. https://doi.org/10.2478/asn-2020-0017

Chioma N., Christie O., Nathaniel O., Aqib F., 2021. Phytochemical analysis and in vitro screening of antifungal activity of *Jatropha multifida*, *Euphorbia hirta*, *Occimum gratissimum* and *Mitracarpus scaber* leaves extract. GSC Biological and Pharmaceutical Sciences, 14 (3), 098-112.

Czerniewicz P., Chrzanowski G., Sprawka I., and Sytykiewicz H., 2018. Aphicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer). Pesticide Biochemistry and Physiology, 145, 84-92.

https://doi.org/10.1016/j.pestbp.2018.01.010

Dane Y., Mouhouche F., Canela-Garayoa R., Delpino-Rius A., 2016. Phytochemical analysis of methanolic extracts of *Artemisia absinthium* L. (Asteraceae), *Juniperus phoenicea* L., and *Tetraclinis articulata* (Cupressaceae) and evaluation of their biological activity for stored grain protection. Arabian Journal for Science and Engineering, 41, 2147–2158. https://doi.org/10.1007/s13369-015-1977-2

Deresa E.M., Diriba T.F., 2023. Phytochemicals as alternative fungicides for controlling plant diseases: a comprehensive review of their efficacy, commercial representatives, advantages, challenges for adoption, and possible solutions. Heliyon, 9 (3), e13810. https://doi.org/10.1016/j.heliyon.2023.e13810

El Mihyaoui A., Esteves da Silva J.C.G., Charfi S., Candela Castillo M.E., Lamarti A., Arnao M.B., 2022. Chamomile (*Matricaria chamomilla* L.): a review of ethnomedicinal use, phytochemistry, and pharmacological uses. Life, 12 (4), 479. https://doi.org/10.3390/life12040479

Elbouzidi A., Taibi M., Ouassou H., Ouahhoud S., Ou-Yahia D., Loukili E.H., Aherkou M., Mansouri F., Bencheikh N., Laaraj S., Bellaouchi R., Saalaoui E., Elfazazi K., Berrichi A., Abid M., Addi, M., 2023. Exploring the multi-faceted potential of carob (*Ceratonia siliqua* var. Rahma) leaves from Morocco: a comprehensive analysis of polyphenols profile, antimicrobial activity, cytotoxicity against breast cancer cell lines, and genotoxicity. Pharmaceuticals, 16 (6), 840. https://doi.org/10.3390/ph16060840

Faraone I., Rai D.K., Chiummiento L., Fernandez E., Choudhary A., Prinzo F., Milella L., 2018. Antioxidant activity and phytochemical characterization of *Senecio clivicolus* Wedd. Molecules, 23 (10), 2497. https://doi.org/10.3390/molecules23102497

Fatehi N., Benmehdi H., Allali H., Sahel N., Oulednecir N., 2021. Evidence-based antifungal potential of some traditional medicinal plants from the Bechar region (Southwest Algeria). Indian Journal of Natural Products and Resources, 12 (1), 68–73. doi:10.56042/ijnpr.v12i1.23936

Gharibi S., Sayed Tabatabaei B.E., Saeidi G., 2015. Comparison of essential oil composition, flavonoid content and antioxidant activity in eight Achillea species. Journal of Essential Oil Bearing Plants, 18 (6), 1382–1394. https://doi.org/10.1080/0972060X.2014.981600

Gharibi S., Sayed Tabatabaei B.E., Saeidi G., Goli S.A.H., Talebi M., 2013. Total phenolic content and antioxidant activity of three Iranian endemic Achillea species. Industrial Crops and Products, 50, 154–158. https://doi.org/10.1016/j.indcrop.2013.07.038

Ghuffar S., Irshad G., Naz F., Khan M.A., 2021. Studies of *Penicillium* species associated with blue mold disease of grapes and management through plant essential oils as non-hazardous botanical fungicides. Green Processing and Synthesis, 10 (1), 21–36. https://doi.org/10.1515/gps-2021-0007

