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Aims and Scope

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Original article

In vitro sensitivity of the tomato early blight disease agent *Alternaria alternata* to some fungicides

Domateste Erken yaprak yanıklığı hastalığı etmeni *Alternaria alternata*'nın bazı fungisitlere karşı *in vitro* duyarlılığı

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ABSTRACT

Early leaf blight is a common fungal disease caused by *Alternaria alternata* (Fr.) Keissler. Various fungicides are used for the chemical control of this disease. As a result of the frequent use of fungicides, decreased susceptibility to the pathogen may be observed. In this study, the susceptibility of early leaf blight (*A. alternata*), a problem in tomato plantations, to azoxystrobin, tebuconazole and mancozeb was determined. Sixty *A. alternata* isolates were obtained from 121 infected plant samples collected from the Antalya, Ankara, Bartın, and Zonguldak provinces in 2013 and 2014. Pathogenicity tests revealed that the disease severity of the isolates varied between 50% and 85% on average. The susceptibility of the isolates to azoxystrobin, tebuconazole, and mancozeb was determined by radial growth tests, and ED_{50} values against azoxystrobin, tebuconazole and mancozeb were determined to be 0.4 ppm, 0.6 ppm and 0.6 ppm, respectively. Polatlı was the most susceptible isolate to the three active substances. Alanya isolate to azoxystrobin (ED50= 452 ppm), Derbent isolate to mancozeb (ED50= 14.45 ppm), Serik and Kayaburnu isolates to tebuconazole (ED50= 33.61 ppm) were determined as the highest resistance isolates. As a result of the study, it was determined that some of the isolates developed resistance to these fungicides

INTRODUCTION

Early leaf blight disease on tomatoes is caused by *Alternaria solani* (Ell. and Mart) Jones and Grout. and *Alternaria alternata* (Fr.) Keissler (Akhtar et al. 2004, Bessadat et al. 2014, Loganathan 2014, Ozan and Maden 2005, Yadav et al. 2020). This disease can cause significant crop losses, especially in greenhouse tomato cultivation and in some potato production areas (Anonymous 2008). As a result of the widespread and excessive use of fungicides and misuse applications to control of disease, the sensitivity of pathogens decreases, and fungicide resistance occurs. When the intended results cannot be obtained from the applications, producers increase both the dose of the fungicide and the

number of applications to achieve the desired success. In this case, the resistance problem deepens, more pesticides are consumed, the cost of control increases, and most importantly, the problems in terms of human health and environmental pollution continue to increase.

Fungus-resistance is the most important problem in the application of site-specific fungicides, now known as modern fungicides, and the market life of members of this class is largely determined by resistance risks (Delen 2008). Resistance can be defined as an irreversible decrease in the susceptibility of an organism to plant protection products used to control that organism as a result of a mutation in its genetic structure. One of the factors that causes resistance is the risk of resistance to the fungicide used. The more specialized the site of action of the fungicide in the fungal cell is, the greater the risk of resistance (Yılmaz et al. 2018).

Fungicide resistance in *Alternaria* spp. has been studied in different plants (cauliflower, cabbage, cultivated rocket, basil, potato etc.) (Ding et al. 2019, Matić et al. 2019). Some Alternaria species have been reported to exhibit resistance to certain fungicides (Farrar et al. 2004). *Alternaria dauci* was found to be resistant to azoxystrobin (Amistar) and trifloxystrobin (Zato) (Surviliene and Dambrauskiene 2006), whereas *A. solani* was reported to be resistant to azoxystrobin (Nuwamanya et al. 2022). In addition, *A. alternata* has been shown to be resistant to pyraclostrobin and boscalid (Avenot et al. 2008).

In a study on the susceptibility of *A. solani* to dithiocarbamate, phthalamide, sulfamide, chlorinated hydrocarbon, dicarboximide, and imidazole fungicides, a decrease in susceptibility to some fungicides was reported, and it was stated that this situation should be checked peryodically (Benlioğlu and Delen 1991).

This study was conducted to determine the susceptibility of *A. alternata* isolates obtained from Ankara, Antalya, Bartın, and Zonguldak provinces, where tomato cultivation was intensively carried out between 2013 and 2016, to azoxystrobin, tebuconazole, and mancozeb under *in vitro* conditions.

MATERIALS AND METHODS

Sample collection and isolation of Alternaria spp.

In 2013 and 2014, 121 symptomatic leaf samples were collected from tomato cultivation areas, including open fields and undercovers, where chemical uses are intense in Ankara, Bartın, Zonguldak, and Antalya. Of the 60 isolates isolated from diseased leaf samples, 15 were obtained from Bartın, 10 from Zonguldak, 17 from Antalya, and 18 from Ankara. For comparison, isolations were also made from samples taken from two tomato fields in the Polatlı and Sincan counties of Ankara, where no plant protection product was applied. The samples were cleaned with tap water in the laboratory. Then, 4-5 mm pieces, including symptomatic and healthy tissue, were cut with a sterile scalpel, kept in 1.5% sodium hypochlorite (NaOCl) for 2 min, and washed in three series of sterile distilled water. After the plant parts were dried on sterile filter papers, they were placed in 9 cm Petri dishes containing 10 ml of potato dextrose agar (PDA; Merck, Darmstadt, Germany) with four plant parts in each petri dish. Petri dishes were incubated in a dark cabinet at 23±1 °C for 7-10 days. After incubation, mycelia from colonies showing similar development as *Alternaria* spp. were transferred to tomato juice agar (Benlioğlu and Delen 1991). Fungal isolates on tomato juice agar for 10-14 days were transferred to 2% water agar (Agar-Agar; Merck, Darmstadt, Germany) to obtain single spore isolates.

Pathogenicity and morphological identification of isolates

Pathogenicity tests were carried out by modifying the method of Ozan and Maden (2005) with 60 isolates obtained in the study. Stalks of tomato leaves were wrapped with cotton soaked in sterile water and placed in blotter medium. A 10 mm diam. agarose discs excised from 7-14-day-old cultures on tomato juice agar were placed on tomato leafstalks and incubated for 1 week at 25–26 °C with three replicates. One week later, the isolates that caused symptoms on the leaves were considered pathogenic. The evaluation was performed on a rating scale 0 to 4 (0= no signs of disease, 1= local and mild yellowing of the leaf, 2= severe yellowing covering the entire leaf surface, 3= lesion formation on the leaf, 4= completely dried leaf) (Ozan and Maden 2005). Disease severity was calculated by using formula given below (Townsend and Heuberger 1943).

Disease Severity (%) = □ (n * V) *100 / x * N

n: number of leaves entering the scale value; V: scale value; x: highest scale value; N: total number of leaves.

As a result of the pathogenicity test, the morphological features of 60 *A. alternata* isolates confirmed to be pathogens were examined under a compound microscope (Leica DM750, Wetzlar, Germany) with a 40x objective, and identification performed according to Elliot (1917), Ellis (1971), Joly (1964) and Gilman (1959).

In vitro susceptibility testing to fungicides

Susceptibility testing for azoxystrobin, tebuconazole, and mancozeb was conducted *in vitro*. For this purpose, fungicides (azoxystrobin, tebuconazole, and mancozeb) were studied at 0 (control), 0.1 ppm, 0.3 ppm, 1 ppm, 3 ppm, 10 ppm, 30 ppm, 100 ppm, 300 ppm, and 1000 ppm doses, as in preliminary studies (Anonymous 2021). PDAs with fungicide added separately for each dose were poured into 9 cm Petri dishes (14 ml per Petri dish). No fungicide was added to the PDA in the Petri dishes used as a control. After the Petri dishes were prepared in four replications for each dose in a laminar cabinet, 5 mm diameter discs from the pathogen culture grown for 7 days on PDA were placed in the middle. The Petri dishes were incubated at 24±1 °C for 7 days in the dark.

The colony diameters were measured in two directions for each individual culture and averaged. Inhibition values were calculated for each replication of the isolates using the following formula: % inhibition value = [(control growth diameter–application growth diameter)/control growth diameter × 100] (Benlioğlu and Delen 1991).

If the ED_{50} value of an isolate ranged between 0 and 1 ppm, it was considered susceptible (S); if it was between 1 and 15 ppm, it was considered reduced susceptibility (RS); if it was between 15 and 100 ppm, it was considered intermediate resistance (IR); and if it exceeded 100 ppm, it was considered resistant (R) (Avenot et al. 2008).

Statistical analysis

After the calculated % inhibition values were converted to arcsine transformation in the MINITAB statistical program, the logarithm of the doses and angle values were written in the MINITAB statistical program, and the regression equation for each isolate for each plant protection product was created separately. The ED50 (50% inhibition of mycelial growth) values of the isolates were determined using regression equations.

RESULTS

A total of 60 isolates of *Alternaria alternata* were obtained from 121 diseased plant samples collected from the Ankara, Bartın, Zonguldak, and Antalya provinces in 2013-2014. The isolates formed colonies ranging from dark green to blackish-brown on PDA media. The conidiophores of the pathogen are single, small, simple or branched, yellowish-brown in colour, and bear one or more conidial scars. The conidia were oval, obpyriform, or elliptical, had 3-5 transverse and several longitudinal septa, with short beak (2-4 µm) and 9-11 x 20-32 µm in size, and were composed of chains of 5-16 (Figure 1).

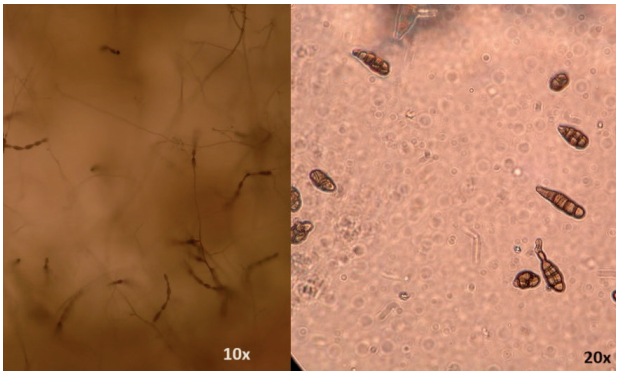

Figure 1. Microscopy images of *Alternaria alternata* conidia

The mean disease severity ranged from 52- 66% for the Bartın isolates, 50–55% for the Zonguldak isolates, 55–75% for the Ankara isolates, and 80–85% for the Antalya isolates. No correlation was found between the susceptibility of *A. alternata* to azoxystrobin, mancozeb, or tebuconazole and disease severity in tomatoes (Table 1).

Reduced sensitivity to azoxystrobin was observed; in all isolates from Bartın, in all except one isolate with moderate resistance from Zonguldak, in all but two sensitive isolates from Polatlı and Malıköy in Ankara, and in six isolates from Antalya. In addition, eight intermediate resistance isolates from Antalya and three high-level resistance isolates obtained from Serik, Kayaburnu and Alanya were identified (Table 1).

All isolates from Beypazarı and Polatlı showed decreased susceptibility to mancozeb, except for two isolates from Ankara, which were sensitive to mancozeb, while no moderate or high-level resistant isolates were obtained. Decreased susceptibility to tebuconazole was detected in all the Bartın and Zonguldak isolates. In Ankara, two isolates from Polatlı and Malıköy were found to be susceptible, whereas the others showed decreased susceptibility. Eight isolates from Antalya developed intermediate resistance, whereas the others showed decreased susceptibility. Isolate number 59 obtained from the Polatlı district of Ankara was susceptible to all three plant protection products, while an isolate obtained from Malıköy was susceptible to azoxystrobin and tebuconazole and showed decreased susceptibility to mancozeb (Table 1).

DISCUSSION

Alternaria solani and *A. alternata* cause leaf blight in tomatoes (Akhtar et al. 2004, Loganathan 2014, Ozan and Maden 2005). Ozan and Maden (2005) reported that *A. alternata* was the causative agent of tomato leaf blight in the Nallıhan, Ayaş and Beypazarı districts of Ankara province

Table 1. Disease severity and sensitivity* to azoxystrobin, mancozeb and tebuconazole of Alternaria alternata isolates obtained different locations

Isolate number	Location	% Disease severity Mean ± SE (Min-Max)	Azoxystrobin ED_{50} (ppm)				Mancozeb ED_{50} (ppm)				Tebuconazole ED_{50} (ppm)			
			S	RS	IR	HR	S	RS	IR	R	S	RS	IR	HR
1	Derbent/BARTIN	60,67±2,6 (58-66)		8,81				12,02				8,92		
2	Derbent/BARTIN	52,67±2,67 (50-58)		8,93				8,07				8,15		
3	Derbent/BARTIN	52,67±2,67 (50-58)		8,52				9,07				9,03		
4	Derbent/BARTIN	63,33±2,67 (58-66)		7,58				4,13				3,44		
5	Derbent/BARTIN	66,00±0,00 (66-66)		8,29				12,69				9,27		
6	Derbent/BARTIN	63,33±2,67 (58-66)		9,90				4,40				4,98		
7	Derbent/BARTIN	60,67±2,67 (58-66)		3,99				3,63				3,43		
8	Derbent/BARTIN	63,33±2,67 (58-66)		4,80				9,70				12,88		
9	Derbent/BARTIN	60,67±2,67 (58-66)		10,42				9,03				9,26		
10	Derbent/BARTIN	58,00±0,00 (58-58)		4,70				7,53				8,15		
11	Derbent/BARTIN	63,33±2,67 (58-66)		5,15				14,45				8,92		
12	Derbent/BARTIN	63,33±2,67 (58-66)		6,51				12,02				3,44		
13	Derbent/BARTIN	58,00±0,00 (58-58)		8,03				9,70				9,03		
14	Derbent/BARTIN	60,67±2,67 (58-66)		6,52				8,07				8,15		
15	Derbent/BARTIN	60,67±2,67 (58-66)		4,46				12,69				8,81		
16	Kayıkçılar/ZONGULDAK	50,00±0,00 (50-50)			17,72			8,50				4,34		
17	Kayıkçılar/ZONGULDAK	55,33±2,67 (50-58)		11,57				7,22				8,15		
18	Kayıkçılar/ZONGULDAK	52,67±2,67 (50-58)		10,17				7,57				8,92		
19	Kayıkçılar/ZONGULDAK	52,67±2,67 (50-58)		14,50				9,03				9,15		
20	Bakacakkadı/ZONGULDAK	52,67±2,67 (50-58)		3,49				10,05				3,44		
21	Bakacakkadı/ZONGULDAK	50,00±0,00 (50-50)		3,42				7,22				8,15		
22	Bakacakkadı/ZONGULDAK	52,67±2,67 (50-58)		9,57				8,68				3,43		
23	Kayıkçılar/ZONGULDAK	50,00±0,00 (50-50)		10,30				7,57				9,03		
24	Bakacakkadı/ZONGULDAK	52,67±2,67 (50-58)		6,84				3,63				8,92		

25	Bakacakkadı/ZONGULDAK	50,00±0,00 (50-50)	10,16			8,50	8,15	
26	Alanya/ANTALYA	80,33±2,67 (75-83)	10,24			7,77	4,93	
27	Serik/ANTALYA	80,33±2,67 (75-83)			239,10	6,69		15,99
28	Serik/ANTALYA	85,67±2,67 (83-91)		28,18		6,33		17,56
29	Serik/ANTALYA	83,00±0,00 (83-83)		33,61		4,87		24,18
30	Serik/ANTALYA	80,33±2,67 (75-83)	14,81			8,81	8,70	
31	Serik/ANTALYA	85,67±2,67 (83-91)		18,28		6,33	12,95	
32	Kumluca/ANTALYA	80,33±2,67 (75-83)	14,57			10,23	11,05	
33	Serik/ANTALYA	80,33±2,67 (75-83)	7,16			6,42	12,92	
34	Serik/ANTALYA	80,33±2,67 (75-83)		33,50		5,56		33,61
35	Turunçova/ANTALYA	80,33±2,67 (75-83)		17,32		5,66		17,64
36	Serik/ANTALYA	80,33±2,67 (75-83)		18,88		6,61		16,90
37	Kayaburnu/ANTALYA	83,00±0,00 (83-83)	14,42			3,63	12,95	
38	Kayaburnu/ANTALYA	80,33±2,67 (75-83)			204,00	4,93		18,94
39	Kayaburnu/ANTALYA	80,33±2,67 (75-83)		54,08		5,52		33,61
40	Kumluca/ANTALYA	83,00±0,00 (83-83)	9,88			9,15	9,57	
41	Alanya/ANTALYA	85,67±2,67 (83-91)			452,00	5,34	7,03	
42	Alanya/ANTALYA	85,67±2,67 (83-91)		64,65		5,52	7,57	
43	Çubuk/ANKARA	69,00±3,00 (66-75)	12,05			4,46	4,30	
44	Çubuk/ANKARA	60,67±2,67 (58-66)	4,20			4,36	4,20	
45	Çubuk/ANKARA	66,00±0,00 (66-66)	10,91			2,40	2,43	
46	Çubuk/ANKARA	60,67±2,67 (58-66)	4,64			1,89	1,85	
47	Çubuk/ANKARA	58,00±0,00 (58-58)	14,57			4,29	4,23	
48	Çubuk/ANKARA	60,67±2,67 (58-66)	8,90			2,40	2,43	
49	Çubuk/ANKARA	60,67±2,67 (58-66)	8,32			4,17	4,14	
50	Ayaş/ANKARA	58,00±0,00 (58-58)	3,93			2,01	2,00	
51	Haymana/ANKARA	58,00±4,62 (50-66)	2,71			2,63	2,56	
52	Haymana/ANKARA	60,67±2,67 (58-66)	6,86			4,18	4,06	

53	Haymana/ANKARA	55,33±2,67 (50-58)		11,76		1,94		1,89
54	Beypazarı/ANKARA	72,00±3,00 (66-75)		2,61	0,73			2,43
55	Akkaya/ANKARA	75,00±0,00 (75-75)		8,50		2,67		3,84
56	Akçakavak/ANKARA	72,00±3,00 (66-75)		4,96		4,31		4,08
57	Dibecik/ANKARA	60,67±2,67 (58-66)		3,63		1,96		2,10
58	Ayaş/ANKARA	60,67±2,67 (58-66)		1,33		1,92		1,89
59	Polatlı/ANKARA	58,00±0,00 (58-58)	0,38		0,59			
60	Malıköy/ANKARA	55,33±2,67 (50-58)	0,50			1,71		

*S=Sensitive (0-1 ppm), RS=Reduced sensitivity (1-15 ppm), IR= Intermediate-resistant (15-100 ppm), HR=Highly resistant (100< ppm)

and had an average prevalence of 12%. The finding that *A. alternata* is a pathogen that causes early leaf blight in tomatoes is consistent with the results of Ozan and Maden (2004, 2005), Gazozcuzade (2010), Mutlu and Üstüner (2017).

