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Original article (Orijinal araştırma)

Nematode damage and management in banana in Turkey[1]

Türkiye muz alanlarında nematod zararı ve mücadelesi

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Abstract

Banana is grown in tropical and subtropical regions of the world, and they are also cultivated in the coastal regions of the Mediterranean Region in Turkey. The present study aimed to determine the losses induced by the plant-parasitic nematodes in Anamur and Erdemli districts of Mersin Province, Turkey. Spiral nematode, *Helicotylenchus multicinctus* Cobb, 1893 (Tylenchida: Hoplolaimidae); root-knot nematodes, *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885) (Tylenchida: Meloidogynidae) were detected in the study greenhouses. Three nematicides, oxamyl, fenamiphos and ethoprophos, were used with two or four application against these nematodes. The experiments were conducted in three replicates in four greenhouses each with nematicide treated and untreated plots. In the study, yield for each treatment was calculated for 15 plants (5 plants/replicate). Nematodes were isolated from soil and root samples with a modified Baermann funnel method and nematode counting was performed under a microscope. In banana plantations with nematode management, the yield was between 28.6 and 53.1 kg/plant and the yield increase ranged between 34 and 117%. The results suggested that use of a single control method would not be effective against these nematodes and additional control methods in integrated management should be used to control nematodes in banana plantations.

Keywords: Banana, integrated management, nematicides, nematodes

Öz

Muz, dünyanın tropikal ve subtropikal bölgelerinde yetişir ve ayrıca Türkiye'nin Akdeniz Bölgesi'nin sahil şeridinde yetiştirilir. Bu çalışmada, Türkiye'nin Mersin İli, Anamur ve Erdemli ilçelerinde bulunan bitki paraziti nematodlarının neden olduğu kayıpların belirlenmesi amaçlanmıştır. Çalışma seralarında Spiral nematodu, *Helicotylenchus multicinctus* Cobb, 1893 (Tylenchida: Hoplolaimidae); kök ur nematodları, *Meloidogyne incognita* (Kofoid & White, 1919) ve *Meloidogyne javanica* (Treub, 1885) (Tylenchida: Meloidogynidae) tespit edilmiştir. Bu nematodlara karşı üç nematisid, Oxamyl, Fenamiphos ve Ethoprophos iki veya dört uygulama olarak kullanılmıştır. Denemeler her biri nematisid uygulanmış ve uygulama yapılmamış parsellere sahip dört serada üç tekerrürlü olarak yürütülmüştür. Bu çalışmada, her uygulama için verim 15 bitki (5 bitki / tekerrür) üzerinden hesaplanmıştır. Nematodlar, Geliştirilmiş Baermann Huni yöntemiyle toprak ve kök örneklerinden izole edilmiş ve nematodların sayımı mikroskop altında yapılmıştır. Muz alanlarında nematod mücadelesi ile verim 28.6 ile 53.1 kg /bitki ve verim artışı % 34 ile 117 arasında değişmiştir. Sonuçlar muz alanlarında bu nematodlara karşı tek bir mücadele yönteminin kullanılmasının etkili olmayacağını ve entegre mücadele yöntemleri içerisinde ilave mücadele yöntemlerini kullanımı ile kontrol edilebileceğini önerilmiştir.

Anahtar sözcükler: Muz, entegre mücadele, nematisid, nematodlar

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Introduction

Banana cultivation has great economic value in subtropical and tropical regions around the world. In Turkey, banana is cultivated in microclimates protected by Taurus Mountains around Gazipaşa, Alanya, Anamur, Bozyazı, Silifke, Erdemli, and İskenderun Districts with 499 kt of bananas produced on 7.6 kha in 2018 (TUİK, 2019).

Plant-parasitic nematodes are common in banana plantations and these are among the most damaging pests in all banana cultivars, leading to severe crop losses in commercial export banana plantations, and also significantly limiting the production and viability of other banana species. Many studies have been conducted on nematodes in bananas (Wardlaw, 1961; Gowen, 1995; Sarah & Fallas, 1996; Gowen et al., 2005; Wang & Hooks, 2009).

The first evidence of nematode damage leading to yield loss in banana was in the Jordan Valley (Minz et al., 1960). Several studies reported that important plant-parasitic nematodes, such as burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949 (Tylenchida: Pratylenchidae); spiral nematode, *Helicotylenchus multicinctus* (Cobb, 1893) (Tylenchida: Hoplolaimidae); lesion nematode, *Pratylenchus* spp. (Tylenchida: Pratylenchidae) and root-knot nematode, *Meloidogyne* spp. (Tylenchida: Meloidogynidae), lead to economic losses in banana plantations (Mant & Hinai, 1996; Brooks, 2004; Chavez & Araya, 2010). In studies conducted in banana plantations in Turkey, *H. multicinctus, Helicotylenchus dihystera* (Cobb, 1893) (Tylenchida: Hoplolaimidae), *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885) (Tylenchida: Meloidogynidae) were identified (Elekcioğlu, 1992; Elekçioğlu & Uygun, 1994; Özarslandan & Elekcioglu, 2010; Özarslandan & Dinçer, 2015). It has been reported that *H. multicinctus* populations were higher in banana greenhouses in Bozyazı District of Mersin Province than *Meloidogyne incognita* and *M. javanica* populations (Kasapoğlu et al., 2015). It has also been found that spiral nematodes were more prevalent in banana plantations in Turkey than root-knot nematodes (Özarslandan & Dinçer, 2015).

In Florida, *H. multicinctus* can cause severe root damage resulting in the toppling of mature plants (McSorley & Parrado, 1986; Gowen, 1995). According to McSorley & Parrado (1986), this nematode is highly prevalent in subtropical banana plantations. In Lebanon, *H. multicinctus* was considered as the most important root parasite of banana (Sikora & Schloesser, 1973). Since the spiral nematode feeds on the cortical cells close to the epidermis, it often forms superficial reddish brown and black lesions. Purple-black lesions can be observed in the root or rhizome, and the root lesions can merge and expand, and finally kill the entire root. Root-knot nematodes form galls in the main and secondary roots and the symptoms are observed frequently in the form of irregular bulges. However, knot formation is not observed in thick primary roots. In addition to the direct damages caused by the nematodes, they can also indirectly enable pathogenic fungal infection. Fungal pathogens (*Fusarium* spp. and *Rhizoctonia* spp.) can infect and kill the roots. Nematodes inhibit nutrient and water intake due to root damage in banana. Thus, the plant collapses due to the loss in root strength and also plants exhibit retarded growth, trunk shrinkage, yellow leaves, decreased in leaf count and size, delayed flowering, delayed harvest, decreased bunch weight, and decreased fruit size and weight (McSorley & Parrado, 1986; Bridge, 1988; Fogain & Gowen, 1997; Araya et al., 1999). As a result of nematode damage, the leaves grow on top of each other and the plant buckles. These plants either do not produce flowers or the yield is reduced. Thus, the market value of the fruit is impacted.

The density of soil and root nematodes has been reported to be important for decision-making (Moens et al., 2001; Pattison et al., 2002). The number of nematodes per gram of root is used to determine the economic threshold for nematicide application. Greater then 1 nematode/g soil in banana plantations has been reported to be the damage threshold (Rajendran et al., 1980). Many studies have also found that when the number of nematodes per 100 g banana roots exceeds 2000, economic losses are likely to occur

(Gowen & Quénéhervé, 1990; Sarah & Dallas, 1996; Wang & Hooks, 2009). There is no universal threshold for economic damage to nematodes in banana plantations around the world.

It is known that root plant nematodes and spiral nematodes, which are important plant-parasitic nematodes in banana areas of Turkey, are widespread. The aim of this study was to determine the damage conditions of nematodes in banana plantations and to determine the methods of combating them.

Materials and Methods

Experiments were conducted in four greenhouses in Erdemli (Greenhouse 1 and 2) and Anamur (Greenhouse 3 and 4) Districts in Turkey. In these greenhouses, the experiments had four treatments: oxamyl 15 ml/plant), fenamiphos 20 ml/plant, ethoprophos 15 ml/plant and untreated control in three replicates. Five plants were selected for each replicate and each treatment was applied to 15 plants and plant yields recorded. Five soil samples were collected from five locations up to 30 cm from the trunk and near the roots of each plant. The soil samples (2 kg) were collected into plastic bags. Root samples were collected from the same locations and placed plastic bags (Mant & Hınai, 1996; Wang & Hooks, 2009). Nematodes were extracted from soil samples (100 g) in a Petri-dish method; a modified Baermann funnel method (Barker, 1985; Southey, 1986). Root samples were washed with water to remove the soil and cut into 5-25 mm long pieces and the nematodes were extracted with a modified Baermann funnel method (Barker, 1985; Southey, 1986). Thus, the nematode number in 100 g of fresh root was determined. Nematicide application dates,13 March, 29 April, 19 June and 12 August 2014. Only two application were treated with nematicide on the first and second dates.

The effect (%) of treatments on yield was determined with Abbott's formula. The yield, root and soil nematode counts were analyzed by variance analysis using SPSS 23.0 (IBM Corporation, Armonk, NY, USA) and the means were compared using the Duncan’s test at 0.05 significance level.

Result and Discussion

Root-knot and spiral nematodes were found in mixed populations in experimental sample and spiral nematode, *H. multicinctus*, *M. incognita* and *M. javanica* were also present. Nematodes were active for 12 months in the greenhouse. In winter, the root-knot nematode population decreased. In Greenhouse 1, the highest yield (31.9 kg/plant) was with four ethoprophos applications (15 ml/plant), an increase of 49.8%. The yields with two and four oxamyl applications, four ethoprophos applications, and four fenamiphos applications were 28.6, 30.1, 31.9, 30.5 and 31.3 kg/plant, respectively, i.e., increases of 34.3, 41.3, 49.8, 43.2 and 46.9% over the control (Table 1). In Greenhouse 2, the highest yield was with four oxamyl 15 ml/plant applications (53.1 kg/plant), an increase of 71.9%. The yield increases with two and four oxamyl applications, and two and four fenamiphos applications were 47.6, 53.1, 44.1 and 48.2 kg/plant, respectively, i,e., increases of 54.1, 71.9, 42.7 and 55.9% over the control (Table 1). In Greenhouse 3 the highest yield was observed with four oxamyl 15 ml/plant applications (49.5 kg/plant), an increase of 117%. The yields with four oxamyl applications, and four ethoprophos applications were 49.5 and 44.4 kg/plant, respectively, i.e. increases of 117 and 94.7% over the control (Table 2). In Greenhouse 4, the highest yield (41.9 kg/plant) was with four oxamyl applications (15 ml/plant), an increase of 63%. The yields obtained with four applications of fenamiphos and ethoprophos were 37.1 and 36.4 kg/plant, respectively, i.e., increases of 44.4 and 41.6% over the control (Table 2). These results show that overall yield in the experiment areas was from 28.6 to 53.1 kg/plant with the nematode management, with yield increases between 34 and 117%.

Table 1. Nematode numbers in root (100 g) and soil (100 g) after 1 and 12 months, banana yield (kg/plant) and effect relative to the control (%) in Erdemli, Mersin Province, Turkey

Application	Root/ Soil	M/H*	Greenhouse 1				Greenhouse 2			
			1 months	12 months	Yield	Effect (%)	1 months	12 months	Yield	Effect (%)
Oxamyl 15ml 4 Application	root	M	1040±301	0±0	30.1±1.6 a	41.3	0±0	1200±427	53.1 ±1.8 a	71.9
		H	2000±415	1600±369			640±138	2400±565		
	soil	M	100±46	40±23			0±0	40±23		
		H	20±20	600±80			400±173	1500±427		
Oxamyl 15ml 2 Application	root	M	0±0	100±46	28.6±1.6 a	34.3	0±0	1700±496	47.6±2.5 ab	54.1
		H	640±80	2700±438			440±138	4500±773		
	soil	M	220±69	200±69			0±0	20±11		
		H	600±127	1580±219			240±69	1520±554		
Ethoprophos15ml 4 Application	root	M	2800±323	0±0	31.9±2.4 a	49.8				
		H	1840±369	400±69						
	soil	M	20±11	20±11						
		H	120±23	440±184						
Fenamiphos 20ml 4 Application	root	M	880±103	0±0	31.3±1.6 a	46.9	0±0	100±46	48.2±1.3 ab	55.9
		H	720±138	2100±254			320±69	1400±334		
	soil	M	280±80	20±11			0±0	0±0		
		H	260±46	240±80			0±0	100±46		
Fenamiphos 20ml 2 Application	root	M	0±0	100±46	30.5±3.2 a	43.2	0±0	200±69	44.1±0.9 b	42.7
		H	400±80	700±173			200±57	2600±450		
	soil	M	160±34	60±23			20±11	0±0		
		H	180±92	780±150			160±34	1400±219		
Control	root	M	60±23	300±103	21.3±3.0 b		160±57	0±0	30.9±4.7 c	
		H	1300±334	4400±542			480±173	2600±496		
	soil	M	20±11	0±0			20±11	0±0		
		H	12034	1820± 323			180±57	400±161		

* H, spiral nematode, *Helicotylenchus multicinctus*; M, root-knot nematodes, *Meloidogyne* spp.

The per plant yield differences between the greenhouses demonstrated that nematicide application alone was not sufficient to increase the yield. In order to increase per plant yield, it is necessary to use integrated nematode management methods. Since banana is monoculture production, nematode populations are advantaged by continuous development. After the nematicide application, spiral nematode population did not decrease, the life cycle was completed, and the nematode population increased. However, the plant trunk thickened, the leaf count and the distances between the leaves increased and the overall plant development improved after the nematicide applications.

Table 2. Nematode numbers in root (100 g) and soil (100 g) after 1 and 12 months, banana yield (kg/plant) and effect relative to the control (%) in Anamur, Mersin Province, Turkey

Application	Root/ Soil	M/H*	Greenhouse 3				Greenhouse 4			
			1 months	12 months	Yield	Effect (%)	1 months	12 months	Yield	Effect (%)
Oxamyl 15 ml 4 Application	root	M	200±103	6900±773	49.5±2.7 a	117.1	0±0	100±46	41.9±1.5 a	63
		H	0±0	0±0			2800±427	900±300		
	soil	M	20±11	2970±271			0±0	240±115		
		H	0±0	0±0			160±34	800±230		
Ethoprophos15 ml 4 Application	root	M	640±98	800±207	44.4±2.1 a	94.7	60±23	100±34	36.4±0.9 b	41.6
		H	0±0	0±0			1040±196	5000±329		
	soil	M	20±11	280±80			40±23	40±23		
		H	0±0	0±0			180±23	1900±334		
Fenamiphos 20 ml 4 Application	root	M					0±0	100±46	37.1±1.5 b	44.4
		H					2400±334	1000±230		
	soil	M					0±0	0±0		
		H					20±11	120±23		
Control	root	M	480±161	120±57	22.8±4.0 b		0±0	0±0	25.7±0.9 c	
		H	0±0	0±0			640±138	1800±323		
	soil	M	120±46	120±23			0±0	0±0		
		H	0±0	0±0			80±23	2000±323		

* H, spiral nematode, *Helicotylenchus multicinctus*; M, root-knot nematodes, *Meloidogyne* spp.

The total number of nematodes (*Helicotylenchus* spp. + *Meloidogyne* spp.) obtained from the root and soil samples collected in August was higher than in May, and the nematode population was more than 2500/100 g in 62% of the root samples collected in August (Özarslandan & Dincer, 2015). Since spiral nematode attacks the root tissues of banana, causing degradation, it prevents the development and reproduction of root-knot nematodes (Araya & Moens, 2003). Thus, it was observed that the spiral nematode population was higher in banana plantations (Tables 1 & 2). Although, it is difficult to determine the damage caused by a specific nematode species in banana, nematicide application provided 61 to 98% yield increase with root-knot and spiral nematode infestation in Nigeria (Caveness & Badra, 1980; Badra & Caveness, 1983) and Ivory Coast (Adiko, 1988). Also, it was reported that nematicides gave 119% and higher yield increases in the banana plantations in Jamaica (Hutton & Chung, 1973) and in Puerto Rico,

nematicides gave 207-275% yield increases for more than 3 years (Roman et al., 1977). A yield loss of 15-50% due to *R. similis* and *H. multicinctus* was reported in East Africa (Speijer & De Waele, 2001). In that study, *Azadirachta indica* A. Juss. (Rutales: Meliaceae) and *Allium sativum* L. (Asparagales: Alliaceae) extracts and ethoprophos were used three times at weekly intervals and Mocap application was determined as the most effective, and gave significant increases in plant growth in all applications (Bartholomew et al., 2014). In a study by Mant & Hinai (1996), oxamyl, fenamiphos and ethoprophos were used for nematode control in banana. They reported an increase in plant growth and 48.8% increase in with three nematicides applications of 2.5-3.5 g ai/plant (Quénéhervé et al., 1991b). It was reported that nematicide application increased the yield by 20-40% through nematode nematode management in banana plantations (Araya & Cheves, 1997). In a study by Araya & Lakhi (2004), 3 g ai/plant nematicide led to an average yield increase of 30.8%. Eissa et al. (2005) reported that 1-2 applications of certain biological agents and oxamyl (15 ml/plant; 240 ai/L) in banana plantations against *Meloidogyne incognita, Helicotylenchus exallus* and *Criconemoides* spp. increased bunch weight and finger count. Two or three non-fumigant applications led to 21-44% yield increase in Australia (Broadley, 1979), 50% yield increase in Cameron and 41% yield increase in Costa Rica (Araya & Cheves, 1997). Studies conducted on nematicide use in various banana producing countries found that yield responses varied significantly between 15 and 275% (Gowen & Quénéhervé, 1990). In the Philippines, yield reductions based on bunch weights that varied between 26.4 and 57.1% were observed after inoculation with the root-knot nematode *M. incognita* (Davide & Marasigan, 1985). In greenhouse experiments, significant reductions in plant growth (Jonathan & Rajendran, 2000) and alteration of the concentration of macro- and micronutrients in leaves (Cofcewicz et al., 2004) were observed after inoculation with root-knot nematodes. In the 1980s, only *H. multicinctus* and *Meloidogyne* spp. were considered important pests in Nigerian (Caveness & Badra, 1980) and Ivory Coast plantains (Adiko, 1988), and yield increases that ranged between 61 and 98% after established plantains infested with these nematode species had been treated with nematicide (Caveness & Badra, 1980; Badra & Caveness, 1983). Plantain yield losses that ranged between 25 and 64% for the first crop and 50-90% for the successive crop cycles were reported in Ghana (Coyne et al., 2005). In a field experiment conducted in Cameroon, the total production losses in the first and second cycles were 60 and 51%, respectively (Fogain, 2000). After chemical treatment, large yield improvements were observed in Jamaica with 119% in one cycle (Hutton & Chung, 1973). The present study findings on yield improvement in banana plantations with nematicide treatment is consistent with previous studies.

Injection of carbofuran and oxamyl in the harvested pseudostem did not suppress nematode numbers per 100 g fresh roots, but bunch weight increased significantly, compared to the untreated control (Araya, 1999). While nematicides are effective against stable endoparasitic root-knot nematodes, they are not effective against motile spiral nematodes in the root. Nematode populations did not decrease significantly after nematicide applications and spiral nematode population increased despite the treatment. Araya & Lakhi (2004) reported that nematicides affected the root-knot nematodes, while the nematodes were motile in the root completed their life cycle despite the nematicide treatment and the nematode population increased. It was reported that aldicarb, fenamiphos, isazophos, carbofuran and cadusaphos applications did not prevent the increase in nematode populations in the root in the banana plantations (Stanton & Pattison, 2000; Quénéhervé et al., 1991a, b).

The causes of the root death in banana plantations are biotic and abiotic factors, and it was reported that these not only included nematode damage but also by fungi, bacteria, soil type and poor drainage, pH, plant species, soil moisture, soil structure and mechanical damage (Kobenan et al., 1997). Due to monoculture of banana, the nematode populations continuously increase and there is a always nematodes present in the soil. The low nematode population in winter months adversely affect the development of banana suckers with increasing soil temperature in spring. It was reported that chemical control has been adopted in plant-parasitic nematode management in banana plantations since 1960, but yield loss due to

plant-parasitic nematodes can be reduced through the application of integrated management approaches (Gowen et al, 2005; Stirling & Pattison, 2008; Roy et al., 2016; Shankar et al., 2016). Another study reported that fertilizer use after nematicide treatment would be beneficial in nematode-inoculated banana plantations (Smithson et al., 2001).

In banana monoculture in Turkey, it was observed that yield and quality decreases due to nematode damage, leaves concentrated on the top and banana cluster weight decreased. Continuous banana cultivation is conducted in certain locations either in fields or in greenhouses. Spiral and root-knot nematode populations in the mother plant prevent sucker root development, which is the basis of the next generation fruit production. The plants in the control plot were exposed to a high nematode population since they were adjacent to the mother plant. Thus, their yield and quality decreased. Banana suckers are exposed to a lower nematode population when they are further away from the mother plant. Therefore, plant growth, yield and quality increase (Özarslandan, 2019). In the present study, nematode damage awareness was observed among producers. Nematicide should be applied in April, May, June and August in infested greenhouses. In the present study, field observations were conducted and found that ethoprophos 20 L/ha and fenamiphos 40-43.5 L/ha applications led to good plants, and no leaf accumulation was observed. Rates of 150 ml/100 L ethoprophos and 150-200 ml/100 L fenamiphos were applied to the suckers. This treatment yielded 28.6-53.1 kg/plants. The difference between the yields demonstrated that production increases cannot be achieved with nematicides alone. In order to increase product growth in banana plantations, it was demonstrated that an integrated nematode management should be adopted. It is also important that the planted suckers are grown using tissue culture. Since the sucker roots produced by the farmers in greenhouses are infected with disease and nematodes, these suckers should not be planted, since the presence of disease and nematodes in roots will affect plant growth, which will subsequently lead to decreases in the yield and quality. Mycorrhiza treatment should be administered to the suckers before planting. Mycorrhizae are effective against soil-based fungi and nematodes and improve the nutrient uptake and root volume. The Increases in root volume and nutrient intake lead to an increase in the yield and quality. Burnt animal manure should be used. The animal manure decreases nematode motility and propagation, thus leading to good root development. Nematode population decreases due to the increases in beneficial microorganisms in the soil induced by the animal manure. Several benefits of animal manure are already known. Nematode population increases due to cultivation in the same location. Thus, when the mother plant is cut, the nematodes in the roots attack adjacent suckers. Since the development of suckers is negatively affected, they do not develop well. Therefore, an alternation system should be implemented by changing the row positions in the greenhouse frequently. When the suckers are planted adjacent or close to the mother plant, the nematodes in the mother plant attack the suckers and adversely affect their growth. We recommend replacing the suckers every three years due to yield and quality decreases observed after the third year of sucker plantation. In banana cultivation, the row spacing is 3 m and the suckers should be planted at a distance of 1.5 m from the rows. Since the nematode density is 0-40 cm around the plant (Özarslandan, 2019), plant development and yield quality are better when they are planted away from the mother plants. The locations of the rows should be changed frequently in greenhouses. Cut suckers or carved suckers should be used. The suckers planted next to the mother plant in April should be cut and carved between June and early July. A new sucker is obtained far from the mother plant. This sucker gives more yields when compared to the suckers adjacent to the mother plant. Shadow dust should be applied in early April, when the greenhouse temperature rises significantly above 40°C. The greenhouse should be checked at 2 pm when the highest temperatures are observed. The greenhouse temperature should be kept at 30°C. The temperature inside the greenhouse should not exceed 36°C. When temperatures exceed 40°C, plants close to greenhouse vents grow well, however the plants in the center of the greenhouse do not develop well at high temperatures. Since these plants cannot tolerate nematode and root diseases, yield and quality losses increase. Weeds increase nematode population and lead to yield and quality losses in banana. Nematode populations can be reduced by weed control. The nematicide

treatment should be conducted in warm periods when the nematodes are active. The banana plant roots develop until flowering. To control the nematodes in these roots, nematicides are applied as two applications in April and May. In August, when decline symptoms are observed in nematode infected roots, the application is necessary if the roots are unhealthy that may cause poor bunches and delayed harvest. Plants with healthy roots form bunches in July and harvest is completed between September and December. Greenhouses should be irrigated in the morning. For plant growth, moisture should be kept around 80% in greenhouses. In certain greenhouses with integrated management, nematicides are used, while they are not required in other greenhouses. Banana could be cultivated without nematicide application. Farmers should utilize all management methods to grow healthy plants that could tolerate the nematodes.

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Original article (Orijinal araştırma)

Culturable bacterial strains isolated from *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) populations of Adana and Mersin Provinces of Turkey and their entomopathogen characteristics[1]

Türkiye'nin Adana ve Mersin illerinden toplanan *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) popülasyonlarıyla ilişkili kültüre alınabilen bakteriler ve entomopatojen özelliklerinin belirlenmesi

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Abstract

Whitefly, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) hosting various bacteria is among the most significant insect pest causing economic losses in agricultural production. A study was conducted to determine the bacterial flora of *B. tabaci* and to identify the efficiency of these bacteria against *B. tabaci*. Samples were collected from sesame and melon plants in Mersin and Adana Provinces of Turkey in 2014. Nine bacterial strains were identified by morphological, MALDI-TOF MS and molecular identification methods to species level. *Bacillus* sp., *Methylobacterium* sp., *Microbacterium* sp., *Serratia marcescens* and *Sphingomonas* sp. were identified from the samples collected from sesame and *Acinetobacter lwoffii*, *Bacillus cereus*, *Staphylococcus hominis* and *Staphylococcus warneri* were identified from the samples collected from melon. To determine biological efficiency against *B. tabaci* adult (biotype B), whiteflies were fed with insect food as control (sucrose + water), insecticidal control (acetamiprid) and bacterial suspensions. The entomopathogenic bacteria *S. marcescens* isolated from *B. tabaci* for the first time in this study yielded an efficiency of 72% against adult whitefly. However, the other strains had efficiencies below 25%. It was concluded, given the efficiency of *S. marcescens,* that further research should be conducted on the pathology of entomopathogenic bacteria in pest insects.

Keywords: *Bemisia tabaci*, biological control, entomopathogen bacteria, *Serratia marcescens*, symbiont

Öz

Bünyesinde çeşitli bakterileri barındıran, Beyazsinek, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) tarımsal üretimlerde ekonomik kayıplara neden olan en önemli zararlı böcek türlerinden birisidir. Bu çalışmada *B. tabaci*'nin bakteriyel florasını belirlemek ve bu bakterilerin etkinliğini ortaya çıkarmak amacıyla 2014 yılında iki farklı ilden (Mersin ve Adana) sırasıyla susam ve kavun bitkilerinden örnekler toplanmıştır. Morfolojik, MALDI-TOF MS ve moleküler tanı yöntemleri kullanılarak yapılan analizler sonucunda toplam 9 adet bakteri türü saptanmıştır. Susam tarlasından toplanan örneklerden *Bacillus* sp., *Methylobacterium* sp., *Microbacterium* sp., *Serratia marcescens*, *Sphingomonas* sp.; kavun bitkisinden toplanan örneklerden *Acinetobacter lwoffii*, *Bacillus cereus*, *Staphylococcus hominis* ve *Staphylococcus warneri* türleri belirlenmiştir. *Bemisia tabaci* erginlerine (B biyotip) karşı yapılan biyolojik etkinlik çalışmalarında beyazsinekler, kontrol olarak böcek besini (sakkaroz+su), ilaçlı kontrol (acetamiprid) ve bakteri süspansiyonlarıyla beslenmişlerdir. İlk defa bu çalışma kapsamında *B. tabaci*'den izole edilen ve entomopatojen bir bakteri olan *S. marcescens* ergin beyazsineğe karşı %72 oranında başarılı olmuştur. Diğer izolatların etki oranları %25'in altında kalmıştır. *Serratia marcescens* ile elde edilen bu başarı, izolatın zararlı böcekler üzerindeki patolojisi ile ilgili çalışmaların devam ettirilmesi gerektiği sonucunu ortaya çıkarmıştır.

Anahtar sözcükler: *Bemisia tabaci*, biyolojik mücadele, entomopatojen bakteri, *Serratia marcescens*, simbiyont

[1] Part of this work was presented as a poster presentation at the VII. Plant Protection Congress of Turkey (14-17 November 2018, Muğla, Turkey).

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Introduction

As is common worldwide, cotton whitefly, *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) feeds on different host plants causing significant economic losses in Turkey. Adult and larval stages of the pest damage plants through sap-sucking, inducing sooty mold by secreting large amounts of honeydew and transmitting important plant viruses. Together with the development of transportation technologies and increasing international plant transfers, the pest has a global distribution and is encountered on every continent of the world, except for Antarctica. *Bemisia tabaci* is defined as a species-complex composed of at least 40 biotypes. Among the biotypes, B (*Bemisia argentifolii* Bellows & Perring, 1994, and MEAM1 = Middle East Asia Minor 1) and Q (MED = Mediterranean) are the most common and invasive. Given the high biotic potential and rapid increase in population size, these biotypes are able to generate economic losses in a short time, consequently chemical control is the most preferred. However, with the use of chemicals, resistant biotypes have become widespread and result in epidemic disease outbreaks generating significant economic losses in Turkey and globally (Henneberry & Faust, 2008; Karut et al., 2017). *Bemisia* biotypes develop resistance to insecticides over time, thus integrated pest control including biopesticides and biological control is required for the long-term management of this pest (Ateyyat et al., 2009).

Microorganisms are colonized insects forming films over of the cuticle and intestines, and also form microbial populations in the hemocoel, body fluids and cells (Jing et al., 2014). Bacteria in their host insects are important for food digestion, pheromone synthesis, pH regulation, vitamin and amino acid synthesis, resistance development against parasitoids and entomopathogens (Dillon & Dillon, 2004; Ateyyat et al., 2010; Zhang et al., 2014). So, bacteria can be symbionts essential for survival of the insect but also entomopathogens that kill them.

Some insects of the Sternorrhyncha suborder of Hemiptera (psyllids, whiteflies, aphids and mealybugs) are in obligate-relationship with primary and secondary endosymbiont bacteria. The individuals of this suborder with piercing-sucking mouthparts in *B. tabaci* complex have seven different bacterial species carried on their body. These are *Portiera aleyrodidarum* (obligate primary endosymbiont), *Arsenophonus*, *Hamiltonella*, *Rickettsia*, *Wolbachia*, *Cardinium* and *Fritschea* (facultative secondary endosymbionts) all of which evolved with Aleyrodidae species. These bacteria either negatively or positively influence vital activities of the insect (Thao & Baumann, 2004).

Majority of entomopathogenic bacteria infect insects through their digestive system. Those living over the surface may penetrate into the body through wounds. In contrast to fungal entomopathogens, bacterial entomopathogens are not able to actively penetrate into outer integument of the insect (Demirbag et al., 2008). The majority of the bacteria obtained from whiteflies form mutual interactions with the insects contributing to digestion and nutrition of the host. These bacteria can occur in intestines of the whitefly and associated sooty mold (Davidson et al., 2000). Although some of these bacteria associated with whitefly were found to be promising in biological efficiency studies, studies on the use of bacteria in biological control of the piercing-sucking mouthparts insects are ongoing (Ateyyat et al., 2009; Roopa et al., 2014).

Demonstration of the interaction between *B. tabaci* and its bacterial entomopathogens and symbionts has a significant potential for developing control practices for the pest. Therefore, there is a need for detailed studies on bacteria-insect interactions and defense mechanisms of whitefly (Zhang et al., 2014). In this study, culturable bacterial strains were isolated from the flora of *B. tabaci* to determine their potential entomopathogenic properties for biological control of the pest. Efficiency of the bacterial stains on whitefly was determined under laboratory conditions.

Materials and Methods

Bemisia tabaci populations

To determine bacterial flora of whitefly, *B. tabaci* infected leaf samples and adult whitefly samples were collected from sesame and melon plants in Tarsus (Mersin Province) and Balcalı (Adana Province), respectively, in 2014. Adults were sampled directly from the plants with a mouth-aspirator to obtain at least 100 insects from each population.

Bacterial isolations

Adult and immature whiteflies were brought to laboratory and bacterial isolations were performed. About 100 pupae and 150 nymphs collected from the lower part of the leaves with a thin-pin insect needle in the laboratory and 100 adults collected from the field were surface sterilized with alcohol. These individuals were then separately crushed in nutrient broth and the suspension diluted to 1 ml to generate stock suspensions. A dilution series of each suspension were prepared, and 100 µl from each dilution was spread on nutrient agar (NA) with a glass rod and incubated at 25ºC. Bacterial isolates were subcultured on NA, yeast extract agar (YEA) and potato dextrose agar (PDA).

Morphological and physiological tests

Morphological and physiological identification of the bacterial strains were performed on NA, SNA (NA+5% sucrose), King's B (KB) medium and yeast dextrose calcium carbonate agar (YDCA), gram reactions by potassium hydroxide and fluorescent pigmentation on KB, and oxidase reaction (Lelliott & Stead, 1987). All these tests were performed at Dr. Hatice Satar, Entomopathogen Laboratory of Adana Biological Control Research Institute, Turkey.

MALDI-TOF MS identification

MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) was used for identification of bacterial strains to genus and/or species level (Wunschel et al., 2005). This was done by a commercial service under the supervision of Prof. Dr. Soner Soylu from Mustafa Kemal University Plant Health and Clinics Research and Implementation Center (Antakya, Turkey).

Molecular identification

Strains not identified by MALDI-TOF MS were identified by molecular methods. For this purpose, DNA isolation was performed by the Dellaporta DNA extraction method (Dellaporta et al., 1983). Genomic DNA was amplified by PCR with 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTAC CTTGTTACGACTT-3′) primers for the 16S region. Sequences of ~1500 bp PCR products were determined (Lane, 1991). The DNA sequences were arranged with the aid of Bioedit Software Version 7.2.5, then subjected to BLAST analysis (National Center for Biotechnology Information).

Bioassays

Biological efficiency of the bacteria against the lab population of *B. tabaci* biotype B adult stage was determined through the modified method of Davidson et al. (2000). In this method, 2 ml Eppendorf tubes were used to feed *B. tabaci* adults. Initially, the bottom of the tubes was cut with a bistoury and covered with 5 cm^2 Parafilm pieces. The cut and Parafilm-covered section were covered again with Parafilm to give a 5 mm thick layer. Finally, the prepared tube was surrounded with duct tape (Davidson et al., 2000; Zhang et al., 2014). Twenty adults (1-2 d old) were then transferred to each tube. To have adult individuals of the same age, the plants with high populations of the pest at pupal stage were selected 2 d before the experiments and all adults were removed. These plants were then placed into Plexiglas cages to obtain newly hatched adults of the same age. The adults on the sides of the cages were used in experiments

because their stylets would not be damaged. After transfer of adults, the tubes were placed upside-down into the cuvettes with Parafilm-covered section upward. Then, Parafilm-covered section was supplemented with 100 µl of only insect food (negative control), insecticide with acetamiprid active ingredient (positive control) or suspensions of the bacterial strains. The insect food was prepared by smashing four discs of cotton leaf (15 mm diameter) in 100 ml distilled water and 30 g sucrose. The positive control was prepared by supplementing insect food with 0.6 g/l of an insecticide with acetamiprid active ingredient, commercially available and registered for whitefly. For bacterial suspensions, the bacteria developed in NA was collected with a loop and supplemented to 10 ml of insect food. The suspension optical density was adjusted to 0.5 at 600 nm using a spectrophotometer.

Experiments were conducted with at least five replicates. Each feeding tube was regarded as a replicate. Counting was performed 48 h after commencing the experiments. The individuals that had fallen to the bottom of the tube and with no evident motion were accepted as dead.

Statistical analysis

In bioassay studies, mortality ratio (%) of bacterial isolates was calculated as the ratio of dead individuals to total number of individuals. Percent values were subjected to arcsine square root transformation before the analysis. Normality of variances was checked using Levene's test. Analysis of variance (ANOVA) followed by Tukey's multiple range test at 5% significance level to determine differences between the means, for parametric data in SPSS (Version 23) software. Also, the percent efficiency of the isolates was calculated with the Henderson-Tilton equation (Karman, 1971).

Results

Bacterial flora of *Bemisia tabaci*

The strains obtained from *B. tabaci* samples collected from sesame were analyzed by MALDI-TOF MS and identified as *Bacillus* sp. (Bacillales: Bacillaceae), *Methylobacterium* sp. (Rhizobiales: Methylobacteriaceae), *Microbacterium* sp. (Actinomycetales: Microbacteriaceae) and *Serratia marcescens* (Enterobacteriales: Yersiniaceae); the strains obtained from the *B. tabaci* samples collected from melon were identified as *Acinetobacter lwoffii* (Pseudomonadales: Moraxellaceae), *Bacillus cereus* (Bacillales: Bacillaceae), *Staphylococcus hominis* and *S. warneri* (Bacillales: Staphylococcaceae). Also, a strain obtained from whiteflies collected from sesame was compared with the data in system library for MALDI-TOF MS, but it was not able to be identified. Molecular methods were used for this bacterial strain and it was 99% similar with *Sphingomonas* sp. (Sphingomonadales: Sphingomonadaceae).

Morphological examination revealed that *Acinetobacter lwoffii* formed cream, smooth circular colonies on four different growth media. *Bacillus cereus* developed matt cream, circular colonies on all growth media and colonies had rough and wavy contours on YDCA and KB media. *Bacillus* sp. strain initially exhibited colorless colonies on all growth media, then turned cream-color on all media, except for YDCA on which it was yellow. This strain formed smooth circular colonies on all media, but had wavy contours on KB medium. *Methylobacterium* sp. strains exhibited quite slow development on all media and formed small circular colonies. The colonies were initially cream-color, then turned into light pink. *Microbacterium* sp. strains formed bright orange, smooth circular colonies on all media, but had a slightly bulbous texture on KB medium. *Serratia marcescens* strains formed red, smooth circular colonies on all growth media, but were light pink on SNA medium. These strains had bulbous development on NA and SNA, but were more bouffant on the other media. *Sphingomonas* sp. formed bright yellow, circular bulbous colonies in all media. *Staphylococcus hominis* and *S. warneri* strains developed in cream colonies on YDCA medium and close to white circular bulbous colonies on the other media (Table 1).

Acinetobacter lwoffii, *S. marcescens* and *Sphingomonas* sp. strains were gram-negative and the others as gram-positive. *Bacillus cereus*, *Methylobacterium* sp. and *Sphingomonas* sp. strains were oxidase-positive and all others oxidase-negative. None of the strains were fluorescent (Table 1).

Table 1. Culturable bacterial species isolated from *Bemisia tabaci* and morphological and physiological characteristics of the strains

Species	Colony development in growth media				Reactions		
	NA*	SNA	KB	YDCA	Gram reaction	Oxidase	F/NF
Acinetobacter lwoffii	Cream, circular, smooth	Cream, circular, smooth	Cream, circular, smooth	Cream, circular, smooth	–	–	NF
Bacillus cereus	Matt cream, circular	Matt cream, circular	Matt cream, circular, wavy contours	Matt cream, circular, wavy contours	+	+	NF
Bacillus sp.	Cream, circular, smooth	Cream, circular, smooth	Cream, circular, wavy contours	Yellow, circular, smooth	+	–	NF
Methylobacterium sp.	Light pink, circular	Light pink, circular	Light pink, circular	Light pink, circular	+	+	NF
Microbacterium sp.	Orange, circular, smooth	Orange, circular, smooth	Orange, circular, slightly bulbous	Orange, circular, smooth	+	–	NF
Serratia marcescens	Red, circular, bulbous	Light pink, circular, bulbous	Red, circular, bouffant	Red, circular, bouffant	–	–	NF
Sphingomonas sp.	Yellow, circular, bulbous	Yellow, circular, bulbous	Yellow, circular, bulbous	Yellow, circular, bulbous	–	+	NF
Staphylococcus hominis	White, circular, bulbous	White, circular, bulbous	White, circular, bulbous	Cream, circular, bulbous	+	–	NF
Staphylococcus warneri	White, circular, bulbous	White, circular, bulbous	White, circular, bulbous	Cream, circular, bulbous	+	–	NF

* NA, nutrient agar; SNA (NA+5% sucrose); KB, King's B; YDCA, yeast dextrose calcium carbonate agar; F/NF, fluorescent/non-fluorescent).

Biological efficiency of bacterial strains against *Bemisia tabaci*

For biological efficiency of bacterial strains against *B. tabaci* adults, mortality and % impact ratios were compared and analyzed accordingly. According to the ANOVA of data obtained 2 d after the treatment, the differences in mortality and % impact ratios of the treatments were found to be significantly different from the control treatment ($df = 10$, $F = 14.8$, $P < 0.005$) (Table 2).

Mortality ratios are shown in Figure 1. After acetamiprid treatment, the greatest mortality ratios were observed in *S. marcescens* (75%), *Microbacterium* sp. (31%), *S. warneri* (29%), *Sphingomonas* sp. (25%), *S. hominis* (23%) and *B. cereus* (24%) strains. Mortality ratios of the other strains were all below 20%.

The mortality ratios of the adults fed with bacterial strains suspensions and insect food (control) were compared and % impact ratios were calculated with the Henderson-Tilton equation (Table 2). Impact ratios of the treatments varied between 9 and 91% and two treatments (*A. lwoffii* and *Methylobacterium* sp.) were found to be ineffective. The greatest impact ratio (91%) was obtained from acetamiprid (positive control) treatment. The greatest impact ratio of the bacterial strains (72%) was obtained from *S. marcescens* and this treatment was placed into the same group with the acetamiprid treatment. *S. marcescens* was followed by *Microbacterium* sp. (24%) (Table 2). Additionally, there was a distinctive color change (reddening) in dead individuals fed with *S. marcescens* strain (Figure 2).

For mortality and impact ratios (%) of the bacterial strains, only *Serratia marcescens* had an impact greater than 70% (Table 2, Figure 1).