Guenane H., Rezzoug M., Bakchiche B., Cheraif K., Mohamed A.S., El-Shazly M.A.M., 2024. Antioxidant properties and mineral contents of different solvent extracts of some medicinal plants cultivated in Algeria. Tropical Journal of Natural Product Research, 8 (1), 5987–5991. https://doi.org/10.26538/tjnpr/v8i1.39

Hassanpour H., Niknam V., Ahmadi-Sakha S., Haddadi B., 2020. Antioxidant activity and flavonoid content of *Matricaria chamomilla* extracts from different populations of Iran. Journal of Botanical Research, 2 (2), 8-13. https://doi.org/10.30564/jrb.v2i2.1909

Hbika A., Daoudi N.E., Bouyanzer A., Bouhrim M., Mohti H., Loukili E.H., Mechchate H., Al-Salahi R., Nasr F.A., Bnouham M., Zaid A., 2022. *Artemisia absinthium* L. aqueous and ethyl acetate extracts: antioxidant effect and potential activity *in vitro* and *in vivo* against pancreatic α-amylase and intestinal α-glucosidase. Pharmaceutics, 14 (3), 481. https://doi.org/10.3390/pharmaceutics14030481

Hendel N., Sarri D., Sarri M., Selloum M., Boussakra F., Driche O., 2021. Screening for in vitro antioxidant activity and antifungal effect of *Artemisia campestris* L. International Journal of Agriculture, Environment and Food Sciences, 5 (3), 251–259. https://doi.org/10.31015/jaefs.2021.3.1

Hendel N., Sarri D., Sarri M., Napoli E., Palumbo Piccionello A., Ruberto G., 2024. Phytochemical analysis and antioxidant and antifungal activities of powders, methanol extracts, and essential oils from *Rosmarinus officinalis* L. and *Thymus ciliatus* Desf. Benth. International Journal of Molecular Sciences, 25 (14), 7989. doi: 10.3390/ijms25147989

Hernández-Ceja A., Loeza-Lara P.D., Espinosa-García F.J., García-Rodríguez Y.M., Medina-Medrano J.R., Gutiérrez-Hernández G.F., Ceja-Torres L.F., 2021. *In vitro* antifungal activity of plant extracts on pathogenic fungi of blueberry (*Vaccinium* sp.). Plants, 10 (5), 852. https://doi.org/10.3390/plants10050852

Kaczorová D., Karalija E., Dahija S., Bešta-Gajević R., Parić A., Čavar Zeljković S., 2021. Influence of extraction solvent on the phenolic profile and bioactivity of two *Achillea* species. Molecules, 26 (6), 1601. https://doi.org/10.3390/molecules26061601

Khennouf S., Benchiekh D., Djidel S., Dahamna S., Amira S., Charef N., Baghiani A., Harzallah D., Arrar L., 2013. Polyphenols and antioxidant properties of extracts from *Mentha pulegium* L. and *Matricaria chamomilla* L. Pharmacognosy Communications, 3 (2), 35–40. https://doi.org/10.5530/pc.2013.2.8

Kumar A., Nirmal P., Kumar M., Jose A., Tomer V., Oz E., Proestos C., Zeng M., Elobeid T., Sneha K., Oz F., 2023. Major phytochemicals: recent advances in health benefits and extraction method. Molecules, 28 (2), 887. https://doi.org/10.3390/molecules28020887

Kursa W., Jamiołkowska A., Wyrostek J., Kowalski R., 2022. Antifungal effect of plant extracts on the growth of the cereal pathogen *Fusarium* spp.—an *in vitro* study. Agronomy, 12 (2), 3204. https://doi.org/10.3390/agronomy12123204