Although there is no record of resistance of *A. alternata* to azoxystrobin in tomato, it has been reported in pistachio (Avenot and Michallides 2007, Ma et al. 2003), apple (Ishii 2008), citrus fruit (Mondal et al. 2009), potato (Fairchild et al. 2013) and leafy vegetable plants (Matić et al. 2019).

The susceptibility to mancozeb was reduced in 96.6% of the isolates, with ED_{50} values between 1 and 15 ppm. There was no intermediate or high-level resistance to mancozeb, and the decrease in susceptibility was less than that of azoxystrobin. Benlioğlu and Delen (1991) reported that the ED_{50} values of mancozeb for 60 *A. solani* isolates ranged from 3-300 µg/l. In that study, *A. alternata* appeared to be more sensitive to mancozeb than *A. solani*. He et al. (2017) reported no resistance of *A. alternata* to mancozeb in China, as in this study.

The ED_{50} values of tebuconazole for 86.6% of the isolates were between 1 and 15 ppm. The decrease in sensitivity to tebuconazole was lower than that to azoxystrobin, as was the case for mancozeb. Moreover, resistance to tebuconazole, a fungicide of the azole group with demethylation inhibiting activity, has been reported to be moderate in many fungi of the ascomycete group, such as black spot (*Venturia ineaqualis*), gray mold (*Botrytis cinerea*), monilia (*Monilinia fructicola*), powdery mildew (*Erysiphe graminis*) (Anonymous 2022). A total of 13.3% of the isolates in the study were found to be moderately resistant to tebuconazole. Malandrakis et al. (2015) reported that 42 *A. alternata* isolates obtained from tomato fields and greenhouses in southern Greece, were resistant to mancozeb (ED_{50} = 2.34 - >100 µg/l), and the ED_{50} values of tebuconazole ranged from 0.43 - 20 µg/l.

In general, the resistance rates to azoxystrobin, mancozeb, and tebuconazole in isolates from greenhouses in Antalya, Bartın, and Zonguldak provinces were greater than those in isolates from open-field tomato-growing areas in Ankara Province. This is thought to be because disease is more common in greenhouses than in open fields, and because plant protection products are applied more often in greenhouses.

It is important to recognize and monitor resistance related to resistance in *A. alternata* and develop resistance management strategies. In this study, the susceptibility of *A. alternata* to azoxystrobin, tebuconazole and mancozeb was determined in tomatoes from Antalya, Bartın, Zonguldak and Ankara provinces. Fungicide-resistant, less susceptible and sensitive strains of *A. alternata* were detected in our country. The results of this study showed that since *A. alternata* strains vary in their sensitivity to fungicides depending on the region, fungicide recommendations may differ according to the areas where resistance occurs rather than countrywide.

It is important to determine the sensitivity of plant pathogenic fungi to plant protection products, and studies should be conducted regularly to determine the decrease in sensitivity in advance and to establish strategies for resistance management.

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presented as a poster presentation titled "Determination of the susceptibility of Early Leaf Blight in Tomatoes (*Alternaria* spp.) to Azoxystrobin" at VI. The Plant Protection Congress held in Konya on 5-8 September 2016. Another part of the study was presented as a poster at the 3rd Agriculture and Food Congress of the Central Anatolia Region held in Sivas on October 26-28, 2017, titled "Determination of resistance to azoxystrobin and mancozeb in tomato early blight (*Alternaria alternata*)."

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Domateste erken yaprak yanıklığı, *Alternaria alternata* (Fr.) Keissler)'nın neden olduğu yaygın bir fungal hastalıktır. Hastalığın kimyasal mücadelesinde çeşitli fungisitler kullanılmaktadır. Fungisitlerin çok sık kullanımı sonucunda patojende duyarlılık azalışı görülebilmektedir. Bu çalışmada, domates ekiliş alanlarında sorun olan erken yaprak yanıklığı hastalığının, azoxystrobin, tebuconazole ve mancozeb etkili maddelerine karşı duyarlılığı belirlenmiştir. Antalya, Ankara, Bartın ve Zonguldak illerinden 2013 ve 2014 yıllarında toplanan 121 enfekteli bitki örneğinden yapılan izolasyonlar sonucunda 60 adet *Alternaria alternata* izolatı elde edilmiştir. Patojenisite testleri sonucunda izolatların hastalık şiddetlerinin ortalama %50-85 arasında değişiklik gösterdiği tespit edilmiştir. İzolatların azoxystrobin, tebuconazole ve mancozeb'e duyarlılık düzeyleri radyal gelişme testi ile belirlenmiş ve azoxystrobin, tebuconazole ve mancozeb'e karşı ED_{50} değerleri sırasıyla 0.4 ppm, 0.6 ppm ve 0.6 ppm olarak belirlenmiştir. Polatlı izolatı üç etken maddeye karşı en duyarlı izolat olmuştur. Alanya izolatı azoxystrobine karşı (ED_{50}= 452 ppm), Derbent izolatı mancozeb'e karşı (ED_{50}= 14.45 ppm) , Serik ve Kayaburnu izolatları tebuconazole'e karşı (ED_{50}= 33.61 ppm) en yüksek dirence sahip izolatlar olarak belirlenmiştir. Çalışma sonucunda izolatlardan bazılarının bu fungisitlere karşı direnç geliştirdiği tespit edilmiştir.

Anahtar kelimeler: *Alternaria alternata*, azoxystrobin, tebuconazole, mancozeb, duyarlılık

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Original article

Nematophagous fungi species from Erzurum and Erzincan provinces in Türkiye

Türkiye'de Erzurum ve Erzincan illerinden elde edilen nematofag fungus türleri

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ABSTRACT

In Erzurum and Erzincan provinces, some nematophagous fungi were determined on roots of alfalfa (*Medicago sativa* L.), potato (*Solanum tuberosum* L.), and strawberry (*Fragaria x ananassa* Duchesne) plants during 2009-2011, and identification of fungi species was performed by classical and/or molecular techniques. Purified 5 isolates were determined as *Arthrobotrys cladodes Drechsler* 1937 (1 isolate), *Arthrobotrys conoides* Drechsler 1937 (1 isolate) and *Arthrobotrys superba* Corda 1839 (3 isolates). Identification of *Arthrobotrys* isolates was also confirmed by ribosomal DNA (rDNA)-ITS (internal transcribed spacer) sequence analysis. *Harposporium* genus was also determined considering the morphological characteristic of fungal spores growing on nematodes found in two samples, but these fungi could not be purified. *Harposporium* species were identified as *Harposporium anguillulae* Lohde emend. Zopf 1888 and *Harposporium crassum* A.M. Sheph 1955 according to the morphological characteristic. To our knowledge, all *Arthrobotrys* and *Harposporium* species identified in this study are reported for the first time in Türkiye.

INTRODUCTION

Plant-parasitic nematodes cause serious damage to many important crops such as alfalfa (*Medicago sativa* L.), potato (*Solanum tuberosum* L.), and strawberry (*Fragaria x ananassa* Duchesne). Fungi are a group of organisms that are highly abundant in soils, and some of these fungi species feed on various nematode species. In contrast, others infect many plant species or survive as saprophytic in the soil. More than 700 species of nematophagous (nematode-destroying) fungi can control plant-parasitic nematodes through antagonistic behavior (Li et al. 2015). Nematophagous fungi are potentially important for biological control, an indispensable component in sustainable agriculture, and play a major role in integrated pest management programs (Eken et al. 2023, Yadav et al. 2023).

Nematophagous fungi were divided into four main groups according to their mechanism of action: nematode-trapping, nematode egg and female parasites, endoparasitic, and toxin-producing fungi (Hyde et al. 2014). Species belonging to the genera *Arthrobotrys, Cystopage, Dactylella,*

Dactylellina, Drechslerella, Hohenbuehelia, Hyphoderma, Monacrosporium, Nematoctonus, Orbilia, Stylopage, Tridentaria, Tripospora and *Zoophagus* are nematode-trapping fungi; *Drechmeria, Harposporium, Hirsutella, Nematoctonus* and *Myzocytium* are endoparasitic fungi; *Lecanicillium, Nematophthora, Paecilomyces* and *Pochonia* are egg and cyst parasitizing fungi; *Coprinus* and *Pleurotus* are toxin-producing fungi (Yang and Zhang 2014, Zhang et al. 2011). These species belong to fungal taxa, including Oomycota, Chytridiomycota, Blastocladiomycota, Zygomycota, Ascomycota, and Basidiomycota (Li et al. 2015, Yang and Zhang 2014).

There are few studies on the isolation of *Arthrobotrys* species in Türkiye. *Arthrobotrys arthrobotryoides* (Berl.) Lindau 1905 was isolated from outdoor air (İmalı 2005, İmalı et al. 2011, Kalyoncu and Ekmekci 2008), *Arthrobotrys oligospora* Fresen. 1850 from egg masses and females of *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 (Tylenchida: Heteroderidae) nematode species (Karakas 2015), and *Arthrobotrys* sp. from wheat and barley seeds (Yurdakul 2019).

The study aims to identify nematophagous fungi by using morphological and/or molecular methods. These fungi were collected during the studies to determine the fungal plant pathogens that cause disease in the roots of alfalfa, potato and strawberry plants in Erzurum and Erzincan provinces.

MATERIALS AND METHODS

Isolation of nematophagous species

Nematophagous fungi were isolated during the studies to determine the fungal plant pathogens that cause disease in the roots of various plants. Diseased alfalfa, potato, and strawberry plants were collected from fields in Erzurum and Erzincan provinces during 2009-2011. The roots of the plants were washed in running tap water to eliminate the soil. Plant parts (1.5 cm long) were disinfected in 1% sodium hypochlorite (NaOCl) for 1 min, immersed with sterile distilled water, and placed on 1.5% Water Agar (WA) supplemented 50 mg/l streptomycin sulfate. After a 3-7 day incubation period at 25 °C in the dark, it was observed that nematodes growing in some Petri dishes were caught by various fungal structures. These plates were periodically examined for growth of nematophagous fungi for an additional 10-15 days. Nematophagous fungi were purified using the single spore isolation method, then transferred to a slant containing Potato Dextrose Agar (PDA) and stored at 5 °C.

Morphological and/or molecular identification of isolates

Nematophagous fungi species were determined using classical and/or molecular methods. In the classical method, fungi were characterized based on their morphological characteristics (Karling 1938, Shepherd 1955, Yu et al. 2014). Five purified isolates were grown on PDA in the dark at 25 °C for 7-14 days to determine differences in conidia size, colony morphology, and radial growth rate. A five mm diameter agar disk from the edge of the colony of the isolate was placed in the center of a 9 cm diameter Petri dish containing PDA, and colony diameter was determined after 7 days. Four Petri dishes were used for each isolate, and the average of the colony diameter measurements made at right angles to each other was taken to determine the measurement value in each replicate. Conidia size was determined for each isolate by measuring conidia (n= 50) at right angles longitudinally and transversely using a phase contrast microscope. Measurements values were recorded as minimum-maximum (average). The morphological features of the fungi in the two unpurified samples were examined only with the phase contrast microscope. All micromorphological features were photographed with an Olympus BH2 microscope.

Purified five isolates were also identified molecularly using ribosomal DNA (rDNA)-ITS (Internal Transcribed Spacer) regions (ITS1, 5.8, ITS2). Mycelia of the isolates to be used in DNA isolation were obtained as described by Genc Kesimci et al. (2022). Genomic DNA isolation, PCR amplification using ITS1 and ITS4 primers (White et al. 1990), and sequence analysis from these isolates were performed by REFGEN (Ankara University Technopolis, Ankara, Türkiye). The sequences of each isolate were edited using BioEdit software, version 7 (Hall 1999), and aligned using the Clustal W algorithm (Thompson et al. 1994). The sequences of isolates were performed by BLAST (Basic Local Alignment Search Tool) analysis and compared with the other sequences in the National Center for Biotechnology Information (NCBI) database. Sequence similarity of 97% or above was taken into account when determining the species. The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei 1987) implemented in MEGA software, version 6 (Tamura et al. 2013) using reference isolates (GenBank MH857992.1, MH179686.1, and MZ427475.1) whose base sequences were obtained from the NCBI database, and *Dactylellina appendiculata* (Anastasiou) M. Scholler, Hagedorn & A. Rubner 1999 (GenBank MH858419.1) as the distant species, and 1000 bootstrap replicates. The sequences of 5 isolates were deposited in the NCBI database and accession numbers were obtained (Table 1).

Table 1. Isolate code, isolation source, sampling year, location, and GenBank accession number of isolates of *Arthrobotrys* species identified by classical and molecular techniques

Species	Isolate	Isolation Source	Year	Location	Genbank Accession Number
Arthrobotrys cladodes	S-KHB-14	Strawberry	2009	Yakutiye-Erzurum	OR149151
Arthrobotrys conoides	A-2	Potato	2011	Pasinler-Erzurum	OR149152
Arthrobotrys superba	NF-1	Strawberry	2009	Yakutiye-Erzurum	OR149153
Arthrobotrys superba	SS10-6	Strawberry	2009	Yakutiye-Erzurum	OR149154
Arthrobotrys superba	MTT-13	Alfalfa	2010	Pasinler-Erzurum	OR149155

RESULTS AND DISCUSSION

Five isolates were obtained from roots of alfalfa (1 isolate), potato (1 isolate), and strawberry (3 isolates) plants in Erzurum province (Table 1). These isolates were identified as *Arthrobotrys* genus by the classical identification techniques (Yu et al. 2014). Based on morphological descriptions, the 5 isolates were defined as *Arthrobotrys cladodes* Drechsler 1937 (teleomorph: *Orbilia cladodes* (Drechsler) E. Weber & Baral) (1 isolate), *Arthrobotrys conoides* Drechsler 1937 (teleomorph: unknown) (1 isolate) and *Arthrobotrys superba* Corda 1839 (teleomorph: *Orbilia auricolor* (A. Bloxam) Sacc.) (3 isolates). The *Arthrobotrys* isolates were examined for their morphological characteristics, including colony features (Figure 1 and Table 2) and conidia (Figure 2 and Table 2). Conidia size was given as minimum-maximum (average) measurements.

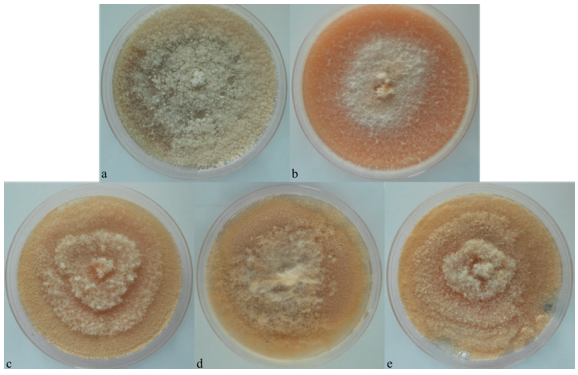

Figure 1. Colony of *Arthrobotrys* spp. on Potato Dextrose Agar media. a) *Arthrobotrys cladodes* (S-KHB-14); b) *Arthrobotrys conoides* (A-2); c-e) *Arthrobotrys superba* (NF-1, SS10-6 and MTT-13, respectively)

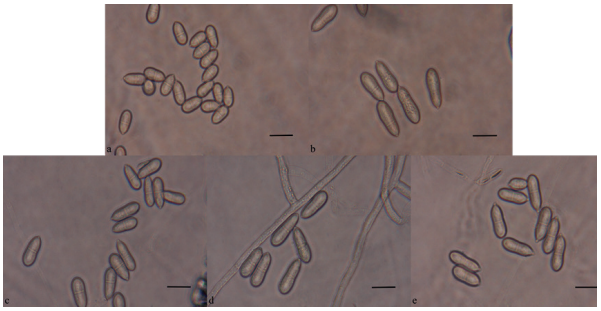

Figure 2. Conidia of *Arthrobotrys* spp. on Potato Dextrose Agar media. a) *Arthrobotrys cladodes* (S-KHB-14); b) *Arthrobotrys conoides* (A-2); c-e) *Arthrobotrys superba* (NF-1, SS10-6 and MTT-13, respectively). Scale bars: a-e= 20 µm

Arthrobotrys cladodes was isolated from strawberry root in Yakutiye-Erzurum, *A. conoides* from potato root in Pasinler-Erzurum, and *A. superba* from alfalfa and strawberry roots in Pasinler-Erzurum and Yakutiye-Erzurum, respectively (Table 1).

Arthrobotrys cladodes isolate (S-KHB-14) on PDA was whitish, then turned greenish-yellow and cottony (Figure 1a), growing rapidly to reach an 85 mm diameter after 7 days in the incubator at 25 °C (Table 2). Conidia ellipsoid, 1-septate at the center of the spore (Figure 2a), measuring 14.2-19.8 (16.6) x 6.6-11.2 (8.5) µm (Table 2).

Arthrobotrys conoides isolate (A-2) on PDA was initially whitish, then turned to reddish-orange, with white superficial hyphae towards the center (Figure 1b), growing rapidly to reach an 80 mm diameter after 7 days in the incubator at 25 °C (Table 2). Conidia elongate obconical, 1-septate about one-third from the basal end, and constricted at the septum (Figure 2b), measuring 21.1-38.4 (30.2) x 6.9-12.4 (9.5) µm (Table 2).

Table 2. Colony diameter, conidia length, and width of *Arthrobotrys* isolates

Species	Isolate	Colony Diameter (mm)	Conidia Length* (µm)	Conidia Width (µm)
Arthrobotrys cladodes	S-KHB-14	85	14.2-19.8 (16.6)	6.6-11.2 (8.5)
Arthrobotrys conoides	A-2	80	21.1-38.4 (30.2)	6.9-12.4 (9.5)
Arthrobotrys superba	NF-1	54	19.2-30.9 (24.2)	6.8-10.2 (8.4)
Arthrobotrys superba	SS10-6	54	21.8-33.8 (28.0)	7.0-10.0 (8.7)
Arthrobotrys superba	MTT-13	44	20.5-28.9 (24.5)	8.0-13.3 (10.1)

*: Minimum-Maximum (Average)

Arthrobotrys superba isolates (NF-1, SS10-6, and MTT-13) on PDA were initially whitish, then turned to yellowish-orange and cottony (Figure 1c-e), growing moderately to reach diameters of 54, 54, and 44 mm after 7 days in the incubator at 25 °C, respectively (Table 2). Conidia elliptical, 1-septate at the center of spore, slightly constricted at the septum (Figure 2c-e), measuring 19.2-33.8 (25.6) x 6.8-13.3 (9.1) µm (Table 2).