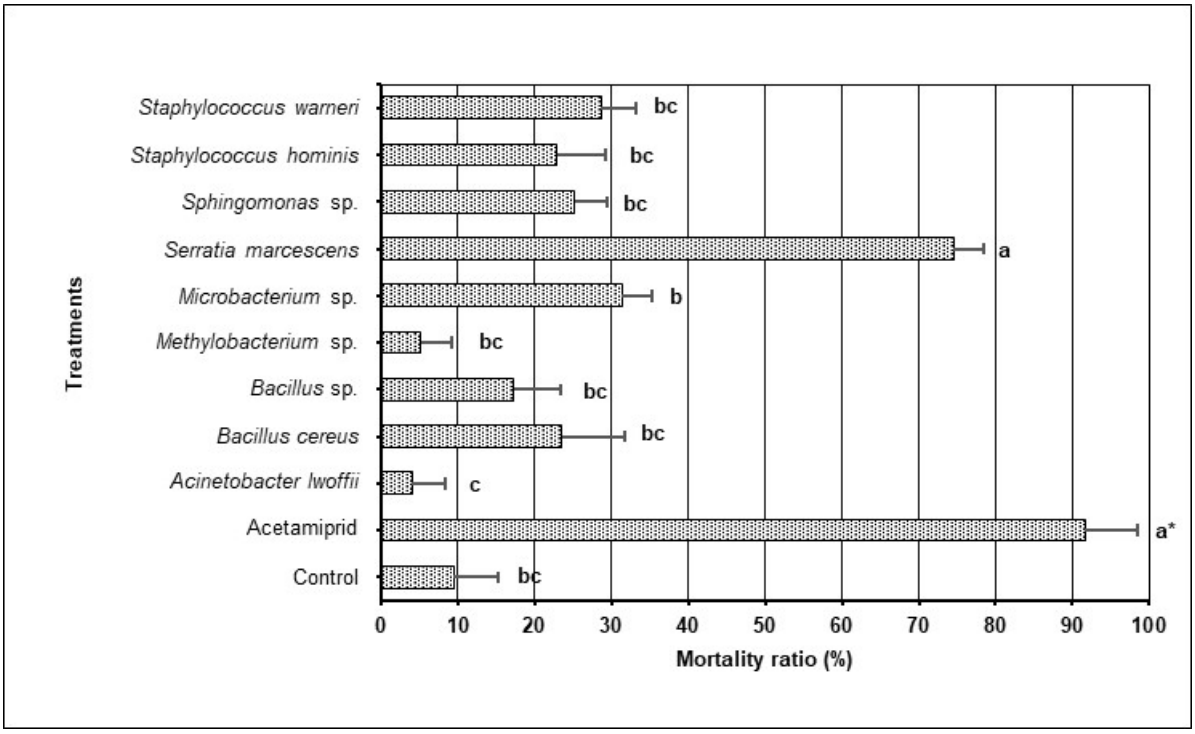

Figure 1. Mortality ratios of *Bemisia tabaci* adults with different bacterial strains treatments (%).
* The means with the same latter is not different significantly by Tukey's Multiple Range Test (P > 0.005).

Table 2. Henderson-Tilton % impact ratios of different bacterial strains applied against *Bemisia tabaci* adults

Treatments	N	Dead	Alive	Impact (%)
Control	138	13	125	
Acetamiprid	107	98	9	91
Acinetobacter lwoffii	100	4	96	0
Bacillus cereus	102	24	78	16
Bacillus sp.	84	17	67	9
Methylobacterium sp.	116	6	110	0
Microbacterium sp.	131	41	90	24
Serratia marcescens	110	82	28	72
Sphingomonas sp.	103	26	77	17
Staphylococcus hominis	109	25	84	15
Staphylococcus warneri	129	37	92	21

N, total number of individuals; control, insect food; acetamiprid, positive control.

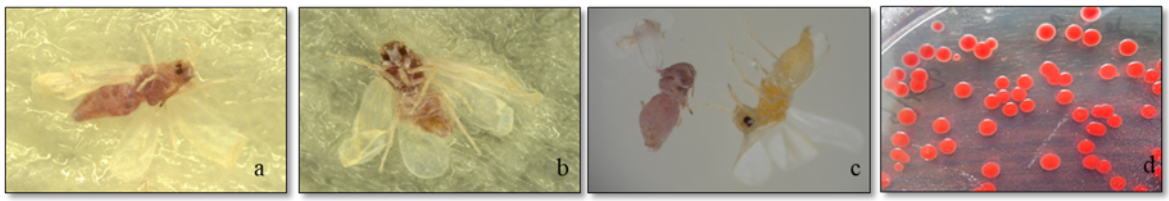

Figure 2. a, b) Adult *Bemisia tabaci* with color change after *Serratia marcescens* strain treatment, c) pathogen-infected and dead individuals in control, d) colony development of the pathogen in Nutrient agar medium.

Discussion

Nine bacterial species were obtained from *B. tabaci* collected from sesame and melon plants. The strains obtained from *B. tabaci* samples collected from sesame were *Bacillus* sp., *Methylobacterium* sp., *Microbacterium* sp., *S. marcescens* and *Sphingomonas* sp., and the strains from melon were *A. lwoffii*, *B. cereus*, *S. hominis* and *S. warneri*. In biological control of insects, bacteria are not as advantageous as fungi because bacteria are not able to penetrate into cuticle. Bacteria directly infect only through digestive system (Demirbag et al., 2008). Zhang et al. (2014) indicated two main approaches for bacterial infection of the insects, feeding and injection into hemocoel, and also indicated that feeding was preferred because it is more natural and does not generate any physical damages on the host. Therefore, the method recommended by Davidson et al. (2000) and Zhang et al. (2014) was used to determine biological efficiency of the bacteria and whiteflies were fed with bacterial suspensions for 48 h.

Two bacterial strains, *Bacillus* sp. and *B. cereus* were isolated from the whiteflies collected from melon. El-Assal et al. (2013) conducted an efficiency study of *B. cereus* against *B. tabaci*, they isolated the bacteria from the soil and applied it to second instar nymphs and reported 18% mortality 10 d after application. Although this mortality was achieved against nymphs, it is consistent with the 16% mortality achieved against adult with *B. cereus*.

Microbacterium species interact with insects. Of these bacteria, *Microbacterium testaceum* and *M. thalassium* were isolated from *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Pyralidae) larvae, *Microbacterium arborescens* from *Sesamia nonagrioides* (Lefèbvre, 1827) (Lepidoptera: Noctuidae) and *M. liquefaciens* were isolated from *Dendroctonus micans* (Kugelann, 1794) (Coleoptera: Curculionidae) (Yaman et al., 2010; Secil et al., 2012; Eski et al., 2015). In the present study, *Microbacterium* sp. was isolated from whiteflies collected from sesame, but the efficiency (24%) against *B. tabaci* was found to be low.

Bacteria of the genus *Serratia* are quite widespread in nature, but rare in humans and animals. The majority of those isolated from humans do not produce pigments, whereas those isolated from insects are generally pigmented (Sikorowski et al., 2001). *Serratia marcescens*, *S. entomophila*, *S. odorifera*, *S. ureilytica*, *S. grimesii* and *S. liquefaciens* are *Serratia* species isolated from the insects (Grimont et al., 1988; Sezen, 1998; Ince et al., 2008; Albayrak Iskender, 2009; Yaman et al., 2010; Sezen et al., 2013). *Serratia marcescens,* non-pathogenic at low-density, pass into hemocoel, multiply there and kill the insect in 1 to 3 d (Sikorowski et al., 2001). *Serratia marcescens* common in insects is commonly isolated from several pests of Lepidoptera and Coleoptera (Cakici et al., 2014; Eski et al., 2015; Pu & Hou, 2016; Bidari et al., 2018). However, there are no records on the isolation of this bacteria from the whiteflies. *Serratia marcescens* was isolated for the first time in this study from *B. tabaci* collected from sesame yielded significant efficiency (72%) against this host. Similarly, *S. marcescens* yielded efficiency of between 50 and 100% against agricultural pests like *Rhynchites bacchus* (L., 1758) (Coleoptera: Rhynchitidae), *Polyphylla olivieri* (Castelnau, 1840) (Coleoptera: Scarabaeidae), *Oberea linearis* (L., 1761). (Coleoptera: Cerambycidae), *Balaninus nucum* (L., 1758) (Coleoptera: Curculionidae), *Rhynchophorus ferrugineus* (Oliver, 1790) (Coleoptera: Curculionidae), *S. nonagrioides*, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) and *O. nubilalis* (Sezen & Demirbag, 1999; Bahar & Demirbag, 2007; Gokce et al., 2009; Secil et al., 2012; Pu & Hou, 2016).

Staphylococcus spp. are commonly isolated from various insect groups including whiteflies (Davidson et al., 2000; Yu et al., 2008; Ateyyat et al., 2010; Cakici et al., 2015; Pu & Hou, 2016). Ateyyat et al. (2009) tested *Staphylococcus gallinarum* isolated from *B. tabaci* against the second instar nymphs of the pest through the leaf dipping method and reported that bacteria generated 19.5 and 28% infection after 3 and 5 d, respectively. These researchers indicated that a toxin secreted by the bacteria might have acted as an antibiotic and this toxin resulted in death of the pest through destruction of the interactions between *B. tabaci* and its symbionts. Davidson et al. (2000) found *Staphylococcus aureus* and *S. epidermidis*

isolated from *B. argentifolii* ineffective against the pest. These researchers indicated that bacteria either never or rarely enter into the esophagus and that bacteria should have a diameter of 0.5 µm to pass through the stylet of a whitefly. In this study, *S. hominis* and *S. warneri* were isolated from *B. tabaci* from melon. *Staphylococcus hominis* and *S. warneri* isolates yielded efficiency of 15 and 21% against *B. tabaci*, respectively, which is similar to the results of Ateyyat et al. (2009).

In conclusion, in biological efficiency experiments conducted with the bacteria, only the *S. marcescens* strain provided an efficiency of greater than 70% in a short period (2 d) in feeding experiments, so this strain is considered to be a potential microbial control agent. Further research on the efficacy of entomopathogenic bacteria *S. marcescens* isolated from *B. tabaci* is needed as such research could make a great contribution to development of alternative methods to chemical control. Genetically-modified plants are now commonly used in pest control. Transgenic cotton, maize and potato plants producing *Bacillus thuringiensis* (Bt) toxins against pests are the best-known (Demirbag et al., 2008). While the plants producing Bt toxins are effective against the pests of Lepidoptera and Coleoptera insects with chewing mouthparts, they are not effective against the pests with piercing-sucking mouthparts. In the present study, *S. marcescens* yielded a high efficiency (72%) against *B. tabaci* adults. With the aid of biotechnological methods, as with Bt toxin-producing plants, transgenic plants able to produce *S. marcescens* toxins could be developed. In this way, with a new generation transgenic plants, a successful biotechnological control method, could be developed against piercing-sucking mouthparts pests as well as those with chewing mouthparts. In addition, testing the activity of the *S. marcescens* strain in chewing mouthparts insects could also provide important information that would be useful to improve biological control.

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Original article (Orijinal araştırma)

Fruit fly (Diptera: Tephritidae) fauna of Çorum and Sinop Provinces with two new records for Turkey[1]

Türkiye için iki yeni kayıt ile birlikte Çorum ve Sinop illerinin meyve sineği (Diptera: Tephritidae) faunası

Vedat GÖRMEZ[2*] **Murat KÜTÜK[2]**

Abstract

The fruit fly (Diptera: Tephritidae) fauna of Çorum and Sinop Provinces in Turkey were determined. Specimens were collected between 2015 and 2018 from possible host plants. The specimens were examined and diagnosed under stereo microscope after preparation of the collected materials in laboratory. Sixty-six species and 24 genera belonging to five subfamilies were determined for the fruit fly fauna of Çorum and Sinop Provinces. The genus *Acidia* Robineau-Desvoidy, 1830 as well as the species *Acidia cognata* (Wiedemann, 1817) and *Carpomya wiedemanni* (Meigen, 1826) are reported for the first time for the fruit fly fauna of Turkey. In addition to wing photographs and information on species determined from the research region, host plants, world distribution, body and female genitalia photographs of new records are also presented.

Keywords: Çorum, fauna, fruit flies, new record, Sinop, Turkey

Öz

Türkiye'de Çorum ve Sinop illerinin meyve sineği (Diptera: Tephritidae) faunası belirlenmiştir. Örnekler 2015 ve 2018 arasında muhtemel konukçu bitkilerden toplanmıştır. Toplanan örnekler laboratuvarda preparasyonları yapıldıktan sonra stereo mikroskopta incelendi ve teşhis edildi. Çorum ve Sinop İllerinin meyve sineği faunası için beş alt familyaya ait 24 cins ve altmışaltı tür tespit edilmiştir. *Acidia* Robineau-Desvoidy, 1830 cinsinin yanı sıra *Acidia cognata* (Wiedemann, 1817) ve *Carpomya wiedemanni* (Meigen, 1826) türleri Türkiye'den ilk defa bildirilmiştir. Araştırma bölgesinden tespit edilen tüm türlerin kanat fotoğrafları ve incelenen materyal bilgilerine ek olarak yeni kayıtların konukçu bitkileri, dünya yayılışları vücut ve dişi genital fotoğrafları da sunulmuştur.

Anahtar sözcükler: Çorum, fauna, meyve sinekleri, yeni kayıt, Sinop, Türkiye

[1] This study is part of first Author's PhD studies. Some parts of it were presented as abstract papers at Ecology 2017 (11-13 May 2017, Kayseri, Turkey), Eurasianbiochem 2018 (26-27 April 2018, Ankara, Turkey), Ecology 2018 (19-23 June 2018, Kastamonu, Turkey) and 24. UBK (10-14 September 2018, Manisa, Turkey) congresses.

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Introduction

Tephritidae is one of the most important family of the order Diptera. It includes about 492 genera and 4716 species (Pape et al., 2011). Many species of Tephritidae, especially the subfamily Tephritinae, develop on the flower heads of Asteraceae species (Freidberg & Kugler, 1989). Currently, there are about 1500 fruit fly species associated with fruits, more than 250 species of which are of economic significance (Li et al., 2013).

Koçak & Kemal (2013) reported 156 species of fruit flies from Turkey. After the report of Koçak & Kemal (2013), four new species (*Urophora turkeyensis* Yaran & Kütük, 2014, *Heringina arezoana* Namin & Korneyev, 2015, *Carpomya liat* (Freidberg, 2016) and *Terellia akguli* Yaran et al., 2018) and seven new records (*Dacus ciliatus* Loew, 1862, *Dioxyna sororcula* (Wiedemann, 1830), *Hemilea dimidiata* (Costa, 1844), *Ictericoides zelleri* (Loew, 1844), *Terellia ivannikovi* Korneyev et al., 2013, *Terellia armeniaca* Korneyev, 1985 and *Urophora trinervii* Korneyev & White, 1996) of fruit flies have been published in Turkey (Yaran & Kütük, 2014, 2015, 2016; Korneyev & Kolcsar, 2015; Namin & Korneyev, 2015; Yaran et al., 2018a, b; Çalışkan Keçe et al., 2019). Considering these references, 167 species of Tephritidae have now been reported in Turkey.

The main purpose of this study was to contribute to the fruit fly fauna of Turkey. In this paper, the genus *Acidia* Robineau-Desvoidy, 1830 as well as the species *Acidia cognata* (Wiedemann, 1817) and *Carpomya wiedemanni* (Meigen, 1826) are reported for the first time for the fruit fly fauna of Turkey.

Materials and Methods

A total of 1854 specimens of fruit flies (721 ♀♀ and 1133 ♂♂) were collected from Çorum and Sinop Provinces between 2015 and 2018. Adult fruit flies were obtained from possible host plants using a sweep net. After these specimens were killed in ethyl acetate killing jars, they were brought to the Gaziantep University Entomology Laboratory. Then, they were prepared as standard museum materials. Diagnosis of the fruit fly specimens followed Hendel (1927), White (1988), Freidberg & Kugler (1989), Korneyev & White (1993, 1999), Merz (1994), Korneyev (2003, 2006, 2013), Kütük (2003), Kütük & Yaran (2011), Korneyev et al. (2013, 2017) and Namin & Nowzari (2015).

Results

Sixty-six species and 24 genera belonging to five subfamilies (Aciurinae, Myopitinae, Tephritinae, Terellinae, Trypetinae) of Tephritidae were determinedy. The genus *Acidia* as well as the species *A. cognata* (Figure 2a-d) and *C. wiedemanni* (Figure 2e-g) were recorded for the first time for the fruit fly fauna in Turkey. Consequently, the number of fruit fly species in Turkey has increased to 169 species.

The identified species are reported in alphabetical order below.

***Acanthiophilus helianthi* (Rossi, 1794); Figure 1a**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 1♀; Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 3♀♀, 2♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 3♀♀, 1♂; Center, Çatak, 40°41' N, 34°50' E, 1185 m, 20.VIII.2017, 1♀; Center, Kadıkırı, 40°27' N, 34°51' E, 715 m, 27.VII.2015, 2♀♀, 2♂♂; Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 3♀♀; Dodurga, Dikenli, 40°49' N, 34°41' E, 1019 m, 2.VII.2015, 1♀, 2♂♂; İskilip, Başmakçı, 40°43' N, 34°35' E, 857 m, 27.VII.2017, 8♀♀; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 1♀, 2♂♂; Mecitözü, Beyözü, 40°34' N, 35°16' E, 985 m, 28.VII.2015, 1♀; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 7♀♀, 2♂♂; Sinop, Boyabat, Doğanburnu, 41°60' N, 34°86' E, 908 m, 28.VI.2016, 5♀♀, 2♂♂; Center, Ahmetyeri, 41°49' N, 35°02' E, 165 m, 1.VII.2015, 5♀♀, 4♂♂; Dikmen, Yenikent, 41°44' N, 35°13' E, 275 m, 1.VII.2015, 1♂; Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 2♀♀, 3♂♂; Gerze, Belören, 41°49' N, 35°09' E, 84 m, 1.VII.2015, 1♀, 1♂.

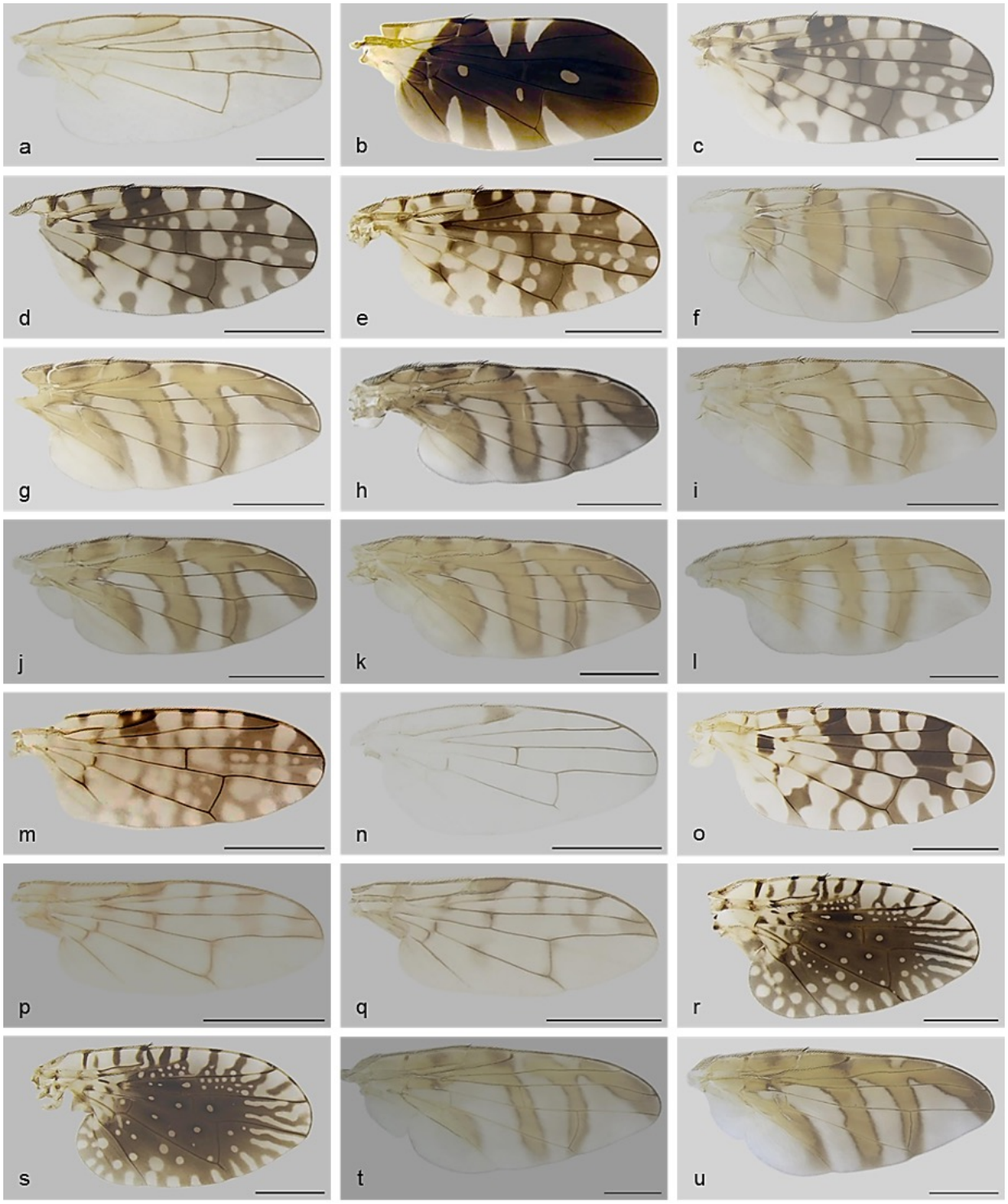

Figure 1. Wings of fruit fly species: a) *Acanthiophilus helianthi*, b) *Aciura coryli*, c) *Campiglossa absinthii*, d) *Campiglossa producta*, e) *Campiglossa tesellata*, f) *Carpomya schineri*, g) *Chaetorellia carthami*, h) *Chaetorellia conjuncta*, i) *Chaetorellia jaceae*, j) *Chaetorellia loricata*, k) *Chaetorellia succinae*, l) *Chaetostomella. cylindrica*, m) *Dioxyna bidentis*, n) *Ensina sonchi*, o) *Euaresta bullans*, p) *Myopites apicatus*, q) *Myopites cypriacus*, r) *Noeeta bisetosa*, s) *Noeeta crepidis*, t) *Orellia falcata*, u) *Orellia stictica*, (Scale: 1 mm).

***Acidia cognata* (Wiedemann, 1817); Figure 2a-d**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1321 m, 31.VII.2017, 1♀, 3♂♂; Bayat, Kunduzlu, 40°45' N, 34°14' E, 1360 m, 26.VII.2017, 3♀♀, 6♂♂; Bayat, Kunduzlu, 40°76' N, 34°25' E, 1346 m, 25.VII.2018, 2♀♀, 3♂♂; İskilip, Ahlatçık, 40°74' N, 34°28' E, 1482 m, 25.VII.2018, 2♀♀, 6♂♂; Sinop, Ayancık, Gökçukur, 41°39' N, 34°40' E, 866 m, 17.VIII.2017, 4♂♂; Ayancık, Gökdere, 41°39' N, 34°40' E, 801 m, 21.VI.2018, 1♀, 9♂♂.

Host plant: *Adenostyles glabra* (Miller)*, Homogyne alpina* Cass.*, Petasites albus* (L.)*, P. hybridus* (L.) and *Tussilago farfara* L. (Merz, 1994).

Distribution: Central Europe, Eastern Europe, Italy, Northern Europe, Ukraine and Yugoslavia (Merz, 1994).

This genus and species are new records for the fruit fly fauna of Turkey.

Figure 2. *Acidia cognata,* a) adult, b) wing, c) aculeus, and d) apex of aculeus (enlarged); and *Carpomya wiedemanni,* e) adult, f) thorax, and g) wing, (Scale: 1 mm).

***Aciura coryli* (Rossi, 1794); Figure 1b**

Material examined: Çorum, Alaca, Fakılar, 40°09' N, 34°50' E, 955 m, 23.VI.2018, 1♂.

***Campiglossa abshinthii* (Fabricius, 1805); Figure 1c**

Material examined: Çorum, Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 1♀; Sungurlu, Çadırhöyük, 40°17' N, 34°05' E, 614 m, 26.VII.2017, 1♂.

***Campiglossa producta* (Loew, 1844); Figure 1d**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1321 m, 31.VII.2017, 1♀; Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 1♀, 1♂; Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♂; Center, Konaklı, 40°38' N, 35°12' E, 963 m, 31.VII.2015, 1♂; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 1♀; Sungurlu, Çadırhöyük, 40°17' N, 34°05' E, 614 m, 26.VII.2017, 1♂; Sinop, Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 2♂♂.

***Campiglossa tesellata* (Loew, 1844); Figure 1e**

Material examined: Çorum, Boğazkale, Hattuşa, 40°01' N, 34°37' E, 1019 m, 16.VIII.2017, 1♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 1♂.

***Carpomya schineri* (Loew, 1856); Figure 1f**

Material examined: Çorum, Center, Konaklı, 40°38' N, 35°12' E, 963 m, 31.VII.2015, 1♀, 1♂.

***Carpomya wiedemanni* (Meigen, 1826); Figure 2e-g**

Material examined: Çorum, Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♂.

Host plant: *Bryonia cretica* L.*, B. dioica* Jacq.*, B. syriaca* Boiss. (Merz, 1994; Smith & Bush, 1999).

Distribution: Armenia, Austria, Belgium, Britain, European Russia; France, Germany, Hungary, Italy, Spain, Switzerland, Netherlands and Ukraine (Korneyev et al., 2017).

This species is new record for the fruit fly fauna of Turkey.

***Chaetorellia carthami* Stackelberg, 1929; Figure 1g**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♂; Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 4♀♀, 2♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 2♀♀, 1♂; Center, Büyükçayır, 40°34' N, 35°24' E, 713 m, 29.V.2016, 1♂; Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 2♂♂; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 1♀; Mecitözü, Beyözü, 40°34' N, 35°16' E, 985 m, 28.VII.2015, 1♀, 3♂♂; Ortaköy, Totali, 40°25' N, 35°23' E, 973 m, 1.VII.2017, 1♂; Sungurlu, Yörüklü, 40°18' N, 34°13' E, 652 m, 26.VII.2017, 2♀♀, 2♂♂.

***Chaetorellia conjuncta* (Becker, 1913); Figure 1h**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♀; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♀, 2♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 10♀♀, 13♂♂; İskilip, Elmalı, 40°46' N, 34°21' E, 1044 m, 26.VII.2017, 1♂; Laçin, Kavaklıçiftlik, 40°47' N, 34°53' E, 504 m, 2.VII.2015, 1♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 2♀♀, 3♂♂; Sungurlu, Turgutlu, 40°10' N, 34°09' E, 635 m, 13.VI.2017, 3♀♀, 12♂♂; Sinop, Boyabat, Gökçeağaçsakızı, 41°36' N, 34°35' E, 462 m, 14.VI.2017, 5♀♀, 6♂♂; Center, 42°01' N, 35°08' E, 20 m, 30.VII.2015, 1♀, 3♂♂; Durağan, Aşağıkaracaören, 41°24' N, 35°05' E, 200 m, 14.VI.2017, 9♀♀, 20♂♂; Erfelek, Gökçebel, 41°52' N, 34°46' E, 678 m, 30.VII.2015, 1♀, 2♂♂.

***Chaetorellia jaceae* (Robineau-Desvoidy, 1830); Figure 1i**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 1♀; Center, Çatak, 40°41' N, 34°50' E, 1185 m, 20.VIII.2017, 1♀, 1♂; Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 1♀, 2♂♂; Laçin, Obruk, 40°47' N, 34°48' E, 483 m, 2.VII.2015, 1♀, 1♂; Mecitözü, Beyözü, 40°34' N, 35°16' E, 985 m, 28.VII.2015, 3♂♂; Ortaköy, Totali, 40°25' N, 35°23' E, 973 m, 1.VII.2017, 1♀, 1♂; Osmancık, Ardıç, 41°03' N, 34°60' E, 398 m, 28.VI.2016, 2♀♀; Sungurlu, Yörüklü, 40°18' N, 34°15' E, 695 m, 13.VI.2017, 3♀♀, 5♂♂; Uğurludağ, Kumçeltiği, 40°34' N, 34°30' E, 784 m, 1.VII.2017, 1♂; Sinop, Boyabat, Kuzveren, 41°26' N, 34°49' E, 403 m, 1.VII.2015, 1♂; Center, Melekşah, 41°52' N, 35°03' E, 171 m, 31.V.2016, 2♂♂; Erfelek, İnesökü, 41°54' N, 34°54' E, 228 m, 31.V.2016, 1♀, 4♂♂.

***Chaetorellia loricata* (Rondani, 1870); Figure 1j**

Material examined: Çorum, Alaca, Karatepe, 40°01' N, 34°57' E, 1080 m, 27.VII.2015, 2♀♀, 3♂♂; Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 1♀; Mecitözü, İbek, 40°20' N, 35°14' E, 680 m, 28.VII.2015, 1♀, 2♂♂; Sinop, Durağan, Erduası, 41°22' N, 35°03' E, 667 m, 31.VII.2015, 1♀.

***Chaetorellia succinae* (Costa, 1844); Figure 1k**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 1♀; Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 3♀♀; Center, Kadıkırı, 40°27' N, 34°51' E, 715 m, 27.VII.2015, 5♀♀, 6♂♂; Laçin, Gökgözler, 40°48' N, 34°50' E, 460 m, 2.VII.2015, 1♀; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 1♀; Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 1♀; Sinop, Boyabat, Kuzveren, 41°26' N, 34°49' E, 403 m, 1.VII.2015, 3♀♀, 3♂♂; Center, Ahmetyeri, 41°49' N, 35°02' E, 165 m, 1.VII.2015, 3♀♀, 2♂♂; Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 1♂; Gerze, Belören, 41°49' N, 35°09' E, 84 m, 1.VII.2015, 1♀.

***Chaetostomella cylindrica* (Robineau-Desvoidy, 1830); Figure 1l**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♀, 9♂♂; Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 1♀, 4♂♂; Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♀, 1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 5♀♀, 7♂♂; Uğurludağ, Kumçeltiği, 40°34' N, 34°30' E, 784 m, 1.VII.2017, 1♀, 2♂♂.

***Dioxyna bidentis* (Robineau-Desvoidy, 1830); Figure 1m**

Material examined: Çorum, Boğazkale, Hattuşa, 40°10' N, 34°37' E, 1070 m, 25.VII.2017, 1♂.

***Ensina sonchi* (Linnaeus, 1767); Figure 1n**

Material examined: Çorum, Kargı, Asarcıkkazaklı, 41°11' N, 34°36' E, 1468 m, 17.VIII.2017, 1♀; Sungurlu, Çadırhöyük, 40°17' N, 34°05' E, 614 m, 26.VII.2017, 4♀♀, 8♂♂.

***Euaresta bullans* (Wiedemann, 1830); Figure 1o**

Material examined: Çorum, Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♂; Center, Altınbaş, 40°39' N, 34°49' E, 899 m, 1.VII.2017, 3♀♀, 2♂♂; Dodurga, Dikenli, 40°49' N, 34°41' E, 1019 m, 2.VII.2015, 1♂; İskilip, Başmakçı, 40°43' N, 34°35' E, 857 m, 27.VII.2017, 3♀♀, 2♂♂; Mecitözü, İbek, 40°20' N, 35°14' E, 680 m, 28.VII.2015, 2♀♀; Sungurlu, Yörüklü, 40°18' N, 34°15' E, 695 m, 13.VI.2017, 38♀♀, 27♂♂; Uğurludağ, Boztepe, 40°41' N, 34°28' E, 636 m, 13.VI.2017, 1♀; Sinop, Boyabat, Gökçeağaçsakızı, 41°36' N, 34°35' E, 462 m, 14.VI.2017, 6♀♀, 1♂♂; Durağan, Aşağıkaracaören, 41°24' N, 35°05' E, 200 m, 14.VI.2017, 6♀♀, 3♂♂.

***Myopites apicatus* Freidberg, 1980; Figure 1p**

Material examined: Sinop, Ayancık, Kovanlık, 41°51' N, 34°44' E, 681 m, 17.VIII.2017, 2♂♂; Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 1♂; Erfelek, Hasandere, 41°52' N, 34°56' E, 346 m, 17.VIII.2017, 4♀♀, 2♂♂.

***Myopites cypriacus* Hering, 1938; Figure 1q**

Material examined: Sinop, Ayancık, Kovanlık, 41°51' N, 34°44' E, 681 m, 17.VIII.2017, 1♀; Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 1♂; Erfelek, Hasandere, 41°52' N, 34°56' E, 346 m, 17.VIII.2017, 3♀♀, 5♂♂.

***Noeeta bisetosa* Merz, 1992; Figure 1r**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1321 m, 31.VII.2017, 4♀♀, 1♂; Sinop, Ayancık, Kovanlık, 41°51' N, 34°44' E, 681 m, 17.VIII.2017, 1♀; Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 1♂.

***Noeeta crepidis* Hering, 1936; Figure 1s**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°15' E, 1605 m, 31.VII.2017, 1♂.

***Orellia falcata* (Scopoli, 1763); Figure 1t**

Material examined: Çorum, Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 1♀, 1♂; Osmancık, Ardıç, 41°03' N, 34°60' E, 398 m, 28.VI.2016, 1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 1♀.

***Orellia stictica* (Gmelin, 1790); Figure 1u**

Material examined: Çorum, Dodurga, Center, 40°48' N, 34°50' E, 983 m, 2.VII.2015, 1♂; Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 2♀♀, 1♂; Sungurlu, Çavuş, 40°19' N, 34°48' E, 863 m, 27.VI.2016, 1♂; Sinop, Saraydüzü, Çalpınar, 41°18' N, 34°59' E, 1440 m, 28.VI.2016, 1♀.

***Oxyna flavipennis* (Loew, 1844); Figure 3a**

Material examined: Çorum, Mecitözü, Elvançelebi, 40°35' N, 35°09' E, 1025 m, 29.VI.2015, 3♂♂.

***Oxyna nebulosa* (Wiedemann, 1817); Figure 3b**

Material examined: Sinop, Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 31.V.2016, 1♀.

***Rhagoletis berberidis* Jermy, 1961; Figure 3c**

Material examined: Çorum, Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♀.

***Sphenella marginata* (Fallen, 1814); Figure 3d**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1360 m, 26.VII.2017, 1♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♂; Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♀, 1♂; İskilip, Başmakçı, 40°43' N, 34°35' E, 857 m, 27.VII.2017, 1♂; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 1♀; Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 1♀; Sinop, Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 3♀♀, 3♂♂.

***Tephritis bardanae* (Schrank, 1803); Figure 3e**

Material examined: Çorum, Dodurga, Yeniköy, 40°82' N, 34°69' E, 1011 m, 28.VI.2016, 2♀♀, 2♂♂.

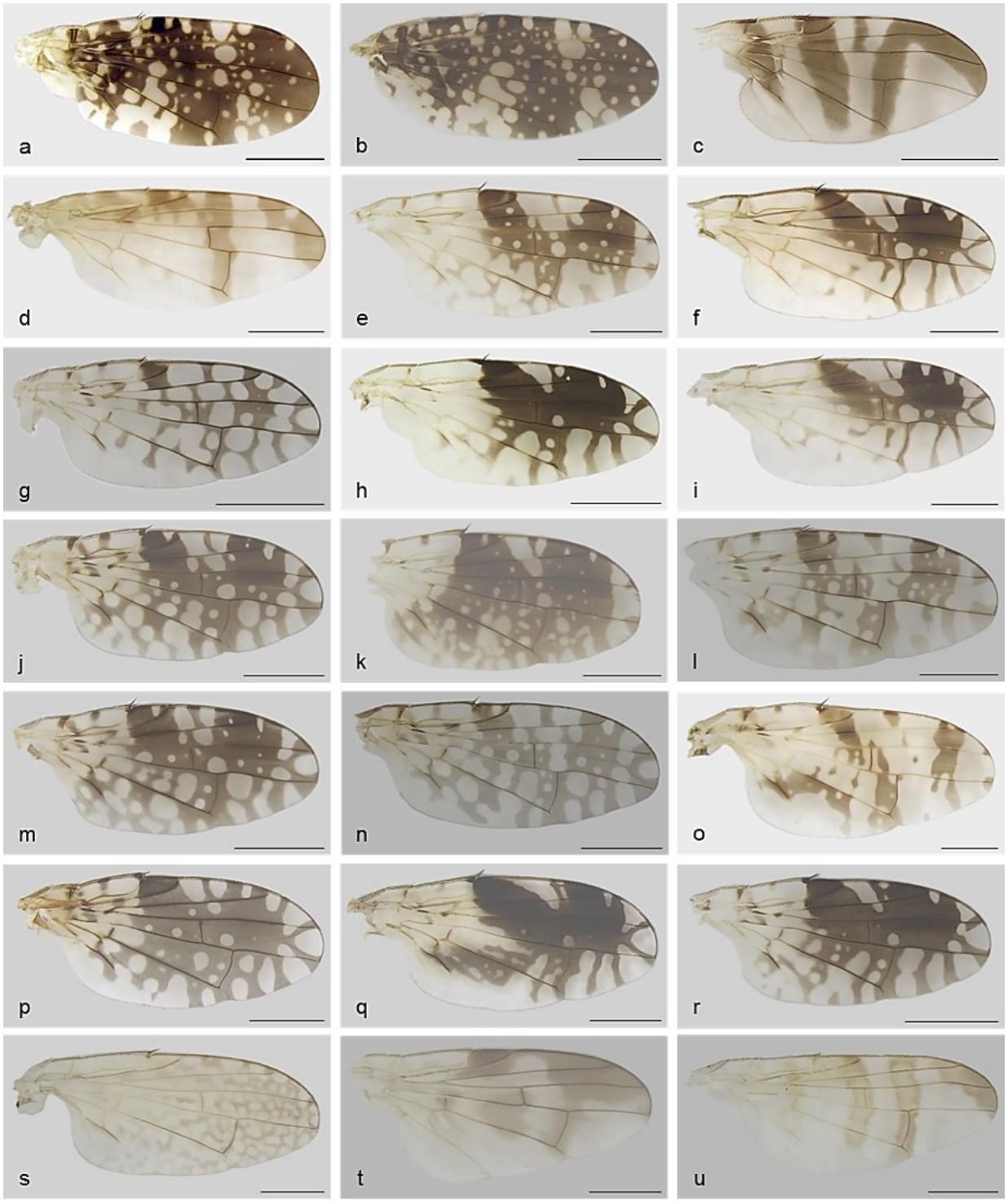

Figure 3. Wings of fruit fly species: a) *Oxyna flavipennis*, b) *Oxyna nebulosa*, c) *Rhagoletis berberidis*, d) *Sphenella marginata*, e) *Tephritis bardanae*, f) *Tephritis cometa*, g) *Tephritis dioscurea*, h) *Tephritis divisa*, i) *Tephritis erdemlii*, j) *Tephritis fallax*, k) *Tephritis formosa*, l) *Tephritis hyoscyami*, m) *Tephritis. matricariae*, n) *Tephritis nigricauda*, o) *Tephritis postica*, p) *Tephritis pulchra*, q) *Tephritis recurrens*, r) *Tephritis seperata*, s) *Tephritomyia lauta*, t) *Terellia colon*, u) *Terellia gynaecochroma*, (Scale: 1 mm).

***Tephritis cometa* (Loew, 1840); Figure 3f**

Material examined: Çorum, Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 1♀, 3♂♂; Dodurga, Center, 40°48' N, 34°50' E, 983 m, 2.VII.2015, 2♂♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 1♂; Kargı, Asarcıkkazıklı, 41°12' N, 34°37' E, 1458 m, 1.VII.2015, 1♀, 5♂♂; Mecitözü, Elvançelebi, 40°35' N, 35°09' E, 1025 m, 29.VI.2015, 1♂; Osmancık, Ovacıksuyu, 40°59' N, 34°40' E, 414 m, 1.VII.2015, 1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 3♀♀.

***Tephritis dioscurea* (Loew, 1856); Figure 3g**

Material examined: Çorum, Mecitözü, Elvançelebi, 40°35' N, 35°09' E, 1025 m, 29.VI.2015, 1♀, 2♂♂.

***Tephritis divisa* Rondani, 1871; Figure 3h**

Material examined: Çorum, Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♀, 2♂♂; Mecitözü, İbek, 40°22' N, 35°03' E, 870 m, 1.VII.2017, 1♂.

***Tephritis erdemlii* Kütük, 2008; Figure 3i**

Material examined: Çorum, Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 2♂♂; Center, Konak, 40°38' N, 35°11' E, 980 m, 28.VII.2017, 4♀♀, 2♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 1♂; Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 4♀♀, 1♂.

***Tephritis fallax* (Loew, 1844); Figure 3j**

Material examined: Çorum, Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 5♀♀, 3♂♂; Sinop, Durağan, Aşağıkaracaören, 41°24' N, 35°05' E, 200 m, 14.VI.2017, 1♀, 1♂.

***Tephritis formosa* (Loew, 1844); Figure 3k**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°15' E, 1605 m, 26.VII.2017, 1♂; Center, Konak, 40°38' N, 35°11' E, 980 m, 28.VII.2017, 1♂.

***Tephritis hyoscyami* (Linnaeus, 1758); Figure 3l**

Material examined: Çorum, Mecitözü, İbek, 40°22' N, 35°03' E, 870 m, 1.VII.2017, 1♀, 2♂♂.

***Tephritis matricariae* (Loew, 1844); Figure 3m**

Material examined: Çorum, Center, Kadıkırı, 40°27' N, 34°51' E, 715 m, 27.VII.2015, 1♂.

***Tephritis nigricauda* (Loew, 1856); Figure 3n**

Material examined: Sinop, Durağan, Çerçiler, 41°24' N, 35°12' E, 634 m, 31.V.2016, 1♀.

***Tephritis postica* (Loew, 1844); Figure 3o**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♀; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♀, 4♂♂; Çorum, Center, Altınbaş, 40°39' N, 34°49' E, 899 m, 1.VII.2017, 1♀; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 4♂♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 1♀, 3♂♂; Sinop, Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 2♀♀, 4♂♂.

***Tephritis pulchra* (Loew, 1844); Figure 3p**

Material examined: Çorum, Dodurga, Center, 40°48' N, 34°50' E, 983 m, 2.VII.2015, 1♂.