Lagnika L., Amoussa A.M.O., Adjileye R.A.A., Laleye A., Sanni A., 2016. Antimicrobial, antioxidant, toxicity and phytochemical assessment of extracts from *Acmella uliginosa*, a leafy vegetable consumed in Bénin, West Africa. BMC Complementary and Alternative Medicine, 16, 34. https://doi.org/10.1186/s12906-016-1014-3

Lebbal S., Benhizia T., Djebaili S., Bouzidi C., Ghassir H., Rahal K., Zeraib A., 2023. Aphicidal activity screening of plant extracts from *Pistacia lentiscus* (Anacardiaceae). Entomologia Hellenica, 32 (2), 12–19. https://doi.org/10.12681/entomologia.35414

Lezoul N.E.H., Belkadi M., Habibi F., Guillén F., 2020. Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. Molecules, 25 (20), 4672. https://doi.org/10.3390/molecules25204672

Li H., Zhao R., Pan Y., Tian H., Chen W., 2024. Insecticidal activity of *Ageratina adenophora* (Asteraceae) extract against *Limax maximus* (Mollusca, Limacidae) at different developmental stages and its chemical constituent analysis. PLOS One, 19 (4), e0298668. https://doi.org/10.1371/journal.pone.0298668

Ljubuncic P., Song H., Cogan U., Azaizeh H., Bomzon A., 2005. The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease. Journal of Ethnopharmacology, 100 (1-2), 198–204. https://doi.org/10.1016/j.jep.2005.03.006

Mammeri A., Bendif H., Bensouici C., Benslama A., Rebas K., Bouasla A., Rebaia I., Souilah N., Miara M.D., 2022. Total phenolic contents, in vitro antioxidant activity, enzymes inhibition, and anti-inflammatory effect of the selective extracts from the Algerian *Lavandula multifida*. Acta Pharmaceutica Sciencia, 60 (1), 1–15. https://doi.org/10.23893/1307-2080.APS.6001

Martin J.H. (1983). The identification of common aphid pests of tropical agriculture. Tropical Pest Management, 29 (4), 395–411. https://doi.org/10.1080/09670878309370834

Mbarga M.J.A., Podoprigora I.V., Anyutoulou K.L.D., Kezimana P., Smolyakova L.A., Tene M.H., Rehailia M., Yashina N.V., Smirnova I.P., Manga I.A.M., Das M.S., 2022. Antimicrobial and antibiotic-resistance reversal activity of some medicinal plants from Cameroon against selected resistant and non-resistant uropathogenic bacteria. Frontiers in Bioscience (Elite Ed), 14 (4), 25. https://doi.org/10.31083/j.fbe1404025

Mehmood A., Javid S., Khan M.F., Ahmad K.S., Mustafa A., 2022. *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chemistry, 16, 64. https://doi.org/10.1186/s13065-022-00858-2

Moawad S.S., Al-Barty A.M.F., 2011. Evaluation of some medicinal and ornamental plant extracts toward pomegranate aphid, *Aphis punicae* (*Passerini*) under laboratory conditions. African Journal of Agricultural Research, 6 (10), 2425–2429. https://doi.org/10.5897/AJAR11.294

Mokhtari R., Fard M.K., Rezaei M., Moftakharzadeh S.A., Mohseni A., 2023. Antioxidant, antimicrobial activities, and characterization of phenolic compounds of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and thyme–sage mixture extracts. Journal of Food Quality, 2023, Article ID 2602454, 9 pages. https://doi.org/10.1155/2023/2602454

Molole G.J., Gure A., Abdissa N., 2022. Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. BMC Chemistry, 16 (1), 48. https://doi.org/10.1186/s13065-022-00841-x

Mourad B., Rachid B., Sihem B., 2018. Antioxidant activity and phenolic content of *Artemisia campestris* from two regions of Algeria. World Journal of Environmental Biosciences, 7 (2), 61–66.