The *Arthrobotrys cladodes*, *A. conoides* and *A. superba* isolates obtained in this study are nematode-trapping fungi and capture nematodes using three-dimensional adhesive networks (Figure 3). According to our observations during the study, when *Arthrobotrys* species and nematodes were co-inoculated into Petri dishes containing 1.5% WA, abundant three-dimensional adhesive networks were formed in the entire colony, and nematode growth was 100% inhibited within two weeks. In the nematode-free Petri dishes, it has been observed that there is no adhesive network formation in the fungal colony or it was very few in the center of the colony, so three-dimensional adhesive network formation occurs in response to the presence of nematodes. The nematode species used in this study were not identified.

In addition to identification based on morphological characteristics, *Arthrobotrys* isolates were also identified based on sequence analysis of rDNA-ITS regions. A sequence similarity of 97% or above was considered for species determination. A neighbor-joining phylogenetic tree of the 5 *Arthrobotrys* isolates, reference isolates, and distant species is shown in Figure 4. Ultimately, *Arthrobotrys* isolates (Table 1) were confirmed as *A. cladodes* (1 isolate), *A. conoides* (1 isolate), and *A. superba* (3 isolates).

The *Harposporium* genus was determined on the morphological characteristic of fungal spores growing on nematodes in two samples from strawberry plants in 2009 (Figure 5), although these fungi could not be purified. *Harposporium* species were classified as *Harposporium anguillulae* Lohde emend. Zopf 1888 and *Harposporium crassum* A.M. Sheph. 1955 based on the morphological characteristics of the conidia and/or conidiophores (Karling

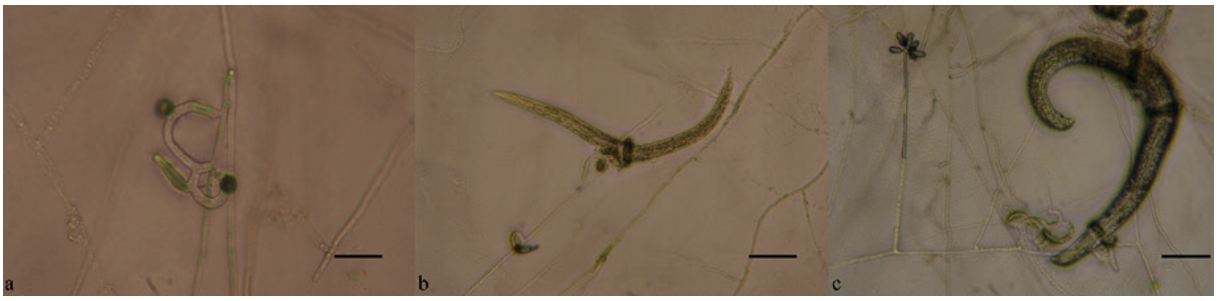

Figure 3. The trap formation process and interaction between the nematophagous fungi *Arthrobotrys* sp. and nematode on Water Agar surface. a) Three-dimensional adhesive network; b) Trapped nematode in three-dimensional adhesive network at a point; c) Conidia and conidiophores of *Arthrobotrys* sp., and nematode trapped in three-dimensional adhesive networks at various points. Scale bars: a= 40 µm; b, c= 80 µm

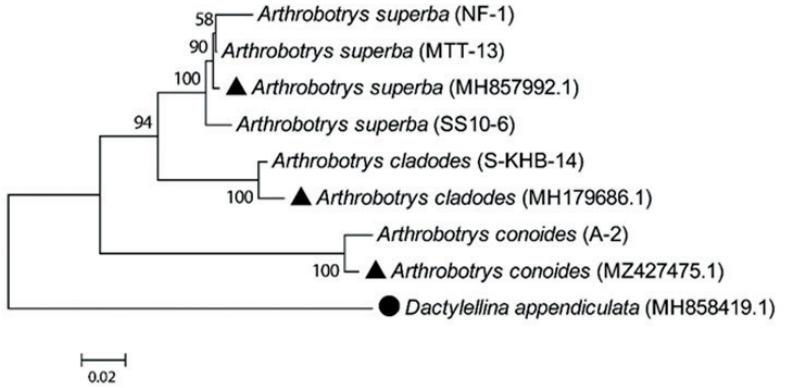

Figure 4. Phylogenetic tree based on rDNA-ITS sequences constructed using the neighbor-joining method for *Arthrobotrys* isolates. The numbers on the tree branches indicate the bootstrap values from 1000 replicates using Mega 6.0. The outgroup, *Dactylellina appendiculata* was used to root the tree. ▲: Reference isolates, ●: Outgroup

1938, Shepherd 1955, Wang et al. 2007). *Harposporium anguillulae* conidia were sickle-shaped, pointed at both ends, and non-septate (Figure 5a), measuring 8-12 (9.7) x 2 µm. *Harposporium crassum* conidia were arcuate, pointed at both ends, non-septate (Figure 5b), measuring 12-20 (14.5) x 2 µm. The spores of *H. anguillulae* are smaller than those of *H. crassum. Harposporium anguillulae* and *H. crassum* were determined in the samples collected from Yakutiye-Erzurum and Üzümlü-Erzincan, respectively.

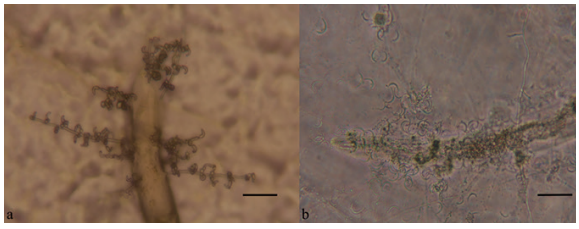

Figure 5. Conidia and/or conidiophores of *Harposporium* spp. on nematode body. a) *Harposporium anguillulae*; b) *Harposporium crassum*. Scale bars: a, b = 40 µm

In this study, *Arthrobotrys* and *Harposporium* species were determined from the roots of various plants or nematodes living on plant roots. These fungi were collected during the studies carried out for the detection of fungal pathogens that cause root rot diseases. In many studies, nematode–fungus disease complexes were reported in several host crops as they occupied the same ecological niche (Back et al. 2002). The fact that they are the most abundant organisms in the soil habitat causes multiple interactions as antagonistic and synergistic interactions between these two groups (Zhang et al. 2020). It is stated that nematophagous fungi are efficient in the biocontrol of parasitic nematodes in antagonistic interaction (De Freitas Soares et al. 2023).

Nematophagous fungi are natural enemies of nematodes and have cosmopolitan distribution (De Freitas Soares et al. 2018). *Arthrobotrys* is the most complex, wide, and dominant group genus of nematophagous fungi in most habitats representing 59 accepted species (Zhang et al. 2022). Also, *Harposporium* are commonly known nematode-trapping fungi genera (Yang and Zhang 2014). In Türkiye, *A. arthrobotryoides* was isolated from outdoor air samples in Çorum, Manisa and Van province (İmalı 2005, İmalı et al. 2011, Kalyoncu and Ekmekci 2008), *A. oligospora* from egg masses and females of *M. incognita* from tomato fields in Ankara province (Karakas 2015) and *Arthrobotrys* sp. from wheat and barley seeds in Konya province (Yurdakul 2019). It was reported that 3 *Arthrobotrys* species obtained in this study were isolated from the field and forest soil (Yu et al. 2014).

Nematode–trapping fungi form specific trapping structures on hypha, such as adhesive networks, adhesive knobs, and constricting rings (Rubner 1996). It is determined that *Arthrobotrys cladodes*, *A. conoides* and *A. superba* are nematode-trapping fungi and capture nematodes by three-dimensional adhesive networks in this study; however, *Harposporium* species are endoparasitic fungi and have specially shaped spores that are ingested by nematodes. Because of their shape, the spores get stuck in the esophagus of the nematodes and start the infection from there (Aschner and Kohn 1958). A nematode containing the species *H. anguillulae* or *H. crassum*, conidia and conidiophores form outside of the nematode cadaver.

Accurate identification of the biological agents is the most important condition for success in biological control. Morphological identification of fungi is a traditional method that has been used for many years. However, distinguishing morphologically similar species in this way

may lead to misconceptions for scientists who do not have sufficient experience. Therefore, in addition to the classical diagnostic method, ribosomal DNA-ITS regions are widely used for molecular identification of fungi species (Li et al. 2014, Wang et al. 2022). In this study, the identification of *Arthrobotrys* isolates was also confirmed by rDNA-ITS sequence analysis. Similar to the previous study, molecular techniques are used to identify fungi (Zhang et al. 2022). In this study, all *Arthrobotrys* and *Harposporium* species were determined for the first time from Türkiye. Studies on nematophagous fungi in Türkiye, which has a potential in terms of biological control, should be increased.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Erzurum ve Erzincan illerinde yonca (*Medicago sativa* L.), patates (*Solanum tuberosum* L.) ve çilek (*Fragaria* x ananassa Duchesne) bitkilerinin köklerinde 2009-2011 yıllarında bazı nematofag funguslar belirlenmiş ve fungus türlerinin tanılanması klasik ve/veya moleküler tekniklerle gerçekleştirilmiştir. Saflaştırılan 5 izolatın *Arthrobotrys cladodes* Drechsler 1937 (1 izolat), *Arthrobotrys conoides* Drechsler 1937 (1 izolat) ve *Arthrobotrys superba* Corda 1839 (3 izolat) türlerine ait olduğu belirlenmiştir. *Arthrobotrys* izolatlarının tanılanması ribozomal DNA (rDNA)-ITS (internal transcribed spacer) baz dizi analizi ile de doğrulanmıştır. İki örnekte bulunan nematodlar üzerinde gelişen sporların morfolojik özellikleri dikkate alınarak *Harposporium* cinsi de belirlenmiş, ancak bu funguslar saflaştırılamamıştır. *Harposporium* türleri morfolojik özelliklerine göre *Harposporium anguillulae* Lohde emend. Zopf 1888 ve *Harposporium crassum* A.M. Sheph 1955 olarak tanılanmıştır. Bildiğimiz kadarıyla bu çalışmada belirlenen tüm *Arthrobotrys* ve *Harposporium* türleri Türkiye'de ilk defa rapor edilmiştir.

Anahtar kelimeler: *Arthrobotrys, Harposporium*, nematofag fungus, rDNA-ITS bölgesi

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Original article

Effect of trap bait and colours, and number of entry holes in monitoring of Drosophilidae (Diptera) species in a fig orchard

Bir incir bahçesinde Drosophilidae (Diptera) türlerinin izlenmesinde tuzak cezbedicisi, tuzak rengi ve giriş delik sayısının etkisi

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ABSTRACT

The study's objective was to evaluate how the capture rate of bottle traps is influenced by their color, number of entry holes, and bait liquids.For this purpose, yellow, blue, green, red, black, white, and transparent (colourless) traps were tested. Apple cider vinegar, grape vinegar, white wine, yeast, and water (control) were used as bait liquids. The number of entry holes was 2, 4, 6, and 8 holes per trap on the side of the bottle traps. The experiments were conducted in a randomised block design with three replicates in a fig orchard (variety Bursa Siyahı) with 437 trees in Aydın province from September 2018 to March 2019. In total, 48 traps were mounted on the experimental trees (1 trap per 1 tree), and counting of the drosophilid individuals in the traps was performed weekly. Red-coloured traps attracted the highest number of drosophilid individuals, followed by yellow-coloured. Regarding different baits, grape vinegar attracted the most drosophilid individuals, followed by white wine. Concerning the number of trap entry holes, the highest number of individuals were caught in traps with the highest number of holes, 8. In all traps, *Zaprionus tuberculatus* Malloch, 1932 was the species caught in the highest numbers, followed by *Drosophila subobscura* Collin, 1936 and *Drosophila suzukii* (Matsumura, 1931). *Z. tuberculatus* was mostly caught in traps containing grape vinegar, while *D. subobscura* and *D. suzukii* were mainly caught in traps containing white wine. In our study, the red-coloured traps containing vinegar with 8 entry holes were the most effective in monitoring drosophilid populations.

INTRODUCTION

Drosophilidae members are minute and fragile flies represented by highly numerous species. Brake and Bachli (2008) stated that the family Drosophilidae comprises 3950 valid species and is distributed worldwide throughout many biogeographic regions. Many drosophilid species inhabit and consume fermenting and decaying organic

materials (Atkinson 1977, Schmitz et al. 2007). Unlike other common drosophilid species, *D. suzukii* is a pest on ripening fruits whose females can insert their highly sclerotised serrated ovipositor into the intact fruit to lay eggs (Walsh et al. 2011). It is an invasive pest species reported in many countries and introduced into many others (Calabria et al. 2012, Depra et al. 2014, Hauser 2011, Kinjo et al. 2014, Lee et al. 2011). Since the first appearance of *D. suzukii* in Turkey (Orhan et al. 2014), the presence of this pest has been reported in many regions, and many studies have been performed in the country. Tozlu et al. (2018) studied the bacterial composition isolated from *D. suzukii*, and the hypersensitivity reaction of these bacteria was determined. In addition, population dynamics (Zengin and Karaca 2019), population development in vineyards (Kasap and Özdamar 2019), its parasitoids (Kaçar 2020), its spread and hosts (Özbek-Çatal et al. 2021), population dynamics and damage in cherry and nectar orchards (Arıdıcı-Kara and Ulusoy 2022) were studied. Moreover, an international project (including Türkiye) on the biology, ecology and control of *D. suzukii* for IPM studies was carried out (Sanches-Ramos et al. 2022).

Recently, the other drosophilid species, *Zaprionus indianus* (Gupta) 1970, has been reported as a serious pest in Turkey (Özbek Çatal et al. 2019). *Z. indianus* has recently introduced into many countries (Commar et al. 2012). Drosophilids are common flies in fruit-growing areas, especially where a mixed culture of fruit trees is available. Their populations can reach high levels where environmental conditions such as climate and availability of breeding sites are favourable for them in Turkey. In three orchards in Aydın (Çakmar village), a total of 11 drosophid species was shown using yellow-banded grape vinegar traps (Başpınar et al. 2022). Drosophilids are flies that typically feed on ripe fruits and have hosts from plants other than agricultural products. They are polyphagous and have numerous generations per year, which makes their control difficult (Bieńkowski and Orlova-Bienkowskaja 2020, Kenis et al. 2016, Lee et al. 2015, Wang et al. 2022). Additionally, there is also insufficient information about the natural enemies of drosophilids (Walsh et al. 2011).

Monitoring the pest population is the first step to determining the time of insecticide application (Ekström and Ekbom 2011). The bait traps are effective tools for monitoring and controlling *D. suzukii* (Cha et al. 2018). However, many factors affect the capture efficiency of food bait traps used for monitoring and mass trapping.

These factors include the type of trap, some physical features such as trap colour, size, shape, the kind and quality of the lure, the position and number of entry holes on the traps and the site and location where the traps are placed (Basoalto et al. 2013, Lee et al. 2012, Renkema et al. 2014). Trap efficacy also depends on ambient temperature and phenological stages of plants; thus, different attractants are suitable to be applied in different phenological periods, localities and hosts (Tonina et al. 2017).

Recently, many studies have focused on developing more effective species-specific and economic traps for drosophilids (Basoalto et al. 2013, Lee et al. 2012, Renkema et al. 2014).

Therefore, it is essential to determine the effectiveness of traps in pest management. Our study aimed to compare the efficacy of different trap baits and colours and the number of entry holes in monitoring drosophilids.

MATERIALS AND METHODS

Site, preparation of traps, and protocols

The study was conducted in a fig orchard (variety Bursa Siyahı) in size of 2 ha with 437 trees in Aydın province, (37°45'25.0"N, 27°46'49.1"E) from September 2018 to March 2019. The efficacy of trap colour, the number of holes for the entry of drosophilids on the side of the bottle trap, and the attractiveness of different liquid baits were studied in separate trials. Transparent plastic bottles of 500 ml were used as bottle traps. They were perforated with holes (0.25 cm in diameter) placed in the upper quarter of the bottle as entries for drosophilids, and 100 ml of liquid bait was added into the traps. The bottle traps were placed in the orchard in the canopy of trees at a 1.5-2.0 metres above the ground on the south side of the trees and replaced with new ones every week. Fly samples were separated and counted under the stereomicroscope. They were then preserved in vials with 70% alcohol and stored in the fridge for identification. Misshaped or incomplete individuals were omitted from the samples.

Experiments

Three successional experiments were conducted at the same orchard to compare the efficacy of different trap baits and colours and the number of entry holes in monitoring drosophilids. First, in the colour experiment, seven different colours (yellow, blue, green, red, black, white, colourless (transparent) (Table 1) were implemented, keeping the bait grape vinegar and the number of 4 entry holes constant. Paint cards were cut in 4 cm width bands and stuck in the surrounding middle of the bottle. In the following bait experiment, five different liquid baits were used (apple cider, vinegar, grape vinegar, white vine, yeast, and

water) (Table 2), keeping the red colour and the number of 4 entry holes constant. Finally, in the hole number experiment, four different entry hole numbers (2, 4, 6, 8) per trap on the side of plastic bottles were tested, keeping grape vinegar as bait and the red colour constant.

Table 1. Characteristics of the paint card colours stuck on the traps

Paint card colour	Colour analysis[1]		
	L* value	a* value	b* value
Yellow	78.90	14.66	80.43
Blue	32.63	1.16	-29.42
Green	59.88	-24.40	45.73
Red	44.12	49.56	25.07
Black	25.31	0.04	-0.31
White	92.25	0.27	0.99
Transparent (=clear)	-	-	-

[1]Paint card colours were analysed with a DOHO DR-10 colourmeter

Table 2. Bait liquids in the traps

Bait liquids	Trade Mark
Apple cider vinegar	KlikyaTM with an acidity of 4-5%
Grape vinegar	TarişTM with an acidity of 4-5%
White wine	DimitriTM with the alcohol of 13%
Yeast	PakmayaTM ingredient is *Saccharomyces cerevisiae*
Water (as control)	Tap water

Experiments were set up in a randomised complete block design with three replicates. Experimental plots were placed in three rows, and each row was represented for each replication, omitting one row between replicates. Traps were placed individually on every other tree in the rows. During the trial period, 16 traps were placed on the experimental tree in each row. Trials were conducted on 48 trees in three replications in total.