***Tephritis recurrens* Loew, 1869; Figure 3q**

Material examined: Çorum, Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 2♀♀, 2♂♂.

***Tephritis separata* Rondani, 1871; Figure 3r**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1360 m, 26.VII.2017, 1♂.

***Tephritomyia lauta* (Loew, 1869); Figure 3s**

Material examined: Çorum, Alaca, Karatepe, 40°01' N, 34°57' E, 1080 m, 27.VII.2015, 1♀; Boğazkale, Center, 40°00' N, 34°38' E, 1190 m, 25.VII.2017, 1♀, 1♂; Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♂; Laçin, Gökgözler, 40°48' N, 34°50' E, 460 m, 2.VII.2015, 1♀; Mecitözü, İbek, 40°20' N, 35°14' E, 680 m, 28.VII.2015, 1♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 1♀; Sinop, Boyabat, Gökçeağaçsakızı, 41°36' N, 34°35' E, 462 m, 14.VI.2017, 2♀♀; Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 3♀♀, 4♂♂.

***Terellia colon* (Meigen, 1826); Figure 3t**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♂.

***Terellia gynaecochroma* (Hering, 1937); Figure 3u**

Material examined: Çorum, Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 2♀♀; İskilip, Elmalı, 40°46' N, 34°21' E, 1044 m, 26.VII.2017, 15♀♀, 29♂♂; Sungurlu, Çavuş, 40°19' N, 34°48' E, 863 m, 27.VI.2016, 3♀♀, 5♂♂; Sinop, Dikmen, Center, 41°40' N, 35°17' E, 140 m, 1.VII.2015, 1♀.

***Terellia ivannikovi* Korneyev et al., 2013; Figure 4a**

Material examined: Çorum, Bayat, Karatepe, 40°74' N, 34°28' E, 1681 m, 25.VII.2018, 3♀♀, 3♂♂.

***Terellia luteola* (Wiedemann, 1830); Figure 4b**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 6♀♀, 5♂♂; Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 7♀♀, 3♂♂; Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 1♂; Dodurga, Dikenli, 40°49' N, 34°41' E, 1019 m, 2.VII.2015, 1♂; Laçin, Berk, 40°48' N, 34°50' E, 438 m, 2.VII.2015, 6♀♀, 11♂♂; Osmancık, Ovacıksuyu, 40°59' N, 34°40' E, 414 m, 1.VII.2015, 4♀♀, 4♂♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 21♀♀, 16♂♂; Sinop, Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 4♀♀, 2♂♂.

***Terellia nigripalpis* Hendel, 1927; Figure 4c**

Material examined: Sinop, Center, Ada, 42°02' N, 35°10' E, 81 m, 1.VII.2015, 2♀♀, 2♂♂.

***Terellia ruficauda* (Fabricius, 1794); Figure 4d**

Material examined: Çorum, Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 2♀♀; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 3♀♀, 3♂♂; Center, Sıklık, 40°34' N, 35°00' E, 960 m, 29.VI.2015, 2♀♀, 2♂♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 37♀♀, 43♂♂; Sinop, Boyabat, Doğanburnu, 41°60' N, 34°86' E, 908 m, 28.VI.2016, 3♀♀, 2♂♂.

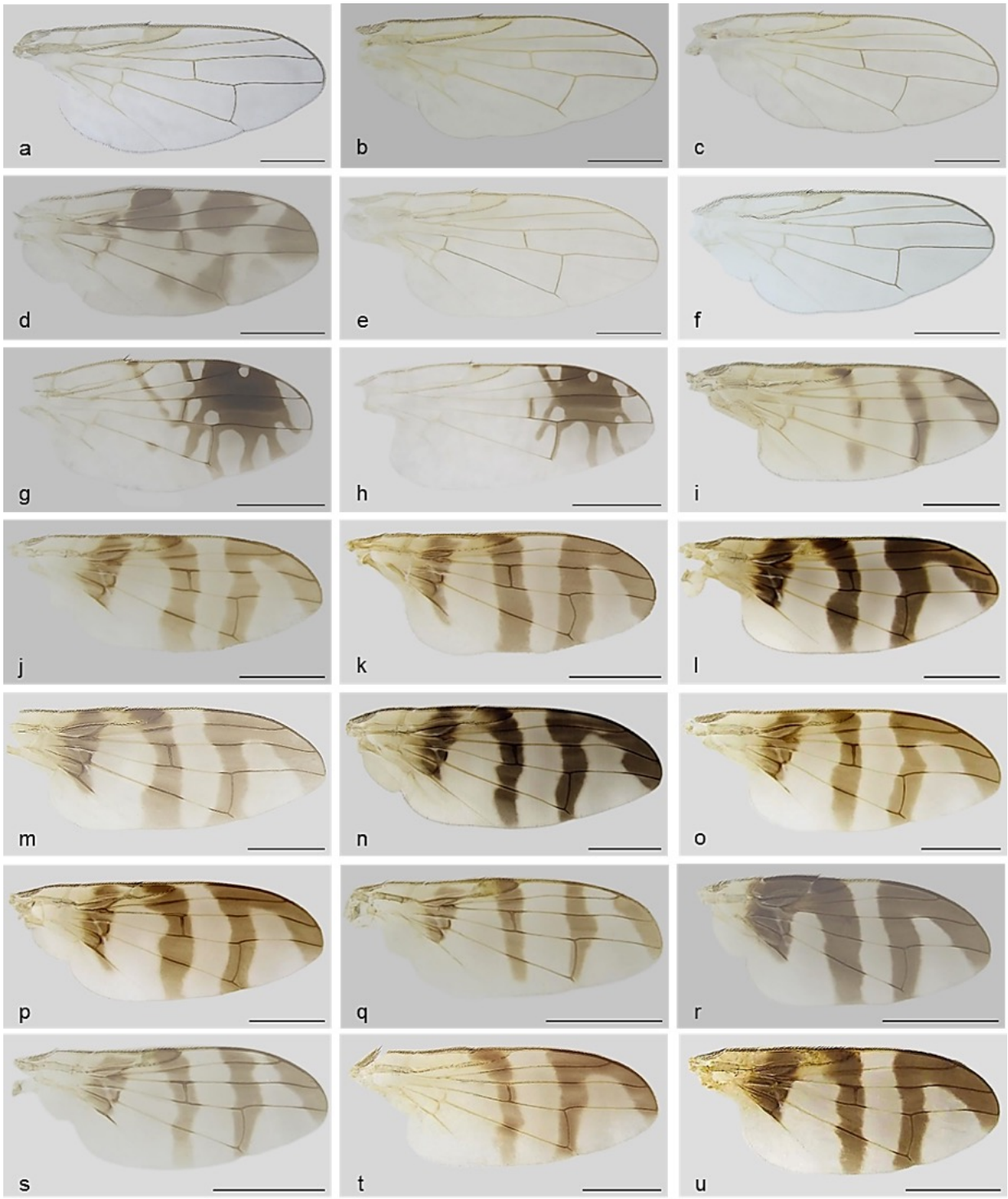

Figure 4. Wings of fruit fly species: a) *Terellia ivannikovi*, b) *Terellia luteola*, c) *Terellia nigripalpis*, d) *Terellia ruficauda*, e) *Terellia serratulae*, f) *Terellia virens*, g) *Trupanea amoena*, h) *Trupanea stellata*, i) *Urophora affinis*, j) *Urophora aprica*, k) *Urophora congrua*, l) *Urophora cuspidata*, m) *Urophora eriolepidis*, n) *Urophora jaceana*, o) *Urophora macrura*, p) *Urophora mauritanica*, q) *Urophora phalolepidis*, r) *Urophora quadrifasciata*, s) *Urophora solstitialis*, t) *Urophora stylata*, u) *Urophora terebrans*, (Scale: 1 mm).

***Terellia serratulae* (Linnaeus, 1758); Figure 4e**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 6♀♀, 7♂♂; Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 4♀♀, 12♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 34♀♀, 45♂♂; Center, Çatak, 40°41' N, 34°50' E, 1185 m, 20.VIII.2017, 25♀♀, 33♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 3♀♀, 9♂♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 2♀♀, 1♂; Kargı, Asarcıkkazıklı, 41°11' N, 34°36' E, 1468 m, 17.VIII.2017, 7♀♀, 5♂♂; Laçin, Kırkdilim, 40°43' N, 34°53' E, 1050 m, 1.VII.2015, 2♀♀, 6♂♂; Mecitözü, İbek, 40°20' N, 35°14' E, 680 m, 28.VII.2015, 9♀♀, 13♂♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 2♀♀, 4♂♂; Sinop, Ayancık, Gökçukur, 41°39' N, 34°40' E, 866 m, 17.VIII.2017, 2♀♀, 2♂♂; Boyabat, Doğanburnu, 41°60' N, 34°86' E, 908 m, 28.VI.2016, 6♀♀, 4♂♂; Dikmen, Yenikent, 41°44' N, 35°13' E, 275 m, 1.VII.2015, 7♀♀, 12♂♂; Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 44♀♀, 67♂♂; Erfelek, Gökçebel, 41°52' N, 34°46' E, 678 m, 30.VII.2015, 1♀, 4♂♂; Saraydüzü, Bahçeköy, 41°20' N, 34°48' E, 492 m, 17.VIII.2017, 1♀, 2♂♂.

***Terellia virens* (Loew, 1846); Figure 4f**

Material examined: Çorum, Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 7♀♀, 10♂♂; Center, Çatak, 40°41' N, 34°50' E, 1185 m, 20.VIII.2017, 2♀♀, 3♂♂; Kargı, Asarcıkkazıklı, 41°11' N, 34°36' E, 1468 m, 17.VIII.2017, 2♀♀, 1♂; Sinop, Ayancık, Gökçukur, 41°39' N, 34°40' E, 866 m, 17.VIII.2017, 1♂; Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 8♀♀, 11♂♂.

***Trupanea amoena* (Frauenfeld, 1857); Figure 4g**

Material examined: Çorum, Center, İnalözü, 40°28' N, 34°34' E, 972 m, 31.VII.2015, 1♀, 1♂; Sungurlu, Kula, 40°28' N, 34°08' E, 526 m, 26.VII.2017, 1♂; Sinop, Center, Ahmetyeri, 41°49' N, 35°02' E, 165 m, 1.VII.2015, 1♂.

***Trupanea stellata* (Fuesslin, 1775); Figure 4h**

Material examined: Çorum, Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 2♀♀; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 1♀.

***Urophora affinis* (Frauenfeld, 1857); Figure 4i**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 2♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♀; Center, Kadıkırı, 40°27' N, 34°51' E, 715 m, 27.VII.2015, 1♀; Dodurga, Dikenli, 40°49' N, 34°41' E, 1019 m, 2.VII.2015, 3♂♂; Laçin, Berk, 40°48' N, 34°50' E, 438 m, 2.VII.2015, 1♀; Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 3♀♀, 2♂♂; Ortaköy, Totali, 40°25' N, 35°23' E, 973 m, 1.VII.2017, 1♀, 1♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 2♀♀, 4♂♂; Sungurlu, Turgutlu, 40°10' N, 34°09' E, 635 m, 13.VI.2017, 18♀♀, 44♂♂; Uğurludağ, Boztepe, 40°41' N, 34°28' E, 636 m, 13.VI.2017, 5♂♂; Sinop, Boyabat, Gökçeağaçsakızı, 41°36' N, 34°35' E, 462 m, 14.VI.2017, 4♀♀, 8♂♂; Center, Melekşah, 41°52' N, 35°03' E, 171 m, 31.V.2016, 3♀♀, 2♂♂; Dikmen, Hacıselli, 41°41' N, 35°17' E, 331 m, 1.VII.2015, 4♂♂; Durağan, Aşağıkaracaören, 41°24' N, 35°05' E, 200 m, 14.VI.2017, 2♀♀, 3♂♂; Erfelek, İnesökü, 41°54' N, 34°54' E, 228 m, 31.V.2016, 3♂♂; Erfelek, Veysel, 41°54' N, 34°57' E, 83 m, 1.VII.2015, 1♀; Gerze, Belören, 41°49' N, 35°09' E, 84 m, 1.VII.2015, 2♂♂.

***Urophora aprica* (Fallen, 1814); Figure 4j**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 2♀♀, 4♂♂; Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 1♂; Boğazkale, Yazılıkaya, 40°01' N, 34°36' E, 1010 m, 2.VII.2017, 5♀♀, 7♂♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 3♂♂; Laçin, Çamlıca, 40°43' N, 34°55' E, 470 m, 1.VI.2016, 1♀; Mecitözü, İbek, 40°22' N, 35°03' E, 870 m, 1.VII.2017,

1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 3♀♀, 3♂♂; Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 5♀♀, 29♂♂.

***Urophora congrua* Loew, 1862; Figure 4k**

Material examined: Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 3♀♀.

***Urophora cuspidata* (Meigen, 1826); Figure 4l**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 2♀♀, 2♂♂; Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 2♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 2♀♀, 4♂♂; Center, Büyükçayır, 40°34' N, 35°24' E, 713 m, 29.V.2016, 4♀♀, 7♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 1♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 1♀, 3♂♂; Laçin, Çamlıca, 40°43' N, 34°55' E, 470 m, 1.VI.2016, 4♀♀, 3♂♂; Mecitözü, Beyözü, 40°34' N, 35°16' E, 985 m, 28.VII.2015, 5♀♀, 4♂♂; Ortaköy, Totali, 40°25' N, 35°23' E, 973 m, 1.VII.2017, 1♀, 2♂♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 2♀♀, 1♂; Sinop, Boyabat, Koçak, 41°36' N, 34°38' E, 350 m, 16.VI.2017, 1♀, 5♂♂; Dikmen, Center, 41°40' N, 35°17' E, 140 m, 1.VII.2015, 1♂; Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 14♀♀, 23♂♂; Gerze, Belören, 41°49' N, 35°09' E, 84 m, 1.VII.2015, 1♀, 5♂♂.

***Urophora eriolepidis* (Loew, 1856); Figure 4m**

Material examined: Çorum, Sungurlu, Şekerhacılı, 40°09' N, 34°34' E, 1430 m, 28.VI.2017, 1♂.

***Urophora jaceana* (Hering, 1935); Figure 4n**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♀, 2♂♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 4♀♀, 8♂♂; Center, Büyükçayır, 40°34' N, 35°24' E, 713 m, 29.V.2016, 3♀♀, 2♂♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 1♀; Laçin, Çamlıca, 40°43' N, 34°55' E, 470 m, 1.VI.2016, 4♂♂; Mecitözü, Center, 40°31' N, 35°17' E, 810 m, 29.VI.2015, 1♀, 1♂; Osmancık, Öbektaşı, 41°03' N, 34°59' E, 1154 m, 15.VI.2017, 1♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 1♀, 3♂♂; Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 1♀, 1♂.

***Urophora macrura* (Loew, 1855); Figure 4o**

Material examined: Sinop, Center, 42°01' N, 35°08' E, 20 m, 30.VII.2015, 1♂.

***Urophora mauritanica* Macquart, 1851; Figure 4p**

Material examined: Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 1♂.

***Urophora phalolepidis* Merz & White, 1991; Figure 4q**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 1♀; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 5♂♂; Center, Büyükçayır, 40°34' N, 35°24' E, 713 m, 29.V.2016, 2♂♂; Mecitözü, Koloğlu, 40°34' N, 35°26' E, 625 m, 2.V.2016, 2♂♂; Osmancık, Çayırköy, 40°59' N, 37°57' E, 540 m, 15.VI.2017, 2♂♂; Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 1♀, 1♂.

***Urophora quadrifasciata* (Meigen, 1826); Figure 4r**

Material examined: Çorum, Alaca, İsahacı, 40°09' N, 34°55' E, 965 m, 27.VII.2015, 1♀; Center, Seydim, 40°32' N, 34°44' E, 1216 m, 31.VII.2015, 1♂; Laçin, Kavaklıçiftlik, 40°47' N, 34°53' E, 504 m, 2.VII.2015, 1♂; Mecitözü, Emirbağı, 40°24' N, 35°13' E, 640 m, 28.VII.2015, 4♀♀, 5♂♂; Sungurlu, Kamışlı, 40°07' N, 34°28' E, 1000 m, 2.VII.2017, 1♂; Sinop, Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 2♂♂.

***Urophora solstitialis* (Linnaeus, 1758); Figure 4s**

Material examined: Çorum, Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 1♀, 10♂♂; Center, Büyükçayır, 40°34' N, 35°24' E, 713 m, 29.V.2016, 4♂♂; Uğurludağ, Boztepe, 40°41' N, 34°28' E, 636 m, 13.VI.2017, 1♂; Sinop, Boyabat, Koçak, 41°36' N, 34°38' E, 350 m, 16.VI.2017, 2♂♂; Center, Melekşah, 41°52' N, 35°03' E, 171 m, 31.V.2016, 1♂; Dikmen, Yenikent, 41°44' N, 35°13' E, 275 m, 1.VII.2015, 1♂; Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 1♀, 1♂.

***Urophora stylata* (Fabricius, 1775); Figure 4t**

Material examined: Çorum, Alaca, Çevreli, 40°13' N, 34°45' E, 1190 m, 28.VI.2017, 2♀♀; Boğazkale, Hattuşa, 40°01' N, 34°37' E, 1019 m, 16.VIII.2017, 7♂♂; Center, Konak, 40°38' N, 35°11' E, 980 m, 28.VII.2017, 4♀♀, 10♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 5♂♂; İskilip, Ahlatçık, 40°46' N, 34°18' E, 1374 m, 31.VII.2017, 2♀♀, 1♂; Kargı, Asarcıkkazıklı, 41°12' N, 34°37' E, 1458 m, 1.VII.2015, 3♂♂; Laçin, Çamlıca, 40°43' N, 34°55' E, 470 m, 1.VI.2016, 1♂; Mecitözü, Koloğlu, 40°34' N, 35°26' E, 625 m, 2.V.2016, 2♀♀, 8♂♂; Osmancık, Öbektaşı, 41°03' N, 34°59' E, 1154 m, 15.VI.2017, 1♀, 4♂♂; Sungurlu, Kula, 40°28' N, 34°08' E, 526 m, 26.VII.2017, 1♀, 4♂♂; Sinop, Boyabat, Kuzveren, 41°26' N, 34°49' E, 403 m, 1.VII.2015, 2♂♂; Center, Melekşah, 41°52' N, 35°03' E, 171 m, 31.V.2016, 1♀, 1♂; Dikmen, Yenikent, 41°44' N, 35°13' E, 275 m, 1.VII.2015, 1♀; Durağan, Aşağıkaracaören, 41°24' N, 35°05' E, 200 m, 14.VI.2017, 1♀; Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 2♀♀, 13♂♂.

***Urophora terebrans* (Loew, 1850); Figure 4u**

Material examined: Çorum, Bayat, Çerkeş, 40°45' N, 34°15' E, 1671 m, 26.VII.2017, 1♂; Boğazkale, Yüksekyayla, 40°00' N, 34°38' E, 1193 m, 13.VI.2017, 3♂♂; Mecitözü, İbek, 40°20' N, 35°14' E, 680 m, 28.VII.2015, 1♂; Sungurlu, Yörüklü, 40°18' N, 34°15' E, 695 m, 13.VI.2017, 1♀; Sinop, Center, Demirciköy, 41°55' N, 35°04' E, 12 m, 1.VII.2015, 1♀, 4♂♂; Durağan, Center, 41°23' N, 35°16' E, 1140 m, 28.VII.2017, 2♀♀, 1♂.

***Xyphosia miliaria* (Schrank, 1781); Figure 5**

Material examined: Çorum, Bayat, Kunduzlu, 40°45' N, 34°14' E, 1360 m, 26.VII.2017, 2♀♀, 9♂♂; Boğazkale, Hattuşa, 40°10' N, 34°37' E, 1070 m, 25.VII.2017, 8♂♂; Center, Konak, 40°38' N, 35°11' E, 980 m, 28.VII.2017, 2♀♀, 13♂♂; Dodurga, Kirenci, 40°49' N, 34°43' E, 968 m, 27.VII.2017, 1♂; İskilip, Elmalı, 40°46' N, 34°21' E, 1044 m, 26.VII.2017, 1♂; Kargı, Asarcıkkazıklı, 41°11' N, 34°36' E, 1468 m, 17.VIII.2017, 7♂♂; Sungurlu, Kula, 40°28' N, 34°08' E, 526 m, 26.VII.2017, 1♀, 1♂; Sinop, Ayancık, Gökçukur, 41°39' N, 34°40' E, 866 m, 17.VIII.2017, 2♂♂; Durağan, Dağdelen, 41°26' N, 34°55' E, 247 m, 31.V.2016, 1♂; Erfelek, Hasandere, 41°52' N, 34°56' E, 305 m, 30.VII.2015, 1♂.

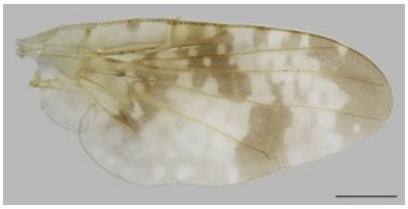

Figure 5. Wing of *Xyphosia miliaria*, (Scale: 1 mm).

Discussion

Fruit flies, Tephritidae, are one of the most important families of the Diptera including 4716 species (Pape et al., 2011). Some species of fruit flies damage fruits and have economic importance. In this study, we determined the fruit fly fauna of Çorum and Sinop Provinces based on specimens collected between 2015 and 2018. As a result of the study, 66 species belonging to 24 genera from five subfamilies of

Tephritidae were determined. The genus *Acidia* as well as the species *A. cognata* and *C. wiedemanni* were determined as new records for the fauna of Turkey. Consequently, the number of fruit flies in Turkey has increased to 38 genera and 169 species.

Çorum and Sinop are adjacent provinces located between the Black Sea Region and Central Anatolia Region of Turkey. Fifty-nine fruit fly species were identified from Çorum Province and 39 from Sinop Province. Çorum Province is richer in species diversity than Sinop Province, possibly because Çorum is under the influence of the Black Sea climate in north and the Central Anatolia climate in south, while Sinop is only under the influence of the Black Sea climate.

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Original article (Orijinal araştırma)

Toxicities of different essential oils to *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) and *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) adults

Farklı uçucu yağların *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) ve *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) erginlerine zehir etkileri

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Abstract

In this study, the toxicities of plant essential oils of *Artemisia absinthium* L.*, Seriphidium santonicum* (L.) Soják, *Seriphidium spicigerum* (K. Koch) Poljakov and *Achillea santolinoides* Lag. (Asteraceae) to two spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) and bean weevil, *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) adults at 10, 15 and 20 µL/Petri dish were investigated. Tests (contact effect for *T. urticae* and fumigant effect for *A. obtectus*) were conducted at 26±1°C, 70±5% RH and 16:8 h L:D photoperiod. Mortality varied depending on plant essential oil. While the mortalities were recorded between 23 and 100% for *T. urticae*, they were between 45 and 100% for *A. obtectus.* After 72 h, when LD_{50} and LC_{50} values are taken into consideration, the most toxic plant essential oil was *A. santolinoides* (7.8 µL/mite for LD_{50}) oil for *T. urticae*. The most toxic plant essential oil after 72 h was *A. santolinoides* (0.001 µL/insect) oil on *A. obtectus* adults. These results showed that the plant essential oils derived from *A. absinthium, S. santonicum*, *S. spicigerum* and *A. santolinoides* may be among one of the most promising alternative methods to control *T. urticae* and *A. obtectus* adults.

Keywords: *Acanthoscelides obtectus,* essential oil, *Tetranychus urticae*, toxicity

Öz

Bu çalışmada, *Artemisia absinthium* L.*, Seriphidium santonicum* (L.) Soják*, Seriphidium spicigerum* (K.Koch) Poljakov ve *Achillea santolinoides* Lag (Asteraceae) bitki uçucu yağlarının 10, 15 ve 20 µL/Petri dozlarında, ikinoktalı kırmızıörümcek, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) ve fasulye tohum böceği, *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) erginleri üzerindeki zehir etkileri araştırılmıştır. Testler (*T. urticae* için kontakt etki ve *A. obtectus* için fumigant etki) 26±1°C, %70±5 RH ve 16:8 h (A:K ışık ortamında) laboratuvar şartlarında yürütülmüştür. Ölüm oranları, uçucu yağlara bağlı olarak farklılık göstermiştir. Bu ölümler *T. urticae* erginleri için %23 ile %100 arasında kaydedilirken, *A. obtectus* erginleri için ise %45 ile %100 arasında bulunmuştur. Uygulamanın 72 saatinden sonra, LD_{50} ve LC_{50} değerleri dikkate alındığında, *T. urticae* için en zehir etkili uçucu yağ olarak *A. santolinoides* (7.8 µL/akar LD_{50}) yağı tespit edilmiştir. Aynı süre içinde, *A. obtectus* üzerinde en fazla zehir etkisi ise *A. santolinoides* (0.001 µL/böcek) yağı için kaydedilmiştir. Bu sonuçlar, *A. absinthium, S. santonicum*, *S. spicigerum* ve *A. santolinoides* bitkilerinden elde edilen uçucu yağların, *T. urticae* ve *A. obtectus* erginlerini kontrol etmek için en umut verici alternatif metotlardan birisi arasında olabileceğini göstermektedir.

Anahtar sözcükler: *Acanthoscelides obtectus,* uçucu yağ, *Tetranychus urticae*, toksisite

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Introduction

Two spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), is an important pest on different crops such as bean, cucumber, eggplant, pepper, strawberry, apple, cotton, jute, tea and various ornamental plants all over the world. It is a polyphagous pest and may cause serious damage to host plants by feeding with leaves and fruits (Jeppson et al., 1975; Zhang, 2003). It can reduce functional leaf surface and cause yellowing, crinkling and twisting of leaves by inserting its stylet into host leaves and absorbing the cell contents. Due to its serious damage, host plant leaves turn brown and die (Jeppson et al., 1975). Also, bean weevil, *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae), is among the most threatening bruchid species of stored legumes seeds, leading to qualitative and quantitative losses, especially in the tropical and subtropical regions. The adults are not harmful. Its larvae feed in *Phaseolus vulgaris* L. (Fabaceae) seeds and lead to serious economic damage in conditions of low humidity. This pest can cause losses of up to 40% in stored legume grains and reduce their nutritional and commercial values (Cardona, 1989; Regnault-Roger & Hamraoui, 1995; Bailey, 2007). The larvae cause damage by continuous cycle feeding on both in storage and in legume seeds in the field (Labeyrie, 1962).

Insects and mites cause important economic losses in storage and in the field. So, there is a need for new insecticides and acaricides for use in both crops and stored products. The general alternatives to control this type of pests are chemical, biological, and physical control or their combinations. Many acaricides and insecticides have been used to prevent damage caused by these pests. However, these chemicals pose risks to environmental pollution, toxicities to other beneficial organisms, pest resistances, pesticide residues, directly toxicities to users and ozone depletion (Barnard et al., 1997; Nauen et al., 2001; Santos et al., 2009; Kumral et al., 2010). Therefore, health authorities are unwilling to allow synthetic chemicals due to their residues in food (Thaung & Collins, 1986). In addition, the new alternative control strategies are needed, because synthetic chemicals have a high toxic effect both on the environment and human health. These disadvantages of the synthetic chemicals have motivated a search for the alternative of the pesticides. For these reasons, studies in recent years have demonstrated that the natural products, such as plant extracts, have minimal effects to environment and the health of warm blooded organism compared to synthetic chemicals, and are preferred by growers to protect their crops from pest incursions (Arnason et al., 1989; Tapondjou et al., 2005). Among these plants derived compounds, plant essential oils are extracted from various parts such as flower, fruit, seed, leaf, stem, trunk, shoot and tuber of plants and known as friendly botanical pesticides for the safety of mammals and the environment. Given that they degrade in nature rapidly, they have almost no toxicity to warm blooded organisms when applied. Also, they are important for managing pest resistance (Daferera et al., 2000; Isman, 2000; Göktürk et al., 2017). However, most importantly are their effects, such as insecticidal, acaricidal, antifeedant and repellant, on many pests in the agricultural fields and stored products (Yıldırım et al., 2013).

Turkey has a high potential in terms of many aromatic and medicinal plant species as most of them are considered to be endemic (Davis, 1982; Baytop, 1999). Among them, genus *Artemisia* L. and *Achillea* L. are two valuable groups of the Asteraceae. This family has approximately 20,000 species belonging to 1,000 genera. The genus *Artemisia* has about 500 species in Asia, Europe and North America. Among them, *Artemisia absinthium* L., *Seriphidium santonicum* (L.) Sojak and *Seriphidium spicigerum* (K. Koch) Poljakov grow naturally in the different regions of Turkey. *Artemisia* species are known in Anatolia as *acı pelin*, *ak pelin*, *yavşan*, *deniz yavşanı* and *acı yavşan*. Most of them are vitally important because of their insecticidal, antifungal, antibacterial, allelopathic and other properties. The members of the genus contain high levels of plant essential oils and bitter substances that include flavone and pinene (Regnault-Roger & Hamraoui, 1995; Usanmaz Bozhüyük et al., 2016). The genus *Achillea* has almost 85 species, 42 species (20 species endemic) of them are found in Turkey (Davis, 1982; Baytop, 1999). *Achillea* species are called *civanperçemi*, *pireotu*, *yılan çiçeği*, *serviotu*, *kardeş kınası*, *ayvadene*, *kardeşkanı*, *kılıç otu*, *paspanos*, *pasvana* and *pesvana* in Anatolia. *Achillea santolinoides* (K. Koch) Lag. is grown naturally in almost all regions of Turkey, and has long been used for its therapeutically and aromatic (Davis, 1982; Kesdek et al., 2014).

Many scientific studies have been conducted on the toxicity of plant essential oils and their use as eco-friendly pesticides, and the vast majority of them have been accomplished (Aslan et al., 2004; Yıldırım et al., 2005; Tripathi et al., 2009; Ebadollahi et al., 2010; Regnault-Roger et al., 2012; Benelli et al., 2017; Üstüner et al., 2018). Some other notable studies were conducted with plant essential oils against mite and insect pests. Abdelgaleil & Badawy (2006) reported that the plant essential oil of *Mentha spicata* subsp. *condensata* (Briq.) Greuter & Burdet (syn. *Mentha microphylla* Koch) (Lamiaceae) gave from 56 to 100% mortality of *T. urticae* in a fumigation bioassay. Kumral et al. (2010) determined that seed and leaf extracts of the thorn apple (*Datura stramonium* L.) had acaricidal, repellent and oviposition deterrent activities on *T. urticae*. Fatemikia et al. (2014) found that *Elettaria cardamomum* L. (Zingiberaceae) plant essential oils had toxic effects on adults and eggs of *T. urticae*. Usanmaz et al. (2016) determined that the plant essential oils of *Seriphidium santonicum* (L.) Soják (syn. *Artemisia santonicum* L.), and *Seriphidium spicigerum* (K. Koch) Poljakov (syn. *Artemisia spicigera* K. Koch) caused high mortalities (85-100%) on the adults of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Bruchidae). Fatemikia et al. (2017) found that *Ferula gummosa* Boiss. (Asteraceae) plant essential oils had high toxicity to eggs and adults of *T. urticae*. Also, they found that all tested concentrations of *F. gummosa* plant essential oils had oviposition activity and repellency of adults of *T. urticae*.

The genus *Artemisia* and *Achillea* species are common and abundant in natural areas of Turkey. *T. urticae* and *A. obtectus* are two important pests. While *T. urticae* causes significant damage to the different cultivated plants in the field, *A. obtectus* causes economic loses in the stored legume seeds. This study was planned to investigate which the tested plant essential oils are more effective on adults of the two pests. Therefore, the aim of the study was to determine acaricidal and insecticidal effects of the plant essential oils obtained from four plant species, *A. absinthium, S. santonicum, S. spicigerum* and *A. santolinoides* from different localities in Turkey, on *T. urticae* and *A. obtectus* adults under laboratory conditions.

Materials and Methods

Plant material and isolation of essential oils

Flowering stages of *A. absinthium, S. santonicum, S. spicigerum* and *A. santolinoides* were collected from different localities in Turkey between June 2016 and August 2017. The identification of plant materials was made by Prof. Dr. Yusuf Kaya, Department of Biology, Faculty of Art and Science, Atatürk University, Erzurum, Turkey. The voucher specimens of these plants have been deposited in the herbarium of Ataturk University, Erzurum, Turkey. Aerial parts of the plants were dried in shade before processing with a grinder. Then 500 g was hydrodistilled for 4 h using a Clevenger-type apparatus (Göktürk et al., 2017). Hydrodistillation of *A. absinthium, S. santonicum, S. spicigerum* and *A. santolinoides* yielded 0.6, 0.8 and 0.5% (w/w) of plant essential oil based on dried parts of the tested plants, respectively. The drying of the plant essential oils was done with anhydrous Na_2SO_4. The plant essential oils obtained were kept under N_2 in a closed glass bottle until needed, and then stored at 4°C during testing.

Insect and mite cultures

In this study, *T. urticae* and *A. obtectus* adults were collected from strawberry, *Fragaria ananassa* Rouzier (Rosaceae) plants without any pesticide exposure and infected bean, *Phaseolus vulgaris* L. (Fabaceae) seeds in a private storage facility, respectively, in (Fethiye, Muğla Province of Turkey in April and May of 2018. The bean seeds were purchased from a local market and kept in a deep-freezer at -15°C for 2 d to control any arthropod pests before use in bioassays. Then, *A. obtectus* adults were reared in 1 L jars containing cowpea seeds. The cultures were reared in the dark in a growth chamber at 26±1°C and 70±5% RH without exposure to any insecticide for several generations. *T. urticae* adults were reared on strawberry plants in the laboratory at 12-13% moisture content in a container (25 cm diameter x 30 cm high) under the same conditions.

Toxicity tests of the essential oils

To test the efficacy of the four plant essential oils against *T. urticae* and *A. obtectus* adults, assays were performed in Petri dishes (9 x 1.5 cm, 120 ml in volume). In order to determine the contact (for *T. urticae* adults) and fumigant effects (for *A. obtectus* adults), the plant essential oils were dissolved with ethanol (20, 30 and 40 µL/Petri dish) and sterile water (1 ml) solution (10%, v/v). The final concentrations of the treatments were 10, 15 and 20 µL/Petri dish. A filter paper was placed at the bottom of each Petri dish.

Contact toxicity test for *T. urticae* adults

To test the contact effects of the plant essential oils, 20 *T. urticae* adults were transferred from infested strawberry leaves to uninfested leaves using a thin brush in each Petri dish. To maintain the turgor of the uninfested leaves during experiments, the petioles of the leaves were placed in a piece of saturated cotton. The plant essential oils were then sprayed on *T. urticae* adults using a hand spray (Manual Potter Spray Tower-Burkard Scientific Limited, Uxbridge, UK). In this way, direct contact of the plant essential oils to *T. urticae* adults were ensured. Each Petri dish was covered with a lid, placed into the incubator, at 26±1°C, 70±5 RH and 16:8 h L:D photoperiod for 3 d. *T. urticae* adults on strawberry leaves were exposed separately the four plant essential oils. In order to determine mortalities at each exposure time, the number of dead *T. urticae* adults was counted under a microscope after 24, 48 and 72 h.

Fumigant toxicity test for *A. obtectus* adults

To test the fumigant toxicities of four plant essential oils on *A. obtectus* adults, the insect adults with 20 bean seeds were placed in Petri dishes. Three doses of the plant essential oils were applied with a pipette onto Whatman papers (No: 1; 2 x 2 cm) attached to underside of each Petri dish lid. Twenty *A. obtectus* adults were added to each Petri dish, which were covered with a lid and incubated for 3 d as above. The *A. obtectus* adults were feed with an appropriate amount of bean seed (1 seed/insect) during the tests. In order to determine mortalities for each exposure time, the number of dead adults was counted for *A. obtectus* adults under the microscope at 24, 48 and 72 h. A group treated with water and ethanol served as a negative control. Three different doses (10, 15 and 20 µL/Petri dish) of Malathion 65 EM (Koruma: malathion 650 g/l) were used as a positive control. All test procedures were performed under the same conditions as the cultures and each test was repeated three times.

Data analysis

To determine whether there was a statistically significant difference between the results obtained, Two-way analysis of variance was performed using SPSS (Statistical Package for Social Sciences 17.0). The differences between means were determined with Duncan's multiple range test. Lethal dose and Lethal concentration (LD_{50} and LD_{90}; LC_{50} and LC_{90}) values after 24, 48 and 72 h were calculated using the Finney method (Finney, 1971). To determine LC and LD values at 95% confidence limits EPA Probit Analysis Program was used. The results showed significant differences at $P < 0.05$ levels.

Results and Discussion

The results of the study are summarized in Tables 1 and 3. The plant essential oils gave different mortality rates and there are statistical differences between them. When the mortality rates of these plant essential oils were compared for 24 h exposure, it was found that there were statistical differences between the treatments for each pest. The highest mortality rate (100%) was with 15 and 20 µL/Petri dish of *A. santolinoides* oil with *T. urticae* adults. The lowest mortality rate (23%) was with 10 µL/Petri dish of *A. absinthium* oil (Table 1). Similarly, when the mortality rates of all doses of these plant essential oils were compared for 48 h exposure, the highest mortality (100%) was with 20 µL/Petri dish of *S. santonicum* and at 10, 15 and 20 µL/Petri dish of *A. santolinoides* oils with *T. urticae* adults, whereas the lowest mortality (55%) was with 10 µL/Petri dish of *A. absinthium* oil (Tables 1 & 3). However, after 48 h, the highest mortality

(97%) was with 20 µL/Petri dish of *A. absinthium* oil with *A. obtectus* adults. The lowest effect (78%) was observed at 10 µL/Petri dish of *S. santonicum* oil for this pest. As a parallel to these, when the mortality rates of 10, 15 and 20 µL/Petri dish caused by four plant essential oils at the end of 72 h were compared, the highest mortality rate (100%) was for *S. santonicum, A. santolinoides* and *S. spicigerum* plant essential oils with *T. urticae* adults. Also, 100% mortality was recorded with 20 µL/Petri dish of *A. absinthium* oil, but it was 90% and 98% with 10 and 15 µL/Petri dish of *A. absinthium* oil, respectively. The lowest mortality effect (90%) was with 10 µL/Petri dish of *A. absinthium* oil after 72 h with *T. urticae* adults. However, after 72 h, the mortality was more than 95% at 10, 15 and 20 µL/Petri dish for all plant essential oils with *A. obtectus* adults (Tables 1 & 3). Also, the toxicities of the plant essential oils on *T. urticae* and *A. obtectus* adults increased with increasing dose and exposure time. Mortality of both pests were significantly different according to treatment dose and time (for *T. urticae* adults, $F_{15,164} = 87.3$, $P < 0.05$; and for *A. obtectus* adults $F_{15,164} = 81.5$, $P < 0.05$). One of the most widely used acaricide and insecticide for *T. urticae* (mite pests) and *A. obtectus* adults (stored product pests) is malathion 65 EM. In this study, 100% toxicity was obtained after 72 h with the highest dose of malathion dose (20 µL/Petri dish) (Tables 1 & 3).

Table 1. The contact toxicity results of multiple comparison with means and standard error of exposure times and doses of plant essential oils of four plant species with *T. urticae* adults

Treatment Essential oils	Dose (µL/Petri)	*Tetranychus urticae* mortality (%)[a] Exposure time (h)[b]					
		24 h		48 h		72 h	
Artemisia absinthium	10	23±3.3	h	55±2.9	f	90.0±0.0	b
	15	32±3.3	gh	70±0.0	e	98±1.7	a
	20	42±1.7	ef	78±3.3	cd	100±0.0	a
Seriphidium santonicum	10	35±5.8	fg	78±1.7	cd	100±0.0	a
	15	63±4.4	d	98±1.7	ab	100±0.0	a
	20	93±4.4	ab	100±0.0	a	100±0.0	a
Seriphidium spicigerum	10	50±2.9	e	73±1.7	de	100±0.0	a
	15	78±4.4	c	80±2.9	c	100±0.0	a
	20	85±2.9	bc	93±3.3	b	100±0.0	a
Achillea santolinoides	10	82±1.7	c	100±0.0	a	100±0.0	a
	15	100±0.0	a	100±0.0	a	100±0.0	a
	20	100±0.0	a	100±0.0	a	100±0.0	a
Positive Control (Malathion 650 g/l)	10	100±0.0	a	100±0.0	a	100±0.0	a
	15	100±0.0	a	100±0.0	a	100±0.0	a
	20	100±0.0	a	100±0.0	a	100±0.0	a
Negative Control (Ethanol + Sterile water)	-	0±0.0	ı	0±0.0	g	2±1.4	c

[a] The values are mean±SE of three replicates, each set up with 20 adults;
[b] Exposure time values followed by the same letters within a column are not significantly different at P≤0.05.

When LD and LC values after 72 h treatment (LD_{50}, LD_{90} and LC_{50}, LC_{90}) of these plant essential oils were compared for their effects on *T. urticae* and *A. obtectus* adults, the most toxic plant essential oils based on LD_{50} and LD_{90} values were for *A. santolinoides* (7.8 µL/mite) and *S. spicigerum* (4.8 µL/mite) plant essential oils with *T. urticae* adults. However, the most toxic plant essential oils, based on LC_{50} and LC_{90}, after 72 h were with *A. santolinoides* (0.001 µL/insect) and *S. santonicum* (8.0 µL/insect) plant essential oils with *A. obtectus* adults. Similarly, the most toxic plant essential oil after 48 h, based on LD_{50} and LD_{90}, was with *A. santolinoides* plant essential oils (7.8 µL/mite and 10.0 µL/mite) with *T. urticae* adults, respectively. Also, the most toxic plant essential oils, based on LC_{50} and LC_{90}, after 48 h was with *A. santolinoides* (6.1 µL/insect and 19.7 µL/insect) plant essential oil with *A. obtectus* adults, and the most toxic plant essential oil after 24 h, based on LD_{50} and LD_{90}, was with *A. santolinoides* plant essential oil (9.4 µL/mite and 11.0 µL/mite) with *T. urticae* adults, respectively. After 24 h, the most toxic LC_{50} and LC_{90} values were with *A. absinthium* plant essential oil (15.7 µL/insect and 28.2 µL/insect) with *A. obtectus* adults. The lowest toxicity after 72 h, based on LD_{50} and LD_{90} values, was with *S. santonicum* and *A. absinthium* plant essential oils (336.9 µL/mite and 14.7 µL/mite) with *T. urticae*. The lowest toxicity after 72 h, based on LC_{50} and LC_{90}, was with *A. absinthium* and *S. spicigerum* (7.8 µL/insect) and *A. santolinoides*

(24.4 µL/insect) plant essential oils with *A. obtectus* adults, respectively (Tables 2 & 4). The lowest toxicity after 24 and 48 h, based on LC_{50} and LC_{90}, was with S*. spicigerum* and *A. santolinoides,* and *S. santonicum* plant essential oils with *A. obtectus*, respectively. The lowest toxicities after 24 and 48 h, based on LD_{50} and LD_{90}, were with *A. absinthium* and *S. spicigerum* plant essential oils with *T. urticae* adults, respectively. These results showed that the acaricidal and insecticidal activities increased with increasing dose and exposure time. All of the plant essential oils gave meaningful mortalities (Tables 1 to 4).