Mwangi R.W., Mustafa M., Charles K., Wagara I.W., Kappel N., 2023. Selected emerging and reemerging plant pathogens affecting the food basket: a threat to food security. Journal of Agriculture and Food Research, 14, 100827. https://doi.org/10.1016/j.jafr.2023.100827

Naimi I., Tastift M.A., Zefzoufi M., Gadhi C., Ba M'hamed T., Bouamama H., 2025. Chemical characterization, anti-cholinesterase and insecticidal activities of Moroccan *Artemisia absinthium* L. leaf extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Journal of Stored Products Research, 112 (1), 102635. https://doi.org/10.1016/j.jspr.2025.102635

Ngegba P.M., Cui G., Khalid M.Z., Zhong G., 2022. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. Agriculture, 12 (5), 600. https://doi.org/10.3390/agriculture12050600

Noureldeen A., Kumar U., Asad M., Darwish H., Alharthi S., Fawzy M.A., Al-Barty A.M., Alotaibi S.S., Fallatah A., Alghamdi A., Albogami B., Alkashgry N., 2022. Aphicidal activity of five plant extracts applied singly or in combination with entomopathogenic bacteria, *Xenorhabdus budapestensis*, against rose aphid, *Macrosiphum rosae* (Hemiptera: Aphididae). Journal of King Saud University – Science, 34, 102306. https://doi.org/10.1016/j.jksus.2022.102306

Onanuga A.O., Oloyede G.K., 2022. Phytochemical analysis and antifungal activity of *Costus lucanusianus* J. Braun & K. Schum aerial and rhizome crude extracts. GSC Biological and Pharmaceutical Sciences, 18 (2), 215-223. https://doi.org/10.30574/gscbps.2022.18.2.0068

Othman A., Mukhtar N.J., Ismail N.S., Chang S.K., 2014. Phenolics, flavonoids content and antioxidant activities of 4 Malaysian herbal plants. International Food Research Journal, 21 (2), 759–766.

Palaiogiannis D., Chatzimitakos T., Athanasiadis V., Bozinou E., Makris D.P., Lalas S.I., 2023. Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. Oxygen, 3 (3), 274–286. https://doi.org/10.3390/oxygen3030018

Pandey G., Rathore H., 2023. Toxicity of strobilurins fungicides: a comprehensive review. Journal of Chemical Health Risks, 13 (2), 207–218. https://doi.org/10.22034/jchr.2023.1960279.1563

Pintye A., Bacsó R., Kovács G.M., 2024. Trans-kingdom fungal pathogens infecting both plants and humans, and the problem of azole fungicide resistance. Frontiers in Microbiology, 15, 1354757. https://doi.org/10.3389/fmicb.2024.1354757

Quezel P., Santa S., 1962. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Éditions du Centre National de la Recherche Scientifique, Paris, 1170 p.

Rahim G., Qureshi R., Hazrat A., Ahmad B., Khan A.A., Aziz T., Alharbi M., Alshammari A., 2023. Phytochemical, antimicrobial, radical scavenging and in-vitro biological activities of *Teucrium stocksianum* leaves. Journal of the Chilean Chemical Society, 68 (1), 5748–5754. https://doi.org/10.4067/S0717-97072023000105748

Ranjbar M., Naghavi M.R., Alizadeh H., 2020. Chemical composition of the essential oils of Artemisia species from

Iran: a comparative study using multivariate statistical analysis. Journal of Essential Oil Research, 32 (4), 361-371. https://doi.org/10.1080/10412905.2020.1750495

Rizwana H., Alwahibi M.S., Soliman D.A., 2016. Antimicrobial activity and chemical composition of flowers of *Matricaria aurea*, a native herb of Saudi Arabia. International Journal of Pharmacology, 12 (6), 576–586. https://doi.org/10.3923/ijp.2016.576.586

Salari E., Ahmadi K., Zamani R., 2010. Study on the effects of acetonic extract of *Otostegia persica* (Labiatae) on three aphid species and one stored product pest. Advances in Environmental Biology, 4 (3), 346–349. https://www.researchgate.net/publication/235799744