Data were transformed by using square root transformation to provide homogenous variances. Then the General Linear Model procedure in the SPSS statistical program was used to fit a linear model for each data set to determine the substantial effect of the treatment groups. After significant effects were identified, differences between treatment means were considered significant at 0.05 based on the Tukey adjustment type I error rate.

RESULTS

Considering the overall species composition, seven species belonging to the family Drosophilidae were detected in the trap trials in the Black variety fig orchard. *Zaprionus tuberculatus* Malloch, 1932 was the most abundant species in terms of the number of individuals caught in the traps, followed by *Drosophila subobscura* Collin, 1936, *Drosophila suzukii* (Matsumura, 1931), *Hirtodrosophila confusa* (Staeger, 1844), *Drosophila melanogaster* Meigen, 1830, *Drosophila busckii* Coquillett, 1901 and *Drosophila immigrans* Sturtevant, 1921 (Table 3, 4 and 6). The number of flies captured in the traps varied according to trap type. In the trap colour trials, the highest number of drosophilids was found in red traps with 804 individuals in sum (Table 3). Yellow-coloured traps ranked second in attractiveness with 683 individuals, followed by white with 582 individuals, transparent with 559 individuals, green with 483 individuals and blue with 419 individuals. The black colour was the least attractive to 265 individuals compared to the others. When considering the catch results by species, many drosophilid species were attracted to the red colour. *Z. tuberculatus* was the most common species caught in the red traps, with a mean of 202.00±56.89 individuals. *D. subobscura* and *D. suzukii* were more attracted to yellow traps (69.00±6.11 and 8.33±2.91 individuals, respectively) than the red ones. *D. subobscura* and *D. suzukii* were more attracted to yellow traps (69.00 ± 6.11 and 8.33 ± 2.91 individuals, respectively) than red ones. In addition, *H. confusa* was caught more in black and transparent traps than in any other coloured traps, with 5.33±0.89 and 5.00±3.10 individuals, respectively. However, there was no statistically significant difference between the effectiveness of different colours (Tukey test, $X>0.05$).

In the bait trials, grape vinegar traps made the highest number of drosophilid catches with 4379 individuals. It was followed by white wine with 4208 individuals and apple cider vinegar with 1818 individuals. While 869 drosophilid individuals were caught in traps containing the yeast, no drosophilid fly was detected in control traps containing tap water (Table 4). In the bait trials, *Z. tuberculatus* was the most numerous species, with 9975 individuals in sum, followed by *D. subobscura* with 1047 individuals and *D. suzukii* with 197 individuals. Considering the weekly catches by species, statistically significant differences were found in the effectiveness of different baits (Tukey test, $P<0.05$). The most common species, *Z. tuberculatus* was

Table 3. The mean number of the sum of drosophilid individuals captured per trap with different colours in the study period (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies							
	Yellow	Blue	Green	Red	Black	White	Transparent	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.67±0.33 (0-1)	0.33±0.33 (0-1)	0.00	0.00	0.33±0.33 (0-1)	6
Drosophila immigrans	0.00	1.33±0.89 (0-3)	0.00	0.00	0.00	0.00	0.33±0.33 (0-1)	5
Drosophila melanogaster	0.00	0.67±0.33 (0-1)	1.00±0.58 (0-2)	0.00	0.33±0.33 (0-1)	1.33±0.89 (0-3)	0.33±0.33 (0-1)	11
Drosophila subobscura	69.00±6.11 (57-77)	44.67±9.13 (31-62)	52.00±2.52 (49-57)	54.67±10.17 (36-71)	38.00±20.52 (16-79)	45.00±2.90 (42-49)	56.33±2.91 (51-61)	1079
Drosophila suzukii	8.33±2.91 (3-13)	6.00±1.00 (4-7)	3.33±1.33 (2-6)	7.67±0.67 (7-9)	5.00±2.52 (2-10)	6.00±2.10 (3-10)	5.33±2.60 (1-10)	125
Hirtodrosophila confusa	3.00±1.00 (2-5)	1.67±1.20 (0-4)	2.33±0.33 (2-3)	3.33±2.40 (0-8)	5.33±0.89 (4-7)	1.67±3.33 (1-2)	5.00±3.10 (1-11)	67
Zaprionus tuberculatus	147.00±41.80 (67-208)	85.00±5.03 (75-91)	101.67±41.29 (55-184)	202.00±56.89 (89-270)	39.67±2.33 (36-44)	140.15±14.15 (116-165)	118.67±35.38 (48-157)	2502
Sum	683	419	483	804	265	582	559	3795

* SE: Standard error

most attracted by grape vinegar traps with 92.22±32.68 individuals/trap/week, followed by apple cider vinegar and white wine, respectively. *D. subobscura*, the second common species, was most attracted by white wine at 13.71±3.47 individuals/trap/week, followed by grape vinegar, apple cider vinegar and yeast, respectively (Table 5) (Tukey test, P<0.05). *D. suzukii*, the third common species, was most attracted by white wine with 1.76±0.54 individuals/trap/week, followed by grape vinegar, apple cider vinegar and yeast; however, the differences in attractiveness among the baits were not statistically significant (Table 5) (Tukey tests, P>0.05).

When the capture efficiency of traps with different numbers of entry holes was analysed, the number of drosophilids captured rose with the number of entry holes and traps with 8 holes provided the most captures (Table 6). The sum

Table 4. Mean of the sum of drosophilid individuals captured in different bait traps (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies					
	Apple cider vinegar	Grape vinegar	White wine	Yeast	Water	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.00±0.00	0.00±0.00	0.00±0.00	2
Drosophila immigrans	1.00±0.00 (1-1)	0.00±0.00	0.33±0.33 (0-1)	0.00±0.00	0.00±0.00	4
Drosophila melanogaster	0.33±0.33 (0-1)	1.67±0.61 (0-4)	1.00±0.58 (0-2)	0.00±0.00	0.00±0.00	5
Drosophila subobscura	87.67±7.33 (95-73)	116.67±19.74 (94-156)	205.67±20.10 (179-245)	24.00±13.50 (10-51)	0.00±0.00	1047
Drosophila suzukii	12.33±4.84 (7-22)	20.33±9.35 (10-39)	26.33±5.61 (18-37)	6.67±1.76 (4-10)	0.00±0.00	197
Hirtodrosophila confusa	2.00±0.58 (1-3)	9.00±0.58 (8-10)	3.66±1.10 (1-8)	0.00	0.00±0.00	44
Zaprionus tuberculatus	502.33±240.98 (147-962)	1398.33±613.67 (177-2114)	1165.67±527.00 (413-2181)	259.00±113.29 (82-470)	0.00±0.00	9975
Sum	1818	4379	4208	869	0	11274

* SE: Standard error

Table 5. The mean number of drosophilids captured weekly per trap with different bait in the study period (individuals/trap/week)

Drosophilid species	Mean* ±SE** number of flies in a trap Trap lure			
	Apple cider vinegar	Grape vinegar	White wine	Yeast
Drosophila subobscura	5.44±1.15b	7.78±1.88b	13.71±3.47a	1.60±0.62b
Drosophila suzukii	0.82±0.24a	1.36±0.62a	1.76±0.54a	0.44±0.23a
Zaprionus tuberculatus	33.49±15.20b	92.22±32.68a	17.71±24.70b	17.27±8.01b

* Means within a row followed by the different letters are significantly different (Tukey test, P<0.05). The means for the first three species and Hirtodrosophila confusa, as well as water as a control bait in Table 4, were not implemented in statistics in this table since the number of overall catches was very low. **SE: Standard error

Table 6. Mean of the sum of drosophilid individuals captured per grape vinegar trap with different numbers of entry holes (individuals/trap/study period)

Drosophilid species	Mean ±SE* (minimum-maximum numbers) number of flies				
	2	4	6	8	Sum
Drosophila busckii	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.33±0.33 (0-1)	0.67±0.33 (0-1)	5
Drosophila immigrans	0.00±0.00	0.67±0.67 (0-2)	0.67±0.67 (0-2)	0.67±0.67 (0-2)	6
Drosophila melanogaster	1.33±0.90 (0-3)	7.00±6.51 (0-20)	0.33±0.33 (0-1)	1.67±0.33 (1-2)	12
Drosophila subobscura	38.00±10.01 (23-57)	73.00±10.26 (53-87)	73.67±8.74 (63-91)	87.67±14.34 (59-103)	817
Drosophila suzukii	8.33±2.03 (5-12)	10.33±2.91 (5-15)	12.00±3.21 (7-18)	13.67±1.76 (11-17)	133
Hirtodrosophila confusa	2.33±0.88 (1-4)	6.67±4.70 (1-16)	6.33±5.84 (0-18)	7.67±3.67 (4-15)	69
Zaprionus tuberculatus	263.67±111.15 (135-485)	323.67±138.10 (167-599)	247.67±84.36 (140-414)	404.33±115.37 (179-560)	3482
Sum	942	1010	1024	1548	4524

* SE: Standard error

of the number of drosophilids was 1548 in the traps with 8 entrance holes. When the numbers were analysed, there were no statistical differences in the attractiveness of the entrance hole numbers (Tukey test; P>0.05). *Z. tuberculatus* was the most numerous drosophilid in the traps, with 3482 individuals, followed by *D. subobscura* and *D. suzukii* with 817 and 133 individuals (Table 6).

DISCUSSION

In recent years, drosophilids such as *D. suzukii* and *Z. indianus*, which are invasive species that damage many soft-textured fruits, have started to attract attention as agricultural pests with their spread in many countries with high agricultural potential (EPPO 2023). The fact that both of these drosophilid species have become critical pests in several fruits, which play an important role in world trade, encourages studies on their control. Within the framework of detecting, monitoring or mass trapping of the drosophilids, traps have gained importance, and their implementations are becoming widespread (Harmon et al. 2019, Joshi et al. 2014, Lee et al. 2013, Özbek-Çatal et al. 2019, Rodriguez-Saona et al. 2020). Trap optimisation is essential for the effective use of traps. For this reason, it is crucial to know the factors affecting the traps' capture efficiency to reduce the catch variation and get more effective results.

Within this context, Basoalto et al. (2013) studied the trapping efficiency of trap colour, volume and number and

width of entry holes. According to the average number of flies landing on coloured cards, red and black, as well as burgundy, were the most effective colours for capturing *D. suzukii*. In laboratory studies, green and blue colours were found to be less attractive. However, in our study, red was the most effective colour for capturing Drosophilidae species in terms of total numerical value. When we evaluated the drosophilids separately by species, it was found that the red colour attracted *Z. tuberculatus* very effectively. On the other hand, yellow-coloured traps attracted more *D. subobscura* and *D. suzukii*. Lee et al. (2013) stated that the yellow colour was the most effective in attracting *D. suzukii* compared to the other colours. Still, there was no statistical difference between yellow and red, similar to the results of the present study. The transparent traps seem to be less effective in terms of the number of individuals captured in total.

Another factor affecting trap efficiency is the trap volume and entry area in the traps. In the trials with plastic jars, colour, number of entry holes, and the ratio of entry area on the bottle were compared concerning the capture efficacy of the traps, and red and black colours and larger entry areas provided more effective capture. Additionally, it was found that transparent traps followed them concerning the number of flies captured (Basoalto et al. 2013). In the present study, similar results were obtained relating to the ranking of the effect of transparent traps concerning the colour, and transparent traps followed the other colours found to be more effective in the experiment (Table 3). Controversially, however, fewer drosophilids were captured in the present study using black traps. The effect of different attractant lures in the traps could be a reason for these different trial results.

Traps with more number and larger diameter of entry holes were found to be more effective in capturing *D. suzukii* (Basoalto et al. 2013). From the results of the study, it can be said that the total number of drosophilids captured increased with the number of trap holes, and the most effective results were obtained in traps with 8 holes. In other words, increasing the number of entry holes on the trap increases the probability of entry by drosophilids.

The results in the present study indicated that the red-coloured traps with grape vinegar and 8 holes were the most effective traps for monitoring and surveillance studies of drosophilids in all respects. However, the yellow-coloured traps with wine and eight entry holes seem to be the most promising for *D. suzukii* in monitoring and mass trapping. Wine traps were the most attractive concerning the number of *D. suzukii* flies in the traps. Grape vinegar, being second in terms of trap efficiency by only a very small margin, may be preferred instead of wine, depending on the price of wine, and whether it will increase the costs of the traps in some countries. Many studies are required to develop more effective traps for catching pest flies. Drosophilid flies have a wide range of habitats in which they live, differ greatly in their feeding habits, and are affected by specific environmental factors that affect the efficiency of the trap. Our results may contribute to the development of more effective and more species-selective traps for different drosophilid pests.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışmanın amacı, farklı cezbedici besin, renk ve delik sayısı içeren tuzakların drosophilidleri yakalama etkinliklerinin belirlenmesidir. Bunun için renk olarak sarı, mavi, yeşil, kırmızı, siyah, beyaz ve şeffaf (renksiz) tuzaklar denemeye alınmıştır. Cezbedici sıvı olarak, elma sirkesi, üzüm sirkesi, beyaz şarap, maya ve su (kontrol) kullanılmıştır. Ayrıca, 2, 4, 6 ve 8 adet/tuzak giriş deliği olan tuzaklar değerlendirilmiştir. Tesadüf blokları deneme deseninde 3 tekerrürlü olarak Aydın ilindeki Bursa Siyahı çeşiti 437 ağaç içeren bir incir bahçesinde Eylül 2018 - Mart 2019 tarihleri arasında yürütülmüştür. Toplam 48 ağaç üzerine (1 tuzak/ağaç) tuzak asılmış ve sayımlar haftalık olarak yapılmıştır. Sonuç olarak, en çok drosophilid bireyini sayısal olarak toplamda kırmızı renkli tuzaklar cezbetmiş, bunu sarı renk izlemiştir. Mavi, yeşil ve şeffaf tuzaklar etkisiz bulunmuştur. Cezbedici olarak ise, üzüm sirkesi içeren tuzaklarda en çok drosophilid bireyi yakalanmış, bunu beyaz şarap içeren tuzaklar izlemiştir. Tuzak giriş deliği sayısı bakımından en çok yakalanma 8 delikli tuzaklarda sağlanmıştır. Tüm tuzaklarda toplamda en fazla *Zaprionus tuberculatus* Malloch, 1932 yakalanmış, onu *Drosophila subobscura* Collin, 1936 ve *Drosophila suzukii* (Matsumura, 1931) takip etmiştir. *Z. tuberculatus* en çok üzüm sirkesi içeren tuzaklarda, *D. subobscura* ve *D. suzukii* beyaz şarap içeren tuzaklarda yakalanmıştır. Drosophilidlerin

popülasyonlarının izlenmesinde içerisinde sirke bulunan, kırmızı renkli ve 8 delikli tuzakların en etkili sonucu verdiği gözlenmiştir.

Anahtar kelimeler: meyve sinekleri, tuzak, cezbedici, *Ficus carica*

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Original article

New knowledge about Megachilidae and Halictidae (Hymenoptera: Apoidea) fauna from Diyarbakır and Bingöl provinces

Diyarbakır ve Bingöl illerinden Megachilidae ve Halictidae (Hymenoptera: Apoidea) faunası hakkında yeni bilgiler

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ABSTRACT

The data about the bee fauna of Diyarbakır and Bingöl provinces of eastern Türkiye is quite limited. This study was conducted between 2016 and 2017 to determine Megachilidae and Halictidae (Hymenoptera: Apoidea) bee diversity of these two provinces. Adult samples were collected by atrap and killed with ethyl acetate. As a result of the evaluation of the collected bee samples, a total of 28 species (17 species from Megachilidae family and 11 species from Halictidae family) were identified from these provinces. Four species of them are known as cleptoparasites. In terms of the plant-bee relationship, eighteen of the species identified are polylectic, while five are oligolectic. Collection localities of all identified species were provided. With the current study, the number of known species belonging to the Megachilidae and Halictidae fauna of both provinces increased from 45 to 65.

INTRODUCTION

Megachilidae (Hymenoptera: Apoidea) family, represented by over 4000 species in the world, is one of the richest long-tongued bee groups (Michener 2007). Unlike other bee groups, pollen collection is carried out by female individuals with dense hairs on the ventral metasoma called scopa. Some *Megachile* and *Osmia* species are used as commercial pollinators in agricultural areas for the second half of the 20th century (Delaplane and Mayer 2000, Maeta and Kitamura 1964, 1965, 1974). Although more than 450 Megachilidae species were recorded in Türkiye (Güler and Çağatay 2006), it is known that only 29 of these species are distributed in Diyarbakır province and 15 of them are in Bingöl province (Kaplan 2022a, 2022b, Özbek and Zanden 1994, Özbek 2013a, 2013b, Warncke 1991).

Halictidae is one of the most diverse families throughout the Apoidea superfamily and it is represented by nearly 70

genera and more than 4000 species worldwide. Besides, members of this family are widely distributed in all around the world (Michener 2007, Packer 2023, Pesenko et al. 2000, Pesenko 2007). In Türkiye, *Halictus* spp. and *Lasioglossum* spp. are the richest genera among other members with consisting nearly 200 species (Dikmen 2018). As a result of their high abundance in nature, they can be considered as an indispensable pollinator group for ecosystems (Dikmen 2007). Despite their abundance and diversity in Türkiye, only a few records had been published about the bee fauna of Bingöl and Diyarbakır so far (Dikmen and Aytekin 2011). While Warncke (1975, 1984) had reported only seven species from these provinces, Kaplan (2022a) reported 17 species so far.

Diyarbakır and Bingöl provinces are two neighbouring cities in the east of Türkiye. Diyarbakır has a hot and dry climate with abundant agricultural land and fewer forest areas, whereas Bingöl province has a cold and rainy climate, minimal agricultural land, and extensive forest and pasture areas.

This is a faunistic study on the Megachilidae and Halictidae fauna of Bingol and Diyarbakır provinces of eastern Türkiye with data about general and local distribution with new location for the recorded species.

MATERIALS AND METHODS

Bee samples were collected by insect net (atrap) with weekly sampling during March-September at various localities in Bingöl and Diyarbakır provinces located in eastern of Türkiye between 2016 and 2017 (Figure 1). After sampling bees were killed using ethyl acetate and then pinned in the laboratory. Meanwhile, all samples were collected by Emin Kaplan, and identified by Yasemin Güler and Fatih Dikmen.