Table 2. Lethal dose (LD) values of plant essential oils of four plants with *T. urticae* adults

Essential oils	Time (h)	LD_{50}[a]	LD_{90}[b]	X^{2*}	Slope±SE	Probability
Artemisia absinthium	24	29.7	70.5	1.5	3.4±6.0	0.2
	48	9.9	34.9	1.2	2.3±2.8	0.6
	72	10.9	14.7	2.6	9.8±15.1	0.9
Seriphidium santonicum	24	15.4	20.0	6.5	11.3±3.2	0.4
	48	11.1	14.0	2.4	12.3±23.5	0.8
	72	336.9	14.4	8.0	0.9±7.2	1.0
Seriphidium spicigerum	24	9.9	23.0	1.8	3.5±3.2	0.5
	48	16.6	20.3	5.1	14.6±6.5	0.7
	72	19.9	4.8	6.3	2.1±4.5	1.0
Achillea santolinoides	24	9.4	11.0	0.2	19.8±42.8	0.8
	48	7.8	10.0	2.1	12.3±36.2	1.0
	72	7.8	10.0	2.1	12.3±36.2	1.0

[a,b] The lethal concentration give rise to 50 and 90% mortality after 24,48 and 72 h;
* Chi square rate.

Table 3. The fumigant toxicity results of multiple comparison with means and standard error of exposure times and doses of plant essential oils of four plant species with *A. obtectus* adults

Treatment Essential oils	Dose (µL/Petri)	*Acanthoscelides obtectus* mortality (%)[a] Exposure time (h)[b]					
		24 h		48 h		72 h	
Artemisia absinthium	10	48±4.4	hı	87±4.4	cde	98±1.7	a
	15	67±1.7	cd	93±1.7	efg	100±0.0	a
	20	82±1.7	b	97±1.9	ab	100±0.0	a
Seriphidium santonicum	10	50±32.9	ghı	78±1.7	f	95±2.9	a
	15	57±1.7	efg	83±1.7	ef	98±1.7	a
	20	72±1.7	c	92±1.7	bcd	100±0.0	a
Seriphidium spicigerum	10	45±2.9	I	85±2.9	def	98±1.7	a
	15	52±1.7	fgh	92±1.7	def	100±0.0	a
	20	58±1.7	ef	95±0 .0	ab	100±0.0	a
Achillea santolinoides	10	55±2.9	efgh	82±1.7	ef	100±0.0	a
	15	62±1.7	de	92±1.7	bcd	100±0.0	a
	20	67±1.7	cd	93±1.7	abc	100±0.0	a
Positive Control (Malathion 650 g/l)	10	100±0.0	a	100±0.0	a	100±0.0	a
	15	100±0.0	a	100±0.0	a	100±0.0	a
	20	100±0.0	a	100±0.0	a	100±0.0	a
Negative Control (Ethanol + Sterile water)	-	0±0.0	j	1.7±1.4	g	3±1.0	b

[a] The values are mean±SE of three replicates, each set up with 20 adults;
[b] Exposure time values followed by the same letters within a column are not significantly different at P≤0.05.

In a previous study, it was established that *A. absinthium* plant essential oil caused mortality from 71 to 100% at 10, 15 and 20 µL/Petri dish after 96 h exposure of *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) (Kesdek et al., 2015). Eight plant extracts caused mortalities between 9 and 96% on *Tetranychus cinnabarinus* (Boisduval, 1867) after 24 h (Chen & Dai, 2015). In the present study, *A. absinthium* plant essential oil caused mortality from 23 to 100% of *T. urticae* adults and 48 to 100% of *A. obtectus* adults. Topuz & Madanlar (2011) reported that *Mentha pulegium* L. (Lamiaceae)*, Foeniculum vulgare* Mill. (Apiaceae) and *Schinus molle* L. (Anacardiaceae) plant essential oils gave up to 50% mortality at 20 ml/l dose after 96 h in *T. cinnabarinus.* Kesdek et al. (2015) found that *Seriphidium santonicum* (L.) Sojak (syn. *Artemisia santonicum* L.) oil caused mortality between 7 and 100% in *L. decemlineata* adults. In another study, it was determined that *S. santonicum* (syn. *A. santonicum*) plant essential oil lead to

mortalities between 20 and 98% in *C. maculatus* adults (Usanmaz Bozhüyük et al., 2016). In the present study, *S. santonicum* (syn. *A. santonicum*) oil caused mortality from 35 to 100% in *T. urticae* adults. These mortality rates ranged from 50 to 100% in *A. obtectus* adults. Similarly, *Seriphidium spicigerum* (K. Koch) Poljakov (syn. *Artemisia spicigera* (K. Koch) oil caused mortalities between 4 and 42% in *L. decemlineata* adults (Kesdek et al., 2015), and from 27 to 98% in *C. maculatus* (Usanmaz Bozhüyük et al., 2016). In the present study, the mortality rates in *T. urticae* adults ranged from 50 to 100% for *S. spicigerum* oil and between 45 to 100% in *A. obtectus* adults. In another study, *Achillea santoliniodes* (K. Koch) Lag. (syn. *Achillea wilhelmsii* K. Koch) plant essential oil caused mortality from 2 to 29% in *L. decemlineata* adults after 96 h (Kesdek et al., 2015). In the present study, *A. santoliniodes* plant essential oil caused mortalities between 82 and 100% in *T. urticae* adults, and from 55 to 100% in *A. obtectus* adults.

Table 4. Lethal concentration (LC) values of plant essential oils acquired from four plants with *A. obtectus* adults

Essential oils	Time (h)	LC_{50}[a]	LC_{90}[b]	X^{2*}	Slope±SE	Probability
Artemisia absinthium	24	15.7	28.2	1.3	5.0±5.2	0.5
	48	9.8	21.5	3.5	3.8±10.8	0.9
	72	7.8	10.0	2.1	12.3±36.2	10.0
Seriphidium santonicum	24	20.9	32.8	0.7	76.6±5.5	0.5
	48	17.6	26.5	0.9	7.3±8.9	0.8
	72	3.6	8.0	5.6	3.7±2.2	1.0
Seriphidium spicigerum	24	27.0	140.0	0.7	1.8±4.12	0.5
	48	7.7	22.3	1.2	2.8±9.1	0.9
	72	7.8	10.0	2.1	12.3±36.2	1.0
Achillea santolinoides	24	19.8	142.4	10.7	1.5±14.5	0.6
	48	6.1	19.7	1.5	2.5±2.1	0.8
	72	0.001	24.4	7.1	0.3±3.3	1.0

[a,b] The lethal concentration give rise to 50 and 90% mortality after 24,48 and 72 h;
* Chi square rate.

In conclusion, natural acaricides and insecticides are a suitable alternative to synthetic acaricides and insecticides due to their low mammalian toxicity, low environmental effects and worldwide acceptability. Therefore, their adoption will contribute to a reduction in the negative sides of synthetic chemicals, such as residues in food products, insect resistance and environmental pollution. In the present study, the results suggest that four plant essential oils have the potential for use in the control of *T. urticae* and *A. obtectus* adults. Among them, *S. spicigerum* and *A. santolinoides* plant essential oils were found to be more toxic against *T. urticae* adults, whereas *A. absinthium* and *A. santolinoides* plant essential oils were more effective against *A. obtectus* adults. Therefore, it is suggested that these plant essential oils be considered as potential new acaricides and insecticides for *T. urticae* and *A. obtectus*. However, further studies need to be conducted to evaluate the mode of action and cost-effectiveness of these plant essential oils in a wider range of pests in agricultural products and storage facilities.

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Original article (Orijinal araştırma)

First record and parasitism of egg parasitoid *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) on eggs of *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae) in Turkey[1]

Türkiye'de *Chilo partellus* Swinhoe, 1885 (Lepidoptera: Crambidae)'un yumurta parazitoiti olarak *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae)'in ilk kaydı ve parazitleme oranı

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Abstract

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), which is currently found in many parts of the world, is a very damaging maize stem borer in Indian subcontinent and South and Eastern Africa. In 2014, it was recorded for the first time in Turkey. This pest is normally controlled by insecticides. Concerns of the negative effects of incessant use of insecticides have necessitated exploration of more benign and natural control methods. This study investigated the occurrence and parasitism rates of some native egg parasitoids of *C. partellus.* In 2018, first and second maize crops were planted in the research field of the Department of Plant Protection, Çukurova University (39º01'50.5"N, 35º21'06.7"E), in Adana, Turkey. Field was scouted once a week for parasitized egg masses. An egg parasitoid was recorded and morphologically identified as *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae). The egg parasitoid was recorded in August-September 2018 for the first time on *C. partellus* in maize (second crop) with rate of parasitism reaching 100% on 16 August. It is suggested that *T. evanescens* can be used for future development of biological control programs against *C. partellus*.

Keywords: Biological control, *Chilo partellus*, egg parasitoid, maize, *Trichogramma evanescens*

Öz

Chilo partellus Swinhoe, 1885 (Lepidoptera: Crambidae), şu an dünyanın birçok bölgesine yayılmış, orijini Hindistan Yarımadası ve Güney ve Doğu Afrika olan oldukça zararlı bir mısır sap kurdudur. Türkiye'de ilk kez 2014 yılında saptanmıştır. Bu zararlının kontrolünde genellikle insektisit kullanılmaktır. Sürekli insektisit kullanımının olumsuz etkileriyle ilgili endişeler daha çevre dostu ve doğal kontrol yöntemlerinin araştırılmasını gerektirmiştir. Bu çalışma ile *C. partellus*'un mevcut yerli parazitoitlerinin varlığı ve parazitleme oranları araştırılmıştır. 2018 yılında Çukurova Üniversitesi, Bitki Koruma Bölümü, Araştırma ve Uygulama Alanı (39º01'50.5"N, 35º21'06.7"E)'nda birinci ve ikinci ürün olarak yetiştirilen mısır tarlalarında haftada bir defa örneklemeler yapılmış ve parazitlenmiş yumurtalar kaydedilmiştir. Morfolojik karakterler incelenerek *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) olarak tanımlanan yumurta parazitoitinin, 2018 Ağustos- Eylül aylarında ilk kez mısırda *C. partellus*'un parazitoiti olarak kaydedildiği ve 16 Ağustos'ta parazitlenme oranının %100 olduğu belirlenmiştir. *Trichogramma evanescens*'in gelecekte *C. partellus*'un biyolojik mücadele programlarında kullanılabileceği düşünülmektedir.

Anahtar sözcükler: Biyolojik mücadele, *Chilo partellus*, yumurta parazitoiti, mısır, *Trichogramma evanescens*

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Introduction

The invasive spotted maize stem borer *Chilo partellus* Swinhoe,1885 (Lepidoptera: Crambidae) is a dangerous maize pest and it is believed to be originated from India (Kfir, 1997), from there it spread to Eastern and Southern parts of Africa (Mathez, 1972; Melaku et al., 2006). Recently *C. partellus* was reported as a serious maize pest in several countries of the Mediterranean Basin, including Turkey (Sertkaya et al., 2014; Yonow et al., 2017). *Chilo partellus* is known to cause very severe damage on maize and sorghum wherever found (Kfir et al., 2002). It damages the vegetative and reproductive parts of the plant, and the losses may range between 24-75% (Kumar, 2002). The severe pest infestation has been reported to cause 80-100% crop losses in Asia and Africa (Overholt et al., 2000). Management of *C. partellus*is principally by insecticide use (Rauf et al., 2017) although other benign methods are also being exploited. The egg parasitoid *Trichogramma chilonis* Ishii, 1941 (Hymenoptera: Trichogrammatidae) and *Bacillus thuringiensis* Berliner, 1915 (Bacillales: Bacillaceae) were used to reduce population of *C. partellus* in India in a joint application (Jalali & Singh, 2006; Shera et al., 2017). Another species, *Trichogrammatoidea lutea* Girault, 1911 (Hymenoptera: Trichogrammatidae), was reported as an egg parasitoid of *C. partellus* in Africa (Kfir, 1990). Many species of the genus *Trichogramma,* including *Trichogramma cacoeciae* Marchal, 1927, *T. chilonis*, *Trichogramma chilotraea* Nagaraja & Nagarkatti, 1969, *Trichogramma dendrolimi* Matsumura, 1926, *Trichogramma brassicae* Bezdenko, 1968, and *Trichogramma japonicum* Ashmead, 1904 (Hymenoptera: Trichogrammatidae), were reported as egg parasitoids of *C. partellus* in the different regions of world (Polaszek, 2010). The egg parasitoid *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) has been recorded to control many lepidopteran pests. Özpınar & Kornoşor (1994) recorded 75.5% parasitism rate of *T. evanescens* on egg masses of *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae) on the second crop of maize in Adana, Turkey. Adarkwah et al. (2015) determined between 65-90% parasitism by *T. evanescens* on eggs of *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) in paper and jute bags. According to Dix et al. (2009), biological control (biocontrol) with native natural enemies and/or coevolved natural enemies of the invasive pest can be very successful in the new habitat. Consequently, it is necessary to investigate possible native natural enemies for *C. partellus* in the agroecosystems in Turkey. Can Cengiz (2016) recorded *Telenomus busseolae* Gahan, 1922 (Hymenoptera: Scelionidae) and *T. brassicae*as egg parasitoids of *C. partellus* in Hatay Province, Turkey. Morphological parameters are successfully being used to identify many insect species. The male genitalia are a very vital diagnostic character of the *Trichogramma* genus (Nagarkatti & Nagaraja, 1971). Pinto (1999) established very concise procedures, terminologies of morphological characters and ratios of male antennae and genitalia of *Trichogramma*. Some of these characters include: length of aedeagus, length of basal part of aedeagus, length of dorsal aperture, width of dorsal lamina, length of genital capsule, length of longest seta of flagellum, maximum width of flagellum and more (Woelke et al., 2019). There are many reports of successful identification of *Trichogramma* spp. using morphological characters. Birova & Kazimirova (1997) used these male genitalia and antenna characters to identify *Trichogramma danubiense* Birova & Kazimirova, 1997 (Hymenoptera: Trichogrammatidae). Woelke et al. (2019) used these characters to describe two new *Trichogramma* species. Sorokina (1993) and Pintureau (2008) used these characters and identified *T. evanescens* and *T. brassicae*.

The main goal of this study was to determine the occurrence and identify native egg parasitoids of *C. partellus* in the southwestern part of Turkey especially in Adana Province, analyze its influence on the host population, and to check the opportunity of its future application for biological control of *C. partellus*.

Materials and Methods

Study site

The study was conducted in the fields of Çukurova University, near Adana, Turkey (39º01’50.5”N, 35º21’06.7”E), in 2018 (Figure 1). Two maize crops per year is the predominant practice in the

Mediterranean areas of Turkey; the first crop in March-June and the second crop in July-October. A maize (Pioneer Hybrid 1/2013) crop of 0.4 ha was established on 15 March 2018 as the first crop of maize. The second crop of maize was planted on 12 June 2018. Interrow and intra row spacing was 45 cm and 20 cm, respectively. No pesticides were applied during the entire period of the study.

Figure 1. Sampling sites (stations) of the study (Anonymous, 2019).

Sampling

Once a week between 08.00-10.00 am, 60 maize stalks were selected randomly and cut at ground level and transported to the Laboratory of Entomology in the Department of Plant Protection, Çukurova University. The leaves were meticulously examined for egg masses. The egg masses of *C. partellus* were incubated and the egg parasitoids that emerged were identified. Preliminary studies conducted in 2017 showed it is easier to find egg masses of *C. partellus* in August and September, when there is a peak in the population. Thus, much of the emphasis in these studies was during this period. A total of 60 egg masses (each containing 35-50 eggs) of maize stem borer *C. partellus* were collected within this period. About 15 females and 10 males of *Trichogramma* sp. were reared from these parasitized eggs. These newly hatched egg parasitoids *Trichogramma* sp. were isolated and supplied with a large number of eggs of laboratory host, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) attached on paper cards. A culture of an egg parasitoid, *Trichogramma* sp., was maintained at the laboratory (25.0±2ºC, 68±2% RH, 16:8 h L:D h photoperiod) on eggs of *E. kuehneilla*. Although *E. kuehniella* is a very destructive pests of stored grains (Tarlack et al., 2015), its eggs and larvae are generally used as laboratory host for rearing of many entomophagous insects (Samara et al., 2008). Two samples containing over 200 dead adult specimens of *Trichogramma* sp. from the laboratory culture were sent to Dr. Victor Fursov (Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Kiev, Ukraine) for detailed morphological study and species identification.

Species identification

Standard methods of slide mounting were used for the preparation of microscope slides on glasses (Pinto, 1999; Platner et al., 1999). About 200 specimens of *Trichogramma* were macerated in 10% liquid of potassium hydroxide, then washed in distilled water and mounted in Faure's liquid on glass slides under small cover slips. Thirty males of *Trichogramma* were dissected, then mounted on slides and used for the identification. Microscope Olympus CX-40 with digital photo-camera Olympus CX4040 was used to receive digital photos of morphological characters. Voucher specimens mounted on microscope slides are deposited at the Department of entomophagous insects and biocontrol, Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kiev, Ukraine).

Rate of parasitism

The egg masses were incubated in the Laboratory of Entomology, Department of Plant Protection, Çukurova University. The egg masses were checked daily under a stereomicroscope (x10) for parasitism. Two days before wasp emergence, parasitized egg masses become black and were easily differentiated from unparasitized egg masses (Woelke et al., 2019). The rate of parasitism (RP) was estimated as the fraction of the number of parasitized egg masses (P) of the total number of egg masses (T) collected per sampling date: RP = (P/T) x 100.

Results and Discussion

Species identification

The species of *Trichogramma* was identified on the base of morphological study of male genitalia and male antennae (Nagarkatti & Nagaraja, 1977; Pinto, 1999). Morphological keys of Pintureau (2008) and Sorokina (1993) were used for the identification. The parasitoid species was identified as *T. evanescens*.

Diagnosis. The species *T. evanescens* can be distinguished from other species by the occurrence of long setae on male antennae. Setae are at least 2.5 to 3 times longer than the width of clava. Male genitalia of *T. evanescens* have a wide, clearly-visible tip and wide lateral lobes of dorsal extension of phallobase of genitalia, and a long, narrow and sharp intervorsellar process, or ventral extension of phallobase. Figure 2 shows these morphological details of the male antennae and genitalia of *T. evanescens*. We consider the species *T. brassicae* to be the morphologically closest species to *T. evanescens,* with only minor morphological differences (Pintureau, 2008).

Trichogramma brassicae has been widely suggested and used as biological control agent for many lepidopterous pests (Moezipour et al., 2008; Lundgren et al., 2009; Thubru et al., 2018). Since *T. brassicae* has been found in different regions of Turkey (Can Cengiz et al., 2016), it appears that local natural enemies can be adaptable for control of *C. partellus*.

Parasitism rate

The egg parasitoid *T. evanescens* was first reared from egg masses of *C. partellus* on 9 August 2018. The rate of parasitism gradually increased from 71.4% (9 August) to 100% (16 August). The rate of parasitism then dropped and rose again as is shown in Table 1. According to the original results, we suggest that the recorded high level of parasitism of *T. evanescens* in the egg masses of *C. partellus* indicates that this parasitoid is an important natural biological control agent of *C. partellus* in the southwestern part of Turkey. In Turkey, *T. brassicae* and *T. evanescens* have been used against lepidopterous pests. *Trichogramma brassicae* has been used in maize fields and hazelnut orchards in Düzce Province to achieve almost complete control of *O. nubilalis* and *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Noctuidae) (Kutuk 2017). Öztemiz et al. (2017) reported that *T. evanescens* reduced *Cydia pomonella* (L., 1758) (Lepidoptera: Tortricidae) in apple fruit to 9.66% compared to 34.0% in control plots in Pozantı. Also, low to medium parasitism rates of *T. evanescens* on eggs of *Sesamia nonagrioides* Lefebvre,1827 (Lepidoptera: Noctuidae) have been recorded in Adana (Sertkaya et al.,1999; Sertkaya & Kornoşor, 2002). Kayapınar & Kornoşor (1992) and Özpınar & Kornoşor (1994) also recorded very high parasitism of *O. nubilalis* by *T. evanescens* in many other parts of Turkey. It is known that excessive use of insecticides is not only damaging for the environment but it is also costly (Achiri et al., 2016). Alternative methods, such as biological control, are actively being investigated in many parts of the world for the control of *C. partellus* (Jalali & Singh, 2006; Shera et al., 2017; Thubru et al., 2018). This study provided the first report of *T. evanescens* in *C. partellus* in Turkey, and this finding along with the previous report of *T. brassicae* in *C. partellus* (Can Cengiz et al., 2016) are supportive of adaptive local biological control of invasive pests. In

the findings of Can Cengiz et al. (2016), parasitism of *C. partellus* was recorded only in the second crop, probably emphasizing the general parasitoid nature of the parasitoids which are often in the field in the second crop of maize alongside native maize stem borers.

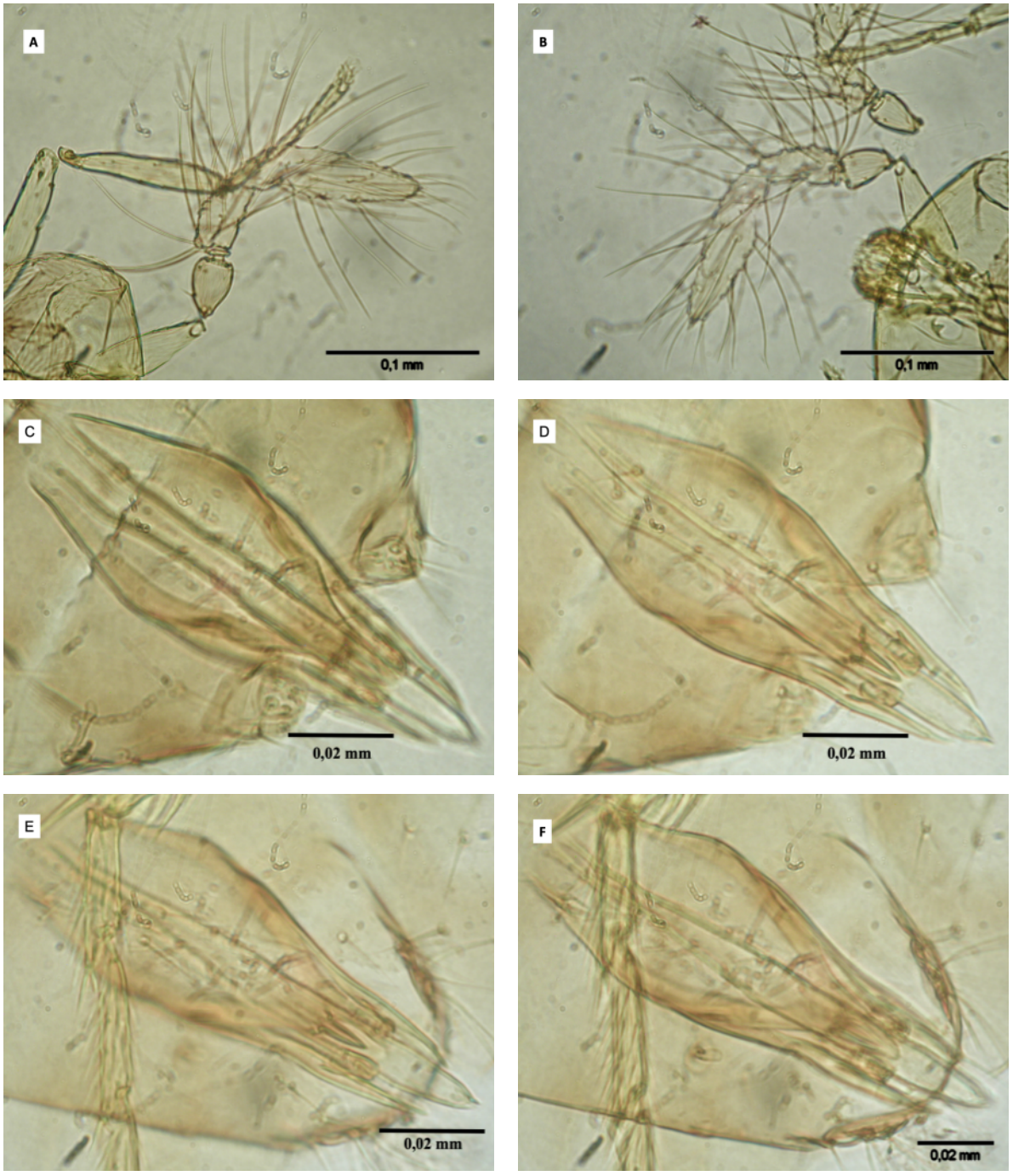

Figure 2. Morphological characteristics of male *Trichogramma evanescens*, reared from eggs of *Chilo partellus* in Adana, Turkey: A) setae at least 2.5 to 3 times longer than the width of clava, B) setae at least 3 times longer than the width of setae, C) wide tip and wide lateral lobes of dorsal extension of phallobase, D) ventral extension of phallobase, E) wide tip and wide lateral lobes of dorsal phallobase enlarged, and F) ventral extension of phallobase enlarged.

Table 1. Rate of parasitism of *Trichogramma evanescens,* reared from eggs of *Chilo partellus* at studied maize fields in Balcali, Adana Province, Turkey in 2018

	Date of collection					
	9 Aug	16 Aug	23 Aug	31 Aug	6 Sep	11 Sep
Egg masses collected	14	6	11	8	12	10
Egg masses parasitized	10	6	0	6	0	10
Rate of parasitism (%)	71.4	100	0.0	75.0	0.0	100

Conclusions

On the base of this study, we suggest that further studies should be conducted, on a large scale, to ascertain the impact of native parasitoids on *C*. *partellus* in the agroecosystems of Turkey. The percentage of egg parasitism and its influence on the population density of *C. partellus* must be taken into the consideration. In addition, an integrated control strategy for *C*. *partellus* should be considered, with natural enemies, such as *T*. *evanescens*, having a pivotal role.

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Original article (Orijinal araştırma)

Distribution and seasonal abundance of predatory bugs, *Orius* spp. (Hemiptera: Anthocoridae) in Adana Province, Turkey[1]

Adana ilinde avcı *Orius* spp. (Hemiptera: Anthocoridae)'nin dağılımı ve mevsimsel yoğunlukları

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Abstract

Orius species are considered effective biological control agents of thrips both in fields and greenhouses worldwide. The seasonal abundance and distributions of *Orius* and thrips species were determined from different arable crops and weeds in Adana Province in 2015-2016. In this study, a total of six species of genus *Orius* namely, *Orius albidipennis* (Reuter,1884), *Orius horvathi* (Reuter,1884), *Orius laevigatus* (Fieber, 1860), *Orius minutus* (L., 1758), *Orius niger* (Wolff, 1881) and *Orius vicinus* (Ribaut, 1923) (Hemiptera: Anthocoridae) were identified. *Orius niger* was the most common predatory Anthocorid species with 1944 specimens, and followed by *O. laevigatus* with 447 specimens. While, *O. laevigatus* and *O. niger* were found to be active throughout the year, *O. vicinus* was only active in spring and summer, and the other *Orius* spp. were collected in summer. The predatory bugs were mostly found with the pestiferous thrips, *Frankliniella occidentalis* Pergande, 1895 and *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae) in diverse agricultural areas. Alfalfa, faba bean and sunflower were determined as a crucial host plant for *Orius* spp. Also, sesame could be potential companion (trap or banker) plant to support the predatory bugs in augmentative and conservative biological control strategies. Of the weeds sampled, *Glebionis segetum* Fourr. and *Sinapis arvensis* L. were the most colonized by *Orius* spp. From results it is concluded that *O. niger* and *O. laevigatus* are well adapted to the geographical conditions and plant biodiversity in Adana Province. Therefore, these predatory bugs could be crucial biological control agents of thrips species in field crops.

Keywords: Abundance, biological control, host plants, *Orius* spp., thrips

Öz

Orius türleri dünyada hem açık alanda hem de seralarda thripslerin önemli biyolojik mücadele ajanı olarak bilinmektedirler. *Orius* ve avları olan thrips türlerinin mevsimsel yoğunlukları ve dağılımları Adana İli'nde 2015-2016 yıllarında belirlenmiştir. Bu çalışma ile *Orius* cinsine bağlı altı tür saptanmış olup, bunlar *Orius albidipennis* (Reuter, 1884), *Orius horvathi* (Reuter, 1884), *Orius laevigatus* (Fieber, 1860), *Orius minutus* (L., 1758), *Orius niger* (Wolff, 1881) ve *Orius vicinus* (Ribaut, 1923) (Hemiptera: Anthocoridae) türleridir. *Orius niger* 1944 adet ile en yaygın avcı tür olurken, *O. laevigatus* 447 adet ile onu izlemiştir. *Orius laevigatus* ve *O. niger* yıl boyunca aktif olarak belirlenirken, *O. vicinus* ilkbahar ve yaz periyodunda, diğer türler ise sadece yaz periyodunda belirlenmişlerdir. Avcı böcekler daha çok tarımsal alanlarda önemli zararlı thripsler olan, *Frankliniella occidentalis* Pergande 1895 ve *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae) ile birlikte örneklenmiştir. Yonca, bakla ve ayçiçeği *Orius* türleri için önemli konukçular olarak bulunmuştur. Bunun yanında, susamın doğal düşmanların korunması açısından potansiyel tuzak ya da banker bitki olabileceği düşünülmektedir. Yabancı otlardan. *Glebionis segetum* Fourr. ve *Sinapis arvensis* L. üzerinde *Orius* türleri daha çok toplanmıştır. Bu sonuçlar ile *O. niger* ve *O. laevigatus*'un Adana ilinin hem coğrafik koşullarına hem de bitki biyoçeşitliliğine iyi uyum sağladığı belirlenmiştir. Bu nedenle, bu avcı türler, açık alanda yetiştirilen kültür bitkilerinde thripslerin biyolojik mücadele ajanı olarak değerlendirilebilir.

Anahtar sözcükler: Bolluk, biyolojik mücadele, konukçu bitki, *Orius* spp., thrips

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Introduction

Chemical control is the most commonly used method for controlling pest organisms that cause economic damage to agricultural products (De Waard et al., 1993; Tyler, 2002). However, intensive and uncontrolled use of pesticides has potentially undesirable effects on ecosystems and human health (Pimentel et al., 1993). Nowadays, the protection of the environment, human health and biological diversity has come into prominence. Consequently, integrated pest management (IPM), including biological control, becomes crucial in controlling of important pest species in agroecosystems. In this context, the importance of predatory bugs belonging to the Anthocoridae family, which feed on many destructive pest Arthropoda species, is increasing in many cultivated areas (Lattin, 1999). Biological control can be described as the utilize of natural enemies to reduce the population density of pest organism than would occur in their absence (Stern et al., 1959; DeBach, 1964).

Conservation biological control is one of the main branches of biological control that can be applied by reducing the use of pesticides or using of selective pesticides with correct application methods and time (DeBach & Rosen, 1991). Thus, using of less toxic methods could preserve natural enemy biodiversity (El-Wakeil et al., 2017). Also, monocultures, reducing the non-crop areas and loss of native habitats, could cause a sharp decline in agroecosystem biodiversity (Wade et al., 2008). The natural enemies may need to utilize alternative plants as pollens-nectars source, oviposition sites or hibernation areas during non-crop periods (Wäckers et al., 2005). Preservation of natural enemies might be achieved by supporting habitat and food resources (i.e., nectar and pollen sources) (Fiedler et al., 2008). Also, winter crops (e.g., alfalfa and faba bean) and weeds usually provide shelter for natural enemies to overwinter. Managing and preventing of these plants or other food sources for natural enemies should be conducted with knowledge of the biology and behavior of the natural enemies and the pests (Bianchi & Wäckers, 2008; Wäckers & van Rijn, 2012). In this way, determination of predatory *Orius* spp. which is considered important biological control agents of many detrimental pest species such as thrips, whiteflies, aphids, and spider mites would be basic study for IPM programs.

In Turkey, *Orius* spp. have been recorded along with various insect pests and mite species in different habitats on cultivated and uncultivated plants (Önder, 1982; Karaat et al., 1986; Göven & Özgür, 1990; Atakan & Uygur, 2004; Büyük, 2008; Bahşi, 2011). Although many studies have been conducted on *Orius* spp., there is no specific information about distribution, seasonal prevalence, habitats, prey (thrips) of *Orius* spp. on the Adana Plain. For this aim, the survey studies have been conducted to determine *Orius* and thrips species from March 2015 to September 2016 in Adana Province.

Materials and Methods

Insect sampling

The abundance and prevalence of *Orius* and thrips species were determined from March 2015 to-September 2016 in Adana Province, Turkey. The survey area was divided into 4 subregions (Region I, Seyhan, Yüreğir, İmamoğlu and Kozan; Region II, Ceyhan, Yumurtalık and Karataş; Region III, Karaisalı and Tarsus; Region IV, Feke, Saimbeyli and Tufanbeyli) as their geographical characteristics and crop biodiversity. The survey areas were visited biweekly, and different cultivated crops (vegetables, orchards and crop fields) and wild plants around these crops sampled (Figure 1). In the sampling areas, there was a wide variety of agricultural practices in which both monoculture and polyculture production were performed as well as irrigated and non-irrigated production systems. Eight to ten randomly chosen plants representing each plant species were tapped into the white trap (34×23×7 cm) for 5-10 s during the flowering period of the crops (Atakan, 2008). *Orius* and thrips species were placed into the plastic tubes (2 ml) containing 70% ethanol using a fine brush. Also, a few of the potential prey of *Orius* spp., such as aphids and leafhoppers, were encountered but these insects were not evaluated in this study. The *Orius*

nymphs collected were reared to obtain adult stages in climatic chambers at 25°C, 60% RH and 16:8 h L:D photoperiod with sterilized *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs and pollen of *Typha latifolia* L. (Typhaceae) provided as food. The samples collected were transferred to Petri dishes (5 cm diameter) and separated as *Orius* spp. and thrips. Thrips species were placed in AGA (9 parts 70% ethanol+1 part glycerin+1 part glacial acetic acid) solution for 2 d to ensure discoloration (Lewis, 1973) and then transferred to plastic tubes (2 ml) containing 70% ethyl alcohol for storage.

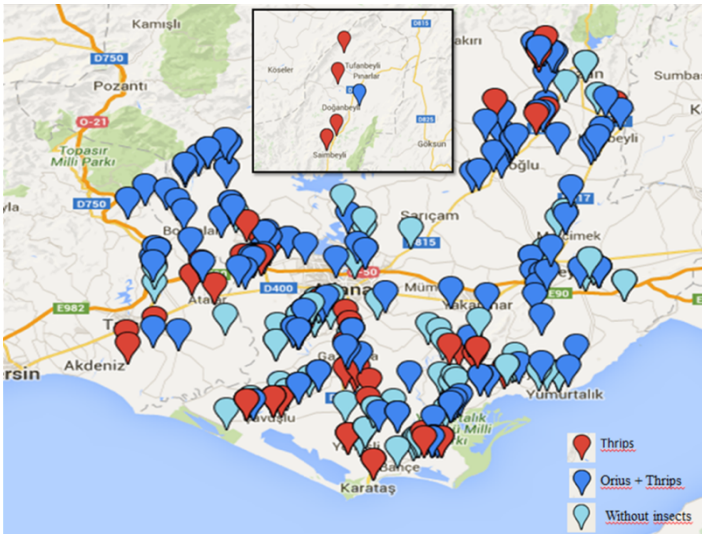

Figure 1. Survey areas of *Orius* and thrips species in Adana Province (Anonymous, 2019).

Insect identification

Microscopic slides of *Orius* spp. were made according to Silveira et al. (2003) and *Orius* adults were identified to species by comparing, the copulatory tube of females and the genital clasper of males according to the key of Péricart (1972). Thrips species were mounted on slides under a stereomicroscope and identified by the second author. These microscope slides were in the collection of the Entomology Laboratory (Department of Plant Protection, Agricultural Faculty, Çukurova University, Adana, Turkey).

Results and Discussion

Distribution of *Orius* species

A total of 2418 specimens of predatory *Orius* were collected. With the survey studies, *Orius albidipennis* (Reuter, 1884), *Orius horvathi* (Reuter, 1884), *Orius laevigatus* (Fieber, 1860), *Orius minutus* (L., 1758), *Orius niger* (Wolff,1881) and *Orius vicinus* (Ribaut, 1923) (Hemiptera: Anthocoridae) were identified. *Orius niger* was the predominant predatory Anthocoridae species (1944 specimens), while it constituted 80.4% of the total *Orius* adults. *Orius laevigatus* was the second most prevalent species with 447 specimens and accounting for 18.5% of the total individuals. Also, small proportions of *O. vicinus* (15 specimens), *O. horvathi* (8 specimens), *O. albidipennis* (3 specimens) and *O. minutus* (1 specimen) were determined (Table 1). *Orius* spp. were mostly collected from Regions I and III where field and horticultural crops are commonly cultivated. *Orius niger* was found mostly from Region I, while *O. laevigatus* was generally determined from the Region III (Table 1). This could be related to the rich crop biodiversity and adaption of both *Orius* spp. to the climatic conditions. Region IV was the highest (average 1000 m) of the areas sampled and only *O. niger* (45 specimens) were determined (Table 1). Region IV has the relatively low plant diversity, cultivating mostly horticultural crops such as stone fruits. *Orius* spp. were collected both in field crops and vegetables in this area. This finding indicates that *O. niger* is better adapted the high-altitude areas.

Table 1. Numbers of *Orius* spp. in Adana Province during 2015 and 2016

Sampling area*	*Orius niger*	*Orius laevigatus*	*Orius vicinus*	*Orius albidipennis*	*Orius horvathi*	*Orius minutus*	Total
Region I	791	107	13	2	3	0	916
Region II	413	62	0	1	5	1	482
Region III	695	278	2	0	0	0	975
Region IV	45	0	0	0	0	0	45
Total	1944	447	15	3	8	1	2418

* Region I: Seyhan, Yüreğir, İmamoğlu and Kozan; Region II: Ceyhan, Yumurtalık, Karataş; Region III: Karaisalı and Tarsus; Region IV: Feke, Saimbeyli and Tufanbeyli.

Orius spp. are considered one of the most effective biological control agents of thrips species both in fields and greenhouses (Lattin, 1999; Funderburk et al., 2000). These results showed that *O. niger* and *O. laevigatus* were the most common Anthocoridae species in Adana Province. These predatory bugs were predominant in vegetables (Zeren & Düzgüneş, 1983; Atakan, 2008), alfalfa (Atakan, 2004), faba bean (Atakan, 2010) and strawberry fields (Atakan, 2011) on the Adana Plain. Moreover, Önder (1982) reported that *O. niger* was widely distributed in all parts of Turkey, while *O. laevigatus* was found generally in the Mediterranean Region of Turkey.

Orius niger and *O. laevigatus* not only identified from cultivated crops but also found on weeds by in these surveys. Atakan & Tunç (2010) informed that *O. laevigatus*, *O. niger* and *Orius majusculus* (Reuter, 1879) (Hemiptera: Anthocoridae) were common Hemipteran predatory species of thrips in weeds in the eastern Mediterranean Region, Turkey. Also, Bahşi (2011) reported that *O. laevigatus* and *O. niger* were predominant species collected in different cultivated and uncultivated plants in the central Mediterranean Region (Antalya). These predatory bugs are widely distributed Anthocorid species in other Mediterranean countries such as Greece (Lykouressis & Perdikis, 1997), Italy (Tommasini, 2004; Bosco & Tavella, 2008) and Spain (Riudavets & Castane, 1994). However, other *Orius* spp., *O. albidipennis*, *O. horvathi*, *O. minutus*, and *O. vicinus* were rarely collected during the survey. These results agree with other studies which conducted in the Mediterranean Basin (Riudavets & Castane, 1994; Lykouressis & Perdikis, 1997; Tommasini, 2004; Bosco & Tavella, 2008; Bahşi, 2011). These results indicate the wide adaptation of *O. niger* and *O. laevigatus* to the ecological conditions and plant biodiversity of the Mediterranean Basin.

Seasonal abundance of *Orius* species

The seasonal abundance of the *Orius* species collected from the survey area are given in Table 2. *Orius niger* and *O. laevigatus* were collected throughout the whole year, while *O. vicinus* was only collected in spring and summer. In contrast, *O. albidipennis, O. horvathi*, and *O. minutus* were only collected in summer.

Table 2. Seasonal abundance of *Orius* species collected from various plants in Adana Province during 2015 and 2016

Seasons	*Orius niger*		*Orius laevigatus*		*Orius vicinus*		*Orius albidipennis*		*Orius horvathi*		*Orius minutus*	
	S.N*	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N
Spring	76	335	20	41	2	3	-	-	-	-	-	-
Summer	201	1281	81	381	11	12	2	3	7	8	1	1
Autumn	28	68	2	4	-	-	-	-	-	-	-	-
Winter	40	260	14	21	-	-	-	-	-	-	-	-
Total	345	1944	117	447	13	15	2	3	7	8	1	1

* S.N: Sample number, I.N: Individual number. The dash indicates that no individuals were collected.