Salem M.Z.M., Behiry S.I., El-Hefny M., 2019. Inhibition of *Fusarium culmorum*, *Penicillium chrysogenum* and *Rhizoctonia solani* by n-hexane extracts of three plant species as a wood-treated oil fungicide. Journal of Applied Microbiology, 126 (6), 1683–1699. doi: 10.1111/jam.14256

Madjitoloum Betoloum S., Talla E., Ngassoum M.B., Tsatsop Tsauge R.K., Nyemb J.N., Mahmout Y., 2018. Optimization of microwave-assisted extraction of total phenol content and total flavonoids content from *Anacardium occidentale* L. (*Anacardeaceae*) using response surface methodology. International Journal of Biochemistry and Biotechnology, 7 (4), 800–809.

Şabanoğlu S., Gökbulut A., Altun M.L., 2019. Characterization of phenolic compounds, total phenolic content and antioxidant activity of three Achillea species. Journal of Research in Pharmacy, 23 (3), 567–576. https://doi.org/10.12991/jrp.2019.164

Silva-Beltrán N.P., Boon S.A., Ijaz M.K., McKinney J., Gerba C.P., 2023. Antifungal activity and mechanism of action of natural product derivatives as potential environmental disinfectants. Journal of Industrial Microbiology and Biotechnology, 50 (1), kuad036. https://doi.org/10.1093/jimb/kuad036

Souto A.L., Sylvestre M., Tölke E.D., Tavares J.F., Barbosa-Filho J.M., Cebrián-Torrejón G., 2021. Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: prospects, applications and challenges. Molecules, 26 (16), 4835. https://doi.org/10.3390/molecules26164835

Šamec D., Karalija E., Šola I., Vujčić Bok V., Salopek-Sondi B., 2021. The role of polyphenols in abiotic stress response: the influence of molecular structure. Plants, 10 (1), 118. https://doi.org/10.3390/plants10010118

Tabet Zatla A., Hammoudi A., Brikci Nigassa N., 2023. Analysis of the chemical composition and evaluation of antioxidant and anti-inflammatory properties of hydrosol extract and its principal component (Carlina oxide) in aerial parts of *Atractylis gummifera* from Western Algeria. Chemical Proceedings, 14 (1), 66. https://doi.org/10.3390/ecsoc-27-16135

Thakshila W.A.K.G., Dammini Premachandra W.T.S., Borgemeister C., 2022. Potential toxic effects of aqueous leaf extracts of *Calotropis gigantea* and *Croton laccifera* against *Aphis craccivora*. International Journal of Tropical Insect Science, 42, 1165–1173. https://doi.org/10.1007/s42690-021-00632-2

Toplan G.G., Taşkın T., İşcan G., Göger F., Kürkçüoğlu M., Civaş A., Ecevit-Genç G., Mat A., Başer K.H.C., 2022. Comparative studies on essential oil and phenolic content with *in vitro* antioxidant, anticholinesterase, antimicrobial activities of *Achillea biebersteinii* Afan. and *A. millefolium* subsp. *millefolium* Afan. L. growing in Eastern Turkey. Molecules, 27 (6), 1956. https://doi.org/10.3390/molecules27061956

Trifan A., Zengin G., Sinan K.I., Sieniawska E., Sawicki R., Maciejewska-Turska M., Skalikca-Woźniak K., Luca S.V., 2022. Unveiling the phytochemical profile and biological potential of five Artemisia species. Antioxidants, 11 (5) 1017. https://doi.org/10.3390/antiox11051017

Yang M., Li M., Chen F., Chen S., 2024. Bioactive components and antimicrobial potential of extracts from Artemisia species and their repellent activities against Aphid (*Macrosiphoniella sanborni*). Ornamental Plant Research, 4:e025. doi:10.48130/opr-0024-0021

Zakaria L., 2024. An overview of *Aspergillus* species associated with plant diseases. *Pathogens*, 13 (9), 813. https://doi.org/10.3390/pathogens13090813

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