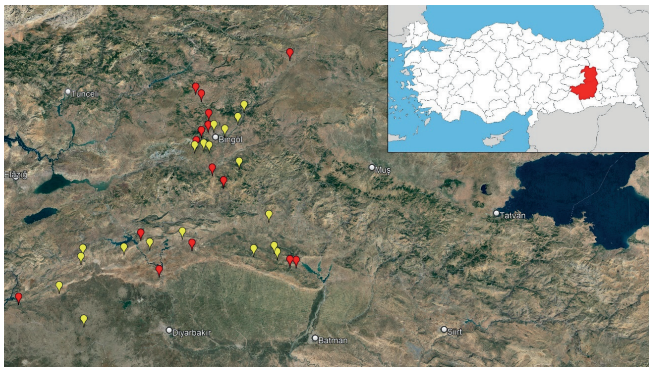

Figure 1. Sampling locations of the study, the map on the top displays the location of Bingöl and Diyarbakır provinces of Türkiye and the pinpoints displays the localities that Megachilidae (yellow) and Halictidae (red) members had been caught

Terminology follows Michener (2007). The materials are deposited in Emin Kaplan's individual collection of the Department of Plant Protection, Faculty of Agriculture, Bingol University (Bingöl, Türkiye) and The Nazife Tuatay Plant Protection Museum of the Plant Protection Central Research Institute, Ankara (Türkiye).

The identification of the species was made according to Warncke (1988, 1991, 1992), Zanden (1992), Banaszak and Romasenko (1998), Michener (2007), Amiet et al. (2004), Scheuchl (2006), and Bogusch (2023) for Megachilidae, and Pesenko (1978, 1984, 1985, 1986), Pesenko et al. (2000), Amiet et al. (2001), and Dikmen (2012) for Halictidae.

RESULTS

A total 34 Halictidae and Megachilidae specimens from 34 locations were collected with two years of sampling. The results of identification of these samples at the species level were given below.

Family Megachilidae

Subfamily Megachilinae

Tribe Dioxyini

Genus *Ensliniana* Alfken, 1938

***Enslinia**na bidentata* (Friese, 1899)**

Material examined: Diyarbakır: Ergani, Sallar, 38° 16' 01.70" N, 39° 38' 59.32" E, 962 m, 11.05.2017, ♀.

Previous records: Nevşehir (Heinrich 1977), Konya, Ağrı, Erzurum as Dioxys bidentata anatolica Heinrich, 1977 (Özbek and Zanden 1993).

General distribution: Algeria, Israel, Jordan, Morocco, Portugal, Spain, Syria, Tunisia, Türkiye (Bogusch 2023).

Tribe Megachilini

Genus Coelioxys Latreille, 1809

Subgenus *Allocoelioxys* Tkalcu, 1974

***Coelioxys (Allocoelioxys) afra* Lepeletier 1841**

Material examined: Bingöl: Altınışık, 38° 49' 29.77" N, 40° 26' 59.07" E, 1605 m, 20.07.2017, ♀.

Previous records: Erzincan, Erzurum, İzmir (Özbek 1979a, Özbek and Zanden 1994), Ankara, Antalya, Bitlis, Bursa, Eskişehir, Hakkâri, Kars, Konya, Kütahya, Nevşehir, Niğde, Van (Warncke 1992), Bilecik (Özbek and Schwarz 2016).

General distribution: South, Eastern and Central Europe, Great Britain, Caucasus, Asia Minor, Central Asian part of the former USSR, North Africa (Banaszak and Romasenko 1998).

***Coelioxys (Allocoelioxys) brevis* Eversmann, 1852**

Material examined: Bingöl: Çiçekyayla, 38° 49' 22.64" N, 40° 28' 22.17" E, 1442, 20.07.2017, ♀.

Previous records: Erzurum (Özbek 1979a), Adana, Ankara, Bayburt, Çankırı, Erzincan, Erzurum, Hakkâri, Kars, Konya, Kütahya, Manisa, Nevşehir, Niğde, Siirt, Sivas, Van (Warncke 1992), Erzincan, Erzurum, Şanlıurfa (Özbek and Zanden 1994).

General distribution: South, Eastern and Central Europe, Caucasus, Algeria (Banaszak and Romasenko 1998).

Genus *Megachile* Latreille, 1802

Subgenus *Creightonella* Cockerell, 1908

***Megachile (Creightonella) albisecta* (Klug, 1817)**

Material examined: Bingöl: Yenibaşlar, 39° 58' 46.96" N, 40° 41' 05.14" E, 1142 m, 7.08.2016, ♀.

Previous records: Antalya, Aydın, Bitlis, Erzincan, Erzurum, Hakkari, İçel, İzmir, Iğdır, Konya, Muş, Sinop (Özbek 1979b, Özbek and Zanden 1994), Ankara, Eskişehir (Güler and Çağatay 2006).

General distribution: South, Eastern and Central Europe, Caucasus, Central Asian part of the former USSR, North Africa (Banaszak and Romasenko 1998).

Subgenus *Megachile* Latreille, 1802

***Megachile (Megachile) apicalis* Spinola, 1808**

Material examined: Bingöl: Balpınar, 38° 50' 06.26" N, 40° 24' 18.26" E, 1829 m, 20.07.2017, ♀.

Previous records: All of Türkiye (Özbek and Zanden 1994), Ankara, Eskişehir, Sivas (Güler and Çağatay 2006).

General distribution: South, Eastern and Central Europe, Caucasus, Central Asian part of the former USSR, North Africa, Canada (Banaszak and Romasenko 1998).

***Megachile (Megachile) deceptoria* Pérez, 1890**

Material examined: Bingöl: Çeltiksuyu, 38° 52' 13.72" N, 40° 34' 06.19" E, 1019 m, 20.07.2017, ♀.

Previous records: Balıkesir (Özbek and Zanden 1994), Ankara, Çankırı, Eskişehir, Kayseri (Güler and Çağatay 2006).

General distribution: South, Eastern and Central Europe, Turkmenistan, West Kazakhstan (Banaszak and Romasenko 1998).

Tribe Osmiini

Genus *Chelostoma* Latreille, 1809

Subgenus *Chelostoma* Latreille, 1809

***Chelostoma (Chelostoma) mocsaryi* Schletterer, 1889**

Material examined: Diyarbakır: Dicle, Bozbaba, 38° 20' 28.52" N, 40° 05' 32.33" E, 834 m, 11.05.2017, ♀; Hazro, Ormankaya, 38° 17' 59.97" N, 40° 46' 48.65" E, 952 m, 14.05.2017, ♀.

Previous records: Adana, Ankara, Aydın, Amasya, Antalya, Artvin, Bursa, Erzincan, Erzurum, Hakkari, Hatay, Kayseri, Konya, Nevşehir, Mersin, Osmaniye, Şırnak, Van (Özbek 2011).

General distribution: South, Eastern and Central Europe, Caucasus, Asia Minor (Banaszak and Romasenko 1998).

Genus *Heriades* Spinola, 1808

Subgenus *Heriades* Spinola, 1808

***Heriades (Heriades) truncorum* (Linnaeus, 1758)**

Material examined: Bingöl: Yenibaşlar, 39° 58' 46.96" N, 40° 41' 05.14" E, 1142 m, 7.08.2016, ♀.

Previous records: Antalya (Özbek 2013a), Bursa, Erzurum, Iğdır, İstanbul (Özbek and Zanden 1992b).

General distribution: Europe, Caucasus, Asia Minor, Central Asian part of the former USSR, North Africa (Banaszak and Romasenko 1998).

Genus *Hoplitis* Klug, 1807

Subgenus *Hoplitis* Klug, 1807

***Hoplitis (Hoplitis) manicata* (Morice, 1901)**

Material examined: Bingöl: Çayboyu, 38° 54' 45.97" N 40° 28' 47.88" E, 1111 m, 7.05.2016, ♀.

Previous records: Amasya (Zanden 1980), Isparta (Özbek and Zanden 1992a), Ankara (Güler and Çağatay 2006), Afyonkarahisar (Güler 2011), Erzincan, Erzurum (Özbek 2013a).

General distribution: Austria, Albania, Algeria, Armenia, Azerbaijan, Bulgaria, Czechia, Corsica, Greece, Hungary, Croatia, Italy, Macedonia, Romania, Southern European Russia, Serbia and Montenegro, Slovakia, Türkiye, Ukraine (Crimea) (Müller 2022).

Subgenus *Alcidamea* Cresson, 1864

***Hoplitis (Alcidamea) praestans* (Morawitz, 1894)**

Material examined: Diyarbakır: Ergani, Bademli, 38° 17' 28.88" N, 39° 55' 57.66" E, 957 m, 12.05.2017, ♀.

Previous records: İçel (Zanden 1980); Antalya, Hakkari, Kars, Konya (Warncke 1991), Antalya, Erzurum, Van (Özbek and Zanden 1992a).

General distribution: Algeria, Austria, Caucasus, Croatia, Egypt, France, France (Corsica), Greece, Hungary, Iraq, Iran, Italy, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Morocco, Portugal, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain, Southern European Russia, Switzerland, Syria, Tajikistan, Tunisia, Türkiye, Ukraine, Uzbekistan (Müller 2022).

Subgenus *Anthocopa* Lepeletier and Serville, 1825

***Hoplitis (Anthocopa) papaveris* (Latreille, 1799)**

Material examined: Diyarbakır: Ergani, Yapraklı, 38° 16' 32.57" N, 39° 38' 39.00" E, 977 m, 11.05.2017, ♀.

Previous records: Anatolia (Alfken 1935), Erzurum (Özbek and Zanden 1992a).

General distribution: Austria, Albania, Belgium, Bulgaria, Caucasus, China, Czechia, France, Germany, Greece, Hungary, Italy, Kazakhstan, Netherlands, Portugal, Romania, Russia, Slovakia, Spain, Switzerland, Türkiye, Ukraine (Müller 2022).

Genus *Osmia* Panzer, 1806

Subgenus *Allosmia* Tkalcu, 1974

***Osmia (Allosmia) rufohirta* Latreille, 1811**

Material examined: Diyarbakır: Hazro, Mutluca, 38° 16' 44.84" N, 40° 53' 50.71" E, 1017 m, 13.05.2017, ♀.

Previous records: Ankara (Alfken, 1935), Antalya, Aydın, Denizli, Karaman (Özbek and Zanden 1992a), Adana, Diyarbakır, Erzincan, Erzurum, Hatay, İzmir (Özbek 2013b).

General distribution: Albania, Austria, Armenia, Azerbaijan, Belarus, Belgium, Bulgaria, Croatia, Czechia, France, France (Corsica), Georgia, Germany, Greece, Hungary, Israel and Palestine, Iran, Italy, Jordan, Kazakhstan, Lebanon, Liechtenstein, Luxembourg, Malta, Macedonia, Morocco, North-western China, Portugal, Romania, Southern European Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, Syria, Türkiye, Ukraine (Müller 2022).

Subgenus *Helicosmia* Thomson, 1872

***Osmia (Helicosmia) aurulenta* (Panzer, 1799)**

Material examined: Diyarbakır: Kulp, Zeyrek, 38° 28' 01.59" N, 40° 51' 22.28" E, 872 m, 28.05.2016, ♀.

Previous records: Eskişehir, Hatay (Fahringer and Friese 1921), Erzurum, Tunceli (Özbek 1979b), Ağrı, Ankara, Bitlis, Hakkari, Karaman, Konya, Nevşehir, Siirt (Warncke 1988), Artvin, Erzincan, Mersin (Özbek and Zanden 1992a), Kayseri (Güler and Çağatay 2006), Bayburt, Bilecik, Burdur, Tokat (Özbek 2013b).

General distribution: Albania, Austria, Andorra, Armenia, Azerbaijan, Belgium, Bulgaria, Bosnia and Herzegovina, Belarus, Croatia, Czechia, Denmark, France, France (Corsica), Germany, Georgia, Greece, Hungary, Iran, Ireland, Italy, Lebanon, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye, Ukraine, United Kingdom (Müller 2022).

***Osmia (Helicosmia) caerulescens* (Linnaeus, 1758)**

Material examined: Diyarbakır: Ergani, Akçoban, 37° 56' 56.21" N, 39° 41' 19.67" E, 970 m, 27.04.2017, ♂.

Previous records: Amasya, İstanbul, Osmaniye (Fahringer 1922), Kahramanmaraş (Fahringer and Friese 1921), Erzurum, Kars, Iğdır (Özbek 1979b), Adana, Ankara, Antalya, Bilecik, Hakkari, Mersin, Karaman, Kars, Konya, Sivas (Warncke, 1988); Aydın, Bursa, Erzincan, Sinop (Özbek and Zanden 1992a), Afyonkarahisar (Güler 2011), Artvin, Eskişehir, Yalova (Özbek 2013b).

General distribution: Albania, Algeria, Austria, Andorra, Armenia, Azerbaijan, Belgium, Bulgaria, Bosnia and Herzegovina, Belarus, Canada, China, Croatia, Cyprus, Czechia, Denmark, Egypt, Estonia, France, France (Corsica), Finland, Germany, Georgia, Greece, Hungary, India, Iran, Israel and Palestine, Italy, Jordan, Kazakhstan, Kyrgyzstan, Latvia, Lebanon, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Morocco, Netherlands, Norway, Pakistan, Poland, Portugal, Romania, Russia, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Tajikistan, Tunisia, Turkmenistan, Türkiye, Ukraine, United Kingdom, USA, Uzbekistan (Müller 2022).

Subgenus *Metallinella* Tkalcu, 1966

***Osmia (Metallinella) brevicornis leucogastra* Morawitz, 1875**

Material examined: Diyarbakır: Çermik, Başarı Bucağı, 38° 06' 03.95" N, 39° 31' 16.19" E, 940 m, 27.04.2017, ♂.

Previous records: Antalya, Artvin, Diyarbakır, Erzurum, Tokat (Özbek 2013b).

General distribution: Afghanistan, Armenia, Azerbaijan, Bulgaria, Caucasus, Cyprus, Georgia, Greece, Iran, Iraq, Kazakhstan, Kyrgyzstan, Macedonia, Southern European Russia, Tajikistan, Turkmenistan, Türkiye, Ukraine (Crimea), Uzbekistan (Müller 2022).

Subgenus *Pyrosmia* Tkalcu, 1975

***Osmia (Pyrosmia) cephalotes longiceps* Morawitz, 1875**

Material examined: Diyarbakır: Silvan, Dolapdere, 38° 18' 51.84" N, 40° 53' 45.96" E, 956 m, 13.05.2017, ♂.

Previous records: Mersin (Zanden 1991), Adana, Antalya, Hakkari, Hatay, Mersin, İzmir, Kayseri, Mardin, Muğla, Nevşehir, Siirt, Şanlıurfa, Van (Warncke 1992), Diyarbakır, Isparta (Özbek and Zanden 1992a), Artvin, Bingöl, Erzurum (Özbek 2013b).

General distribution: Armenia, Azerbaijan, Bosnia and Herzegovina, Croatia, Cyprus, Georgia, Greece, Hungary, Iran, Israel and Palestine, Italy, Jordan, Lebanon, Romania, Serbia and Montenegro, Slovenia, Southern European Russia, Syria, Turkmenistan, Türkiye, Ukraine (Müller 2022).

***Osmia (Pyrosmia) gallarum* Spinola, 1808**

Material examined: Diyarbakır: Hani, Süslü, 38° 23' 27.97" N, 40° 19' 13.30" E, 1072 m, 12.05.2017, ♀.

Previous records: Muş (Özbek 1979b), Ankara, Antalya, Aydın, Eskişehir, Gümüşhane, Hakkari, Kars, İstanbul, Mersin, İzmir, Konya, Muğla, Nevşehir, Niğde, Siirt, Şanlıurfa, (Warncke 1992), Artvin, Bilecik, Erzincan, Erzurum, Isparta (Özbek 2013b).

General distribution: Albania, Algeria, Austria, Bulgaria, Croatia, Czechia, Germany, France, Greece, Hungary, Italy, Luxembourg, Macedonia, Morocco, Poland, Portugal, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, Tunisia, Türkiye, Ukraine (Müller 2022).

Family Halictidae Thomson, 1869

Subfamily Nomioidinae

Tribe Nomioidini

Genus *Ceylalictus* Strand, 1913

***Ceylalictus variegatus* (Olivier, 1789)**

Material examined: Diyarbakır: Hani, Çardaklı, 38° 18' 56.31" N, 40° 24' 06.29" E, 1057 m, 12.05.2017, ♀; Silvan, Babakaya, 38° 15' 09.18" N, 41° 01' 25.08" E, 777 m, 12.05.2017, ♀.

Previous records: Erzurum (Özbek 1979c); Antalya, Mersin, Kahramanmaraş (Dikmen 2012)

General distribution: Common in the Western Palaearctic (Pesenko et al. 2000), Iran, Israel, Cyprus, Egypt, Libya, Türkiye, Greece (Grace 2010).

Subfamily Nomiinae

Tribe Nomiini

Genus *Nomiapis* Cockerell, 1919

***Nomiapis diversipes* (Latreille, 1806)**

Material examined: Diyarbakır: Çüngüş, Şeyhandede, 38° 01' 31.26" N, 39° 16' 39.54" E, 740 m, 28.04.2017, ♀.

Previous records: Erzurum (Özbek 1979c), Ankara (Dikmen ve Çağatay 2007), Afyon (Güler et al. 2011), Kahramanmaraş (Dikmen 2012).

General distribution: Western Palaearctic, From Spain to Kyrgyzstan (Pesenko et al. 2000).

Subfamily Halictinae

Tribe: Halictini

Genus *Evylaeus* Robertson, 1902

***Evylaeus marginatus* (Brulle, 1832)**

Material examined: Bingöl: Çiçekdere, 38° 56' 57.98" N, 40° 27' 04.84" E, 1379 m, 27.05.2017, ♀.

Previous records: Adapazarı, Ankara, Antalya, Balıkesir, Bilecik, Bursa, Erzincan, Erzurum, İstanbul, Karaman, Muğla, Nevşehir, Samsun, Sivas, Trabzon, Uşak (Warncke 1975), Afyon (Güler et al. 2011), Niğde, Mersin, Isparta, Burdur, Antalya (Dikmen 2012)

General distribution: Germany, Czech Republic, Morocco, France, Hungary, Spain, Switzerland, Italy, Poland, Russia, Slovakia, Slovenia, Greece, Eastern Palaearctic, (Polaszek 2004), Armenia, Israel, Pakistan, Nepal (Pauly 2007).