Many Anthocorid species are well adapted to different climatic conditions to synchronize their life cycle with favorable environmental conditions over a whole year (Tommasini & van Lenteren, 2003). Therefore, some predatory *Orius* spp. can be active throughout the year (Van de Veire & Degheele, 1992; Bahşi & Tunç, 2008), while the others undergo reproductive diapause (Ruberson et al., 1991, van den Meiracker, 1994; Ito & Nakata, 1998). The results of the present study indicate that *O. laevigatus* and *O. niger* which were the most prevalent predatory bugs, were found year-round. Also, *O. vicinus* was collected in spring and summer. Other *Orius* spp. were collected only in the summer in Adana Province. These results are consistent with the findings of previous studies indicating *O. laevigatus* and *O. niger* are active throughout the whole year on alfalfa in Adana (Atakan, 2004) and on certain weeds in the eastern Mediterranean Region (Atakan & Tunç, 2010). Bahşi (2011) reported that these predatory bugs were found on cultivated and uncultivated crops throughout the year in Antalya Province. Also, *O. horvathi* and *O. minutus* were collected during the summer, whereas *O. vicinus* was collected only in the winter. In Italy, *O. laevigatus* and *O. niger* did not enter overwintering reproductive diapause during the whole year (Tavella et al., 1996; Bosco & Tavella, 2013). During the summer, favorable climatic conditions and plant biodiversity may positively affect the number of *Orius* spp. collected. Some important pollen-rich plants, such as cotton, sesame and sunflower, may be important for *Orius* species, attracting *Orius* prey, such as thrips, and providing alternative food sources (nectar and pollen for *Orius* species) in summer in Adana. Considerable numbers of *Orius* spp. mainly *O. niger* were also collected in the hard winter conditions of Adana. Faba bean which is generally grown during the winter, appeared to be important for providing food, shelter, and ovipositon areas for predatory bugs in this region.

Plant species on which *Orius* were collected

The plant species on which *Orius* spp. were collected and the numbers of the *Orius* are shown in Table 3. The predatory *Orius* spp. was collected from mostly sunflower (*Helianthus annuus* L.; 1251 specimens). Otherwise, most predatory Anthocorids were collected from an important winter host, faba bean (*Vicia faba* L.; 339 specimens), and from evergreen alfalfa (*Medicago sativa* L.; 336 specimens). Besides, *Orius* spp. were collected in relatively high numbers from cotton (*Gossypium hirsutum* L.; 175 specimens), sesame (*Sesamum indicum* L.; 113 specimens) and pepper (*Capsicum annum* L.; 36 specimens). In addition, a few *Orius* spp. were collected from field crops, such as soybean (*Glycine max* L.; 9 specimens), okra (*Abelmoschus esculentus* L.; 8 specimens), peanut (*Arachis hypogaea* L.; 6 specimens), vetch (*Vicia sativa* L.; 4 specimens), maize (*Zea mays* L.; 4 specimens), cowpea (*Vigna sinensis* L.; 1 specimen) and on some vegetables like cucumber (*Cucumis sativus* L.; 5 specimens), potato (*Solanum tuberosum* L.; 3 specimens), bean (*Phaseolus vulgaris* L.; 2 specimens) and eggplant (*Solanum melongena* L., 1 specimen). However, in the fruit orchards, there was only two predatory bugs were collected, both from apple (*Malus domestica* Borkh). *Glebionis segetum* Fourr. (40 specimens) and *Sinapis arvensis* L. (25 specimens) supported relatively high numbers of *Orius* spp. Also, some *Orius* spp. were also found on *Anthemis arvensis* L. (19 specimens), *Ochtodium aegyptiacum* DC. (15 specimens) and *Daucus carota* L. (11 specimens).

Table 3. Abundance and composition of *Orius* spp. on different plants in Adana Province, Turkey during 2015 and 2016

Family / Species Name	*Orius niger*		*Orius laevigatus*		*Orius vicinus*		*Orius albidipennis*		*Orius horvathi*		*Orius minutus*	
	S.N*	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N
Asteraceae												
***Anthemis arvensis* L.**	4	19	-	-	-	-	-	-	-	-	-	-
****Glebionis segetum* Fourr.**	11	39	1	1	-	-	-	-	-	-	-	-
Helianthus annuus L.	110	914	64	335	2	2	-	-	-	-	-	-
Apiaceae												
****Dacus carota* L.**	7	11	-	-	-	-	-	-	-	-	-	-
Brassicaceae												
****Ochtodium aegyptiacum***	5	14	1	1	-	-	-	-	-	-	-	-
****Sinapis arvensis* L.**	11	22	2	3	-	-	-	-	-	-	-	-
Cucurbitaceae												
Cucumis sativus L.	1	5	-	-	-	-	-	-	-	-	-	-
Cucurbita pepo L.	1	1	-	-	1	1	-	-	-	-	-	-
Fabaceae												
Arachis hypogaea L.	4	4	1	2	-	-	-	-	-	-	-	-
Glycine max L.	7	9	-	-	-	-	-	-	-	-	-	-
Medicago sativa L.	28	278	14	56	2	2	-	-	-	-	-	-
Phaseolus vulgaris L.	2	2	-	-	-	-	-	-	-	-	-	-
Vicia faba L.	63	319	13	20	-	-	-	-	-	-	-	-
Vicia sativa L.	1	4	-	-	-	-	-	-	-	-	-	-
Vigna sinensis L.	1	1	-	-	-	-	-	-	-	-	-	-
Malvaceae												
Abelmoschus esculentus L.	1	7	1	1	-	-	-	-	-	-	-	-
Gossypium hirsitum L.	36	159	10	14	3	3	-	-	-	-	-	-
Pedaliaceae												
Sesamum indicum L.	35	85	6	10	5	7	2	3	7	8	1	1
Poaceae												
Malus domestica Borkh	1	1	1	1	-	-	-	-	-	-	-	-
Zea mays L.	4	6	4	4	-	-	-	-	-	-	-	-
Solanaceae												
Capsicum annuum L.	11	35	1	1	-	-	-	-	-	-	-	-
Solanum melongena L.	1	1	-	-	-	-	-	-	-	-	-	-
Solanum tuberosum L.	1	3	-	-	-	-	-	-	-	-	-	-
Total	345	1944	117	447	13	15	2	3	7	8	1	1

* S.N: Sample number, I.N: Individual number.
** These plant species are weeds. The dash indicates that no individuals were collected.

Plant diversity can affect the distribution of the predatory bugs directly and indirectly (Russell, 1989; Moreira et al., 2016). The present results show that sunflower was the most attractive plant to *Orius* spp. The sunflower is known as an excellent plant to attract beneficial insects including important predators or parasitoids of agricultural insect pests (e.g., minute pirate bugs, ladybird beetles, lacewings, and several parasitoids) (Jones & Gillett, 2005). In this study, faba bean and alfalfa were the important plants bearing relatively high numbers of the predatory bugs, and also providing food source (nectars, pollens), shelter, mating and oviposition sites especially during the winter period. These results agree with findings of previous studies on faba bean and alfalfa in Mediterranean Region (Atakan & Tunç, 2004; Atakan, 2010; Atakan & Malik, 2018; Bahşi, 2011). Also, faba bean have been utilized as a cover crop in vineyard areas to protect the beneficial insects during winter times in the Northern Italy (Burgio et al., 2016). In the USA, when faba bean was grown with the other plants (alyssum, buckwheat, phacelia, and chamomile), it was the more preferred as an oviposition site for *Orius insidiosus* (Say, 1832) (Hemiptera: Anthocoridae) females (Pumariño et al., 2012). Atakan & Tunç (2004) reported that *O. niger* and *O. laevigatus* were the most common on alfalfa in Adana. Ban et al. (2010) suggested that alfalfa plants could help to create a natural balance between predators and pests inside a crop and in the surrounding area of greenhouses from June to mid-August in northern Hungary. On the Adana Plain, sesame, cotton, pepper, strawberry and rapeseed were also alternative host for *Orius* spp. (Atakan, 2010, 2011, 2017; Atakan et al., 2009; Atakan & Bayram, 2011). In the current study, all *Orius* spp. were collected from sesame plants. Sesame plants may be considered to be more attractive to *Orius* spp. in the agroecosystems. Biondi et al. (2016) suggested that sesame could be used for *Nesidiocoris tenuis* (Reuter, 1895) (Hemiptera: Miridae) in augmentative and conservative biological control strategies in tomato crops as a companion (trap or banker) plants. Also, many other studies reported that nectars of sesame greatly improved longevity, fecundity and handling time of parasitoids (Lou et al., 2014; Lu et al., 2015) and predatory mirids (Zhu et al., 2013). In the light of these studies, this plant may have a potential for enhancing the beneficial insect diversity in agroecosystems.

On the weedy flora, *O. niger was* the most prevalent species followed by *O. laevigatus*. *Orius albidipennis*, *O. horvathi*, *O. minutus* and *O. vicinus* were seldom recorded from the uncultivated plants during the survey. This may be related to adaptation of *Orius* spp. to different ecological conditions and differences in habits of predatory bug species. Some researchers indicated that *O. niger* and *O. laevigatus* were commonly found anthocorids in weeds in the Mediterranean Region of Turkey (Atakan & Uygur, 2004; Atakan & Tunç, 2010; Bahşi, 2011). Also, in Italy, this was the most common species found. Also, *O. majusculus*, *O. minutus* and *O. laevigatus* were collected from some weeds (Tommasini, 2004; Bosco & Tavella, 2008). Therefore, some weed species may be important as alternative plants providing food sources, oviposition substrates, hibernation sites, shelter, and refugia for *Orius* spp.

Orius species collected together with thrips species

In the present survey a total of 6725 pest thrips were collected. *Frankliniella occidentalis* Pergande, 1895 (Thysanoptera: Thripidae) was the most prevalent species followed by *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae). Predatory thrips species *Aeolothrips* spp., and phytophagous thrips species such as *Haplothrips* spp., *Melanthrips* spp. and *Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae) were also collected. Most thrips were collected from Region I where most field and horticultural crops are grown (Table 4). This could be related to rich plant biodiversity and well adaptation of the thrips species to the ecological conditions of this region. Fewer thrips were collected in Region IV which had the highest altitude and mostly only horticultural crops are grown (Table 4).

Table 4. Numbers of commonly collected thrips species in survey areas of Adana Province, Turkey during 2015 and 2016

Sampling area*	*Frankliniella occidentalis*	*Thrips hawaiiensis*	*Thrips tabaci*	*Haplothrips* spp.	*Melanthrips* spp.	*Aeolothrips* spp.	Other thrips species	Total
Region I	2170	557	112	230	138	231	25	3463
Region II	891	77	33	85	88	65	5	1244
Region III	1129	194	68	67	193	182	38	1871
Region IV	12	6	30	37	41	21	0	147
Total	4202	834	243	419	460	499	68	6725

* Region I: Seyhan, Yüreğir, İmamoğlu and Kozan; Region II: Ceyhan, Yumurtalık, Karataş; Region III: Karaisalı and Tarsus; Region IV: Feke, Saimbeyli and Tufanbeyli.

Orius spp. collected with thrips during the survey are shown Table 5. Although the *Orius* spp. are considered as the generalist predators, it is well known that they mostly fed upon thrips (Riudavets & Castane, 1998; Baez et al., 2004). *Orius niger*, which was the most common during the survey, was mostly collected with *F. occidentalis* (1598 specimens) but also with thrips, such as *T. hawaiiensis* and *Aeolothrips collaris* (Priesner, 1919) (Thysanoptera: Aeolothripidae) (594 and 570 specimens, respectively). The second most common species, *O. laevigatus*, was collected with *F. occidentalis* (414 specimens), *T. hawaiiensis* (171 specimens) and *A. collaris* (167 specimens). The other predatory bugs, *O. vicinus*, *O. horvathi* and *O. albidipennis,* were mostly collected with *F. occidentalis*. *Frankliniella occidentalis* and *T. hawaiiensis* were the most common pest thrips species and were collected with all predatory bugs (Table 5).

Occurrence of *Orius* spp. with thrips species may be important in management of pest thrips species in field crops and weeds (Bosco & Tavella, 2013). In this study, *F. occidentalis* and *T. hawaiiensis* were the common pest thrips species found with predatory bugs. In many studies, *F. occidentalis* was found to be widespread across the USA (Kirk & Terry, 2003; Morse & Hoddle, 2006), Europe (zur Strassen, 1986; Tommasini, 2003), and Turkey (Tunc & Gocmen, 1995; Atakan, 2003; Tunç & Hastenpflug-Vesmanis, 2016). However, *T. hawaiiensis* was the first reported in 2015 and spread over the eastern Mediterranean Region of Turkey within one year (Atakan et al., 2015). Atakan & Özgür (2001) reported that *O. niger* was one of the prevalent predatory bugs in cotton fields on the Adana Plain, and it appeared before the population increase of *F. occidentalis* during flowering and then the thrips population declined sharply after the peak in the *O. niger* population. Also, Funderburk et al. (2000) reported that *O. insidious* was an effective predator of *Frankliniella* spp. during the spring when thrips were rapidly colonizing pepper flowers in the field.

Bahşi (2011) found that populations of *O. niger* and *O. laevigatus* were related to *F. occidentalis,* but were collected with the other thrips species, such as *A. collaris*, *Aeolothrips intermedius* Bagnall, 1934 (Thysanoptera: Aeolothripidae), *Thrips major* Uzel, 1895 (Thysanoptera: Thripidae) and *T. tabaci*. Collecting *Orius* spp. with the predatory thrips such as *A. collaris* on the same plants could be explained by the competition of predators for the same food source (phytophagous thrips). In this situation, predatory bugs may have negative effects on the densities of *Aeolothrips* spp. due to intraguild predation. Fathi et al. (2008) reported that at low density of *T. tabaci*, the intraguild predation of *O. niger* on *A. intermedius* occurred when these predators were used in combination against this pest thrips.

Table 5. *Orius* spp. collected together with thrips in survey areas of Adana province, Turkey during 2015 and 2016

Thysanoptera species	*Orius niger*		*Orius laevigatus*		*Orius vicinus*		*Orius albidipennis*		*Orius horvathi*		*Orius minutus*	
	S.N*	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N	S.N	I.N
Aeolothripidae												
Aeolothrips collaris Priesner	90	572	38	167	2	2	1	1	1	1	-	-
Aeolothrips ericae Bagnall	3	28	1	9	-	-	-	-	-	-	-	-
Aeolothrips fasciatus (L.)	2	3	-	-	-	-	-	-	-	-	-	-
Aeolothrips intermedius Bagnall	4	8	2	2	-	-	-	-	-	-	-	-
Aeolothrips propinquus Bagnall	1	1	1	1	-	-	-	-	-	-	-	-
Melanthrips fuscus (Sulzer)	47	229	10	14	1	1	-	-	1	1	-	-
Melanthrips pallidior Priesner	-	-	-	-	-	-	1	1	-	-	-	-
Phlaeothripidae												
Haplothrips aculeatus (Fabricius)	13	45	4	11	-	-	1	1	-	-	-	-
Haplothris distinguendus (Uzel)	8	66	3	8	-	-	-	-	-	-	-	-
Haplothrips gowdeyi (Franklin)	11	64	3	8	1	2	-	-	-	-	-	-
Haplothrips reuteri (Karny)	63	402	28	146	1	1	-	-	-	-	-	-
Thripidae												
Anaphothrips sudanensis Trybom	1	20	1	9	-	-	-	-	-	-	-	-
Chirothrips africanus Priesner	1	1	-	-	-	-	-	-	1	1	-	-
Frankliniella occidentalis (Pergande)	295	1598	103	414	13	15	2	3	6	8	1	1
Limothrips ceralium Haliday	3	4	1	1	-	-	-	-	-	-	-	-
Rhipidothrips gratiosus Uzel	4	24	-	-	-	-	-	-	-	-	-	-
Tenothrips frici (Uzel)	1	5	1	8	-	-	-	-	-	-	-	-
Thrips hawaiiensis (Morgan)	111	594	41	171	5	7	2	3	1	2	1	1
Thrips meridionalis (Preisner)	8	48	3	8	-	-	-	-	-	-	-	-
Thrips pillichi Priesner	1	13	0	0	-	-	-	-	-	-	-	-
Thrips tabaci Lindeman	51	347	21	99	-	-	-	-	-	-	-	-
Thrips trehernei Priesner	1	34	1	1	1	1	-	-	-	-	-	-

* S.N: Sample number, I.N: Individual number. The dash indicates that no individuals were collected.

In conclusion, *O. niger* and *O. laevigatus* were the predominant species in different cultivated and weedy plants in Adana Province. These predatory Anthocorids were collected mostly with the pest thrips, *F. occidentalis* and *T. hawaiiensis* in diverse agricultural habitats. Although *O. niger* is one of the most common species in the survey area, it is known that rearing of *O. niger* under laboratory conditions is not efficient (Bahşi & Tunç, 2008). However, *O. laevigatus* has been commercially used to control *F. occidentalis* in European greenhouses (van Lenteren, 2012) as well as in Turkey. Alfalfa, faba bean and sunflower were determined as important host plants supporting both *Orius* and thrips species. Also, sesame

may be an important companion plant to promote predatory bugs for augmentative and conservative biological control strategies in agroecosystems. The protection of natural populations of predatory bugs is crucial to create a balance between pest and beneficial insects in agricultural areas. Presence of *Orius* spp. in agroecosystems may be a key factor for maintenance of the suitable IPM programs.

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Original article (Orijinal araştırma)

New records of the parasitoids of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in newly invaded areas in Turkey: molecular identification

Türkiye'de yeni istila edilen alanlarda *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae)'nin yeni parazitoit kayıtları: moleküler tanımlamaları

Gülay KAÇAR[1*]

Abstract

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) is an invasive pest species of various fruit crops in the USA and Europe. Although *D. suzukii* has been recently reported in strawberry in Erzurum and other newly invaded areas in Turkey (e.g., Ankara, Bolu, Çanakkale and Düzce), there is only limited information on its indigenous parasitoids. In this study, four hymenopteran parasitoids, the larval parasitoids of *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault, 1979), *Leptopilina heterotoma* (Thomson, 1862) (Figitidae) and the pupal parasitoids of *Pachycrepoideus vindemmiae* (Rondani, 1875) (Pteromalidae) and *Trichopria drosophilae* (Perkins, 1910) (Diapriidae), were collected from frugivorous drosophilid species. *Leptopilina boulardi* and *T. drosophilae* were found for the first time in Turkey. *Leptopilina heterotoma* and *P. vindemmiae* were the most common parasitoid species, reared from field-collected fruit samples in this study. The laboratory assays revealed that both pupal parasitoids developed from *D. suzukii* pupae, but the association of *L. heterotoma* and *L. boulardi* with *D. suzukii* is yet to be confirmed. The PCR amplification of the cytochrome c oxidase subunit I loci of mtDNA of the representative four parasitoid samples produced different lengths of DNA fragments, ranging from 633 bp to 658 bp. BLASTn queries based on the COI of the parasitoid samples showed that the sequences were 99-100% identical to those of the corresponding species in the GenBank database.

Keywords: Molecular diagnostic, new parasitoids, spotted wing drosophila, Turkey

Öz

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), Avrupa ve Amerika'da çeşitli meyvelerde zarara neden olan istilacı bir türdür. *Drosophila suzukii*, son zamanlarda Türkiye'de Erzurum'da çilekte rapor edilmesine ve o zamandan beri yeni alanları (Ankara, Bolu, Çanakkale ve Düzce vs.) istila etmesine rağmen, onun yerli parazitoitleri çok az bilinmektedir. Bu çalışmada, dört hymenopter parazitoit, larval parazitoitler *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault, 1979), *Leptopilina heterotoma* (Thomson, 1862) (Figitidae) ve pupal parazitoitler *Pachycrepoideus vindemmiae* (Rondani, 1875) (Pteromalidae) ve *Trichopria drosophilae* (Perkins, 1910) (Diapriidae) türleri, meyvelerde bulunan drosophilid türlerinden elde edilmiştir. *Leptopilina boulardi* ve *T. drosophilae* türlerinin Türkiye'de ilk defa varlığı belirlenmiştir. *Leptopilina heterotoma* ve *P. vindemmiae,* toplanan örneklerden en fazla bulunan türler olmuştur. Laboratuvar denemeleri iki pupa parazitoitinin *D. suzukii* pupalarından geliştiğini belirlenmesine rağmen, *L. heterotoma* ve *L. boulardi*'nin *D. suzukii*'den geliştiklerini hala doğrulanamamıştır. Dört parazitoit türü temsil eden örneklerin mitokondrial DNA'sının cytochrome c oxidase subunit I lokusunun PCR ile çoğaltılması sonucu 633 bp ile 658 bp arasında DNA segmentlerini ürettiği belirlenmiştir. Bu ürünlerin BLASTn analizi sonucu GenBank'daki referans bireylerin sekanslarıyla %99-100 benzerliğe sahip olduğu tespit edilmiştir.

Anahtar sözcükler: Moleküler tanımlama, yeni parazitoitler, noktalı kanatlı drosophila, Türkiye

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Introduction

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), which is primarily known as spotted wing drosophila in the USA and indigenous to southeast Asia (Kanzawa, 1939), has rapidly spread to Europe, South and North America (Bolda et al., 2010; Hauser, 2011; Walsh et al., 2011; Berry, 2012; Depra et al., 2014; Emiljanowicz et al., 2014). After the fly was first found in Spain and Italy in 2008 (Walsh et al., 2011), *D. suzukii* has increasingly spread into many other countries of Europe, such as Croatia, France, Portugal and Slovenia (Milek et al., 2011; Withers & Allemand, 2012). In Turkey, it was first recorded in Erzurum in 2014 (Orhan et al., 2016; Tozlu et al., 2016), and further found Ankara in 2015 (Önder et al., 2016), Bolu and Düzce in 2016 (Kaçar & Koca, 2017), Karaman in 2017 (Ögür et al., 2018) and Çanakkale in 2018 (Efil, 2018; Kasap & Özdamar, 2019). *Drosophila suzukii* appears to be spreading rapidly in Turkey. The most probable reason for this rapid spread is the international trade of infested fruits (EPPO, 2002; Westphal et al., 2008). *D. suzukii* is an extremely polyphagous invasive pest which principally damages soft-skinned fruits (Lee et al., 2011). The female of *D. suzukii* lays eggs in undamaged fruit owing to the serrated female ovipositor (Sasaki & Sato, 1995). Thus, it usually lays eggs on ripe fruit (Mitsui et al., 2007). *Drosophila suzukii* causes physical damage to fresh fruit by means of secondary pathogen infections, which can access the damaged skin of the fruit and cause faster deterioration, resulting in the yield losses of 30-100% in fruit production (Bolda et al., 2010).

Drosophila species are attacked by about 50 hymenopteran parasitoids which have an important function in controlling the drosophilids (Carton et al., 1986). Hymenopteran parasitoids mostly from the genera *Asobara* (Braconidae), *Leptopilina* and *Ganaspis* (Figitidae), *Trichopria* (Diapriidae), and *Pachycrepoideus* (Pteromalidae) commonly parasitize drosophilids (Carton et al., 1986; Hertlein, 1986). Several larval parasitoids, including *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault, 1979), *Leptopilina heterotoma* (Thomson, 1862), *Leptopilina japonica* Novković & Kimura, 2011*, Leptopilina japonica formosana* Novković & Kimura, 2011*, Asobara japonica* Belokobylskij, 1998*, Asobara brevicauda* Guerrieri, Giorgini, Cascone, Carpenito & Achterberg, 2016*, Asobara leveri* (Nixon, 1939)*, Asobara tabida* (Nees, 1834)*, Ganaspis xanthopoda* (Ashmead, 1896) and *Ganaspis brasiliensis* (Ihering, 1905)*,* and pupal parasitoids*;* e.g., *Pachycrepoideus vindemmiae* (Rondani, 1875) (Pteromalidae) and *Trichopria drosophilae* (Perkins, 1910) (Diapriidae), are commonly associated with *Drosophila* species (Fleury et al., 2004; Mitsui et al., 2007; Chabert et al., 2012; Novkovic et al., 2011; Poyet et al., 2013; Kimura & Novkovic, 2015; Daane et al., 2016; Girod et al., 2018a, b, c; Giorgini et al., 2019). Recently, several studies conducted in the USA and European countries have shown that most of the larval drosophila parasitoids are unable to successfully develop in *D. suzukii* (Chabert et al., 2012; Poyet et al., 2013; Rossi Stacconi et al., 2013; Girod et al., 2018a, b, c; Giorgini et al., 2019*). Asobara japonica* successfully developed in *D. suzukii* compared with other widespread larval parasitoids (*A. tabida* and *G. xanthopoda*) although *L. heterotoma* may be country-specific (Mitsui et al., 2007; Kimura & Novkovic, 2015; Rossi Stacconi et al., 2015; Girold et al., 2018). Only two pupal parasitoids, *P. vindemmiae* and *T. drosophilae*, readily attack *D. suzukii* (Rossi Stacconi et al., 2015, 2017; Kaçar et al., 2017). Although *T. drosophilae* is polyphagous parasitoid, it may be one candidate for controlling populations of *D. suzukii* (Girod et al., 2018c; Rossi Stacconi et al., 2018).

The parasitoids of the order Hymenoptera are generally identified according to their morphological characteristics, which leads to challenges in diagnosis due to their body size and undefined characteristics (Tomanovic et al., 2003). The molecular analysis of DNA sequences is considered as a complementary tool for the morphological identification of insects (Farrokhzadeh et al., 2014). Molecular identification has allowed quick recognition, distinction and identification of diverse species based on the DNA sequencing of single samples. However, since researchers perform the identification of species using only key morphological characteristics, the results may not be definitive and they are mostly at genus level. The molecular techniques recently developed allow identification of insects at the species level. For example, the cytochrome c oxidase subunit I (COI) locus of mitochondrial DNA has been employed as a reliable tool to accurately identify parasitoid species (Fre´zal & Leblois, 2008; Linares et al., 2009).

The current study aimed to explore and identify drosophilid parasitoids in different geographical regions of Turkey and present an alternative identification technique for these parasitoids based on phenotypic characteristics to support diagnoses.

Materials and Methods

Collection areas and methods

The survey for the parasitoids of frugivorous drosophila was conducted in two provinces in Turkey, Bolu and Düzce, from May 2016 to December 2018. The samples of drosophilids and parasitoids were collected from infested fresh and fallen decaying fruit (including apples, blackberries, cherries, figs, grapes, pears, persimmons, plums, raspberries and strawberries) in the field. Samples were collected during or after the harvesting seasons for each fruit. The fruit samples were placed in a cooler box to be transferred to a climate room. The location, date and collector were recorded, and each fruit sample was labeled accordingly. The fruit samples collected in the field were separately put in transparent containers with ventilated lids on a layer of moist filter paper until pupation.

All fly pupa reared from the field-collected larvae were inspected and separately placed in 5-cm Petri dishes until the appearance of drosophilid adults or parasitoids which were preserved in alcohol (95%) for later identification. The samples were kept in a climate cabin at 40-70% RH, 22±3°C and 12:12-h L:D photoperiod with natural light in the laboratory. Four parasitoid species were paired and maintained on *D. suzukii* and *Drosophila melanogaster* (Meigen, 1830) supplied with artificial food of 10% honey-water provided for the parasitoids with wet paper towel in plastic container as additional water source. The flies were collected from the field fruits in Düzce and provided an artificial corn meal (Dalton et al., 2011). Petri dishes (9 cm) were filled with 35 g of artificial diet were placed in cages as an oviposition media and changed in every 2 d. These were used for rearing drosophilids and their parasitoids. The parasitoid species were tested in the laboratory to confirm their suitability as a host for *D. suzukii*. Emerged parasitoids were separated in *L. heterotoma* and *L. boulardi* males and females. Each parasitoid pair were individually released, as soon as they were collected, in rearing tubes with drosophila medium and 10 *D. suzukii* 1-2-d old larvae or pupae and honey-water droplets for confirming tests. After all parasitoids had emerged, all dead unemerged pupae were dissected. The majority of emerged flies (notably those from the same dishes where were parasitoids have emerged) were preliminary identified.

Molecular identification of the parasitoids

The fly species were identified using the identification key according to Markow & O'Grady (2006). *D. suzukii* pupae were distinguished considering the existence of a couple of distinct breathing tubes on the anterior end (Kanzawa, 1939). The parasitoids species were identified using the identification key described by Legner et al. (1976), Boucek & Rasplus (1991) and Carton et al. (1986). The identification of the four parasitoids were confirmed by all experts. The figitids were identified by Dr. Mattias Forshage and deposited with the Entomology Collection of the Swedish Museum of Natural History (Stockholm, Sweden). *Trichopria drosophilae* was confirmed or identified by Dr. Ovidiu Gavrilovici (Department of Psychology and Education Sciences, Universitatea Alexandru Ioan Cuza, Iaşi, Romania). *Pachycrepoideus vindemmiae* were confirmed or identified by Dr. Habil Mircea-Dan Mitroiu (Biology Faculty, Alexandru Ioan Cuza University, Romania). All parasitoid samples were also deposited at Bolu Abant Izzet Baysal University, Agriculture and Science Faculty, Bolu, Turkey.

The extraction of the nucleic acids of all specimens either dried or preserved in 95% ethyl alcohol was conducted using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The DNA extracts were quantified spectrophotometrically by a DS-11 FX series spectrophotometer (DeNovix Inc., Wilmington, DE, USA) and ultimately diluted to 10 ng/µl using sterile ddH_2O. The PCR amplification based on part on the mitochondrial protein-coding gene and COI

gene was performed using the universal primer pair LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al., 1994). Amplification reactions were conducted in a 50 µl reaction mixture containing 5 µl 10× PCR reaction buffer, 0.4 µM of each primer, 50 ng DNA template, 0.2 mM each dNTPs, and 1.25 unit Taq DNA Polymerase (New England BioLabs, MA, USA; Neb #M0320S). The amplification program for the COI locus was as follows: an initial denaturation step at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 52°C for 30 s and 72°C for 1 min, and a 5-min final extension at 72°C. All the PCR products were confirmed electrophoretically using agarose gel (1.2% w/v) and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) following the manufacturer's instructions. The DNA fragments were sequenced by the Sanger nucleotide sequencing method provided by a commercial company (Macrogen Inc., Seoul, South Korea).

The resulting sequences were aligned with ClustalW, which is a multiple sequence alignment method (Thompson et al., 1994). The sequences were analyzed and BLAST-searched against GenBank for the identification of the closest presented reference sequences in the NCBI nucleotide collection (http://blast.ncbi.nlm.nih.gov/Blast). The phylogenetic analyses of the sequences and reference sequences available in GenBank were performed using the MEGA 7 software (Kumar et al., 2016). A neighbor-joining tree was constructed using Tamura & Nei's (1993) model with 1000 bootstrap replicates. The sequence of *Trichogramma evanescens* Westwood, 1833 (Hymenoptera: Trichogrammatidae) was included as the out group to root the phylogenetic tree.

Results and Discussion

The adult parasitoid species were determined in field-collected drosophilid specimens from various fruit samples. In the present study, four drosophilid parasitoid species were collected from Bolu and Düzce and identified as *L. boulardi* and *L. heterotoma*, and *P. vindemmiae* and *T. drosophilae*, which are new to the fauna of Turkey (Figure 1). Both pupal parasitoids are usually solitary; *P. vindamiae* is ectophagous but *T. drosophilae* is endophagous (Legner et al., 1976). Four parasitoid species were determined from the field-collected fruit samples from Düzce: *L. boulardi* from strawberry, *P. vindemmiae* from wild blackberry in 2016, and *L. heterotoma* and *T. drosophilae* from pear in 2017 (Figure 1). *Leptopilina heterotoma* from a yellow plum in 2017 and *P. vindemmiae* from apple in 2016 in Bolu. While the laboratory assays confirmed that *P. vindemmiae* and *T. drosophilae* successfully parasitized *D. suzukii* pupae, there was no evidence that the growth of the larval parasitoid, *L. heterotoma*, was associated with *D. suzukii* in the laboratory or field.

Leptopilina species are common parasitoids of Drosophilidae throughout the world (Allemand et al., 2002). *Leptopilina* individuals are solitary koinobiont endoparasitoid that attacks a single host that continues feeding and growing during parasitism (van Noort et al., 2015; Harvey et al., 2016). The typical *Leptopilina* are often superficially similar to *Ganaspis*. *Leptopilina* males have the third and fourth antennomeres slightly curved and have a distinct hair tuft on the metapleural corner (van Noort et al., 2015). *Leptopilina heterotoma* and *L. boulardi* are the most known parasitoids in Africa, Asia, Europe and North America, attack drosophilid hosts, especially *D. melanogaster*, but neither developed from the immune resistance of SWDs under laboratory conditions (Chabert et al., 2012; Poyet et al., 2013; van Noort et al., 2015). However, *T. drosophilae* and *P. vindemmiae* attack the pupae of *D. suzukii* and can effectively parasitize in the laboratory (Kaçar et al., 2017; Rossi Stacconi et al., 2017). *Pachycrepoideus vindemmiae* was reported that the generalist solitary ectoparasitoid which attacked more than 60 fly species (Carton et al., 1986; Hanson & Gauld, 1995; Wang & Messing, 2004). The parasitism rate of *P. vindemmiae* was found over 80% in raspberries in laboratory tests (Chabert et al., 2012; Gabarra et al., 2015). *Pachycrepoideus vindemmiae* was also highly reproduced in *D. suzukii* in the laboratory (Rossi Stacconi et al., 2015). *Trichopria drosophilae* is an idiobiont endoparasitoid specialized in fruit drosophilids (Carton et al., 1986; Chabert et al., 2012; Wang et al., 2016). Of the parasitoids, *T. drosophilae* has been reported several times in the USA and Europe and proven to attack either *D. melanogaster* or *D. suzukii* (Gabarra et al., 2015;

Rossi Stacconi et al., 2015; Mazzetto et al., 2016). Currently, this species is considered to be one of the best candidate parasitoids for the biological control of *D. suzukii.* Field trials in Italy showed that *T. drosophilae* is widely spread all over the country and presents as the promising candidate for the augmentative control of *D. suzukii* in fields (Mazzetto et al., 2016; Rossi Stacconi et al., 2018).

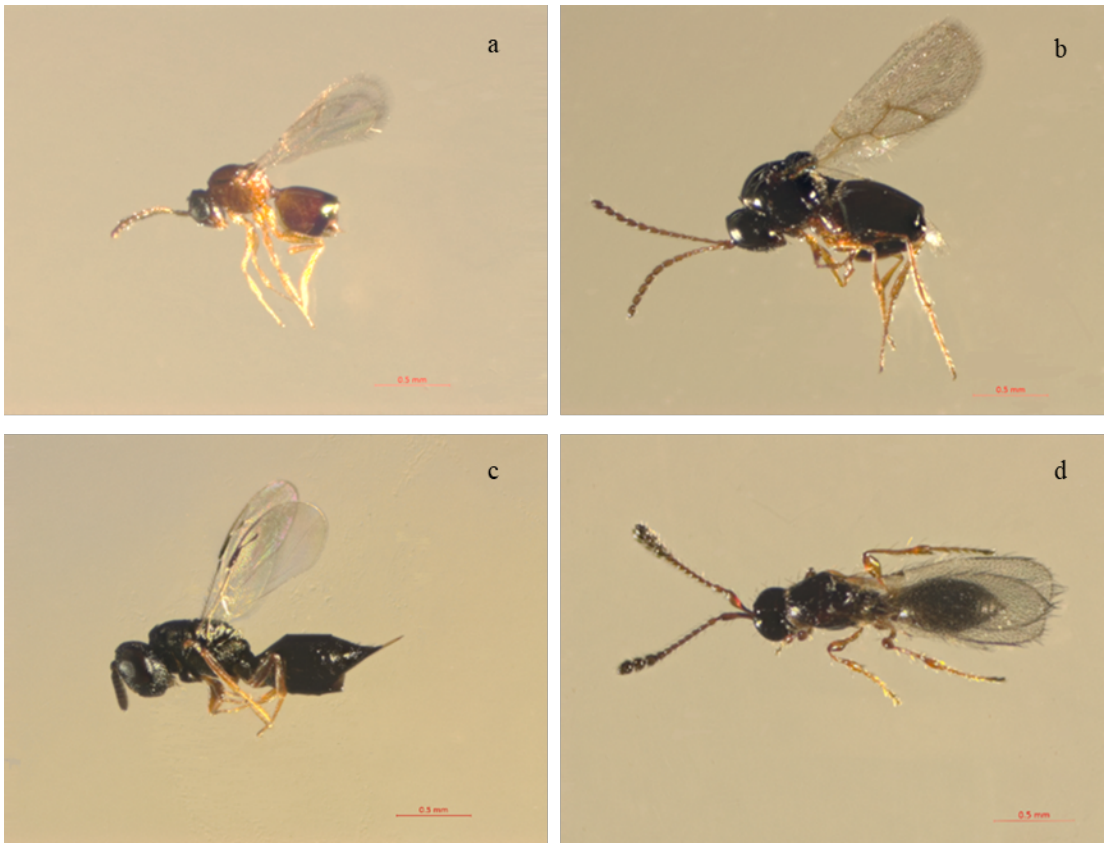

Figure 1. a) *Leptopilina boulardi* Barbotin, Carton & Keiner-Pillault ♀; b) *Leptopilina heterotoma* Thomson ♂; c) *Pachycrepoideus vindemmiae* (Rondani) ♀; d) *Trichopria drosophilae* Perkins ♀.

The PCR amplification of the COI loci of the mtDNA of the representative parasitoid samples produced different lengths of DNA fragments, ranging from 633 to 658 bp. The BLASTn queries based on the COI of the samples showed that the sequences were 99-100% identical to those of the corresponding species in the database of GenBank. There were two sequences of *Trichopria* sp. matching the current sequence at 641/642 sequence identity. The sequences data were deposited in the GenBank with the accession numbers of MK798163, MK813907, MK798164 and MK798165 for the *T. drosophilae, P. vindemmiae, L. boulardi*, and *L. heterotoma* samples, respectively. Morphologically cryptic species are known in *L. heterotoma* (Novkovic et al., 2011). The phylogenetic tree was included these cryptic species. Dzc06 was on a branch with a clone from Sapporo, Japan (AB583568). Phylogenetic analyses based on the COI sequences of the samples obtained from this study and the reference sequences of the corresponding species found in the GenBank indicated that the samples belonging to the same species were clearly separated from each other and *T. evanescens* with a bootstrap support of 100% (Figure 2).

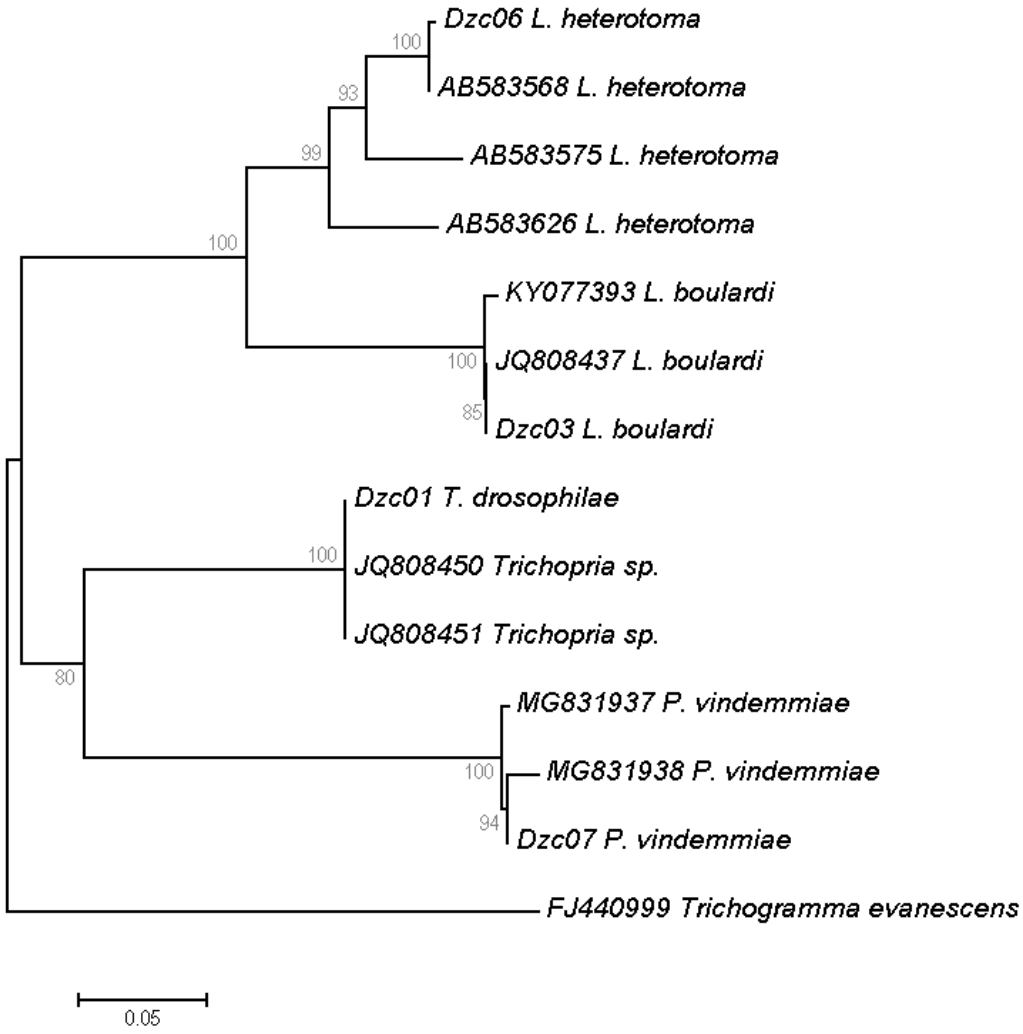

Figure 2. Phylogeny of the parasitoids associated with *Trichogramma evanescens* Westwood based on the COI locus (neighbor-joining). The bootstrap values (percentage, based on 1000 replicates) are shown on the branches.