***Evylaeus laticeps* Schenck, 1868**

Material examined: Bingöl: Genç, Şehitköy, 38° 39' 48.18" N, 40° 29' 31.00" E, 1308 m, 26.05.2017, ♀.

Previous records: Amasya, Ankara, Ardahan, Bursa, Erzurum, İstanbul, Kırıkkale, Konya, Kütahya, Nevşehir, Sinop, Tekirdağ, Tunceli, Zonguldak, (Warncke 1975), Antalya, Denizli, Mersin (Dikmen 2012).

General distribution: Germany, Austria, Belgium, Czech Republic, France, England, Spain, Switzerland, Italy, Hungary, Macedonia, Poland, Russia, Slovakia, Slovenia, Greece (Polaszek 2004).

***Evylaeus politus* (Schenck, 1853)**

Material examined: Diyarbakır: Dicle, Yeşilsırt, 38° 20' 25.58" N, 40° 03' 30.70" E, 797 m, 12.05.2017, ♀.

Previous records: Antalya (Ascher and Pickering 2020), Afyon (Güler et al. 2011), Adana, Hatay, Mersin, Muğla, Niğde (Dikmen 2012).

General distribution: Germany, Austria, Belgium, Czech Republic, France, Spain, Switzerland, Italy, Hungary, Macedonia, Poland, Russia, Slovakia, Slovenia, Greece, North

Africa (Polaszek 2004), Iran, Israel, Egypt, Türkiye, Turkestan (Pauly 2007).

Genus *Halictus* Latreille, 1804

***Halictus resurgens* Nurse, 1903**

Material examined: Bingöl: Kurudere, 38º 54' 41.84" N, 40º 27' 24.10" E, 1127 m, 13.07.2016, ♂; Genç, Yaylabağı, 38º 37' 45.24" N, 40º 30' 45.83" E, 1248 m, 21.07.2017, ♀; Sancak, Sudüğünü, 39° 03' 32.78" N, 40o 24' 11.34" E, 1582 m, 5.08.2016, ♂.

Previous records: Adana, Adıyaman, Ankara, Antalya, Balıkesir, Bilecik, Gaziantep, Hakkari, Hatay, İstanbul, Konya, Mardin, Siirt, Şırnak, Şanlıurfa, Van (Dikmen and Aytekin 2011), Afyon, Burdur, Hatay, Denizli (Dikmen 2012).

General distribution: Northeastern Africa to Central Asia (Polaszek 2004).

***Halictus sajoi* Blüthgen, 1923**

Material examined: Bingöl: Karlıova, Toklular, 39° 16' 10.81" N, 40º 59' 88.91" E, 1804 m, 12.07.2016, ♀.

Previous records: Adana, Ağrı, Ankara, Ardahan, Erzurum, Konya, Giresun (Warncke 1975), Erzurum (Özbek 1979c), Antalya, Hakkari, Şırnak, Van (Warncke 1984), Ankara (Dikmen and Çağatay 2007), Bitlis, Kars, Niğde, Erzincan (Dikmen and Aytekin 2011).

General distribution: Austria, Germany, Hungary, Italy, northwestern Russia, Slovenia, eastern Palaearctic (Polaszek 2004).

Genus *Lasioglossum* Curtis, 1833

***Lasioglossum quadrinotatum* (Kirby, 1802)**

Material examined: Bingöl: Balpınar, 38º 50' 05.26" N, 40º 24' 18.26" E, 1830 m, 27.05.2017, ♀; Diyarbakır: Lice, Gürbeyli, 38º 26' 54.31" N, 40º 42' 48.20" E, 854 m, 14.05.2017, ♀.

Previous records: Edirne, Erzurum (Warncke 1975), Afyon (Güler et al. 2011), Antalya, Muğla (Dikmen 2012).

General distribution: Common in the Western Palaearctic (Pesenko et al. 2000).

Genus *Seladonia* Robertson, 1918

***Seladonia seladonia* (Fabricius 1794)**

Material examined: Bingöl: Kurudere, 38º 54' 41.84" N, 40º 27' 24.10" E, 1127 m, 13.07.2016, 2♀♀.

Previous records: Adana, Karaman, Sivas, Erzurum (Warncke 1975), Ankara (Dikmen and Çağatay 2007), Afyon (Güler et al. 2011).

General distribution: Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Türkiye, France, Greece, Hungary, Italy, Macedonia, Poland, Portugal, Slovakia, Slovenia, Spain, Switzerland, Ukraine, East Palaearctic (Polaszek 2004).

***Seladonia smaragdula* (Vachal, 1895)**

Material examined: Diyarbakır: Eğil, Selmanköy, 38º 11' 45.04" N, 40º 08' 46.13" E, 809 m, 12.05.2017, ♀.

Previous records: Adana, Antalya, Bursa, İstanbul, Samsun (Warncke 1975), Erzurum (Özbek 1979c), Ankara, Hakkari (Warncke 1984), Afyon (Güler et al. 2011), Hatay, Isparta, Karaman, Kahramanmaraş, Mersin, Niğde (Dikmen 2012).

General distribution: Western Palaearctic (Pesenko et al. 2000), Eastern Palaearctic (Polaszek 2004).

Genus *Sphecodes* Latreille, 1804

***Sphecodes longulus* Hagens, 1882**

Material examined: Diyarbakır: Silvan, Ormandışı, 38º 14' 37.69" N, 41º 01' 16.34" E, 760 m, 12.05.2017, ♀.

Previous records: Adıyaman, Antalya, Aydın, Bursa, Erzurum, Hakkari, Kars, Konya, Şanlıurfa (Özbek et al. 2015).

General distribution: From Europe to Asia, it reaches through Russian Far East (Özbek et al. 2015).

DISCUSSION

As a result of the identification of the specimens collected during the two-year study, the presence of 17 Megachilidae and 11 Halictidae species was determined in the study area. All Megachilidae species except *Osmia brevicornis leucogastra, O. cephalotes longiceps* and *Osmia rufohirta* and all Halictidae species except *Ceylalictus variegatus, Halictus sajoi, Lasioglossum quadrinotatum, Seladonia seladonia* and *Sphecodes longulus* species are new records for the fauna of provinces (Diyarbakır or Bingöl) where they were collected.

Among Megachilidae species, three species were known as cleptoparasite: One of them, *Ensliniana bidentata* is cleptoparasite on *Hoplitis (Anthocopa) zaianorum* (Benoist, 1927) (Müller 2022). Two other species, *Coelioxys (Allocoelioxys) afra* Lepeletier and C. *(A.) brevis* Eversmann, are cleptoparasites on some species as *Megachile apicalis, M. leachella* and *M. pilidens* in the subgenus *Eutricharaea* (Megachilidae: Megachile) (Grace 2010). Although they were collected from many provinces of Türkiye, both were recorded from Bingöl province for the first time. Eight of other Megachilidae species (*Hoplitis papaveris, Megachile*

albisecta, M. apicalis, M. deceptoria, Osmia rufohirta, O. aurulenta, O. caerulescens and *O. cephalotes longiceps*) are polylectic species, i.e. visiting more than one plant family (Banaszak and Romasenko 1998). *Osmia brevicornis leucogastra, Chelostoma mocsaryi, Heriades truncorum, Hoplitis manicata, H. praestans* and *Osmia gallarum* are among the oligolectic bees because they have a narrower flower preference (Müller 2022).

For the Halictidae members reported from this region, all members are known to be polylectic species (Dikmen 2012) except *Nomiapis diversipes*. Moreover, while the members of the genus *Seladonia* are known as social, all the other species are known as solitary or subsocial (Pesenko et al. 2000). Only the members of the genus *Sphecodes* are cleptoparasites within this group.

In both provinces, vegetation was shaped by different habitats such as steppe, bush, forest, rocky, water and meadow. To date, more than 1000 plant taxa were identified in the area, 10% of which are endemic (Anonymous 2021). Generally, due to co-evolution, bee diversity is expected to be high in parallel with plant diversity. However, the number of Megachilidae and Halictidae species recorded from the study area to date does not sufficiently demonstrate this potential. In the future, if field studies are carried out at regular intervals in both provinces, this number will increase to reveal actual fauna of the region.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Türkiye'nin doğusundaki Diyarbakır ve Bingöl illerinin arı faunası hakkındaki veriler oldukça sınırlıdır. Bu çalışma, iki ilin Megachilidae ve Halictidae (Hymenoptera: Apoidea) arı çeşitliliğini belirlemek amacıyla 2016-2017 yılları arasında yürütülmüştür. Ergin örnekler atrap yardımıyla toplanmış ve etil asetat yardımıyla öldürülmüştür. Toplanan arı örneklerinin değerlendirilmesi sonucunda, bu illerden toplam 28 tür (Megachilidae familyasından 17 tür ve Halictidae familyasından 11 tür) tespit edilmiştir. Bunlardan dört tür kleptoparazit olarak bilinmektedir. Bitki-arı ilişkisi açısından, tespit edilen türlerin 18'i polilektik, beşi ise oligolektiktir. Tanımlanan tüm türlerin toplama lokaliteleri de belirtilmiştir. Bu çalışma ile her iki ilin Megachilidae ve Halictidae faunasına ait bilinen tür sayısı 45'ten 65'e yükselmiştir.

Anahtar kelimeler: Hymenoptera, tozlayıcı, fauna, arı çeşitliliği, Diyarbakır, Bingöl

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Original article

Investigation of the effect of *Beauveria bassiana* (Balsamo) Vuillemin against potato beetle (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*)

Beauveria bassiana (Balsamo) Vuillemin'in Patates böceği (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*)'ne karşı etkisinin araştırılması

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ABSTRACT

In this study, the objective was to determine the efficacy of entomopathogenic fungi on the Colorado potato beetle [*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)]. To reach this goal, surveys were conducted in potato fields, and dead *L. decemlineata* and soil samples were collected to isolate entomopathogenic fungi. Pathogenicity tests were carried out using 30 entomopathogenic fungi that were obtained as a result of the analyses. According to the biological activity results, EP-1 isolate was identified and diagnosed as having 75% efficacy against *L. decemlineata*. *Beauveria bassiana* (EP-1), was identified as the most effective isolate. In the dose determination studies, 10^6, 10^7, and 10^8 conidia ml/l doses of *B. bassiana*'s most effective isolate were used. The experiments were set up with five replications for each dose and control group. The spore suspension of the entomopathogenic fungus was sprayed on fully-grown potato plants cultivated in pots, targeting mature and 2nd or 3rd instar *L. decemlineata*. The number of live individuals was recorded on the 1st, 3rd, 5th, 7th, and 9th days after application to calculate the percentage of mortality. The most effective dose of *B. bassiana* isolate was determined to be 85% mortality on the 7th day after application with a dose of 10^8 conidia ml/l. According to the obtained data, it was observed that the mortality rates increased with the increase in dose on the 1st, 3rd, 5th, 7th, and 9th days after application. The highest impact was observed in applications with a dose of 108 conidia ml/l. In conclusion, the entomopathogenic fungus *B. bassiana* isolate, which is less harmful to humans and the environment, is considered suitable for use as a biological control agent against *L. decemlineata*.

INTRODUCTION

Potatoes are cultivated in 79% of countries worldwide, ranking 4th in production after wheat, corn, and rice (TUİK 2022). Various processed forms of potatoes, such as canned, frozen, chips, puree, granules, and powder, are marketed in developed countries. Additionally, potatoes are used as raw materials in alcohol, starch, and animal feed production

(Alisdair et al. 2001, Yüceer 2011). Potato cultivation is widespread in almost every province in Türkiye, with key production areas including Niğde, Nevşehir, İzmir, Bolu, and Afyonkarahisar, contributing to 57.9% of the national production (TUİK 2022). The Colorado potato beetle [(*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)] is a significant pest affecting potato productivity, causing 70%-80% product loss. This beetle not only feeds on leaves but also acts as a carrier for diseases like potato brown rot, spindle tuber viroid, and potato ring rot (Oerke et al. 1994, Yüceer 2011).

Traditionally, chemical pesticides have been used to control the Colorado potato beetle; however, due to the associated risks to human health, the environment, and pest resistance, there is a growing interest in biological control methods (Erdoğan 2015). Entomopathogenic fungi, such as *Beauveria bassiana*, have emerged as a promising alternative. These fungi directly penetrate the insect cuticle, eliminating the need for ingestion by the pest. To date, over 700 entomopathogenic fungi species from at least 90 genera have been identified (Rath 2000). Commercially produced *B. bassiana* and *Metarhizium anisopliae* isolates have effectively controlled plant-feeding insects without harming bees (Uzuner et al. 2017).

Despite the potential of *B. bassiana* for biological control, there is considerable variation in pathogenicity among isolates. Selection criteria often focus on observed insect mortality rates, neglecting considerations of environmental suitability and the ability to persist in the intended application environment (Meyling and Eilenberg 2007). Recent evidence suggests that *B. bassiana* may exhibit an opportunistic endophytic strategy, prompting further investigation into isolate variation in plant tissue colonization (Kia et al. 2017, McKinnon et al. 2018, Vidal and Jaber 2015). Understanding whether different *B. bassiana* isolates vary in their ability to colonize leaf and root tissues is crucial, as this can affect the effectiveness of biological control strategies.

In addition to insect pathogenicity, some entomopathogenic fungi, particularly *Metarhizium* species, form associations with plant roots in the rhizosphere. These fungi may contribute to nutrient cycling by translocating nitrogen from insect cadavers to plants (Behie et al. 2012). However, the potential effects of entomopathogenic fungi on soil microbial communities, especially in terms of carbon utilization, remain poorly understood. Following the application of entomopathogenic fungi to the rhizosphere, an assessment of community-level physiological profiles (CLPPs) using techniques such as BiologTM and MicroRespTM can shed light on microbial functional diversity and soil functioning (Calbrix et al. 2005).

The purpose of this study was to assess the impact of different concentrations (10^6, 10^7, and 10^8 conidia ml/l) of the most effective *B. bassiana* isolates on the Colorado potato beetle. The study aimed to determine if entomopathogenic fungi could be utilized as part of pest management strategies.

MATERIALS AND METHODS

Production of potato beetle

The cultivation of potato plants utilized Marabel variety seeds and took place in the climate rooms of the Adana Biological Control Research Institute. Sterilized soil was filled halfway into pots, and potato tubers were planted. The pots were then moved into hygienic climate chambers, provided with water, and subjected to regular irrigation at 2-3 day intervals. No fertilizers or pesticides were applied during the potato cultivation process.

The production of potato beetle individuals for the experiment involved rearing adults and larvae on potato plants in climate chambers, maintaining conditions of 25±1 °C and 60±5% relative humidity. On potato seedlings, potato beetle larvae and egg packets were transferred from stock culture.

Isolation and culture of entomopathogenic fungi and preparation of spore suspensions

The primary focus of this study was on the potato plant (*Solanum tuberosum*) and the adults of *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae). Additionally, soil samples were collected from potato cultivation fields in the Niğde province. *Beauveria bassiana* isolates were acquired from these soils using the "Galleria trap method" (Zimmermann 1986, 2007). Isolations of *B. bassiana* were also conducted from deceased potato beetle adults found in these agricultural fields.

To evaluate the activity of entomopathogenic fungi on the potato beetle (*L. decemlineata*), experiments were conducted in a climate chamber at 25±2 °C and 60±5% relative humidity. The entomopathogenic fungal isolates used in the experiment were cultured on a Potato Dextrose Agar (PDA) medium. PDA (39 g/l, Merck) was prepared with distilled water, sterilized in an autoclave at 121 °C for 20 minutes, and then poured into Petri dishes (9 cm) to establish a new fungal culture. The entomopathogenic fungi obtained were identified under a stereomicroscope. The spores of the entomopathogenic fungi were collected from pure cultures and spread on Petri dishes containing 12-15 ml of medium

to initiate a fresh culture under aseptic conditions. Petri dishes were incubated at 20-25 oC with 75% humidity in the dark. Spores collected from 14-day-old fungal cultures incubated in the dark at 25±1 °C and 60±5% humidity in Petri dishes containing PDA were gently scraped into 50 ml sterile distilled water containing 0.05% Tween 80, and spore suspensions were prepared.

Spore suspensions of entomopathogenic fungi were then sprayed on the leaves of potato plants grown in climate chamber against the 2nd, 3rd, and 4th stage larvae and adults of the potato beetle, with 10 individuals per treatment, and on the control group with sterile distilled water. In plastic containers weighing 1 kg, two layers of sterile blotting paper were placed and moistened with sterile pure water. Ten *L. decemlineata* 2nd, 3rd, and 4th instar larvae and adults were transferred to the leaves of potato plants grown in pots using a brush, and the pots were covered to prevent the escape of the pests. In the spraying method, spore suspensions of entomopathogenic fungi were sprayed three times (2 ml) as fine particles with a hand sprayer at a distance of 20 cm from the larvae and adults placed on the leaves. The pots were kept in four replicates and exposed to 16 hours of light and 8 hours of darkness in a laboratory setting, with a temperature of 25±2 °C throughout the experiment (Saruhan et al. 2015). The first count was performed 24 hours after the start of the experiment, and subsequent counts were performed every 24 hours for 7 days. The study was monitored daily and deceased individuals were recorded and re-incubated again at 25±2 °C to promote fungal growth. To confirm that mortality was caused by the fungus, dead individuals were transferred to Petri dishes with moist blotting paper and incubated to allow for spore development.

Following the experiment, trials were conducted to optimize the dosage using the most effective isolate. The concentrations of the suspensions prepared for this purpose were calibrated as 10^6, 10^7, and 10^8 conidia/ml for application to *L. decemlineata*, utilizing a Thoma slide and light microscopy. For all three doses of the two most effective isolates of *B. bassiana* (10^6, 10^7, and 10^8), sterile distilled water containing 0.05% Tween 80 was applied as the control treatment. Consequently, 40 individuals were allocated to each treatment, and the experiment was structured with four replications, using one pot for each replication. The number of surviving individuals on the 3rd, 5th, 7th, and 9th day after treatment was recorded separately for each treatment. To observe the growth of entomopathogenic fungi, deceased potato beetles were transferred to slides in Petri dishes containing moistened blotting paper, and fungal growth was examined under a binocular stereo microscope. The experiment was conducted in climatic chambers at 25±1 °C and 60±10% humidity under a 16-hour light and 8-hour dark cycle.

Data analysis

The experiment followed a single-factorial randomized experimental design with a total of four replications, with each pot representing one replication. Percent mortality values, calculated by enumerating the number of live individuals in the entomopathogenic and control groups each day, underwent homogeneity and Shapiro-Wilk normality tests. A one-way analysis of variance (One-Way ANOVA) was applied following Arc Sin angle transformation, identified as nonparametric. Subsequently, to identify similar and distinct groups, Duncan's multiple comparison tests was conducted at a 5% significance level. All statistical analyses were performed using the SPSS (Version 23) software package.

RESULTS

The "Galleria trap method" was employed to extract entomopathogenic fungi from soils collected from potato cultivation areas in Niğde. Additionally, deceased adult potato beetles collected from agricultural fields were sterilized and then cultured on Potato Dextrose Agar (PDA) to isolate entomopathogens (Figure 1). Entomopathogenic fungi isolates that developed on deceased potato beetles were purified and cultured. The isolated fungi code and source material were detailed in Table 1. While numerous fungi were obtained through the isolations, only 30 were confirmed to be entomopathogenic. These fungi exhibited efficacy against the potato beetle, as indicated in Table 2. The fungi were further identified, revealing them to be distinct isolates of *Beauveria bassiana, Simplicillium lamellicola, Lecanicillium muscarium* and *Fusarium subglutinans*.

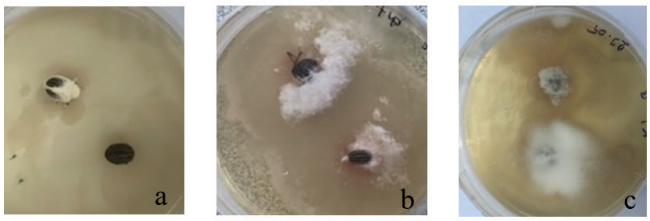

Figure 1. Entomopathogenic fungus development observed in dead potato beetle collected from agricultural areas (*a-Beauveria bassiana b-Fusarium subglutinans c-Lecanicillium muscarium*)

Table 1. The isolate code of entomopathogenic fungi obtained as a result of surveys and the material isolated in the study

Fungal isolate No	Isolated material
EP-1	Dead potato beetle
EP-2	Soil
EP-3	Dead potato beetle
EP-4	Soil
EP-5	Dead potato beetle
EP-6	Dead potato beetle
EP-7	Soil
EP-8	Soil
EP-9	Soil
EP-10	Dead potato beetle
EP-11	Soil
EP-12	Dead potato beetle
EP-13	Dead potato beetle
EP-14	Soil
EP-15	Dead potato beetle
EP-16	Soil
EP-17	Dead potato beetle
EP-18	Soil
EP-19	Dead potato beetle
EP-20	Soil
EP-21	Soil
EP-22	Soil
EP-23	Dead potato beetle
EP-24	Soil
EP-25	Soil
EP-26	Soil
EP-27	Dead potato beetle
EP-28	Soil
EP-29	Dead potato beetle
EP-30	Dead potato beetle

Experiments were conducted to assess the efficacy of the obtained isolates. In each experiment, 10 live insect adults and larvae were introduced, and a 200 ml spore suspension solution of fungi was prepared and sprayed onto them. The experiment was set up with four replications (Table 2, Figure 2).

Table 2. Efficacy rates of various entomopathogen

Isolate	Average Number of Dead Insects ±SS*	Average Impact Rate ±SS*
EP-1	7.50±1.29 g	75.00±12.91 g
EP-2	5.75±1.71 f	57.50±17.08 f
EP-3	5.00±0.82 f	50.00±8.16 f
EP-4	2.00±0.82 bcd	20.00± 8.16 bcd
EP-5	2.75±0.96 cde	27.50± 9.57 cde
EP-6	3.00±1.41 de	30.00±14.14 de
EP-7	5.00±0.82 f	50.00± 8.16 f
EP-8	5.00±0.82 f	50.00± 8.16 f
EP-9	3.00±0.82 de	30.00± 8.16 de
EP-10	2.75±1.71 cde	27.50± 17.08 cde
EP-11	1.25±0.50 ab	12.50± 5.00 ab
EP-12	0.50±0.58 ab	5.00± 5.77 ab
EP-13	0.75±0,96 ab	7.50± 9.57 ab
EP-14	3.00±1.83 de	30.00± 18.26 de
EP-15	1.25±0.50 ab	12.50± 5.00 ab
EP-16	0.50±0.58 ab	5.00± 5.77 ab
EP-17	0.75±0.50 ab	7.50± 5.00 ab
EP-18	3.50±1.29 e	35.00± 12.91 e
EP-19	1.50±0.58 abc	15.00± 5.77 abc
EP-20	0.00 a	0.00 a
EP-21	0.25±0.50 a	2.50±5.00 a
EP-22	0.00 a	0.00 a
EP-23	0.00 a	0.00 a
EP-24	0.00 a	0.00 a
EP-25	0.50±0.58 a	5.00± 5.77 a
EP-26	0.75± 0.50 ab	7.50± 5.00 ab
EP-27	0.50± 1 a	5.00± 10.00 a
EP-28	0.00 a	0.00 a
EP-29	0.75± 0.50 ab	7.50± 5.00 ab
EP-30	0.00 a	0.00 a
Mean	1.92± 2.16	19.17±21.60

* Values marked with different letters are in different groups
**SS – Standard Deviation

Pathogenicity trials were conducted to determine the entomopathogenic nature of the obtained fungi, and the impact of the isolates on insects was measured by calculating effect values (Figure 2).

Figure 2. Pathogenicity trial of the obtained entomopathogenic fungi

The efficacy rates of 30 different entomopathogenic fungi used against the potato beetle exhibited a range from 75% to 20%. To assess the significance of differences between the entomopathogenic fungal isolates, One-way ANOVA and the Duncan test were applied to the data. The statistical analyses resulted in the categorization of entomopathogenic isolates into three distinct groups based on their impact levels. These groups were designated as highly effective (a), moderately effective (b), and low (c), as outlined in Table 2.

Subsequently, the most highly effective isolate against the potato beetle was identified as isolate number 1, characterized as *Beauveria bassiana*.

Efficiency trials of the isolates of *B. bassiana* were established and the efficacy of the most effective isolate on potato beetle in different doses was determined. For this purpose, 10^6-10^7-10^8 is set as conidia/ml. The obtained application results were found to be statistically different compared to the control (Table 3, Figure 3).

The disparities in death rates among *L. decemlineata* individuals on the 1st, 3rd, 5th, 7th, and 9th days following administration, in comparison to the death rates observed in the control group, were determined to be statistically significant. Analysis showed a 70% mortality rate for individuals exposed to 10^8 conidia/ml of entomopathogenic fungus isolates on the 5th day. Subsequently, the mortality rates on the 7th and 9th days were calculated to be 85%, and these values showed a statistically significant contrast to the death rates in the control group (Figure 3). The percentage mortality values for *L. decemlineata* Say. individuals (Coleoptera: Chrysomelidae) are graphically depicted in Figure 3.

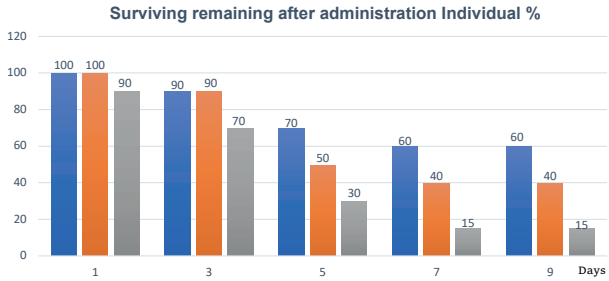

Figure 3. Percent survival rate (%) in *Leptinotarsa decemlineata* individuals exposed to different doses of EP-1 isolates of Beauveria bassiana

→10^6 → 10^7 → 10^8 different doses

DISCUSSION

In the 1980s, there were significant advancements in these studies, particularly in France around 1970 and in the United States of America. In Türkiye, Çam et al. (2002) conducted the first-ever test of an entomopathogenic fungus (*B. bassiana*) isolated from the potato beetle against the same insect. The study reported up to 100% mortality in 2nd, 3rd, and 4th instar larvae treated with entomopathogenic fungi by the end of the seventh day. While adult beetles are generally less susceptible to these fungi, the isolate BMAUM-LDE-001 caused 86.2% mortality. A study has revealed that

Table 3. Mortality rates occurring on different days as a result of application of different doses of entomopathogen (*Beauveria bassiana*) p≤0.05

Application	1. Day	3. Day	5. Day	7. Day	9. Day
10^6	10.00±0.00 b	9.25±0.24 b	6.50±0.33b	6.00±0.49 c	6.00±0.49 c
10^7	10.00±0.24 b	9.00±0.00 ab	5.75±0.31 b	4.50±0.39 b	4.50±0.39 b
10^8	9.50±0.33 a	8.50±0.29 a	3.00±1.62 a	1.50±0.68 a	1.50±0.68 a

* The differences between the means (± standard errors) carrying different letters in the same column, separately for each isolate, are statistically significant (SPSS (Version 23) package program, p> 0.05; each application was conducted on 40 individuals)

distinct entomopathogenic fungal species and isolates may demonstrate different levels of pathogenicity in a range hosts (Butt et al. 1994). Todorova et al. (2000) assessed 10 different *B. bassiana* isolates against *L. decemlineata, Myzus persicae,* and their predator *Coleomegilla maculata lengi*. Six isolates demonstrated high efficacy against all three insect species, while four isolates exhibited high pathogenicity against the two pest species but low pathogenicity against predators. One advantage of employing entomopathogenic fungi for pest control is their compatibility with insecticide spraying equipment. Direct spraying onto pests enhances the mortality rate (Boucias et al. 1998, Fernandez et al. 2001), ensuring rapid adhesion and germination of spores on the insect cuticle (Fernandez et al. 2001). Our study confirmed these findings, showing that directly spraying entomopathogenic fungus spore solutions was highly effective against both larvae and adults of the pest. Our study confirmed these findings, showing that directly spraying entomopathogenic fungus spore solutions was highly effective against both larvae and adults of the pest. Applying entomopathogens on plant leaves significantly influences pest control (Fernandez et al. 2001), depending on the spores' ability to withstand environmental conditions until they penetrate the pest. Wraight and Ramos (2015) examined the *B. bassiana* GHA strain against *L. decemlineata* larvae using two methods. In the first, *B. bassiana* conidia were directly applied, resulting in approximately 58% mortality. In the second, conidia were applied to the leaves, resulting in less than 10% mortality of potato beetle larvae. In summary, direct applications proved more effective against the potato beetle, aligning with the findings of other researchers.

Recent studies have focused on the extraction of entomopathogenic fungi and their application against pests, with specific attention given to investigating the lethal effect of *F. subglutinans* 12A. Uysal et al. conducted a study in 2022, exploring the potential effectiveness of this fungus against aphids and thrips. Notably, studies have revealed the lethal impact of *F. subglutinans* 12A on Coleoptera species. In the case of *L. decemlineata*, the proportion of *F. subglutinans* 12A was determined to be 8% in adults, and 16% and 18% in the 1st and 2nd instar larvae, respectively. Furthermore, the application of *F. subglutinans* 12A on the 3rd and 4th larval stages led to mortality rates of 64% and 84%, respectively. However, our study revealed that the majority of *Fusarium* species obtained exhibited saprophytic characteristics or demonstrated low pathogenicity.

The trial results obtained through this study are very hopeful, especially for the larval stage, and it has been concluded that it will serve as the basis for more comprehensive studies in the future. In the future, it is necessary to investigate the interactions of this isolate with other pesticides used in potato farming and to expand it to include direct soil application of fungi. In addition, the development of the use of entomopathogenic fungus isolates in the fight against potato beetle will be beneficial in terms of organic agriculture, good agricultural practices and integrated control. Wraight and Ramos (2015) tested *B. bassiana* GHA strain against *L. decemlineat*a larvae using two different methods. In the first method, *B. bassiana* conidia were applied directly against potato beetle larvae and approximately 58% mortality occurred. In the second method, conidia were applied to the leaf and less than 10% mortality was observed in potato beetle larvae. As a result, direct applications against potato beetle were more effective. Several inferences can be made in light of the current findings. This study unequivocally demonstrates the efficacy of entomopathogenic fungi. The research establishes that *L. decemlineata* exhibits highly lethal effects on both larval stages and adults, a phenomenon attributed to the intensive and indiscriminate use of broad-spectrum drugs. Recognizing the drawbacks associated with conventional methods, this study emphasizes the potential of entomopathogenic fungal isolates as effective alternatives for managing *L. decemlineata* in agricultural settings. The remarkably successful results obtained in the development of methods utilizing these entomopathogenic fungal isolates highlight their potential as effective tools in biocontrol strategies. This research not only contributes valuable insights but also serves as a foundation for future studies investigating the use of these isolates as biocontrol agents in agriculture.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Entomopatojen fungusların Patates böceği (*Leptinotarsa decemlineata*) üzerindeki etkinliğini belirlemek amacıyla bu çalışma yapılmıştır. Bu amaçla patates ekim alanlarında sürveyler yapılarak entomopatojen fungusların izolasyonu için ölü patates böcekleri ile toprak örnekleri toplanmıştır. Yapılan analizler sonucunda elde edilen 30 adet entomopatojen fungus ile patajonisite testleri

gerçekleştirilmiştir. Biyolojik etkinlik sonuçlarına göre *L. decemlineata*'ya karşı %75 etki oranında EP-1 izolatı belirlenmiş ve tanısı yapılmıştır. *Beauveria bassiana*'nın (EP-1) en etkili izolat olduğu belirlenmiştir. Doz belirleme çalışmalarında ise, *B. bassiana* izolatından 10^6, 10^7 ve 10^8 konidi ml/l dozları kullanılmıştır. Yapılan çalışmalarda, her doz ve kontrol grubu için beş tekrarlı deneme kurulmuştur. Kontrollü koşullarda saksılarda yetiştirilen patates bitkileri üzerindeki ergin, 2. ve 3. dönem Patates böceği larvalarına entomopatojen fungusların spor süspansiyonu püskürtülmüştür. Canlı bireylerin sayısı, uygulamadan sonraki 1., 3., 5., 7. ve 9. günlerde kaydedilmiş ve ölüm yüzdesini hesaplamak için kullanılmış, *B. bassiana* izolatının en etkili dozu, 10^8 konidi ml/l dozuyla uygulamanın 7. gününde %85 ölüm olarak belirlenmiştir. Elde edilen verilere göre, ölüm oranlarının dozun artmasıyla 1., 3., 5., 7. ve 9. günlerde arttığı gözlemlenmiştir. En yüksek etki, 108 konidi ml/l dozuyla yapılan uygulamalarda gözlenmiştir. Sonuç olarak, insanlar ve çevre için daha az zararlı olan entomopatojen fungus izolatı *B. bassiana*'nın, *L. decemlineata*'ya karşı biyolojik kontrol ajanı olarak kullanılması uygun görülmektedir.

Anahtar kelimeler: patates, patates böceği, biyolojik kontrol, ölüm oranı

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Original article

Wild mustard (*Sinapis arvensis* L.) resistance to tribenuron methyl in wheat fields of Diyarbakır province, Türkiye

Diyarbakır ili buğday tarlalarında yabani hardalın (*Sinapis arvensis* L.) tribenuron methyle karşı dayanıklılık durumu

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ABSTRACT

Chemical weed control is unavoidable in the fields where weeds cause problems in wheat production. However, resistance problems in weeds arise a few years later especially using herbicides with the same mode of action continuously. Frequent and inappropriate use of herbicides leads to various environmental, health and economic issues, as well as labour losses. Recently, growers complained that tribenuron-methyl (acetolactate synthase - ALS), commonly used to control wild mustard (*Sinapis arvensis* L.-SINAR), which is causing problems in the wheat fields of Diyarbakır province, is ineffective. To determine the extent of the problem, wild mustard seeds were collected from 56 suspected wheat fields in 16 districts of Diyarbakır. As a result of applications carried out with the method under controlled conditions, resistance was suspected in the population of 28 fields. The results of the dose-response experiments indicated that no resistance (RI<3) was observed in 20 biotypes, low resistance (3≤ RI<5) was detected in 5 biotypes, and moderate resistance (5 ≤ RI<10) was detected in 3 biotypes.

INTRODUCTION

Wheat is the most widely grown crop in the world. According to the FAO, in 2020 about 760 million tonnes of wheat were produced in 123 countries on an area of about 242 million hectares. In terms of production volume, China ranks first with 17.6% of total wheat production, followed by India (14.1%), Russia (11.3%) and the USA (6.5%). Türkiye ranks 10th with wheat production of 20.5 million tonnes and a production rate of 2.7% (FAO 2022).

Although the wheat plant is highly adaptable, its yield can vary according to ecological and climatic conditions. In addition, the size and characteristics of farms, access to irrigation water and inputs and equipment used in agricultural production, producers' education level, and their attitudes and behaviours in carrying out agricultural activities are also important factors affecting wheat yield (Ateş 2022). Additionally, biotic and abiotic factors can

also affect wheat yield during production Depending on the climate, weeds, especially biotic factors, can cause productivity losses of up to 52% (Ateş 2022, Chaudhary et al. 2008). Yield losses have been reported to be as high as 100% when broadleaf weeds, such as *Sinapis arvensis* L. (SINAR), completely cover the wheat plant and prevent harvest in seasons of heavy infestation (Ateş and Üremiş 2020). SINAR is one of the species that is widely distributed in the wheat-growing areas of Türkiye and has a large coverage area (Ateş and Üremiş 2020, Gökalp and Üremiş 2015, Gürbüz et al. 2018, Özaslan 2011). Herbicides with different modes of action are used to control SINAR in wheat fields. There have been complaints in recent years regarding the failure of chemical applications to yield the intended outcomes. Although the basis of these complaints was the method and timing of application, there have been reports that SINAR is a species susceptible to developing resistance to herbicides (Gherekhloo et al. 2018, Gürbüz et al. 2018). According to the International Herbicide-Resistant Weed Database, 901 cases of herbicide resistance in weeds have been reported in 72 countries and more than 40% of these reports come from wheat fields. Six cases were recorded in SINAR species, with five attributed to tribenuron-methyl (Heap 2024). Tribenuron methyl belongs to the group of acetolactate synthase (ALS) enzyme inhibitors. ALS herbicides inhibit the biosynthesis of branched-chain amino acids such as valine, leucine, and isoleucine, which are required for the synthesis of some essential proteins in the chloroplasts of plants. Without essential amino acids, no proteins can be made and the plant slows down or dies (Ateş 2021, Ross and Lembi 1999). Weed resistance to herbicides can occur because the herbicide cannot bind to the target site due to mutation. After all, the herbicide cannot bind to the target site due to intense protein synthesis in the enzyme region, or because the plant metabolises the herbicide very rapidly and tolerates the phytotoxic effect of the herbicide (Preston and Mallory-Smith 2001). Particularly in areas where monoculture has been practiced for many years, weed resistance to herbicides can result from continuous use of herbicides with the same mode of action. This study aimed to determine, under controlled greenhouse and laboratory conditions, resistance to tribenuron-methyl, one of the ALS-inhibitor herbicides approved in Türkiye and widely used in the wheat-growing areas of Diyarbakır province to control SINAR.