Drosophila suzukii is a new quarantine fly pest attacking all types of fruit throughout the world. This fly is one of the serious economic pests that especially damage soft fruit. The native parasitoids of *D. suzukii* significant contribute to its management worldwide, but they are not fully known in Turkey. In the current study, two drosophilid parasitoids were determined in geographical areas that had not been previously surveyed. In this study, rather than morphological identification, the molecular diagnostic method was used for the drosophilid parasitoid species of *D. suzukii* since it is a faster and easier alternative identification technique. The pupae of *D. suzukii* were successfully parasitized by *P. vindemmiae* and *T. drosophilae* according to the laboratory experiments. However, further investigations are needed to offer more definite results related to larval parasitoids.

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Original article (Orijinal araştırma)

Development of methodology for resistance screening of chickpea genotypes collected in Turkey to the root lesion nematode, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae)[1]

Türkiye'den toplanan nohut genotiplerinin kök yara nematoduna, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) karşı taranması

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Abstract

The root lesion nematode, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) are considered economically important plant parasitic nematodes affecting chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] production. A major strategy to develop resistance to root lesion nematodes in chickpea is to assess and exploit their natural variation. Therefore, nine accessions of wild *Cicer reticulatum* Ladiz. (Fabales: Fabaceae), *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) and domesticate *C. arietinum* were assessed for resistance to *P. thornei* according to multiplication rate of the nematode. This study was conducted during 2014-2015 to detect the suitable initial inoculum density (150, 225 and 300) per plant duration of experiment (16 and 20 weeks) for resistance test to *P. thornei* in chickpea cultivars. There was no significant difference between growing times 16 and 20 weeks and between the initial inoculum density of 225 and 300 nematodes. The only significant difference was observed at a low initial inoculum density of 150 nematodes in all tested cultivars. Therefore, the initial inoculum density of 225 and the growing time of 16 weeks were selected to access of chickpea genotypes for resistance study to *P. thornei*.

Keywords: Chickpea, optimization, *Pratylenchus thornei*, resistance, screening method

Öz

Kök yara nematodu, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) dünyada nohut [*Cicer arietinum* L. (Fabales: Fabaceae)] üretimini etkileyen, ekonomik açıdan önemli bitki paraziti nematod türü olarak kabul edilmektedir. Nohutta kök yara nematodlarına karşı direnç geliştirmenin temel stratejisi, doğal çeşitliliklerini değerlendirmek ve kullanmaktır. Bundan dolayı *P. thornei*'ye karşı dayanıklılık çalışmalarında, üreme oranı dikkate alınarak, dokuz adet yabani *Cicer reticulatum* Ladiz. (Fabales: Fabaceae), *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) ve yerli *C. arietinum* türleri değerlendirilmiştir. Bu çalışma, nohut çeşitlerinin *P. thornei*'ye karşı dayanıklılık denemeleri için, uygun hasat süresi (16 ve 20 hafta) ve bitki başına başlangıç inokulum yoğunluğunu (150, 225 ve 300) belirlemek için 2014-2015 yılları arasında yürütülmüştür. Hasat zamanı 16 ve 20 hafta ile 225 ve 300 başlangıç inokulum yoğunluğu arasında anlamlı bir fark olmadığı, buna karşın bitki başına 150 inokulum yoğunluğunun istatistiki olarak daha düşük etki gösterdiği saptanmıştır. Bu nedenle, nohut çeşitlerinde *P. thornei*'ye karşı dayanıklılık çalışmaları için en uygun deneme parametrelerinin, 225 başlangıç inokulum yoğunluğu ve 16 hafta hasat zamanı olduğu belirlenmiştir.

Anahtar sözcükler: Nohut, optimizasyon, *Pratylenchus thornei,* dayanıklılık, tarama yöntemi

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Introduction

Chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] has an important place in total legume production in the world. The most important chickpea producing countries are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, USA, and Canada (FAO, 2019). Turkey is ranked fifth in the world for chickpea production (FAO, 2019). It may have been grown and widely cultivated as a food legume in Turkey 7500 years ago (Singh & Ocampo, 1997). More species of plant parasitic nematodes have been found in the root and rhizosphere of the chickpea production areas in the world. Plant parasitic nematodes generally feed on different parts of the plant, especially on roots and other subterranean plant structures such as rhizomes in legume crops. Sasser & Freckman (1987) reported that yield losses in chickpea caused by several plant parasitic nematode species was about 13.7% worldwide. Also, Sikora et al. (2005) showed nematode damage to chickpea can make plant sensitive to disease and other stress. Similarly, Atkinson et al. (1995) showed that they are major pests of agriculture crops and have global economic effects on crops of more than 100 billion USD every year by the way. The root lesion nematodes, *Pratylenchus* spp. [*Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* Rensch, 1924 (Tylenchida, Pratylenchidae)], are the most important constraint to legume production and have a wide distribution in in many regions in Turkey (82% of chickpea fields) and affect many agricultural crops around the world (Tanha Maafi et al., 2009; Behmand et al., 2019). Root lesion nematodes in chickpea need to be surveyed periodically and conducted risk analysis, based on its economic importance.

Pratylenchus thornei, P. neglectus, *Pratylenchus penetrans* Cobb, 1917 and *Pratylenchus crenatus* Loof, 1960 (Tylenchida: Pratylenchidae), are the most important root lesion nematodes in the world (Vanstone et al., 1998). Among the root lesion nematodes, *P. thornei* and *P. neglectus* are globally distributed and they enter the root tissue of host plant for feeding and reproduction (Nicol et al., 2004). Also, some studies indicated that in the terms of damage caused by these nematodes is second importance as a nematode problem in the world after root-knot nematodes (Barker & Noe, 1987; Jatala & Bridge, 1990). *Pratylenchus thornei* is one of the most important plant parasitic nematodes causing yield losses of up to 40% in cereals and legumes in dryland cropping areas of southeastern Australia (Thompson et al., 1995; Vanstone, 1998). These nematodes have been found widely distributed in a wheat fields in Turkey (Behmand et al., 2019). However, study of resistance screening methods for chickpea is limited in Turkey. Standardization of factors is important for development of a screening methodology that is stable and able to distinguish various levels of resistance. Thompson et al. (2010) reported that among the root lesion nematodes, *P. thornei* was a major problem in the Australian grain area. Similarly, Taylor et al. (2000) showed that both *P. thornei* and *P. neglectus* are important in chickpea in Australia. Chickpea, *C. arietinum* infested with root lesion nematodes showed symptoms of stunted growth and leaf chlorosis. *Pratylenchus* spp. infection causes symptoms of reduction in root hairs or nodules, and causes yield losses greater than 50% in chickpeas (Castilo & Jimenez, 1998; Castilo & Vovlas, 2007). Thompson et al. (2000) reported that an integrated pest management strategy including rotation with non-host crops or fallow period, and use of resistant cultivar is the best method to control nematode population in the cropping system involving grain legumes and cereals. Similarly, Trudgill (1992) indicated that the use of tolerant cultivars can grow and yield well in the extremely infested region with plant parasitic nematodes and can keep the population density below damage threshold levels.

Population density of nematodes is influenced by the reproduction potential and various factors such as initial population density and growing period. Identification of factors such as initial population density of nematodes and harvest time to keep the nematode population below damage threshold levels is very important in a screening study. Estimating threshold levels and calculating economic thresholds for most nematode/crop problems is not yet possible. Consequently, information on the relationship between initial population densities of nematodes and crop performance is necessary to obtain such data. An

understanding of the information in these relationships is basic to being able to predict yield reductions from estimates of pre-planting nematode population densities (Pi).

There is little diversity of resistance genes in *C. arietinum* cultivars to plant parasitic nematodes (Smýkal et al., 2015). New collections of *Cicer reticulatum* Ladiz. and *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) have been found to have high genetic diversity compared to domestic chickpea (Thompson et al., 2011). A similar study by Thompson et al. (2000) with a limited number of the accession of wild (*C. reticulatum* and *C. echinospermum*) and domesticated *C. arietinum* showed that the wild *Cicer* species can grow and have better tolerance in soil heavily infested with nematodes, and can be used in chickpea breeding programs for nematode resistance.

The aim of the study was to establish a reliable methodology for assessing wild and domesticated chickpea accessions for resistance to *P. thornei* by using different initial inoculation density and time of assessment.

Materials and Methods

Chickpea accessions

In this study, the accessions evaluated included three landraces (Eğil, Kalkan and Şırnak) of *C. reticulatum*, three landraces (Destek, Karabahçe and Ortance) of *C. echinospermum* and three cultivars (Azkan, Çağatay and Gökçe) of *C. arietinum* collected from Diyarbakır, Şanlıurfa and Şırnak Provinces of Turkey by the Department of Field Crops, Harran University. These were assessed in the laboratory for resistance to *P. thornei* under controlled conditions.

The seeds were scarified by making a small cut in the seed coat before germinating to improve water absorption and germination in the wild genotypes. The individual chickpea seeds were disinfected with hypochlorite (4%) and alcohol (30%) pre-germination and placed on the surface of wet filter paper in sterile Petri dishes and seeds were germinated at 21°C for 3 d.

Nematode source

Pratylenchus thornei was cultured on carrot discs by using the method described of Nicol and Vanstone (1993). Nematodes used this study were originally collected from a chickpea production area in Şanlıurfa Province (Harran District) located in the Southeastern Anatolia Region of Turkey and reproduced in the nematology laboratory at Çukurova University. A total of root and soil samples were collected from chickpea field between June and July 2014. Each root and soil samples included 5-10 samples (taken at a depth of 10-20 cm), with a total of 1-2 kg of soil/sample. Nematodes were extracted from both of the roots and soil with using the Baermann funnel technique in the laboratory (Hooper, 1986).

Experimental design

The study was conducted in completely randomized block with four replicates. One germinated seed each was sown into small open-ended tubes (16 cm high, 2.5 cm diameter) that contained of 60 g field soil, (73% clay, 16.5% silt and 10% river sand) that had been autoclaved for 2 h at 121°C.

One week after planting, the nematodes were transferred to room temperature at 25°C and plants were inoculated with either 150, 225 or 300 nematodes/tube in 1 ml water.

Experiment was conducted at the same time with all of the plant accessions and grown in a growth room at 25°C and 50% RH under a 16:8 h L:D photoperiod provided by high pressure sodium lamps.

Assessment of nematode multiplication

After 16 and 20 weeks, plant shoots were removed and the nematodes extracted from roots and soils by the Baermann funnel technique (Hooper, 1986). Then 1 mL of a suspension including nematodes was counted with four replicates in a counting slide under a light microscope and the total number of the nematodes extracted from plant and soil calculated. Multiplication rate (MR) of *P. thornei* was calculated MR = Pf/Pi, where Pf is the final and Pi is the initial nematode population density. For this purpose, the initial and final populations were the number of nematodes/tube.

Statistical analysis

The number of nematodes/tube from the experimental plots were analyzed using a completely randomized design ANOVA in Genstat (V13). Significant differences among treatments and replication of data were calculated at $P < 0.001$. Outliers and variance distribution were assessed using residual plots.

Results

Effect of harvest time on nematode multiplication

There was no significant difference between testing period time (16 and 20 weeks) and *Cicer* species $P > 0.001$ (Table 1). It means the multiplication rate of *P. thornei* was not differentiated among chickpea cultivars when the time of harvest changed from 16 to 20 weeks. However, the development of population density of *P. thornei* in *C. arietinum* was higher than the population density of *C. reticulatum* and *C. echinospermum*. Also, the population density of *P. thornei* in *C. echinospermum* except for 16 weeks when inoculated with 300 nematodes as Pi was lower than the population density of *C. reticulatum* and *C. arietinum* in both growing times of 16 and 20 weeks under laboratory condition (Figure 1).

Effect of initial Inoculum density on nematode multiplication

There was a significant difference between the initial inoculation densities of 150, 225 and 300 nematodes/tube ($P < 0.001$). The nematode density had a major effect on final numbers ($P < 0 .001$) and there is some indication of an interaction between chickpea species and the linear effect of nematode density ($P < 0.001$) (Table 1). Figure 2 shows the final population density of *P. thornei* increasing at initial inoculum densities of 150-225 nematodes/plant, but not at 300 nematodes/plant, and that *C. echinospermum* was more sensitive to the initial nematode density, because it responded more steeply to the 150-225 change ($P = 0.087$). Thus, *C. echinospermum* had lower final nematode counts than the other two species at 150 nematodes/plant, while at 225 and 300 nematodes there is no significant differences observed. Therefore, the data indicate that 225 nematodes is the optimal level. Development population density of *P. thornei* was different in the inoculation density of 150 nematodes in all species. It means the population density of nematodes changed in all species when initial inoculum density was 150 nematodes/plant. In order, the highest population density of *P. thornei* in the inoculation density of 150 nematodes was observed in *C. arietinum* (Gökçe MR = 3, Çagatay MR = 2.9 and Menemen MR = 2.8) and the lowest population density observed in *C. echinospermum* (Karabahçe MR = 2.5, Destek and Ortance MR = 2.6) (Figure 2). Also, there was no significant difference between *Cicer* species and species with an initial inoculation density of 225 and 300 nematodes except initial inoculum density of 150 nematodes. Likewise, there was no difference observed in the population density of *P. thornei* in the inoculation density of 225 and 300. Also, the final nematode counts rose to a peak at 225 nematodes/plant in all species, with no change as the population rises to 300 nematodes/plant (Figure 2).

Table 1. Analysis of variance of nematode multiplication rates of accessions and their interactions

Period. Density. Rep stratum	Source of variation*				
	d.f.	s.s	m.s	v.r	F pr
Period	1	0.7241	0.7241	0.57	0.458
Density	2	14.1543	7.0771	5.62	0.013
Lin	1	11.2589	11.2589	8.94	0.008
Quad	1	2.8954	2.8954	2.30	0.147
Period.Density	2	0.4220	0.2110	0.17	0.847
Period.Lin	1	0.3451	0.3451	0.27	0.607
Period.Quad	1	0.0769	0.0769	0.06	0.808
Residual	18	22.6785	1.2599	8.86	
Period.Density.Rep.*Units* stratum					
Species	2	0.3471	0.1735	1.22	0.298
Period.Species	2	0.6081	0.3041	2.14	0.122
Density.Species	4	0.8025	0.2006	1.41	0.234
Lin.Species	2	0.7065	0.3533	2.48	0.087
Quad.Species	2	0.0960	0.0480	0.34	0.714
Species.Coll_site	4	0.2728	0.0682	0.48	0.751
Period.Density.Species	4	0.4039	0.1010	0.71	0.587
Period.Lin.Species	2	0.0734	0.0367	0.26	0.773
Period.Quad.Species	2	0.3305	0.1653	1.16	0.316
Period.Species.Coll_site	4	0.0684	0.0171	0.12	0.975
Density.Species.Coll_site	8	0.2868	0.0358	0.25	0.980
Lin.Species.Coll_site	4	0.0992	0.0248	0.17	0.951
Quad.Species.Coll_site	4	0.1876	0.0469	0.33	0.858
Species.Coll_site.Var	2	0.4048	0.2024	1.42	0.244
Period.Density.Species.Coll_site	8	0.7664	0.0958	0.67	0.714
Period.Lin.Species.Coll_site	4	0.3056	0.0764	0.54	0.709
Period.Quad.Species.Coll_site	4	0.4608	0.1152	0.81	0.521
Period.Species.Coll_site.Var	2	0.4120	0.2060	1.45	0.238
Density.Species.Coll_site.Var	4	1.0689	0.2672	1.88	0.117
Lin.Species.Coll_site.Var	2	0.4619	0.2310	1.62	0.201
Quad.Species.Coll_site.Var	2	0.6070	0.3035	2.13	0.122
Period.Density.Species.Coll_site.Var	4	0.6446	0.1612	1.13	0.344
Period.Lin.Species.Coll_site.Var	2	0.1766	0.0883	0.62	0.539
Period.Quad.Species.Coll_site.Var	2	0.4681	0.2340	1.64	0.197
Residual	144	20.4873	0.1423		
Total	215	64.5524			

* df: contains degree of freedom which are measure of how much information is contained in each variance;
s.s: Means squares, which are calculated by multiplying the mean square and degree of freedom in the same row;
ms (Means squares): The variance between treatment;
v.r: The ratio of the between treatment variance to the within treatment variance;
F pr or P value: Significance value $P < 0.001$.

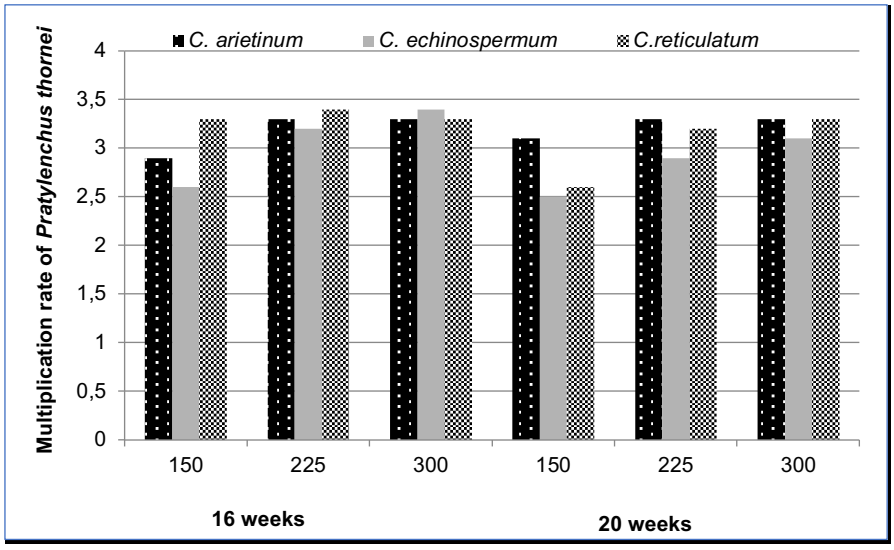

Figure 1. Multiplication rate of *Pratylenchus thornei* on *Cicer* species at two harvest time.

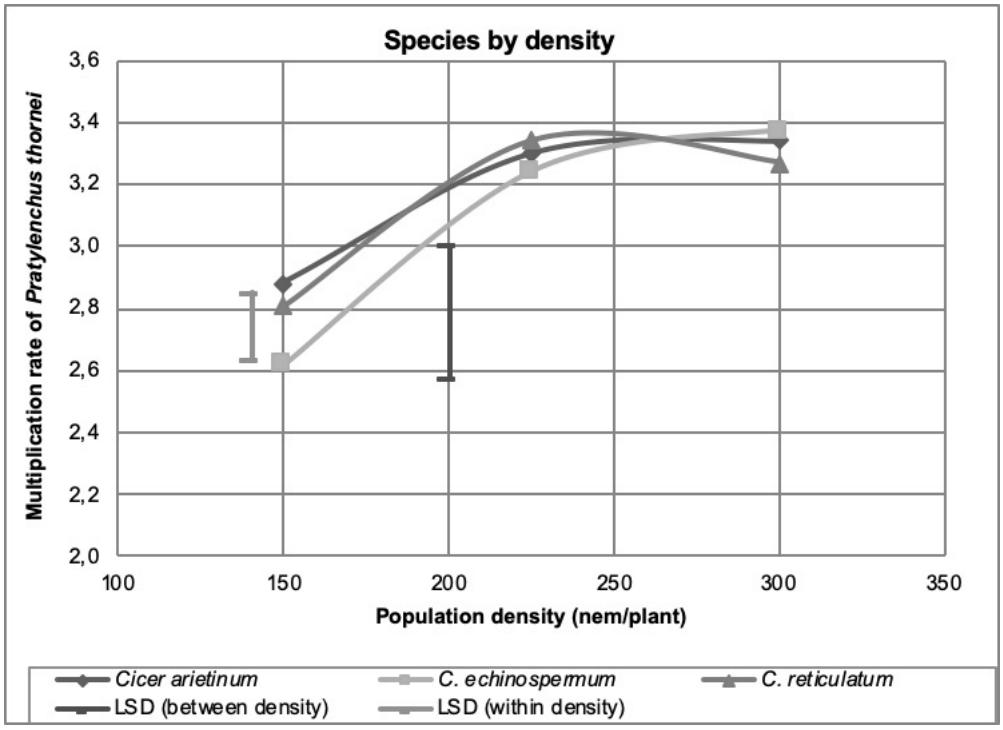

Figure 2. Multiplication rate of *Pratylenchus thornei* on chickpea species at different initial nematode densities.

The reproduction factor of *P. thornei* among chickpea genotypes is shown in Figure 3. The initial density of *P. thornei* clearly had an effect on nematode multiplication and that there is some indication of an interaction between species and the linear effect of nematode density. Statistically significant differences among cultivars with 150 nematodes/tube indicated that *C. echinospermum* has lower nematode multiplication than the other *Cicer* species, whereas at 225 and 300 nematodes/tube there is no changing observed among chickpea genotypes. Also, the population densities of *P. thornei* in plots of *C. arietinum* and *C. reticulatum* were more than *C. echinospermum* at 225 and 300 nematodes/tube. In order, among the *C. arietinum* and *C. reticulatum* genotypes, population development of *P. thornei* at Menemen and

Kalkan plots was less than Şırnak and Gökçe plots (Figure 3). Both were similarly responsive to *P. thornei* and, development of populations was more than any *C. echinospermum* genotype. Also, the relationships between wild and domesticated *C. arietinum* indicated that multiplication rate of *P. thornei* at wild *Cicer* spp. plots (*C. reticulatum* and *C. echinospermum*) were statistically significantly less than domesticated cultivars (*C. arietinum*).

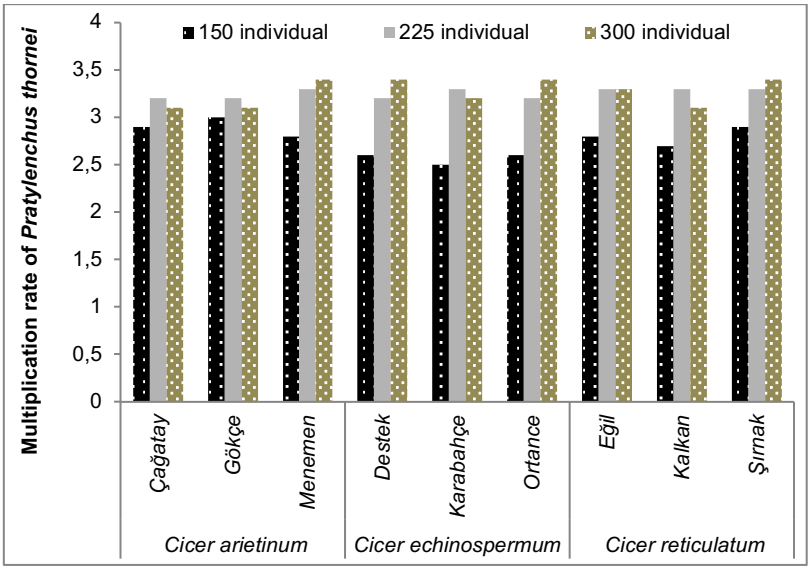

Figure 3. Nematode multiplication ratio of the chickpea cultivars in different initial inoculum densities of *Pratylenchus thornei*.

Discussion

Factors such as soil texture, soil temperature application technique, growing time and relationship between initial and final population density of plant can affect the population density of nematodes (Seinhorst, 1965; Oostenbrink, 1966; Toktay et al., 2012). These factors need to be carefully optimized. Other factors can also have an effect on the presence or absence of nematodes in the soil at given times during the growing time of a crop. Also, determination of the relationship between the initial and final population density of nematodes to keep the nematode population below the damage threshold level is important in chickpea breeding programs. Schomaker and Been (2006) suggested that the information on initial population density and determination of damage level under specific conditions for specific crops are essential for nematode pest management programs.

According to the results of this screening study with limited accessions of *Cicer* species, *C. echinospermum* was more resistant to *P. thornei* than *C. arietinum* and *C. reticulatum*. In other words, *C. arietinum* and *C. reticulatum* genotypes were the most susceptible to *P. thornei*. Both *C. arietinum* and *C. reticulatum* genotypes responded similarly to *P. thornei*, and were more susceptible than any *C. echinospermum* genotype. A similar study by Thompson et al. (2011) indicated that there were species differences in nematode sensitivity and multiplication of root lesion nematodes has been found to be different for each *Cicer* species.

The statistically significant difference of nematode multiplication between wild and domesticated genotypes indicated that wild species (*C. reticulatum* and *C. echinospermum*) were more resistant to *P. thornei* than domesticated *C. arietinum*. Singh and Ocampo (1997) reported that the use of *C. echinospermum* and *C. reticulatum* from the wild gene pool for chickpea is a practical option.

In this study, an efficient optimized method was developed for screening chickpea cultivars to *P. thornei*. In conclusion, this study indicated that inoculation each plant with an initial density of 225 nematodes/tube was the best selection to test chickpea cultivars for resistance to *P. thornei*. Similarly, Toktay et al. (2012) compared nematode inoculum density in wheat and reported that the best-inoculating density was 400 because their study did not find a significant difference between 400 and 600 nematodes. For chickpea, an optimized screening method has been successfully developed. It allows the identification of resistance against the root lesion nematode, *P. thornei* at harvesting time and can be useful to the study of resistance mechanisms. Also, the results indicated that there was no change in the population density of *P. thornei* during the growing time (16 and 20 weeks) in *Cicer* species. Singh and Ocampo (1997) noted that the chickpea is a cool-season crop and required the growing time of 100 d to reach maturity. Reen & Thompson (2009) reported that a longer growth period was required to maximize differences in *P. thornei* densities between cultivars of chickpea (18-20 weeks) than in wheat (16-18 weeks) under laboratory conditions. Taylor et al. (2000) showed that the mean final population density of *P. neglectus* of twenty wheat genotypes was lower than that of six chickpea genotypes 21 or 26 weeks after sowing.

The optimized screening technique will be useful to test chickpea genotypes for resistance to *P. thornei* under laboratory conditions. This study is the first to assess chickpea genotypes collected from Turkey for resistance to *P. thornei*, some of which offer new sources of *P. thornei* resistance and genetic diversity useful for international chickpea breeding programs.

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Original article (Orijinal araştırma)

Histopathological changes of some resistant and susceptible wheat cultivars caused by plant parasitic nematodes[1]

Bitki paraziti nematodlarının neden olduğu bazı dayanıklı ve hassas buğday çeşitlerinin histopatolojik değişiklikleri

Ece B. KASAPOĞLU ULUDAMAR[2] Şenay KARABIYIK[3] İbrahim Halil ELEKCİOĞLU[2]

Abstract

Root lesion nematodes, *Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae), and cereal cyst nematode, *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) are economically important migratory and sedentary endoparasites on host plant roots. In this study, cellular variations in roots of resistant and susceptible wheat cultivars infected by *P. thornei*, *P. neglectus* and *H. avenae* were investigated between 2017 and 2018. Wheat cultivars were infected with *P. neglectus, P. thornei,* and *H. avenae,* separately and *H. avenae* and *P. thornei* together. Twelve weeks after inoculation, roots were washed, embedded in paraffin, cut by microtome. The root cell slides were examined under light microscope for histopathological changes in the root cells. The root lesion nematodes moved from epidermal to cortical cells by damaging the cells along the feeding path. Due to the nematode infection, cortical cells of most wheat cultivars were destroyed by the development of many cavities in the root tissue. Also, it was observed that many root lesion nematodes fed collectively on the same site either stretched out or coiled in different cell layers. *Heterodera avenae* fed on the cortex cells as did root lesion nematode.

Keywords: Cereal cyst nematodes, *Heterodera avenae,* histopathology, root lesion nematodes, wheat

Öz

Kök lezyon nematodları, *Pratylenchus thornei* Sher & Allen, 1953 ve *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) ve Tahıl kist nematodları *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) konukçu bitki köklerinde beslenen ve ekonomik olarak önemli hareketli ve hareketsiz endoparazitlerdir. Bu çalışmada, *P. thornei, P. neglectus* ve *H. avenae* ile bulaştırma yapılan dayanıklı ve hassas buğday çeşitlerindeki hücresel değişimler 2017-2018 yılları arasında incelenmiştir. Buğday çeşitleri, *P. neglectus, P. thornei* ve *H. avenae* türleri ile ayrı ayrı olarak ve *H. avenae* ile *P. thornei* birlikte inokule edilmiştir. İnokulasyondan on iki hafta sonra, buğday çeşitlerinin kökleri yıkanıp parafin içerisine sabitlenmiş, mikrotom ile kesilip kök hücre preparatları hazırlanmış ve kök hücrelerindeki histopatolojik değişimlerin ışık mikroskobu altında incelenmiştir. Kök lezyon nematodlarının, epidermal hücrelerden kortikal hücrelere doğru beslenme yolu boyunca hücrelere zarar vererek hareket ettiği gözlenmiştir. Nematod infeksiyonundan dolayı çoğu buğday çeşidinin, korteks hücrelerinde birçok oyuğun oluşumuyla kök dokusu tahrip olmuştur. Ayrıca, farklı hücrelerde düz ya da kıvrılmış şekilde, aynı bölgede toplu bir şekilde beslenen birçok kök lezyon nematodları gözlenmiştir. *Heterodera avenae* bireyleri de kök lezyon nematodları gibi korteks hücrelerinden beslenmiştir.

Anahtar sözcükler: Tahıl kist nematodları, *Heterodera avenae,* histopatoloji, kök lezyon nematodları, buğday

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Introduction

Wheat is one of the most important grains in the world. Global wheat consumption in 2019/20 was forecasted to reach around 757 Mt. The 2019 cereal harvest is forecast at 34.2 Mt. in Turkey (FAO, 2019 a, b). The production of wheat is impacted by many plant parasitic nematodes worldwide.

The genus *Pratylenchus* Thorne, 1949 (Tylenchida: Pratylenchidae) may include the broadest host range of plant parasitic nematodes. *Pratylenchus* spp. distributed worldwide and while most species are of little or no economic importance, some species are responsible for substantial yield losses in plants (Duncan & Moens, 2006). Plant parasitic nematodes are categorized into groups based on feeding behavior. Root lesion nematodes feed on roots as migratory endoparasitic nematodes. Migratory endoparasitic nematodes cause mechanical damage to the root system by moving through and destroying the cells on which they feed. Once they reach suitable host cells, they start extract water and nutrients. These nematodes move intercellular causing extensive rupturing of cell walls or necrosis (Vovlas & Troccoli, 1990).

Pratylenchus thornei Sher & Allen, 1953 and *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae) are commonly found in wheat fields in Turkey (Elekcioğlu, 1992; Mennan & Handoo, 2006; İmren et al., 2017). Rotation and resistant cultivars are more practical and cheaper ways of controlling these nematodes than other methods. Information on these subjects is of importance for the interaction and use of resistant cultivars as management strategies. Management of root lesion nematodes requires adequate information. Nowadays, most information in all areas of plant parasitic nematodes is related to the development of host-parasite interaction. Many studies have been published about resistance of lesion, cyst and root knot nematodes, yield loss and control of plant parasitic nematodes (Pariyar et al., 2016; Fard et al., 2018; Özdemir & Gözel, 2018; Dababat et al., 2019).

Since host-parasite relationships are important for control of plant parasitic nematodes, so understanding host-parasite relationships is valuable. The histopathology of infection by *Pratylenchus penetrans* (Cobb, 1917) (Tylenchida: Pratylenchidae) and structural change of *Heterodera glycines* Ichinohe, 1952, *Heterodera schactii* Schmidt, 1871 and *Heterodera avenae* Wollenweber, 1924 (Tylenchida: Heteroderidae) have been studied on several crops (Oyekan et al., 1972; Townshend & Stobbs, 1981; Kim et al., 1987, 2010; William & Fisher, 1993; Holtmann et al., 2000). There are few studies on the histopathology and structure of *P. thornei*, *P. neglectus* and *H. avenae* in wheat. The objective of this study was to investigate the migration, feeding, reproduction of cereal cyst nematode and root lesion nematodes in roots of resistant and susceptible wheat cultivars. Susceptible cv. Seri-82 and resistant cvs Adana-99, Ceyhan-99, Porsuk-2000, Atlı-2002 and Silverstar, which have *Cre1* and *Cre3* genes providing resistance to cereal cyst nematode, were selected (Kasapoğlu et al., 2016). This information would be important for determining the mechanisms of nematode damage in these cultivars. Given the importance for control of plant parasitic nematodes, host-parasite relationships ought to be understood thoroughly in nematology.

Materials and Methods

Nematode inoculum

Heterodera avenae was collected from infested soil in Sarıçam/Adana (Elekcioğlu, 1992; İmren, 2013). Cyst nematodes were separated from the soil using a court device, a modified form of the Fenwick (1940) method. The cysts were kept at 4°C for hatching of juveniles to get enough juvenile population (İmren, 2013). The root lesion nematodes were grown on carrot and the inoculum was obtained for the experiments according to Moody et al. (1973). To obtain inoculum, the infected carrot discs were extracted on a modified Baermann funnel method (Hooper, 1986). After extracting the nematodes, they were surface sterilized for 30 min in 0.01% streptomycin sulfate and rinsed several times in sterilized water. Each plant was inoculated with 175 *H. avenae* (juveniles), *P. thornei* and *P. neglectus* (mixed stages).

Wheat cvs Adana-99, Ceyhan-99, Porsuk-2000, Atlı-2002, Silverstar and Seri-82 were used in this study (Table 1). Wheat seeds were surface-disinfested (70% alcohol 30 s, 0.05% sodium hypochlorite 1 min) before germination. These seeds were placed on surface of wet filter paper at 21°C for 3 d in sterile Petri dishes then the germinated seeds were planted in small tubes (13 cm high and 3 cm diameter, 70 g soil) that contained autoclaved soil, (29% clay, 70% river sand and 1% organic matter) with 5 replicates in a climate chamber. Plants were grown in the climate chamber for 12 weeks at 21°C after inoculation, all plant roots were harvested, washed and fixed by hand.

Table 1. List of reactions of wheat cultivars used in experiments with *Pratylenchus thornei, P. neglectus* and *Heterodera avenae*

Cultivar	Wheat Type	Resistance	Reactions*	Reference	Resistance	Reactions*	Genes	Reference
Adana-99	Bread	*P. thornei,* *H. avenae*	R R	Toktay (2008) İmren (2013)	*H. avenae*** *P. thornei*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Ceyhan-99	Bread	*P. thornei,* *P. neglectus,* *H. avenae*	R S S	Toktay et al. (2015) İmren (2013)	*P. neglectus*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Porsuk-2000	Bread	*P. neglectus*	R	İmren (2015)	*H. avenae*** *P. thornei*	R	*Cre 1*	Kasapoğlu Uludamar (2018)
Atlı-2002	Bread	*P. thornei,* *P. neglectus*	R	İmren (2015)	*H. avenae*** *P. thornei*	R	*Cre 1* *Cre 3*	Kasapoğlu Uludamar (2018)
Silverstar	Bread	*H. avenae,* *H. latipons*	R	İmren (2013, 2014)	*H. avenae*	R	*Cre 1* *Cre 3*	Kasapoğlu Uludamar (2018)
Seri-82	Bread	*P. thornei,* *P. neglectus,* *H. avenae*	S	Toktay et al. (2015) İmren (2013)	*H. latipons* *H. avenae*	S	*Cre 1*	Kasapoğlu Uludamar (2018)

* R: Resistant, S: Susceptible;

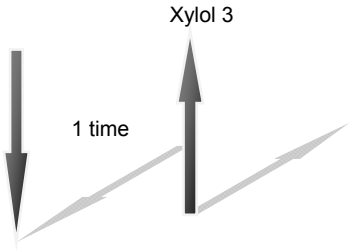
** Mixed inoculation of nematodes.

Histological studies

Root samples were kept in FPA 70 (formaldehyde-propionic acid-alcohol) solution for fixing the structure of the roots. Afterwards, they were dehydrated using tertiary butyl and ethyl alcohol series (70, 85, 95 and 100%) for 4 h at each step, air was evacuated for each sample via desiccator. The samples were kept in pure tertiary butyl alcohol overnight and then embedded in paraffin in 65°C. After 2 d, the samples were put on ice for hardening and placed on wood blocks for cutting process (Eti, 1987). The prepared samples were cut 10 µm thick with LEICA RM 2245 rotary microtome. Sections were emplaced on slides with 1:1 glycerine and egg white mixture, and left on a hot plate to dry and later transferred into an incubator (30°C) for 2 d. Then the slides with root samples were stained with 0.125% hematoxylin as shown in Table 2 and mounted in Entellan (Karabıyık et al., 2018). Slides were examined for infection sites under a LEICA DM 4000B light microscope equipped with LEICA DMC 4500 camera.

Table 2. Staining period of root cell preparations and time schedule

Solutions	Time (Min)
Xylol-1	10
Xylol-2	10
Isopropyl alcohol-1	5
Isopropyl alcohol-2	5
96% ethyl alcohol	3
70% ethyl alcohol	3
40% ethyl alcohol	3
20'% ethyl alcohol	3
Distilled water	15
Hematoxylin	30



Results

The objective of this study was to provide information on the histopathological changes of *P. thornei*, *P. neglectus* and *H. avenae* including penetration, migration to a feeding site, feeding, egg laying, and molting in resistant and susceptible wheat cultivars by using light microscopy to examine living nematodes in the roots. Histological observations indicated that many *P. thornei* larvae had reached the endodermis and cortex where they fed and reproduced (Figure 1a, b). Cavity of cortical layer follows the path of root lesion nematodes, also small nuclei, necrosis and cell wall thinning was observed in the adjacent cells. Most of the *P. thornei* moved together intracellularly. So, pictures were taken of the root damage caused by collective nematodes as shown in Figure 1c, d. Since *H. avenae* did not develop in cv. Atlı-2002 with *Cre1* and *Cre3* genes (Table 1), related pictures were not be able to taken (Figure 1a). Large cavities, necrosis and thickened walls occurred both on vertical and transverse sections. Necrosis were evident of nematode feeding. No evidence was found of nematodes feeding in endodermis.

Mixed inoculation of *H. avenae* and *P. thornei* showed low reproduction in Porsuk-2800. In the experiment, a few plant parasitic nematodes were found in vertical and transverse sections. So, some of cells did not show damage from *P. thornei* and *H. avenae* (Figure 2a, d) but the damage was seen in other sections (Figure 2b, c, e). *Heterodera avenae* and *P. thornei* did not feed in the xylem and phloem. Occasional damage and hyperthyroid nucleus were formed in the cortex (Figure 2d). The movement of *H. avenae* and *P. thornei* was limited within the cortex, resulting in undisturbed cells. Nevertheless, it allowed identification of eggs of nematodes and cytoplasm contained in intensely osmophilic grains and egg laid in cortical cells (Figure 2d). After hatching, females and juveniles moved on parenchymatous cells and caused enlargement of the lesion. Cortical cells adjacent to infected sites showed dense granular cytoplasm with large cavities and damage.

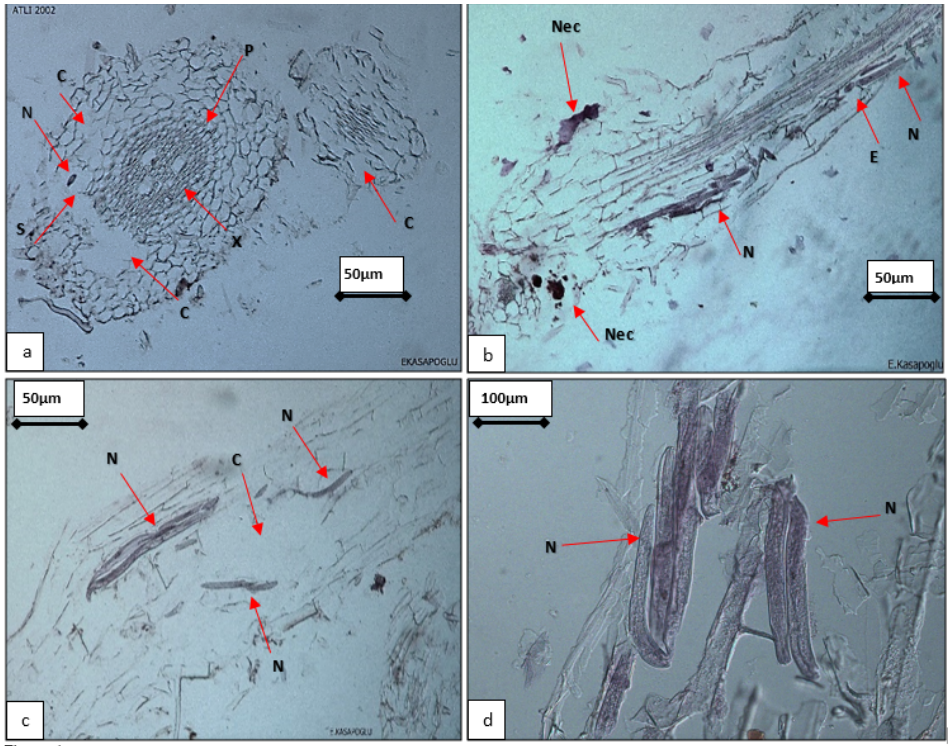


Figure 1. Structure of syncytia induced in the roots of Atlı-2002 by *Heterodera avenae* and *Pratylenchus thornei*: a) extensive cell; b & c) extensive damage of the cortex. Thickened and disrupted cell wall: d) root cell feeding of nematodes.
E: nematode egg, N: Nematode, Nec: Necrosis, S: Syncytium C: Cavity, X: Xylem, P: Phloem, H: Hypertrophied nuclei.