MATERIALS AND METHODS

The field studies were conducted in 2015-2016 in the wheat-growing areas of Bağlar, Bismil, Çermik, Çınar, Dicle, Eğil, Ergani, Hani, Hazro, Kayapınar, Kocaköy, Kulp, Lice, Silvan, Sur and Yenişehir districts of Diyarbakır province, Türkiye (Figure 1).

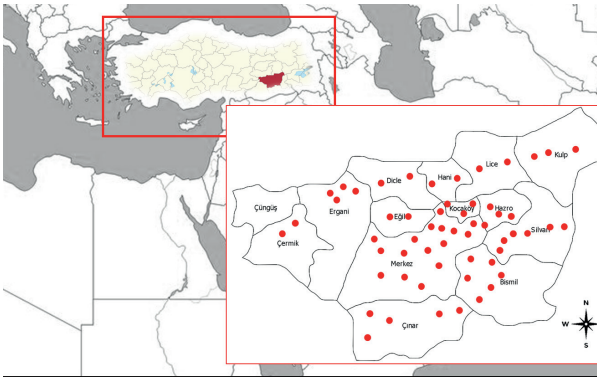

Figure 1. Survey areas where SINAR seeds were collected

SINAR seed samples were taken from the first wheat field seen at random every 5 km along the selected routes (Yıldız et al. 2017). During sampling, 20 plants that had reached seed maturity were pulled from the soil at different points in the field and placed with their root zone in fine-meshed polyethylene bags. The sensitive populations used for the study were collected from the mountainous, non-agricultural area of the Kulp district (Table 1). The plants were transported to the laboratory and dried under shade. Seed populations were obtained from the plants using mechanical methods.

Screening tests

Each population of SINAR seeds collected from wheat fields was placed in a 50% NaOH solution for 10 minutes to break the seed dormancy. The seeds were then kept in a 3% NaCIO solution for 1 min, washed with sterile pure water, and surface sterilised (Ateş et al. 2017). The planting medium, a mixture of peat, sand, and soil (1:1:1), was placed in 10x12 cm pots. A total of 20 pots were planted with three seeds each. The herbicide was applied at a rate of 10 g per hectare (licensed dose) of the active herbicide tribenuron-methyl (75%) using a flat fan nozzle type delivering 300 litres per hectare at 3 atm at a height of 50 cm from the pot surface in a spraying cabinet when the SINAR had 2-4 true leaves. After herbicide application, the pots were watered regularly, taking into account the moisture status of the pots. The pots were watered regularly, taking into account the moisture status of the pots. The trials were terminated on day 28 after herbicide application (HRAC GLOBAL 2024).

Dose-response experiments

Dose-response experiments were conducted on 28 populations suspected of resistance. A total of ten seeds were collected from each suspected resistance seed population. Pre-germinated three seeds were placed in each pot. After thinning at the cotyledon stage, one healthy plant was retained in each pot.

Table 1. Location information of SINAR populations collected from wheat fields in Diyarbakır province

Sampling codes	Districts	Coordinates	Sampling code	Districts	Coordinates
Bağ3	Bağlar	37.908171, 40.160361	Kay86	Kayapınar	37.937161, 40.162938
Bağ55	Bağlar	37.904104, 40.172790	Koc18	Kocaköy	38.291128, 40.498953
Bis37	Bismil	37.493837. 40.403873	Koc36	Kocaköy	38.289713, 40.530035
Bis39	Bismil	37.828221, 40.541256	Koc38	Kocaköy	38.314969, 40.499111
Bis40	Bismil	37.812369, 40.345241	Kul25	Kulp	38.493768, 41.007043
Bis59	Bismil	38.146033, 41.205882	Kul80	Kulp	38.636018, 41.164378
Bis61	Bismil	37794465, 40.773310	Kul82*	Kulp	38.468816, 40.935940
Bis87	Bismil	37.808958, 40.436701	Lic12	Lice	38.486006 40.994365
Çer5	Çermik	38.146531, 39.480153	Lic33	Lice	38.453055, 40.634566
Çer30	Çermik	38.202427, 39.587638	Sil1	Silvan	38.110976, 41.125367
Çın10	Çınar	37.627298, 40.493062	Sil13	Silvan	38.133068, 40.887937
Çın24	Çınar	37.751097, 40.389226	Sil16	Silvan	38.194642, 40.148446
Çın29	Çınar	37.732933, 40.406822	Sil21	Silvan	38.137349, 40.820672
Çın34	Çınar	37.808977, 40.439482	Sil56	Silvan	38.154770, 40.992443
Çın72	Çınar	37.749450, 40.391478	Sil84	Silvan	38.123469, 41.148979
Çın81	Çınar	37.728519, 40.416556	Sur32	Sur	37.923603, 40.296891
Dic7	Dicle	38.352431, 40.070774	Sur42	Sur	37.946437. 40.313619
Dic88	Dicle	38.382999, 40.300850	Sur44	Sur	38.018615, 40.480798
Eği2	Eğil	38.232212, 40.061278	Sur50	Sur	38.127997, 40.441455
Eği16	Eğil	38.194642, 40.148446	Sur51	Sur	37.911120, 40.239542
Erg20	Ergani	38.146968, 39.862059	Sur52	Sur	38.127977, 40.441468
Erg48	Ergani	38.253701, 39.726081	Sur53	Sur	37.870004, 40.304166
Erg57	Ergani	38.273173, 39.845364	Sur62	Sur	37.932387, 40.386099
Erg63	Ergani	38.284300 39.899219	Sur75	Sur	38.005128, 40.448022
Han47	Hani	38.408129, 40.354200	Yen6	Yenişehir	37.994626, 40.221267
Han79	Hani	38.410715, 40.387970	Yen17	Yenişehir	37.990666, 40.161969
Haz.11	Hazro	38.152238. 40.747905	Yen26	Yenişehir	37.962431, 40.222597
Haz. 15	Hazro	38.218530, 40.768873	Yen41	Yenişehir	37.956476, 40.223880
Kay71	Kayapınar	37.943461, 40.140090			
		Total	16 Districts	57 Fields	

*Sensitive population

The development of the plant was monitored regularly. New-generation seeds were collected from plants that had reached harvest maturity. Dose-response studies were carried out with seeds of a new generation (F2) obtained from populations of doubtful resistance (Serim et al. 2022). Application doses were prepared at 0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 times the labelled field rate of 10 g ha-1 for tribenuron-methyl (75%). After adding 0.1% litter adjuvants (Trend 90 EC) to the post-sowing, herbicide applications were made when SINAR had 2-4 true leaves. All trials were replicated 4 times and repeated. Plants in pots were harvested at the point closest to the soil surface on the 28th day after herbicide application, and dried in an oven at 60 °C for 72 hours then weighed (Durigon et al. 2020). Dry biomass weight was determined by weighing on a precision scale and the data was recorded.

Dose-response analyses were evaluated using the log-logistic analysis model with the following formula. GR50 values were determined for each biotype (Equation 1).

$Y = C + ((D–C) / (1 + exp(b^0 (log(X) – log(GR_{50})$ (Equation 1)

Where Y is the response, C is the lower limit, D is the upper limit, b is the slope of the dose-response curve at the GR_{50} point, X is the herbicide dose, GR_{50} refers to the herbicide dose at which the dry biomass weight of the plants is reduced by 50% (Seefeldt et al. 1994). All data were calculated and analysed using the R package programme. As a result of the analysis, the biotypes were accepted as resistant to twice the recommended dose according to the GR_{50} value. In addition, the resistance index (RI) was determined by dividing the GR_{50} of the resistant biotypes by the GR_{50} of the susceptible biotype (Mennan et al. 2012) (Equation 2).

Resistance index(RI) = GR_{50} (Resistant)/GR_{50} (Susceptible) (Equation 2)

The resistance levels of the SINAR populations to tribenuron-methyl were defined according to a standard (Yang et al. 2021). This standard was as follows: no resistance (RI < 3), low resistance (3 ≤ RI < 5), moderate resistance (5 ≤ RI < 10), and high resistance (RI ≥ 10).

RESULTS AND DISCUSSION

As a result of the study conducted to determine the resistance of SINAR to tribenuron methyl, it was determined that 28 out of 56 populations exhibited suspected resistance (Table 2). The results of dose-response experiments conducted with F2 biotypes of SINAR populations with suspected resistance are presented in Table 3.

The results of the dose-response experiments showed that no resistance (RI<3) was observed in 20 biotypes, low resistance (3≤ RI<5) in 5 biotypes and moderate resistance (5 ≤ RI<10) in 3 biotypes (Table 3). 16.6% of the samples from Bismil district exhibited low resistance, while 16.6% displayed moderate resistance. In Çınar district, 33.3% showed low resistance, 11.1% showed low resistance and 22.2% showed moderate resistance, while in Sur district, 22.2% showed low resistance and 25% showed moderate resistance (Figure 2).

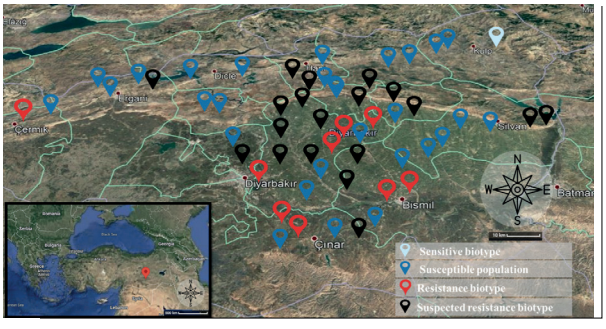

Figure 2. Resistance status in areas where SINAR was sampled

Table 2. Number of SINARs sampled and number of populations with suspected resistance

Districts	Sampling numbers	Number of suspected populations
Bağlar	2	1
Bismil	6	4
Çermik	2	1
Çınar	6	3
Dicle	2	0
Eğil	2	1
Ergani	4	1
Hani	2	1
Hazro	2	2
Kayapınar	2	1
Kocaköy	3	2
Kulp	2	0
Lice	2	0
Silvan	6	2
Sur	9	5
Yenişehir	4	4
Control (Kulp)	1	0
Total	57	28

In the wheat fields of Diyarbakır, tribenuron-methyl is the active ingredient most preferred by growers because it has low phytotoxicity in tank-mixes with monocotyledonous herbicides and is more economical than herbicides with other modes of action. Wheat cultivation in Diyarbakır province is usually done to utilise dry agricultural land. As monoculture is prevalent in the province, herbicide rotation is quite limited, which can naturally lead to cases of resistance. Many studies report that SINAR, which occurs in almost all regions of the world except the poles and has invasive potential in many ecologies, is causing problems in agricultural areas and developing resistance to herbicides (Christoffers et al. 2006, Gherekhloo et al. 2018, Gürbüz et al. 2018, Ntoanidou et al. 2017, Peniuk et al. 1993, Şin 2022, Topuz 2007, Turgut 2023, Veldhuis et al. 2000). In Southeast Anatolia, no study has been reported on SINAR's herbicide resistance. Şin (2021) found that 13 (Amasya 2, Çorum 1, Tokat 7 and Yozgat 3) out of 310 populations collected from wheat fields in the

Table 3. The mean number of the sum of drosophilid individuals captured per trap with different colours in the study period (individuals/trap/study period)

Biotypes	I. experiment			II. experimets			Resistance Categories
	GR_{50}	SE±	Resistance Index (RI)	GR_{50}	SE±	Resistance Index (RI)	
Bağ55	0.22	0.03	1	0.53	0.17	1	no resistance
Bis37	0.96	0.6	4	1.92	1.1	4	low resistance
Bis40	0.4	0.05	1	0.86	0.32	1	no resistance
Bis59	0.11	0.03	1	0.38	0.08	1	no resistance
Bis87	1.22	1.21	5	2.54	0.82	5	moderate resistance
Çer5	0.47	0.3	2	1.06	0.21	2	no resistance
Çın29	0.06	0.04	1	0.19	0.07	0	no resistance
Çın72	0.67	0.09	3	1.39	0.24	3	low resistance
Çın81	0.71	0.13	3	1.49	0.23	3	low resistance
Eği2	0.23	0.08	1	0.53	0.23	1	no resistance
Erg20	0.02	0.02	1	0.24	0.1	1	no resistance
Han47	0.36	0.07	1	0.53	0.13	1	no resistance
Han79	0.12	0.04	1	0.38	0.1	1	no resistance
Haz11	0.17	3.47	1	0.48	2.19	1	no resistance
Kay86	0.11	0.04	1	0.34	0.19	1	no resistance
Koc18	0.33	0.08	1	0.48	0.15	1	no resistance
Koc38	0.15	0.09	1	0.38	0.02	1	no resistance
Sil39	0.08	0.02	1	0.24	0.05	1	no resistance
Sil84	0.25	0.05	1	0.62	0.15	1	no resistance
Sur44	0.14	0.04	1	0.38	0.3	1	no resistance
Sur50	0.77	0.29	3	1.42	3.4	3	low resistance
Sur51	1.51	1.83	6	3.07	2.2	6	moderate resistance
Sur62	0.39	0.11	1	0.38	0.26	1	no resistance
Sur75	1.15	1.02	5	2.41	2.9	5	moderate resistance
Yen6	0.79	0.14	3	1.44	0.16	3	low resistance
Yen26	0.3	0.05	1	0.53	0.11	1	no resistance
Yen41	0.02	0.04	1	0.14	0.09	0	no resistance
Yen65	0.35	0.03	1	0.34	0.03	1	no resistance
Sensitive	0.25	0.01	-	0.48	0.06	-	Susceptible

*RI < 3: no resistance, 3 ≤ RI < 5 low resistance, 5 ≤ RI < 10 moderate resistance, RI ≥ 10: high resistance

Black Sea region developed resistance to tribenuron-methyl. That finding supports our study. Regarding SINAR's ability to develop resistance to ALS group herbicides, Topuz (2007) detected resistance to chlorsulfuron in 4 biotypes in wheat fields in Balıkesir and Çanakkale provinces. In the Çukurova region, Gürbüz et al. (2018) found resistance to pyroxsulam + cloquintocet sodium in 22 populations and to mesosulfuron-methyl + iodosulfuron-methyl sodium in 26 populations in Adana. The resistance status of SINAR to these compounds, which belong to the same class (ALS) as tribenuron-methyl, is similar to the results obtained in our study in Diyarbakır province. As a result of the resistance study conducted by Turgut (2023) on a total of 139 SINAR populations from wheat fields in Samsun, Amasya and Çorum provinces, resistance to 2,4-D+dicamba was detected in 9 biotypes and to 2,4-D+florasulam in 16 biotypes. These studies show that SINAR has developed resistance to several modes of action in Türkiye. Tribenuron-methyl of SINAR, a problem in wheat fields in Iran, was detected in 18 biotypes of 38 populations in Kermanshah province in studies conducted in different regions (Mehdi et al. 2017). Another study detected it in 30 biotypes of 80 populations in the same region (Khaledi et al.

2019). They discovered resistance in 14 biotypes out of 33 populations in Gulistan province (Gherekhloo et al. 2018), and 3 biotypes out of 16 populations in Ramiyan province (Heravi et al. 2018). Herbicide resistance is assumed to occur at the field level rather than at the regional level in these studies. In the study we conducted in Diyarbakır province, resistance was not observed in all fields of the districts where resistance was found, and in this respect, it is similar to the studies of Gherekhloo et al. (2018), Heravi et al. (2018), and Mehdi et al. (2017). These data indicate that the effectiveness of the herbicide containing the active ingredient tribenuron-methyl in controlling wild mustard in wheat fields does not cause problems and that there are problems with resistance in regions where growers' complaints are particularly severe. It is recommended to raise awareness among producers about overcoming weed problems by organizing workshops, informational meetings, field days, and similar educational activities on issues such as the decision-making process for herbicide applications, herbicide selection, maintenance and calibration of tools and equipment used in applications.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Buğday tarımında yabancı otların sorun oluşturduğu alanlarda kimyasal mücadele kaçınılmazdır. Ancak aynı etki mekanizmasına sahip herbisitlerin uzun yıllar kullanılması sonucunda yabancı otlarda dayanıklılık sorunu ortaya çıkabilmektedir. Herbisitlerin sık ve yanlış kullanılması çeşitli çevre ve sağlık sorunlarının yanı sıra iş gücü ve ekonomik kayıpların da yaşanmasına yol açmaktadır. Diyarbakır ili buğday alanlarında sorun oluşturan yabani hardalın (*Sinapis arvensis* L.- SINAR) kontrolünde yoğun kullanılan tribenuron-methyl (asetolaktat sentaz - ALS)'in etkisiz olduğu üreticiler tarafından bildirilmektedir. Bu bildirimlerin boyutlarını belirlemek amacıyla Diyarbakır'ın 16 ilçesinde buğday ekim alanlarından 56 SINAR tohum popülasyonu toplanmıştır. Kontrollü koşullarda klasik test metoduyla yapılan uygulamalar sonucunda 28 tarlaya ait popülasyonda dayanıklılık şüphesi tespit edilmiştir. Doz-etki denemeleri sonucunda SINAR'ın 20 biyotipinde dayanıklılık (RI<3) görülmezken, 5 biyotipte düşük seviyede (3≤ RI<5), 3 biyotipte ise orta düzeyde dayanıklılık (5 ≤ RI<10) tespit edilmiştir.

Anahtar kelimeler: acetolactate synthase (ALS) inhibitörü, herbisit dayanıklılığı, *Triticum aestivum* L.

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