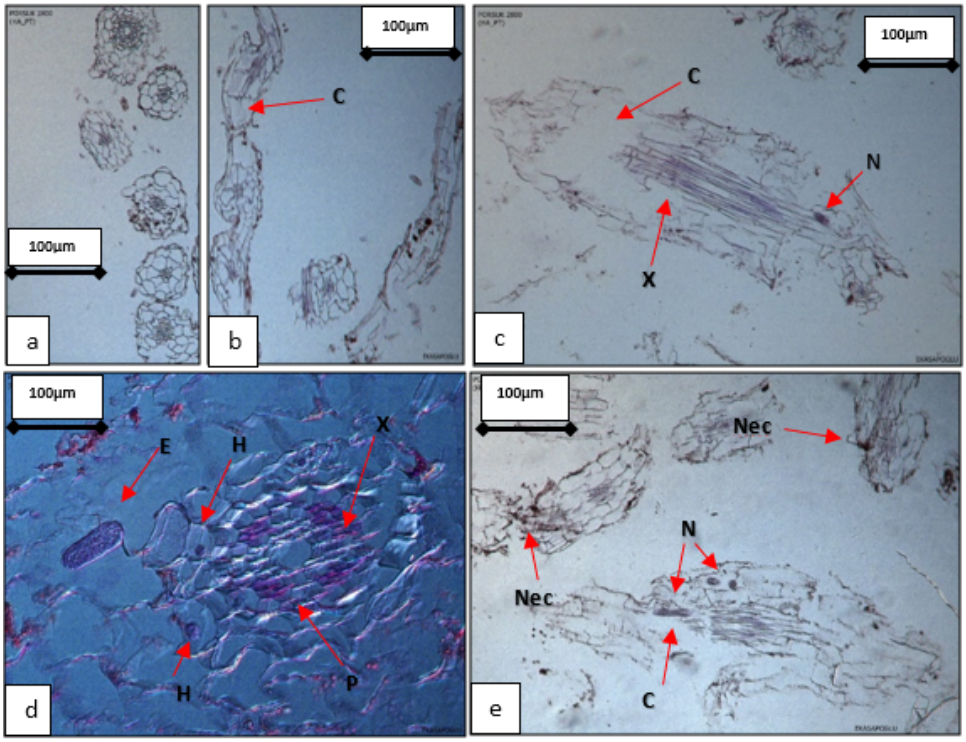


Figure 2. a) Healthy root cell image of cv. Porsuk 2800; b & c) extensive damage of the cell walls in the cortex of cv. Porsuk-2800 by *Heterodera avenae* and *Pratylenchus thornei*, showing hypertrophied cells, cell wall breakdown on transverse section and large cavity in old infections; d & e) transverse section of roots showing damaged endodermis, epidermis and cortex.
E: Nematode egg, N: Nematode, Nec: Necrosis, C: Cavity, X: Xylem, P: Phloem, H: Hypertrophied nuclei.

Figure 3 shows that root cells responded to *P. neglectus* individuals broken through in the susceptible cv. Ceyhan 99. Usually, nematodes affected three or four cells in each region like tunnels (Figure 3b, e, f, g). Feeding of *P. thornei* and *P. neglectus* caused large cavities in the cortex with their walls. These histological observations demonstrated feeding of root lesion nematodes. Seri-82 was susceptible to *P. thornei* and *P. neglectus* (Table 1). So, there were several straight and coiled nematodes in cell tissue. Especially, several nematodes fed together in damaged cells. It was observed that *P. neglectus* moved in the endodermal cells whereas *P. thornei* were found frequently in the cortex (Figure 3d, g). There were large vacuoles; nucleus. The cortex had very extensive cavities and cell walls were thickened.

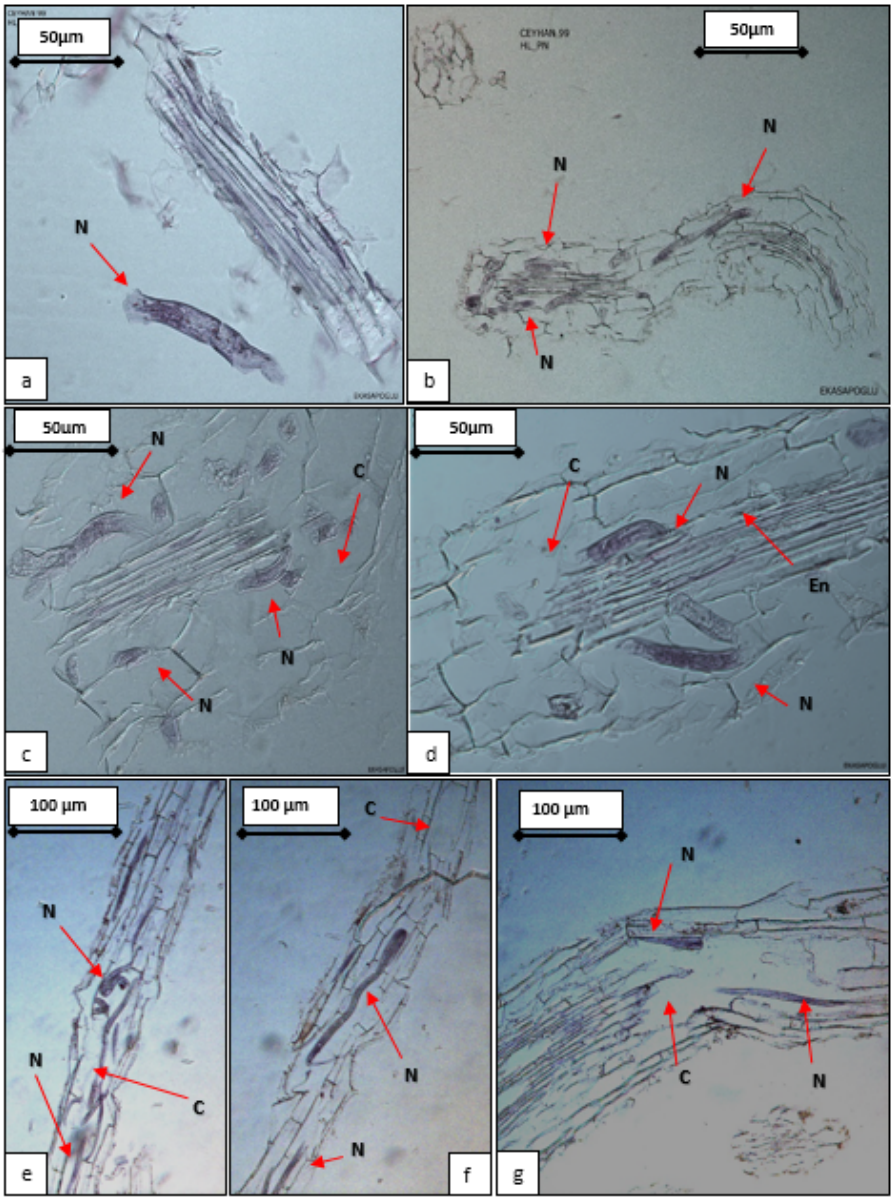


Figure 3. Histopathological changes induced by migration and feeding of the root lesion nematode *Pratylenchus neglectus* in root cell of Ceyhan-99: a) vulva region of *Pratylenchus neglectus* in Ceyhan 99; b,c & d) head, body, tail of *Pratylenchus neglectus* in root cell, feeding of nematodes in the cortex layer in Ceyhan 99; e & f) coiled *Pratylenchus thornei* occupying on different cell in Seri- 82; g) extensive damage (large cavity) in cortical layer in Seri-82.
En: Endodermis, N: Nematode, C: Cavity.

Wheat cv. Adana-99 is resistant to *H. avenae*. Syncytia had large vacuoles and hyperthyroid nuclei (Figure 4b, d). Syncytia developed longitudinally along root axis. Membrane thinning and degeneration was observed. Also, juveniles invaded the endodermal and cortical layers in Adana-99 (Figure 4a, b). Epidermis, exodermis and endodermis were damaged by the nematodes. Cell integrity deteriorated in many layers (Figure 4a, c) of root tissue. According to feeding strategy, some parts had intensive necrosis and discoloration in epidermis and exodermis (Figure 4c, d) and it covered major regions. Since Adana-99 has *Cre1* and *Cre3* genes (Table 1), syncytia occurred in limited numbers and consisted of only few cells (Figures 1a and 4b, d) on this cultivar. Figure 4d shows juveniles migrated in root cell of the resistant cv. Silverstar. They had large vacuoles and hyperthyroid cell around syncytia. Syncytia joined more cells as wall disintegrated but xylem and phloem were unaffected. Compared to wheat with *Cre1* and *Cre3* genes, Silverstar had smaller syncytia than Adana-99.

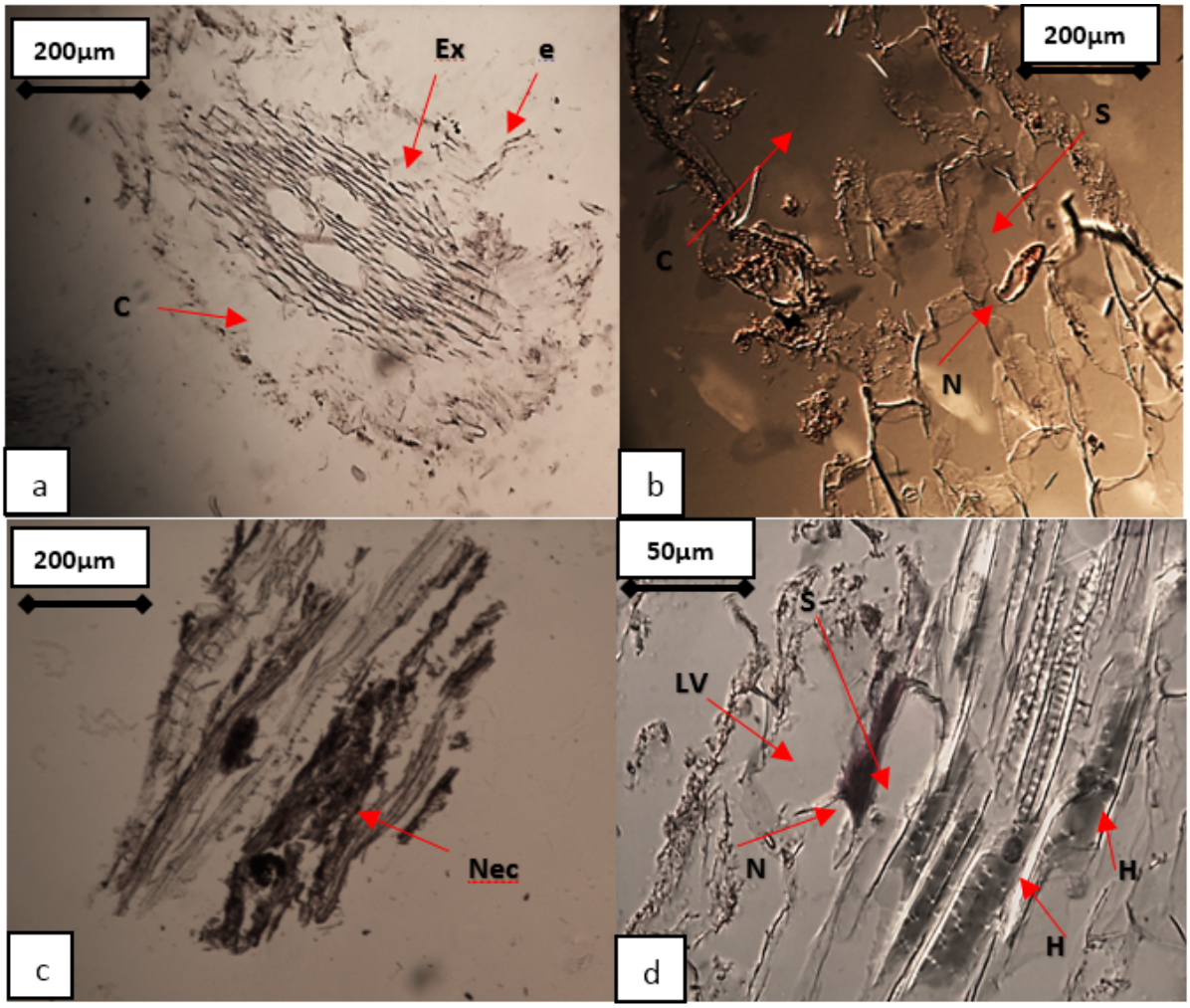


Figure 4. a &b) Cellular reaction to *Heterodera avenae* on Adana-99; c & d) Silverstar.
LV: Large vacuole, E: epidermis, Ex: exodermis, N: Nematode, Nec: Necrosis, C: Cavity, H: Hypertrophied nuclei, S: Syncytium.

Discussion

Juveniles penetrated roots cells of resistant and susceptible wheat cultivars in this study. *H. avenae and P. thornei* avoided the endodermis after infection. However, *P. neglectus* juveniles fed on the endodermal layer. It is known that *P. thornei* always migrates through the epidermal and cortical layers in chickpea (Castillo et al., 1998). No nematodes were found in the stele. Based on these observations, these nematodes infected Adana-99, Ceyhan-99, Porsuk-2800, Atlı-2002 and Seri-82. However, Adana-99, Porsuk-2800, Atlı-2002 were resistant to cyst and root lesion nematodes (Table 1).

Pratylenchus thornei and *P. neglectus* are included into the group of migratory endoparasitic nematodes. Motions of *P. thornei* and number of deposited eggs were limited in resistant wheat cultivars. It is known that nematode multiplication was greatly inhibited by resistance because only 10% juveniles developed in roots of resistant compared to the susceptible plants. Egg deposition was up to 30%, which was lower in resistant cultivars than susceptible cultivars. In addition, the effects of root exudates inhibited migration, hatching and reproduction (Linsell et al., 2014). In our study, it was observed that *P. thornei* and *P. neglectus* damaged the cells in tunnel shapes. The resistant genotype Porsuk-2800 sustained different cavity sizes. However, there were no coiled or straight individuals of *P. thornei*. Nevertheless, *P. thornei* and *P. neglectus* developed colonies in Atlı-2002, Ceyhan-99 and Seri-82. Moreover, Porsuk-2800 retained hyperthyroid nuclei and eggs. It was reported that *P. penetrans* lay eggs in cortical tissues as colonies form (Vovlas & Troccoli, 1990).

Histological studies indicated that *H. avenae, P. thornei* and *P. neglectus* readily penetrated these wheat cultivars. Nematodes were never observed to move in the stele. *Pratylenchus neglectus* penetrated roots in groups and clustered in the cortex. *Pratylenchus neglectus* penetrated equally in resistant and susceptible cultivars. However, the number of nematodes in resistant wheat was lower than in susceptible wheat cultivars due to initial population in soil (Farsi et al., 1996). Although wheat cultivars were resistant to nematodes, it was observed that *P. thornei* and *H. avenae* fed in the cells. Most probably, some of juveniles were not able to develop and were unsuccessful in syncytium zone. However, other juvenile development appeared in syncytial root cells in susceptible and resistant wheat cultivars (Williams & Fisher, 1993). It is known that degeneration of syncytia started 13 d after inoculation in host tissue. Also, the syncytia in wheat with *Cre1* or *Cre3* genes were extensively vacuolated and less metabolically active; syncytia developed later than in susceptible wheat cultivars (Grymaszewska & Golinowski, 1991; Seah et al., 2000). Silverstar has *Cre1* and *Cre3* genes, as a result small syncytium and less activity was observed. Cell wall thickenings and breakage were found in syncytia close to nematode heads.

Plant parasitic nematode damage to cells also allows pathogens such as fungi and bacteria to infect the same sites. This can result in large necrotic lesions in the cortex. The damage of cells which the nematode fed and migrate can result from both biochemical and physical factors (Farsi, 1996; Gheysen & Mitchum, 2011; Linsell et al., 2014; Göze Özdemir et al., 2018). Plants can release secretions as reaction or defense mechanism to kill plant parasitic nematodes (Kepenekçi et al., 2016; Aydınlı et al., 2019). Therefore, the different strategies may have developed in the resistance response in wheat or other crops. This study provides information on the histopathological of the interaction of nematodes with the host plants. It will be helpful in understanding the mechanisms of nematode damage.

Acknowledgments

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Original article (Orijinal araştırma)

Effects of larval crowding on some biological characteristics of the blowfly, *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae)

Larva yoğunluğunun leş sineği, *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae)'nın bazı biyolojik özelliklerine etkileri

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Ünal ZEYBEKOĞLU[2] **Kamil ALAKUŞ[3]**

Abstract

Blowflies are well known necrophagous insects and usually the first insects to discover and colonize a body after death. Thus, postmortem interval (PMI) can be estimated from the length or stage of development of blowfly larvae collected from a corpse. Abiotic and biotic factors influence multiple traits of a population, including body size, fecundity, survival and development rate. Larval crowding is one of the factors affecting blowfly population dynamics. The purpose of this study was to analyze the effect of larval mass on some life history parameters of *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae). Experiments were conducted at the Animal Physiology Research Laboratory, Ondokuz Mayıs University during 2017. Five, 25, 50, 100, 500 or 1000 newly hatched *C. vicina* larvae were introduced into a plastic cup containing fresh chicken liver and kept at 22°C and 70% RH under a 12:12 h L:D photoperiod. They were checked at 12-h intervals and development period, survival rate, adult eclosion time, sex ratio, adult size and pupal and adult weights were recorded. The development periods for larval and pupal stages were positively affected by larval crowding. However, larval and pupal survival rate and the percentage of individuals reaching adulthood were very low in the crowded groups. The results also indicated that pupal and adult weight and adult size negatively affected by increasing larval density. It is concluded that larval crowding has an important effect on life history parameters of *C. vicina* and this need to be considered more reliable estimation of PMI.

Keywords: *Calliphora vicina,* larval density, nutrition, survivorship

Öz

Leş sinekleri iyi bilinen nekrofaj böceklerdir ve genellikle ölümden sonra vücudu keşfeden ve kolonize olan ilk böceklerdir. Bu nedenle, ölüm sonrası geçen zaman aralığı (PMI), cesetten toplanan leş sineği larvalarının gelişim evresine veya uzunluğuna bakılarak belirlenebilir. Abiyotik ve biyotik faktörler bir popülasyonun vücut büyüklüğü, verim, hayatta kalma ve gelişim oranı gibi farklı özelliklerini etkiler. Larva yoğunluğu, leş sineklerinin popülasyon dinamiğini etkileyen en önemli faktörlerden biridir. Bu çalışmanın amacı, larva yoğunluğunun *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae)'nın bazı yaşamsal parametreleri üzerindeki etkisini belirlemektir. Denemeler 2017 yılında Ondokuz Mayıs Üniversitesi, Hayvan Fizyolojisi Araştırma Laboratuvarı'nda yapılmıştır. Yumurtadan yeni çıkan 5, 25, 50, 100, 500 veya 1000 *C. vicina* larvası içerisinde taze tavuk ciğeri bulunan plastik kap içerisine yerleştirilmiş ve 22°C, %70 bağıl nem ve of 12:12 (A:K) s foto periyot koşullarında tutulmuştur. Bu kaplar 12 saat aralıkla kontrol edilerek gelişim süresi, hayatta kalma oranı, ergin çıkış zamanı, eşey oranı, ergin büyüklüğü, pupa ve ergin ağırlıkları kaydedilmiştir. Larva ve pupa dönemlerinin gelişme süreleri larva yoğunluğundan olumlu etkilenmiştir. Buna karşın, larva ve pupaların hayatta kalma oranı ve ergin hale ulaşan bireylerin yüzdeleri kalabalık gruplarda oldukça düşüktür. Sonuçlar ayrıca pupa ve ergin ağırlıkları ile ergin büyüklüğünün larva yoğunluğundaki artıştan olumsuz etkilendiğini göstermiştir. Larva yoğunluğunun *C. vicina*'nın yaşamsal parametreleri üzerinde önemli bir etkiye sahip olduğu ve bu durumun daha güvenilir PMI ölçümü için dikkate alınması gerektiği sonucuna varılmıştır.

Anahtar sözcükler: *Calliphora vicina,* larva yoğunluğu, beslenme, hayatta kalma

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Introduction

Forensic entomology is the scientific study of the use of insects and related arthropods in legal cases including crime scene investigation, abuse and neglect cases, accidents and insect infestation (Amendt et al., 2011). The most important contribution of this science is the estimation of the postmortem interval (PMI), the time between death and the discovery of a corpse (Ireland & Turner, 2006; Verma & Reject, 2013). Shortly after death, the body begins to decompose and many chemicals released by the body attract different types of insects which lay their eggs on the bodily orifices and open wounds (Brown et al., 2015). Insect colonization of carrion has been demonstrated to occur in a predictable manner called insect succession. Blowflies (Calliphoridae) are usually the first insects to discover and colonize a body (Amendt et al., 2004, 2011).

There are two main methods of using insects to evaluate the elapsed time since death (Catts & Haskell, 1990; Anderson, 1995) using successional waves of insects and based on maggot age and development. The first method is to analyze the predictable, successional colonization of insects on the corpse. Insect succession is used when the victim has been dead for a month or longer. Decomposition is a continuous process and each of these stages is associated with the arrival of different suites of insect species (Amendt et al., 2007; Joseph et al., 2011; Brown et al., 2015; Sharma & Kumar, 2015; Layla et al., 2016). The succession of insect species varies according to the regional climatic conditions and geographical location (Reed, 1958; Payne, 1965). Correct species identification is the major step and knowledge of regional insect succession is required for the success of this method (Joseph et al., 2011).

The second method relies on the determination of the age of the oldest immature insect on the corpse, assuming colonization occurred after death (Catts & Goff, 1992; Joseph et al., 2011). The main entomological approaches to age determination are the use of the species-specific time required for an immature fly to reach developmental landmarks such as length, weight and stages of the life cycle, dependent upon the temperature. By measuring the length or weight of the oldest larva and comparing it with the reference data, the age of the fly larva or maggot may be estimated. Another approach is based on the accumulation of degree hours or degree days that are required for larvae to reach a particular stage of development (Amendt et al., 2004; Ireland & Turner, 2006).

Size and developmental stage of the larvae collected from a body provide a major indication of the PMI. Larval size is affected by factors such as temperature, larval crowding, drugs and quantity of food (Fantinou et al., 2008; Niederegger et al., 2013; Khaliq et al., 2014; Jordan & Tomberlin, 2017).

Vertebrate bodies are an ephemeral and limited nutritional source for insects (Grassberger & Frank, 2004; Shiao & Yeh, 2008) and adult female blowflies may lay eggs on the corpse. The eggs quickly hatch into maggots which consume the corpse. The presence of conspecific individuals may influence the selection of oviposition site by females (Ireland & Turner, 2006; Fantinou et al., 2008; Thiéry et al., 2014). Density-dependent competition for food during the larval stages is considered to be one of the most important factors affecting insect population dynamics (Ireland & Turner, 2006). The competitive feeding environment within more crowded larval cultures resulted in increased or decreased development rates and the production of undersized larvae (Saunders & Bee, 1995).

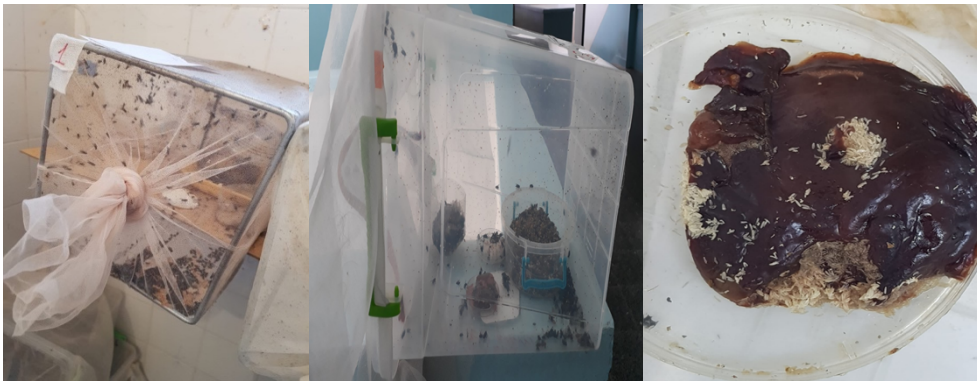
The larvae of blowflies are used to estimate the minimum PMI stated earlier. Larval length is a factor that can reduce confidence in the accuracy of the estimation of PMI (Weatherbee et al., 2017). Therefore, factors which may affect larval size should be considered to the reliable PMI determinations (Saunders & Bee, 1995; Fantinou et al., 2008; Horváth & Kalinka, 2016; Weatherbee et al., 2017). Larval crowding studies are important for investigating how larval size may lead to inaccurate estimation of the PMI. *Calliphora vicina* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae) is a Holarctic necrophagous blowfly

species that is found throughout the world (Bonacci et al., 2009). In Turkey, *C. vicina* is the dominant fly species found on a carcass in the winter and autumn (Kökdener & Polat, 2016).

The effects of larval crowding have been investigated with *Calliphora vomitera* (Linnaeus, 1758) (Diptera: Calliphoridae) (Ireland & Turner, 2006) and *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) larvae (Zheng et al., 2017). To our knowledge, there have been few studies on the effects of larval crowding and competition on *C. vicina*; one such study was performed by Saunders et al. (1999). Separately, the influence of different food substrate on the development of *C. vicina* was determined (Niederegger et al., 2013). Against that background, this study was undertaken to explore the effects of larval overcrowding on some life history parameters of *C. vicina*.

Materials and Methods

A laboratory colony of *C. vicina* was established from adults collected from the campus of Ondokuz Mayıs University, Samsun (41°15′ N, 36°19′ S), Turkey in 2016. The colony was maintained in gauze covered cages (30 x 30 x 30 cm) at 22°C and 70% RH under a 12:12 h L:D photoperiod. This study was conducted in 2017 at the Animal Physiology Research Laboratory, Ondokuz Mayıs University. Newly emerged adults from the colonies were provided with granulated sugar and water ad libitum. The cages contained a maximum of 300 flies at an approximate 1:1 ratio of males and females (Figure 1). When eggs were required, a plastic beaker containing about 50 g of fresh chicken liver was placed inside a rearing cage and monitored hourly for oviposition. Newly deposited eggs (<1 h old) were transferred into sterile Petri dishes covered with Kimwipes (Eczacıbaşı, Turkey) soaked with deionized water. All Petri dishes were kept in an incubator (Sanyo 36VL) maintained at a constant temperature of 22°C and 70% RH under a 12:12 h L:D photoperiod throughout the experiment. The incubator was monitored hourly and newly hatched larvae were used in the subsequent experiments (Figure 1).



a b c

Figure 1. a & b) Rearing cages for *Calliphora vicina*; c) *Calliphora vicina* eggs on liver in petri dishes.

To determine the effects of larval crowding on some life history traits of *C. vicina*, newly hatched larvae were divided into groups at six different densities (5, 25, 50, 100, 500 and 1000 newly hatched larvae). This procedure was repeated four times for each larval density. Post-hatching, the larvae were transferred to a 20 g piece of fresh chicken liver in a plastic cup (15 x 10 x 5 cm). The experimental cups were placed in a larger 500 mL plastic container with a plastic lid with six small air holes and maintained in the incubator at 22°C and 70% RH under a 12:12 h L:D photoperiod. They were checked at 12-h intervals and the development time of larvae in each cup was recorded. As larvae finished feeding and reached the wandering phase, they left the food. At that stage, plastic cups containing the non-feeding larvae and any remaining food were removed from the container and non-feeding larvae were transferred to 1 L glass jars containing about 10 g of dry sawdust for pupation. Jars were sealed with a fine mesh cover and maintained

at 22°C and 70 % RH under a 12:12 h L:D photoperiod for adult emergence. Pupation and eclosion times, pupation success, pupal and adult weight and sex ratio were recorded. Adult flies were collected and killed by freezing at -20°C. They were then removed from the freezer and separated by gender. The size of each adult was measured using a stereomicroscope equipped with a digital camera (Leica MZ 12.5, LAS Version 3.8.0, Leica Microsystems, Switzerland) at a magnification of 10x. Three measurements were taken, namely the length of the posterior cross vein (dm-cu) of the left wing, costa distance between the R_{2+3} and R_1, and the length of the mesothoracic tibia (Laparie et al., 2016).

Statistical analysis

All statistical analysis was performed with the SPSS software, version 22.0. The effects of population density on the larval and pupal development time and pupal weight of *C. vicina* were subjected to one-way analysis of variance (ANOVA). The Tukey-Kramer HSD test at the level of significance P=0.05 was used to determine the significance of means. Adult weight and adult size differences were analyzed by using two-way ANOVA, with the variables being density and gender. In addition, post-hoc multiple comparisons were performed by using Fisher's least significant difference test.

Results and Discussion

The time required for the development of *C. vicina* larvae and pupae at different levels of larval density are given in Table 1.

Table 1. Development time of *Calliphora vicina* immature life stages at different larval densities

Larval density (n)	Stages (h; mean±SE)			
	Larva		Pupa	
5	192.0±0.70	a*	379.0±2.48	a
25	192.6±0.40	a	377.0±4.07	a
50	192.8±0.37	a	374.0±4.59	a
100	168.6±0.40	b	371.0±2.52	b
500	167.8±0.37	b	338.4±2.92	b
1000	160.0±1.14	b	336.0±4.11	b

*Means in the same column followed by the same letter are not significantly different (P=0.05).

The data indicated that increasing larval density led to changes in the larval and pupal development time of *C. vicina*. For their larval development period, the mean values for 100, 500 and 1000 larvae were significantly different (F=570, P=0.0001) and for pupal development time, mean values over a density of 50 were all significantly different (F=21.2, P=0.0001). These results are similar to those obtained by Goodbrod & Goff (1990) for *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart, 1843) (Diptera: Calliphoridae). Similarly, Zayed (2004) reported that the duration of development in the immature stages of *Culex pipiens* (Linnaeus, 1758) (Diptera: Culicidae) decreased as larval density increased. The development period for *C. vicina* shortened with increased larval density (Saunders & Bee, 1995). Also, Ireland & Turner (2006) demonstrated that crowded larval cultures reared on liver and muscle had shorter developmental periods than those reared at low densities. In nature, animal cadavers are important food resources and each female blowfly lays many eggs on a corpse. Large numbers of larvae hatch from these eggs, and then feed on the carcass. Larval aggregation is beneficial for feeding larvae because it causes the secretion of proteolytic enzymes and ammonia by larvae (Reis et al., 2001). These secretions macerate the food externally and lead to more efficient feeding for larval mass. Charabidze et al. (2011) and Kotzé et al. (2016) also observed that gregarious feeding

behavior leads to a local temperature increase among blowfly larvae and this situation affect larval development. These previous studies could help to explain the reasons for faster development in the more crowded cultures. Decreased development time is usually accompanied with the reduction of body size (Saunders & Bee, 1995; Zayed, 2004). In the present study, the response of the blowflies to food competition during larval stage is to exhibit plasticity in the range of size, and thus smaller-sized individuals can be produced in pupal and adult stages. These smaller adults can still lay normal-sized and viable eggs, but usually in smaller numbers; however, an otherwise high risk of predation during immature stages is moderated. Contrary to our findings, Al-Misned (2002) reported a significant increase in the larval development time of *Wohlfahrtia nuba* (Wiedemann, 1830) (Diptera: Calliphoridae) at increasing larval densities. Similarly, Manorenjitha & Zairi (2012) showed that overcrowding and starvation of the larvae of the mosquito *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) prolonged larval growth up to 36 d. Sokal & Sullivan (1963) also reported that the length of the development period of the immature stages of the house fly increased as larval density increased. Taken together, these data indicate that larval crowding affects the development of immature stages of insects differently.

In the present study, the rearing density of the larvae of *C. vicina* affected the survival rate of immature stages (Table 2). Larval and pupal survival and the percentage of individuals reaching adulthood were very low in the crowded cultures. There was a significant effect of population density on the number of surviving pupa and larvae (for pupae F=519, P=0.002; for larvae F=289, P=0.001) and adults (F=21.7, P=0.001). In a previous study, Saunders & Bee (1995) reported that about 1 g of minced beef muscle is sufficient for the full development of a larva of *C. vicina*. Here, 20 g of fresh chicken liver was used for each group. When the initial larval density is above 25 larvae per 20 g of diet, an important decrease was observed in the survival rates of larvae in our study. This result appears to be consistent with the observations of Saunders & Bee (1995)'s. Thus, intraspecific competition for limited food among larvae may lead to high mortality. In addition, higher larval density may induce more stress and then aggressive contacts increase among larvae, this could increase their mortality. Our findings are consistent with those reported for *C. vomitera*, *C. pipiens, Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) and *A. albopictus* (Zayed, 2004; Ireland & Turner, 2006; Arnaldo, 2009; Manorenjitha & Zairi, 2012). Fantinou et al. (2008) showed that larval crowding had a significant effect on larval survival of *Sesamia nonagrioides* (Lefebvre, 1827) (Lepidoptera: Noctuidae). On the contrary, Reigada & Godoy (2006) found that the survival of *C. megacephala* was not affected by larval densities (200 or 1000 larvae) at the same temperature. Likewise, Al-Misned (2002) demonstrated that the percentages of pupal and total survival were not significantly correlated with population density in *W. nuba.*

Table 2. Survival rate of *Calliphora vicina* at different larval densities

Larval density (n)	Survival rate				Eclosion		Adult sex ratio			
	Larvae		Pupa				Female		Male	
	n	%	n	%	n	%	n	%	n	%
5	5	100.0	5	100.0	5	100.0	11	56.0	9	44.0
25	24	96.0	24	96.0	24	96.0	59	61.0	37	29.0
50	30	60.0	30	60.0	25	50.0	56	56.0	44	44.0
100	59	59.0	58	58.0	49	49.0	100	51.0	96	49.0
500	282	56.4	268	53.6	161	32.2	300	47.0	344	53.0
1000	393	39.0	305	30.5	265	26.5	508	48.0	552	52.0

The effects of larval density on the pupal and adult weight of *C. vicina* are presented in Table 3. Statistical analysis revealed a significant difference in pupal weight among different larval densities (F=31.2, df=5, P=0.0001). In addition, both density (F=325, df=5, P=0.001) and sex (F=16.2, df=1, P=0.001) significantly affected the adult weight of *C. vicina*. Similarly, Agnew et al. (2000) found that mosquitoes emerged with lighter starved dry adult weight as larval density increased. Al-Misned (2002) also reported that the increasing larval population density resulted in a decrease of pupal and adult weights of flesh fly, *W. nuba.* It is known that overcrowding during the larval stages of development results in a competitive feeding environment. As more larvae competed for the same amount of food, the larvae may force to metamorphose with an insufficient food reserve, resulting in pupae of reduced weights and these smaller pupae gave rise to smaller adult blowflies.

Table 3. Weights of pupal and adult stages of *Calliphora vicina* at different larval densities

Larval density (n)	Pupal weight (mg; mean±SE)		Adult weight (mg; mean±SE)			
			Female		Male	
5	772±45	a*	737±94	aA**	649±218	aA
25	750±45	a	261±15	bA	257±99	bA
50	750±16	a	143±50	cA	134±40	cA
100	587±92	b	115±33	cdA	109±43	cdB
500	266±55	c	86±46	deA	78±46	deA
1000	180±48	d	54±23	eA	53±222	eA

* Means in the same column followed by the same letter are not significantly different (P=0.05).
** The same uppercase letter in the same line indicate that the means are not significantly different.

Analysis of data for the effects of larval population density on the adult size of *C. vicina* is shown in Table 4. This study determined that density (F=0.253, df=5, P=0.939) and sex (F=0.23, df=2, P=0.978) did not significantly affect the mean length of the posterior cross vein (dm-cu) in *C. vicina*. In contrast, for costa distance (between the R_{2+3} and R_1) the effects of density were significant (F=62.9, df=5, P=0.001). However, sex had no effect on the costal distance (between the R_{2+3} and R_1) of adults (F=0.376, df=1, P=0.540). Larval density also significantly affected the length of the tibia (F=7.86, df=5, P=0.001) but no significant difference was observed among females and males (F=2.89, df=1, P=0.089). In a previous study, Saunders & Bee (1995) reported similar results for *C. vicina*. Similarly, Smith & Wall (1997) showed that for both *L. sericata* and *C. vicina*, the size of male and female adults declined with increasing initial larval number. In addition, Zayed (2004) also found that the female wing length of *C. pipiens* increased as the larval density decreased. All these authors have emphasized the importance of increasing levels of exploitative competition for limited resources among larvae.

In conclusion, our results revealed that larval crowding can have a considerable effect on various life history traits of *C. vicina.* The immature development of *C. vicina* accelerated at high densities which could mislead the forensic entomologist during criminal investigation procedures. Separately, survival percentage of the immature stages, pupal and adult weights and the body size of the *C. vicina* was correlated negatively with increasing larval density. This would explain the lower eclosion percentage associated with higher density rearing. Furthermore, larval crowding affects the size of *C. vicina* and development times and causes undersized individuals. These adverse effects of overcrowding were probably due to differences in food availability and the reduced living space of immature stages. There may also be a buildup of toxic metabolic byproducts.

Table 4. The effect of larval population density on adult size of *Calliphora vicina*

Larval density (n)	Sex	Cross vein length (mm; mean±SE)	Costa distance R_{2+3} and R_1 (mm; mean±SE)	Tibia length (mm; mean±SE)
5	M	2582±109 a*	4381±380 a	3360±282 a
	F	2629±94 a	4470±232 a	3549±263 a
25	M	2533±64 a	4279±118 a	3535±113 ab
	F	2559±83 a	4577±122 a	3679±103 ab
50	M	2519±66 a	4137±149 ab	3482±100 ab
	F	2577±61 a	4337±137 ab	3565±86 ab
100	M	2109±42 a	3933±78 b	3400±63 ab
	F	2027±42 a	3962±78 b	3497±65 ab
500	M	1755±27 a	3466±39 c	3119±39 b
	F	1790±29 a	3667±45 c	3158±42 b
1000	M	1669±49 a	3316±32 c	3001±30 b
	F	1555±22 a	3461±34 c	3169±121 b

*Means in the same column followed by different letters are significantly different.

These results highlight the necessity of a better comprehension of the effects of larval mass on PMI estimation in the context of forensic entomology. Some authors when evaluating larval masses do not mention the effect on the accuracy of postmortem interval estimates (Benecke, 1998; Introna et al., 1998). Heaton et al. (2014) stated that the temperature of maggot masses differ significantly from ambient and elevated mass temperatures may influence larval development rates. However, the effects of larval crowding generally on PMI estimation are unclear and much work is still required to untangle the complex relationships and reveal deeper forensic insights. Future experiments with the different size larvae of different species in combination with field studies would be useful to the understanding of variation in PMI estimates.

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Original article (Orijinal araştırma)

Comparative toxicity of two neonicotinoids and a pyrethroid to forager honeybees (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) by different exposure methods[1]

Toplayıcı bal arılarının (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) farklı maruz kalma yöntemleri ile iki neonikotinoid ve bir piretroidin karşılaştırmalı toksisitesi

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Abstract

Honeybees are exposed to insecticides by direct contact with spray droplets or residues on plant, or through ingestion of contaminated pollen or nectar. Direct contact with foliar spray might be the most common exposure route and contact bioassays are preferred as they better simulate field situation. Bioassays were conducted during 2018 at Sultan Qaboos University, Oman. The acute contact and oral toxicity of commercial formulations of deltamethrin 2.5 EC, thiamethoxam 25 WG and acetamiprid 20 SL to *Apis mellifera* subsp. *lamarckii* Cockerell 1906 (Hymenoptera: Apidae) foragers were measured by three exposure methods (contact by a 1-µL droplet on thorax, contact by Potter spray tower and oral ingestion). Potter tower exposure gave significantly higher mortality at lower concentration of deltamethrin than contact exposure by single droplet on thorax. Thiamethoxam showed significantly higher mortality through oral exposure at all concentrations. HQ_{oral} values were also calculated. Acetamiprid did not give more than 50% mortality even with the highest concentration. Potter tower produced fine droplets (0.286±0.071 µm) and a total of 0.829 µL was deposited on a single honeybee. Forager honeybees are more likely be exposed to the very fine droplets in field and toxicological results obtained by Potter tower or similar devices will be more realistic than a single droplet on thorax.

Keywords: Acetamiprid, *Apis mellifera lamarckii,* deltamethrin, exposure methods, thiamethoxam, toxicity

Öz

Bal arıları sprey damlamaları veya bitkilerdeki kalıntılarından doğrudan temas ile, ya da bulaşık polen veya nektar alımı ile insektisitlere maruz kalmaktadır. İlaçlama ile doğrudan teması en yaygın maruz kalma şeklidir ve arazideki durumu daha iyi simüle ettiği için temas biyolojik denemeleri tercih edilmektedir. Biyolojik denemeler, 2018 yılında Umman Sultan Qaboos Üniversitesi'nde yürütülmüştür. Deltamethrin 2.5 EC, thiamethoxam 25 WG ve acetamiprid 20 SL'nin ticari formülasyonlarının *Apis mellifera* subsp. *lamarckii* Cockerell 1906 (Hymenoptera: Apidae) toplayıcılara akut teması ve ağızdan zehirlenmesi üç yöntem (thoraks üzerinde 1-µL damlacık ile temas, Potter sprey kulesi ile temas ve oral alınım) ile ölçülmüştür. Potter kule uygulaması, daha düşük deltametrin konsantrasyonunda, thoraks üzerindeki tek damlacık ile temasta etkilenmeye göre önemli ölçüde daha yüksek ölüm oranı sağlamıştır. Thiamethoxam, tüm konsantrasyonlarda oral yoldan maruz kalma ile önemli ölçüde daha yüksek ölüm oranı göstermiştir. HQ_{oral} değerleri de hesaplanmıştır. Acetamiprid, en yüksek konsantrasyonda bile %50'den fazla ölüm oranı vermemiştir. Potter kulesi, ince damlacıklar (0.286 ± 0.071 µm) üretmiştir ve tek bir bal arısı üzerinde toplam 0.829 µL biriktirilmiştir. Toplayıcı bal arıları, tarladaki çok ince damlacıklara maruz kalmaya daha yatkındır ve Potter kulesi veya benzer cihazları kullanarak elde edilen toksikolojik sonuçlar, thorakstaki tek bir damlacığa göre daha gerçekçi olacaktır.

Anahtar sözcükler: Acetamiprid, *Apis mellifera lamarckii,* deltamethrin, maruz kalma yöntemleri, thiamethoxam, toksisite

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Introduction

Honeybees (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) produce valuable commercial products (honey, pollen, royal jelly, propolis and wax) and crop pollination largely relies on managed colonies of honeybees (Free, 1993; Gallai et al., 2009). Insecticides which are normally designed to control insect pests can also affect non-target organisms, including the honeybees.

Neonicotinoid insecticides are used on several crops including pome fruits, stone fruits, citrus, grape, other horticultural and ornamental plants to control different insect pests, for example, aphids, whiteflies, plant hoppers, scale insects, moths and soil insects (Muccinelli, 2008). Neonicotinoid insecticides permanently bind to the nicotinic acetylcholine receptors which result in blocking the passage of nerve impulses (Tomizawa & Casida, 2005). Neonicotinoids, applied foliar or seed coating, translocate to pollen and nectar and are consumed by foraging honeybees.

Pyrethroids are also widely used in agriculture and primary target is the voltage-dependent sodium channel (Soderlund & Bloomquist, 1989). The neonicotinoids have higher selectivity factor for insects versus mammals while pyrethroids are non-selective (Tomizawa & Casida, 2005). Both classes of insecticides show high toxicity to pollinating insects particularly the honeybees (Meled et al., 1998; Laurino et al., 2011).

Honeybees may be exposed to insecticides in several ways, including direct contact with spray residues on plants or through ingestion of contaminated pollen or nectar, whether from the crop plants or from the weeds around the fields (Sanchez-Bayo & Goka, 2014). Direct contact of insecticides to honeybees occurs when the spray droplets directly deposit on honeybees. This can occur when applications are made while honeybees are actively foraging on blooming crops, cultivated understory, weeds, cover crops, or habitat areas. Direct contact with foliar spray may be the most obvious exposure route for honeybees.

The dose-response laboratory toxicity bioassays and assessing the toxicity of pesticides to adults by establishing oral and contact LD_{50} and calculate hazard quotients (HQ) is suggested as risk assessment process (EFSA, 2013). Contact bioassays with a 1-µL droplet on the thorax of a honeybee is generally used. However, Potter spray tower produces a droplet size which is closer to the recommended size produced by spray equipment. Oral toxicity is measured by feeding honeybees with pesticide-contaminated honey or sucrose solution. The contact bioassays with a droplet on thorax and Potter tower have not been compared. In this study we measured the acute contact and oral toxicity of three commonly used insecticides to forager honeybees and compared the measured toxicity using three exposure methods (contact by a droplet on thorax, contact by Potter spray tower and oral ingestion).

Materials and Methods

Source of forager honeybees

Bioassays to assess both acute contact and oral toxicity to honeybee foragers were conducted during 2018. Forager honeybees (*Apis mellifera* subsp. *lamarckii* Cockerell, 1906) used in these bioassays were collected from one well-fed, healthy and disease-free colony maintained at the Agriculture Experiment Station, Sultan Qaboos University, Muscat, Oman. The forager honeybees were collected from a single colony.

Insecticides

Commercial insecticide formulations available in Oman were used. The insecticides used in the study were: Delta (deltamethrin) 2.5 EC from Arab Pesticides and Veterinary Drugs Mfg. Co, Jordan, Actara (thiamethoxam) 25 WG from Syngenta, India and Clipper (acetamiprid) 20 SL from Hexter chemicals Sdn. Bhd, Malaysia (Table 1). All preparations were made using deionized (DI) water as solvent. The concentrations (ai) used were: deltamethrin 1.11, 3.33, 10, 30 and 90 µg/mL; thiamethoxam 0.04, 0.12, 0.37, 1.11, 3.33, 10, 30 and 90 µg/mL; acetamiprid 0.37, 1.11, 3.33, 10, 30 and 90 µg/mL active ingredient. Five to eight concentrations were used for each insecticide to obtain mortality between 15 to 85%.

Table 1. Characteristics of commercial insecticide formulations available in Oman and used in the bioassays

Active ingredient	Trade name	Formulation (ai)	Label concentration	Insect pest
Deltamethrin	Delta 2.5EC	2.5 w/w emulsifiable concentrate	80 mL/100 L (a.i. 10 mg/L)	Aphids, thrips, beetles and others
Thiamethoxam	Actara 25WG	25 w/w water dispersible granules	8 g/20 L (a.i. 100 mg/L)	Aphids, psyllids, leaf miners and others
Acetamiprid	Clipper 20SL	20 w/w soluble liquid	10 mL/20 L (a.i. 100 mg/L)	Whiteflies, thrips, and others

Contact and oral bioassays

Contact and oral bioassays were carried out using three methods of exposure to insecticides (Figure 1). A set of 10 forager honeybees was placed in a Petri dish and immobilized by placing them on a chilling pad for contact bioassays. Acute contact toxicity was measured by either placing a 1-µL droplet on thorax using micropipette or spray using a Potter tower. In Potter tower (Burkard Scientific, Uxbridge, UK) bioassay, 2 mL of each insecticide concentration was sprayed at 70 kPa. Deionized water treatment served as control for contact bioassays.

The diameter of droplets (on the honeybee body) from the Potter tower spray was measured by a stereomicroscope, and the volume of droplets was calculated by following formula (Cunha et al., 2013):

$$Vg = \pi Dg^3 / 6$$

where Vg is the volume of each droplet (µL) and Dg, the droplet diameter (µm). This calculated droplet volume was used to calculate the amount (µL) received by individual honeybee. The volume median diameter (VMD) was calculated using a spreadsheet. The number of droplets deposited on head, thorax, abdomen, legs and wings of a honeybee were also recorded.

In acute oral bioassay, 2 mL of each insecticide concentration (prepared with 20% honey) was applied to a cotton ball which was kept in a small lid placed inside a container (9 cm upper diam., 6 cm lower diam., 7 cm high) (Figure 1). Honeybees were starved for 1 h and then let to feed for 2 h (www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm). In this setup, honeybees place front legs on the edge of the lid and use proboscis to feed thus avoiding any unnecessary contact with the insecticides. After 2 h, honeybees were transferred to new containers and provided with only 20% honey solution. A 20% (w/v) honey solution was provided to honeybees in control for oral bioassay. Each concentration for each insecticide was replicated four times.

The lids with cotton balls were weighed before and after 2 h to measure the amount of each insecticide at each concentration consumed by honeybees during oral exposure. The ingestion LD_{50} values were obtained from the relative LC_{50} values by multiplying with the amount of food consumed in 2 h (Laurino et al., 2011).

The prepared containers with honeybees were kept at 24±2°C and 60±5% RH inside a box in complete darkness. Data about number of dead and live honeybees were recorded at 24, 48 and 72 h after treatment. A honeybee was considered dead when it remained motionless for ten seconds after touching it gently by a fine brush (Laurino et al., 2011). Two hundred, 320 and 240 forager honeybees were used for deltamethrin, thiamethoxam and acetamiprid, respectively, and 40 forager for each control.

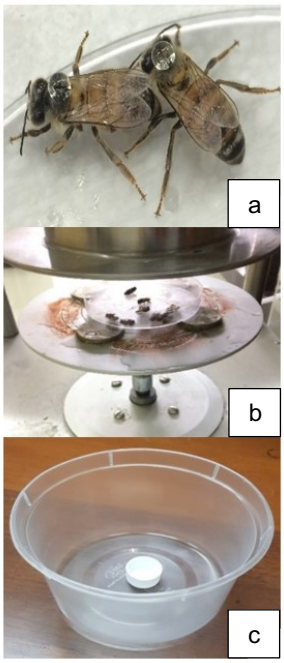


Figure 1. Different methods of honeybee exposure to insecticides: a) a 1-µL droplet on thorax, b) potter spray tower, and c) container used for acute oral bioassay and honeybee feeding.

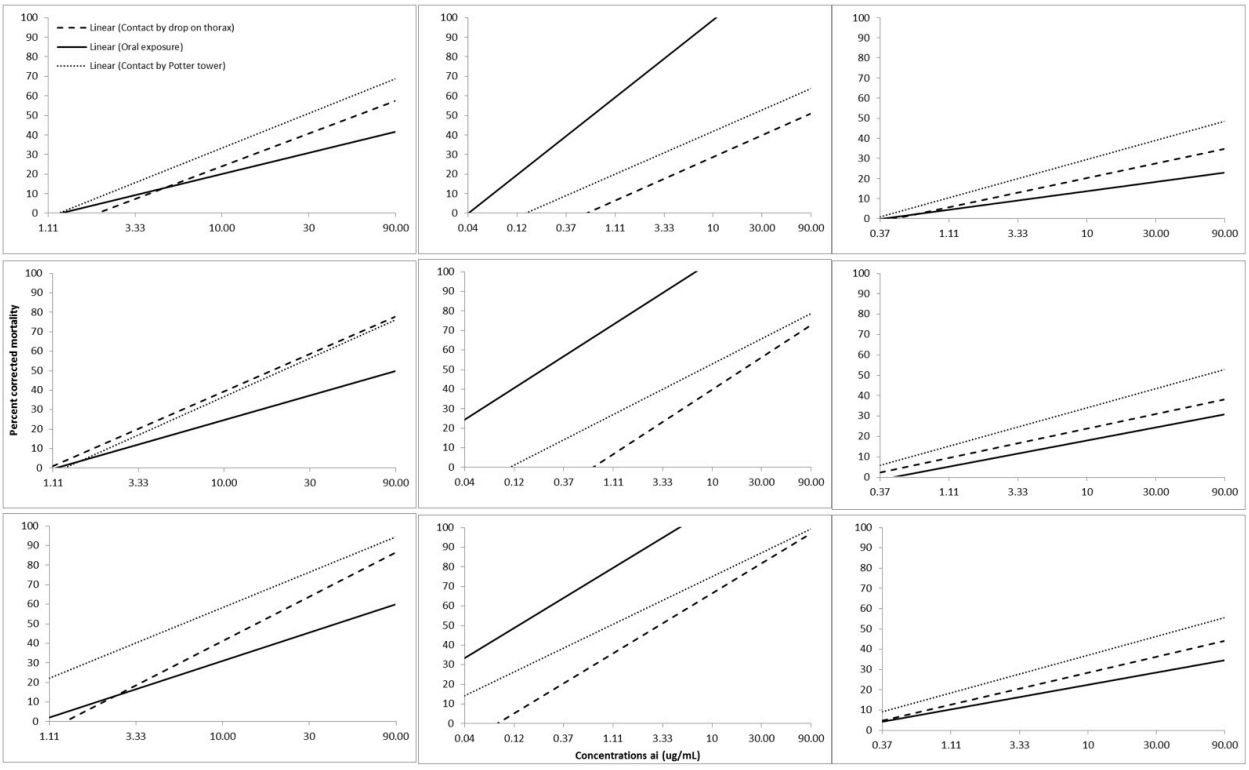
Data analysis

Mortality data was corrected for control using Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} were calculated using PoloPlus software. Probit regressions were plotted by SPSS ver. 18. The number of drops deposited on different body parts of a honeybee after spray by Potter tower was recorded. The number of drops on different body parts was analyzed by ANOVA and Tukey's test was used for means separation in SPSS. A two factor ANOVA was done for the exposure methods for each insecticide at different concentrations; separately for 24, 48 and 72 h. LD_{50} were used to calculate the HQ as field application rate (g/ha) divided by oral LD_{50} (µg/bee) relative to the field application adopted for field concentration determination (Table 1) (OEPP/EPPO, 2010).

Results

Direct observation of the behavior of the honeybees in containers during the trials showed symptoms of poisoning, such as tremors, uncoordinated and uncontrolled movements, and prolonged frenetic movement of the legs at field concentration (30 µL/mL) of deltamethrin and lower concentration (1.1 µL/mL) of thiamethoxam.

After 24 and 48 h of exposure, deltamethrin percent corrected mortality was similar between the exposure methods at lower concentrations. At higher concentrations oral exposure mortality was lower than the contact mortality (Figure 2). At 72 h, Potter tower exposure gave significantly higher mortality ($F = 12.1$, $P = 0.021$) at lower concentrations than contact exposure by single droplet on thorax or oral exposure. This difference was not significant ($F = 1.04$, $P = 0.71$) at the highest concentration from single droplet on thorax exposure (Figure 2).



a b c

Figure 2. Percent corrected mortality caused by a) deltamethrin, b) thiamethoxam and c) acetamiprid by different exposure methods at 24, 48 and 72 h.

Thiamethoxam showed significantly higher mortality through oral exposure at all concentrations than both the contact methods at 24 h ($F = 34.1$, $P = 0.003$), 48 h ($F = 24.3$, $P < 0.001$) and 72 h ($F = 14.2$, $P=0.003$). Potter tower exposure, after 48 and 72 h, gave significantly higher mortality ($F = 16.7$, $P = 0.002$) than droplet on thorax exposure at lower concentrations; the difference was non-significant at higher concentrations. Acetamiprid did not give more than 50% mortality even with the highest concentration at 72h which did not allow calculation of LC_{50} values. Potter tower exposure gave higher mortality which was not significantly different than other exposure methods at 24, 48 and 72 h at lower concentrations. At higher concentration the Potter tower mortality was significantly higher ($F = 7.63$, $P = 0.03$) than oral exposure (Figure 2).

Deltamethrin LC_{50} measured by acute contact exposure (both a droplet on thorax and Potter tower) was significantly lower (95% CI did not overlap) than acute oral exposure, however, LC_{90} values were not different between the exposure methods (Table 2). Thiamethoxam gave low acute oral LC_{50} when fed in treated honey. Thiamethoxam oral LD_{50} and LD_{90} values were significantly lower than both of the contact exposure methods (Table 2).

Table 2. Comparison of LC_{50} and LC_{90} of deltamethrin and thiamethoxam to *Apis mellifera lamarckii* forager after 48 h of exposure by different methods. Six concentrations of deltamethrin and eight concentrations of thiamethoxam were used

Insecticides	Method of exposure	Slope ± SEM	Het[a]	LC_{50}[b]	95% CL[c]	LC_{90}	95% CL
Delta 2.5EC (deltamethrin)	Acute contact (1µL drop on thorax)	1.28±0.22	0.069	19.44	11.76-30.92	195.5	99-669
	Acute contact (Potter tower spray)	1.38±0.26	0.071	22.35	12.19-34.38	189.5	99-663
	Acute oral	0.95±0.23	0.044	70.99	37.77-217.91	1564.0	401-53100
Actara 25WG (thiamethoxam)	Acute contact (1µL drop on thorax)	1.73±0.43	0.996	23.36	12.10-34.54	132.0	76-554
	Acute contact (Potter tower spray)	0.77±0.13	0.699	8.05	3.85-15.61	367.0	127-2690
	Acute oral	1.24±0.18	0.816	0.22	0.11-0.36	2.4	1.42-5.33

[a] Het, heterogeneity adjustment factor;
[b] LC, lethal concentration expressed as µg/mL;
[c] 95% confidence limits.

The slopes of regression lines for deltamethrin acute contact (drop on thorax) and acute oral exposure methods were equal and the hypothesis of parallelism was accepted (P = 0.344). For thiamethoxam the slopes were not equal and the hypothesis of parallelism was rejected (P = 0.002). The lethal dose ratio (LDR) 95% confidence limits were <1 for deltamethrin and only acute contact (drop on thorax) was significantly different than acute oral exposure and there was no significantly different between the two contact exposure methods (Table 3). The calculated LDR for thiamethoxam was not meaningful since the hypothesis of parallelism was rejected. However, regression analysis by PoloPlus showed that acute oral regression line was widely separated from the contact lines indicating that oral LC_{50} values for thiamethoxam were significantly lower than both contact LC_{50} (Table 3).

Table 3. Parallelism hypothesis and lethal dose ratio of deltamethrin and thiamethoxam at LC_{50} after 48 h acute contact (drop on thorax). Two hundred and forty, and 320 honeybees were used for acute contact (deltamethrin) and acute oral (thiamethoxam), respectively

Method of exposure	df	Parallelism (Chi-square)	Lethal Dose Ratio (95% CI)	
			Acute contact (Potter tower)	Acute oral (Feeding honey)
Deltamethrin	2	2.13 (P = 0.344)[a]	0.870 (0.450-1.68)	0.274 (0.111-0.677)[b]
Thiamethoxam	2	12.2 (P = 0.002)	2.90 (1.30-6.50)	105 (51.6-215)

[a] Parallelism hypothesis is not rejected at P > 0.05;
[b] If 95% confidence interval does not include 1, then LD_{50} is significantly different between exposure methods and between insecticides.

In total, 1070±106 droplets were deposited on a single worker honeybee after spray by the Potter tower (Table 4, Figure 3). The average number of droplets deposited on head and thorax of a honeybee were similar but significantly greater than abdomen, legs and wings (F = 23.3, P < 0.001) (Table 5). To the best of our knowledge this information was not available before. The average droplet diameter was 0.286±0.071 µm. The total calculated volume of an insecticide deposited on a honeybee was 0.829 µL (0.669-0.990 µL) and was used in contact LD_{50} calculation. About 66% of the total spray volume was deposited on head and thorax (Table 4).

Table 4. Amount (ng/bee±SEM) of contaminated food (20% honey solution) consumed by individual forager bee. Two hundred and forty honeybees were used for each insecticide

Concentration (µg/mL)	Thiamethoxam	Acetamiprid	Deltamethrin
90	--*	722±81.8	2480±1870
30	--	188±32.1	6750±1190
10	90.0±12.4	40.0±12.8	1750±328
3.33	63.3±2.8	14.5±2.5	500±47
1.11	3.36±0.8	7.4±1.7	167±38
0.37	2.13±0.7		
0.12	2.82±0.2		
0.04	0.72±0.04		

* All honeybees were dead in 30 and 90 µg/mL concentration in thiamethoxam treatment.

Table 5. Number of droplets and droplet volume deposited on different body parts of forager honeybee when sprayed under Potter tower at 70 kPa. In brackets lower and upper limits at 95%. Tukey's test was used for means separation

Body part	Average number of droplets	Total volume (µL)	Percentage
Head	305a* (222-388)	0.236a (0.172-0.300)	28.5a (25.8-30.6)
Thorax	403a (311-494)	0.312a (0.241-0.383)	37.6a (35.8-39.1)
Abdomen	113b (98-127)	0.087b (0.076-0.099)	10.5b (8.3-13.4)
Legs	108b (98-117)	0.083b (0.076-0.091)	10.1b (8.9-11.5)
Wings	143b (106-179)	0.130b (0.082-0.139)	13.3b (10.9-15.7)
Total	1070 (863-1277)	0.829 (0.669-0.990)	

* Values followed by the same letters within column are not significantly different at $\alpha_{0.05}$.

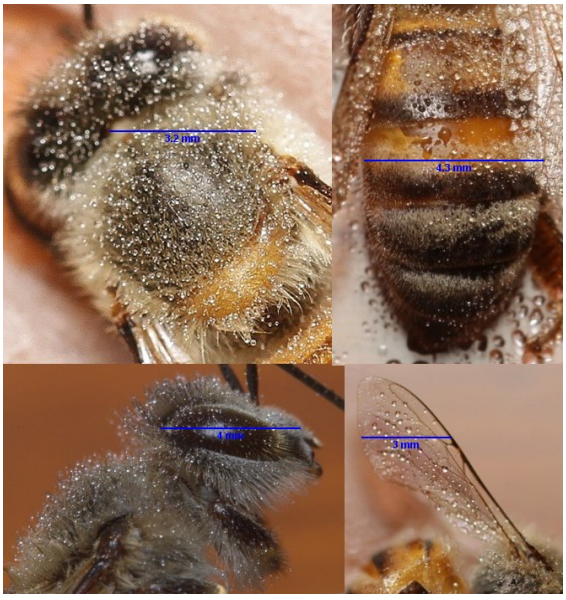


Figure 3. Deposition of droplets on different body parts of a forager honeybee through Potter spray tower at 70 kPa. [Actual sizes: head (4.0±0.5 x 1.0±0.1 mm); thorax (4.7±0.3 x 4.3±0.3 mm); abdomen (6.0±0.5 x 4.3±0.3 mm) and wings (8.3±0.3 x 3.0±0.5 mm)].

The amount of honey solution consumed by a single worker honeybee in 2 h varied according to the concentration and type of insecticide (Table 3). The amount (ai) of deltamethrin consumed decreased from 2480±1870 to 167±38 ng/bee with decrease in concentration. Acetamiprid consumed reduced from 722±81.8 to 7.44±1.7 ng/bee between the highest and the lowest concentration. The consumption of thiamethoxam was very low (90.0±12.4 and 0.72±0.04 ng/bee) compared to the other two insecticides. There was no consumption data for the 30 and 90 mg/mL concentrations of thiamethoxam recorded because of very low non-measurable quantities consumed. The uncontaminated control group consumed 27000±2350 ng of 20% honey/bee (Table 4).

The hazard quotients ($HQ_{contact}$ and HQ_{oral}) for deltamethrin and thiamethoxam (Table 6) were calculated based on the average amount of received by a single honeybee or food consumed by a single forager honeybee contaminated with each insecticide. The average (all concentrations) deltamethrin and thiamethoxam contaminated consumed food was 9.87±1.3 and 12.0±1.7 µg/bee, respectively. The $HQ_{contact}$ values were low and the tested insecticides are less harmful to foraging honeybees. HQ_{oral} values indicate that thiamethoxam is extremely toxic to honeybees when ingested (Table 6). Hazard quotients provide an estimate of the risk in comparing the application rate of an insecticide and its intrinsic toxicity and they aim at deciding whether high tier testing is needed (pp1.eppo.int/standards/PP1-170-4).

Table 6. Lethal concentrations (LC_{50}, LD_{50}) and hazard quotients (HQ) for deltamethrin and thiamethoxam 48 h after exposure. In brackets lower and upper limits at 95%

Insecticide	Method of exposure	LC_{50} (µg/mL)	LD_{50} (ng/bee)	HQ
Deltamethrin	Acute contact (drop on thorax)	19.4	19.4 (11.8-30.9)	0.62 (0.38-1.02)
	Acute contact (Potter tower)	22.4	19.0[a] (10.1-28.5)	0.65 (0.51-1.43)
	Acute oral (feeding honey)	71.0	710[b] (378-2180)	16.9 (5.51-31.8)
Thiamethoxam	Acute contact (drop on thorax)	23.4	23.4 (12.1-34.5)	0.51 (0.35-0.99)
	Acute contact (Potter tower)	8.05	6.68 (3.19-13.0)	1.79 (0.92-3.76)
	Acute oral (feeding honey)	0.222	2.66[c] (1.32-4.32)	3750 (2310-7580)

[a] Potter tower deposited 0.83 µL/bee;
[b] Deltamethrin average oral consumption of 9.87 µg/bee;
[c] Thiamethoxam average oral consumption of 12.0 µg/bee.

Discussion

Contact bioassays are preferred as they better simulate the situation in the field. The standard contact bioassay requires a 1-µL droplet placed on the thorax of an insect (e.g., honeybee). The diameter of a 1-µL droplet recorded on honeybee thorax was 1200±150 µm. A medium spray (VMD of 350 µm) droplet size is recommended for insecticides in field which can be achieved by using cone and fan nozzles (Hewitt et al., 1997; Hanna et al., 2009). The Potter tower spray produced a VMD of 16±3.7 µm. The optimum size for insecticide spray droplets for the highest efficacy was about 20 µm in diameter, whereas droplets 50-100 µm in diameter were marginal in efficiency (Himel, 1969). The average droplet size produced by the Potter spray tower at 70 kPa was similar to the most effective droplet size (20 µm). The size of the droplet on thorax was three times larger whereas the droplets produced by the Potter tower were closer to the field spray droplet sizes. The medium spray droplet size reduced to very fine (VMD of 50 µm) soon after application due to environmental conditions. Forager honeybees are more likely experience the very fine droplets. The fine droplets produced by the Potter tower gave good coverage of the body and all body parts, including eyes were covered which would contribute to quicker/greater

absorption and higher mortality with all insecticides. Whenever possible, honeybee toxicology studies should use Potter tower or similar devices instead of a single droplet on thorax.

Mortality of forager honeybee when exposed to different concentrations of deltamethrin, thiamethoxam and acetamiprid varied between the different exposure methods. Contact exposure mortality was higher than oral exposure mortality in deltamethrin and acetamiprid treated honeybees while oral exposure was higher in thiamethoxam treated honeybees because deltamethrin is a contact and thiamethoxam is a systemic insecticide. Acetamiprid is a systemic insecticide with translaminar activity and cause mortality both by contact and ingestion (PPDB, 2019). The small spray droplets by Potter tower could have been easily and rapidly absorbed. Potter tower exposure gave higher mortality than droplet on thorax exposure for all the three insecticides. The nitro-substituted neonicotinoids like thiamethoxam are found to be the most toxic to the honeybee in laboratory studies, however, the cyano-substituted neonicotinoids like acetamiprid exhibited a much lower toxicity (Iwasa et al., 2004). Although acetamiprid is a neonicotinoid insecticide, it is much safer to forager honeybees than thiamethoxam (Laurino et al., 2011).

The 48-h oral LD_{50} of deltamethrin was significantly higher (710 ng/bee) compared to either contact exposure by a droplet on thorax (28.3 ng/bee) or Potter tower (18.6 ng/bee). Deltamethrin can be more toxic to honeybees if directly exposed during spray operations, which is a more likely scenario for forager bees. There is a range of acute contact LD_{50} values found in literature. The topical (1-µL droplet on thorax) LD_{50} of 24 ng/bee, 50.7 ng/bee and 677 ng/bee have been reported (Mayer, 1999; Carvalho et al., 2013; Sanchez-Bayo & Goka, 2014). Our calculated acute contact LD_{50} values for deltamethrin, although on the lower side, are within the reported range. An acute oral LD_{50} of 270 ng/bee and 850 ng/bee of deltamethrin have been reported (Carvalho et al., 2013; Sarto et al., 2014).

Thiamethoxam gave significantly lower oral LC_{50} (0.222 µg/mL) compared to both contact exposure methods. The calculated oral LD_{50} was 2.66 ng/bee based on the average consumption of 12 µL/bee. An oral LC_{50} of 0.150 µg/mL and an LD_{50} of 4.41 ng/bee was calculated based on the average consumption of 35 µL/bee (Laurino et al., 2011). An oral LD_{50} of 5.0 ng/bee has also been reported (Tomlin, 2003; Decourtye & Devillers, 2010). In our control group the average consumption was 29±2.9 µL/bee but consumption of treated honey was different. Since neonicotinoid insecticides are systemic, they have less contact activity. Thiamethoxam acute contact LD_{50} (by a droplet on thorax) of 23.36 ng/bee, 24 ng/bee and 30 ng/bee are also available in literature (Senn et al., 1998; Iwasa et al., 2004; Decourtye & Devillers, 2010).

The thiamethoxam slopes for exposure methods were not parallel and the hypothesis of parallelism was rejected, therefore, the calculated LDR was not meaningful. However, the acute oral regression line was widely separated from the contact lines, which explains the significant difference between oral and contact exposure methods.

The droplets deposited with the Potter spray tower gave good coverage of the body of a bee. The presence of larger number of droplets on head and thorax were because of the presence of hair. The total calculated volume deposited on body was <1 µL which is a standard droplet size on thorax but the recorded mortality was usually higher. The higher mortality can be attributed to even spread and quicker absorption of smaller droplets. The authors could not find any reference for comparison.

At the same highest and lowest concentrations, the consumption of acetamiprid decreased 34 and 22 times, respectively, compared to deltamethrin. There was 34 times and 22 times decrease in the consumption of acetamiprid compared to deltamethrin when offered the same highest and lowest concentrations, respectively. A fiftyfold decrease was recorded in the thiamethoxam consumption at the concentration of 1.11 µg/mL compared to deltamethrin. Acetamiprid can act as a repellent and this repellency effect may increase at lower concentrations (Laurino et al., 2011). Food regurgitation and vomiting by poisoned honeybees at higher concentrations of thiamethoxam can occur (Laurino et al., 2011). The lowest concentration of thiamethoxam 0.12 µg/mL caused 44% mortality. Ninety-one and 6.7 ng/mL

of deltamethrin, and 127 and 17 ng/mL of thiamethoxam have been found in the pollen and nectar, respectively (Scott-Dupree et al., 2001; Chauzat et al., 2011; Stoner & Eitzer, 2013). These low concentrations of thiamethoxam can be toxic to forager honeybees through oral ingestion. The very high HQ_{oral} value of 3750 (2310-7580) indicates that thiamethoxam is extremely toxic to honeybees when ingested. HQ value of 22700 for thiamethoxam with an LD_{50} of 4.41 ng/bee has been reported (Laurino et al., 2011). The higher HQ is due to the higher value LD_{50} which is dependent on the amount consumed.

Bees are generally active from sunrise until a couple of hours before sunset, and pesticide risk exposure to honeybees can be reduced by spraying the crops in the evening when honeybees are not foraging. Some countries, for example Canada and USA, have strict drift prevention protocols and use specific devices, and spray droplets in air may not be of concern. There must be proper communication between the applicators, farmers and beekeepers, and beekeepers should be informed of any spraying operations so they can protect their beehives.

Forager honeybees are more likely experience the very fine droplets in field and acute contact toxicological results obtained by Potter tower or similar devices will be more realistic than a single droplet on thorax. Insecticide-contaminated food consumed by forager honeybees was significantly lower than the control, and the actual amount of food consumed at each concentration could be used in LD_{50} calculations.

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Original article (Orijinal araştırma)

Mite diversity and population dynamics of eriophyid mites on olive trees in Western Turkey[1]

Türkiye'nin batısında zeytin ağaçlarında akar çeşitliliği ve eriophyid akarların popülasyon dalgalanması

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Abstract

A study was conducted to determine the diversity of phytophagous mites and their predators in olive orchards in Balikesir, Izmir and Manisa Provinces, Turkey from 2015 to 2017. Also, the population density of eriophyid mites was detected in four olive orchards in Izmir and Manisa in 2016 and 2017. As a result, sixteen species were collected, namely, *Aceria oleae* (Nalepa, 1900), *Tegolophus hassani* (Keifer, 1959) (Eriophyidae), *Cenopalpus lineola* (Canestrini & Fanzago, 1876), *Brevipalpus* sp. (Tenuipalpidae), *Amblyseius andersoni* Chant, 1957*, Euseius stipulatus* (Athias-Henriot, 1960)*, Neoseiulus barkeri* Hughes, 1948*, Paraseiulus talbii* (Athias-Henriot, 1960)*, Typhlodromus athenas* Swirski & Ragusa, 1976*, Typhlodromus athiasae* Porath & Swirski, 1965, *Typhlodromus psyllakisi* Swirski & Ragusa, 1976*, Typhlodromus rarus* Wainstein, 1961*, Typhlodromus recki* Wainstein, 1958 (Phytoseiidae), *Raphignathus gracilis* (Rack, 1962) (Raphignathidae), *Agistemus duzgunesae* Koc, Cobanoglu & Madanlar, 2005 (Stigmaeidae) and *Tydeus californicus* (Banks, 1904) (Tydeidae). *Aceria oleae* and *T. hassani* were found throughout the growing period from April to November in all orchards and they reached their highest population densities on buds during April, and leaves and fruit during May and June. *Typhlodromus athenas* was observed throughout the year in the majority of the orchards, and this is a first record of this species for the Turkish fauna. It was observed that *A. duzgunesae* also fed on eriophyid mites. Further studies are needed to investigate prey range of the predatory mites, *T. athenas* and *A. duzgunesae* and their potential as biological control agents of eriophyid mites.

Keywords: Eriophyidae, mites, olive, Phytoseiidae, Turkey

Öz

Bu çalışmada 2015-2017 yıllarında Balıkesir, İzmir ve Manisa illerinde zeytinde zarar yapan akar türleri ve predatörlerinin belirlenmesi amaçlanmıştır. Ayrıca, eriophyid akarların popülasyon yoğunlukları 2016 ve 2017 yıllarında Izmir ve Manisa'da dört adet zeytin bahçesinde belirlenmiştir. Sonuç olarak on altı tür saptanmıştır: *Aceria oleae* (Nalepa, 1900),*Tegolophus hassani* (Keifer, 1959) (Eriophyidae), *Cenopalpus lineola* (Canestrini & Fanzago, 1876) and *Brevipalpus* sp. (Tenuipalpidae), *Amblyseius andersoni* Chant, 1957*, Euseius stipulatus* (Athias-Henriot, 1960)*, Neoseiulus barkeri* Hughes, 1948*, Paraseiulus talbii* (Athias-Henriot, 1960)*, Typhlodromus athenas* Swirski & Ragusa, 1976*, Typhlodromus athiasae* Porath & Swirski, 1965, *Typhlodromus psyllakisi* Swirski & Ragusa, 1976*, Typhlodromus rarus* Wainstein, 1961*, Typhlodromus recki* Wainstein, 1958 (Phytoseiidae), *Raphignathus gracilis* (Rack, 1962) (Raphignathidae), *Agistemus duzgunesae* Koc, Cobanoglu & Madanlar, 2005 (Stigmaeidae) ve *Tydeus californicus* (Banks, 1904) (Tydeidae). *Aceria oleae* ve *T. hassani*'ye örnekleme yapılan tüm bahçelerde nisan ayından kasım ayına kadar tüm vejetasyon boyunca rastlanılmış ve en yüksek popülasyon yoğunluğu nisan ayında tomurcuklarda, mayıs ve haziran aylarında yaprak ve meyvelerde saptanmıştır. *Typhlodromus athenas*, örnekleme yapılan bahçelerin büyük çoğunluğunda yıl boyunca yaygın olarak gözlenmiş ve Türkiye'de varlığı ilk kez bu çalışmayla saptanmıştır. Bununla birlikte *A. duzgunesae*'nin eriophyid akarlarla beslendiği gözlenmiştir. Daha sonra yapılacak çalışmalarda avcı akarlar, *T. athenas* ve *A. duzgunesae*'nin av yelpazesinin belirlenmesi ve bunların eriophyid akarlar üzerinde biyolojik mücadele etmeni olarak potansiyellerinin ortaya çıkarılması gerekmektedir.

Anahtar sözcükler: Eriophyidae, akar, zeytin, Phytoseiidae, Türkiye

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Introduction

Due to the positive effects of olive oil on human nutrition and health, olive production is increasing in Turkey as well as all over the world. Olive is produced economically in 37 countries around the world, especially in Spain, Greece, Italy, Turkey, Morocco, Egypt and Tunisia (FAO, 2017). Olive trees are a major agricultural crop in the Aegean Region, Turkey where they are mostly grown in Balikesir, Izmir and Manisa Provinces for olive oil and table olive production. There are many factors, including pests and diseases, that limit the production of olives.

Mites have been found to cause significant damage in olive production areas over the last decade (Kacar et al., 2010; Leiva et al., 2013). They are found on the leaves, buds, flowers and fruit of the olive tree and cause greenish-yellow spots on old leaves and deformations on young leaves; dark green depressions and rust spots on buds; abnormal formations, brown cracked areas and whitish, silvery colored parts on fruit (Cetin & Alaoglu, 2006; Kacar et al., 2010). Jardak et al. (2007) showed that mites, which were previously regarded as secondary pests, caused significant damage in some olive regions for the last 20 years, reduced the olive oil yield by up to 46%, and increased the acidity of olive oil by decreasing the chlorophyll and polyphenol content. Leiva et al. (2013) reported that mites in olives cause up to 20% economic loss due to deformation in leaves and fruit.

Some members of the superfamilies Eriophyoidea and Tetranychoidea (Acari: Prostigmata) are pests of olives and are either monophagous or oligophagous. Thirty species, including 12 species in Eriophyidae, 17 species in Tenuipalpidae and one species in Tetranychidae, have been detected in olive trees worldwide to date (Tzanakakis, 2003). Eriophyid mites are economically pest species in olive (Jardak et al., 2007). Many species of eriophyid mites are known to be specific to their host and 10 species have been recorded only on olives (Tzanakakis, 2003). *Phytoptus* (*Aceria*) *oleae* (Nalepa, 1900) (Acari: Eriophyidae) was determined in the first studies on the mite species in olive trees in Turkey (Iyriboz, 1938; Bodenheimer, 1941; Iyriboz, 1968). Kumral & Kovancı (2004) reported seven mite species (2 phytophagous, 2 predators and 3 neutral) were found in olive orchards in Bursa Province and *A. oleae*, *Typhlodromus involutus* Livshitz & Kuznetsov, 1972 (Acari: Phytoseiidae) and *Brevipalpus oleae* Baker, 1949 (Acari: Tenuipalpidae) were present at high densities. *Aceria oleae* and *Aculus olearius* Castagnoli, 1977 (Acari: Eriophyidae) were found in olives in Mersin (Mut) (Cetin & Alaoglu, 2006). Kacar et al. (2010) found *A. oleae* and *Tegolophus hassani* (Keifer, 1959) (Acari: Eriophyidae) in olive trees in the Eastern Mediterranean Region of Turkey. Kumral et al. (2010) found two phytophagous mites, *B. oleae* and *A. oleae*, and seven predatory mites, *Typhlodromus athiasae* Porath & Swirski, 1965, *Typhlodromus recki* Wainstein, 1958, *T. involutus*y (Phytoseiidae), *Cheletogenes ornatus* (Canestrini & Fanzago, 1876) (Cheyletidae), *Zetzellia* sp. (Stigmaeidae), *Pronematus ubiquitus* (McGregor, 1932) (Tydeidae), and *Erythraeus* sp. (Erythraeidae) on olive trees in Bursa, Turkey. Also, *A. oleae* and *A. olearius* were determined on fruit of olive in the Aegean Region of Turkey (Cetin et al., 2012). Very few studies have been conducted on mites in olive trees in Turkey and only a small number of mite species have been reported in Turkey compared to the literature (Tzanakakis, 2003; Jardak et al., 2007). Therefore, this study was conducted to determine the diversity of phytophagous mites, their predators and the population dynamics of eriophyid mites that occur in olive orchards in the Balikesir, Izmir and Manisa Provinces, Turkey.

Materials and Methods

Phytophagous mites and their predators on olive trees

A survey was conducted to determine phytophagous mites and their predators on olive orchards in Balikesir, Izmir and Manisa Provinces, Turkey from 2015 to 2017. Orchards were regularly visited every month between March and December (twice a month in spring and autumn) in 2015. Additional, non-periodic samplings were performed in 2016 and 2017. Each orchard was sampled by collecting 150 leaves, fruit and buds from different sides of the trees (Table 1). Samples were placed into polyethylene bags and brought to the laboratory within an ice chest. Each sample was divided into three sets of plant material

(buds, leaves and fruit) that were either directly examined under the stereomicroscope (30X, Leica EZ4, Wetzlar, Germany) or put into Berlese funnels to extract mites. All mites found in the samples were placed in Eppendorf tubes containing 70% ethanol. Afterward, mites were cleared in a lacto-phenol solution, and then mounted in Hoyer's medium (Dizlek et al., 2019). Slides were dried at 45-50°C for 3-4 d on a hot plate (Termal N11153C, Istanbul, Turkey). Identification of the mite species was done by Ismail Doker (Turkey) for Phytoseiidae, Farid Faraji (The Netherlands) for Phytoseiidae, Tenuipalpidae, Tydeidae, Salih Dogan (Turkey) for Raphignathidae, Stigmaeidae and one of the authors (Evsel Denizhan) for Eriophyidae. The voucher specimens of species were deposited in the mite collection of Ibrahim Cakmak at the Department of Plant Protection, University of Aydin Adnan Menderes, Turkey.

Table 1. Number of olive trees and orchards sampled in the surveyed provinces and districts in Turkey

Province	District	Number of trees ($x10^6$)	Number of orchards sampled
Balikesir (I)*	Ayvalik (a), Burhaniye (b), Edremit (c), Gomec (d), Havran (e)	9.2	19
Izmir (II)	Aliaga (a), Bergama (b), Bayindir (c), Bornova (d) Dikili (e), Foca (f), Kemalpasa (g), Menderes (h), Odemis (i), Selcuk (j), Seferihisar (k), Tire (l), Torbali (m), Urla (n)	15.7	31
Manisa (III)	Akhisar (a), Ahmetli (b), Belen (c), Kirkagac (d), Merkez (e), Saruhanli (f), Soma (g), Salihli (h)	17.3	35
Total		52.2	85

* Codes in parentheses are used to reference these districts in the below.

Population density of eriophyid mites

The population density of eriophyid mites was determined in four olive (cv. Ayvalik) orchards, two orchards in Izmir (Bornova and Kemalpasa), two orchards in Manisa (Akhisar and Saruhanli). Weekly sampling was done between March and November in 2016 and 2017. In each orchard, 30 leaves, 30 buds and 30 fruit were randomly collected from different sides of the trees at 1-1.25 m height. The collected leaf, bud and fruit samples were wrapped with paper and put in polyethylene bags. They were brought in an icebox to the laboratory and kept in the refrigerator at 4°C. Eriophyid mites on the leaves, buds and fruit were counted separately under a stereomicroscope (Soif MD90, Shanghai, China). All sampled orchards were pesticide-free. The average temperature and relative humidity in the four districts sampled in 2016 and 2017 are given in Figure 1.

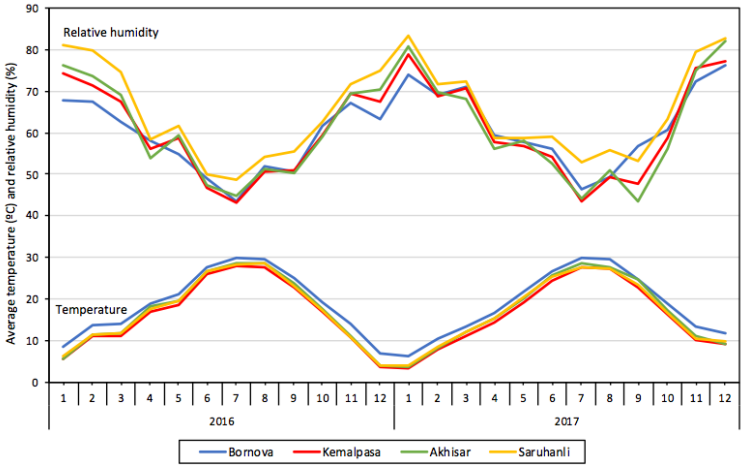

Figure 1. Mean temperature and relative humidity values in four districts sampled in 2016 and 2017.

Results

Phytophagous mites and their predators on olive trees

Four phytophagous mites, *Aceria oleae*, *T. hassani* (Eriophyidae), *C. lineola* and *Brevipalpus* sp. (Tenuipalpidae); eleven predatory mites, *Amblyseius andersoni* Chant, 1957, *Euseius stipulatus* (Athias-Henriot, 1960), *Neoseiulus barkeri* Hughes, 1948, *Paraseiulus talbii* (Athias-Henriot, 1960), *Typhlodromus (Anthoseius) athenas* Swirski & Ragusa, 1976, *T. (Anthoseius) psyllakisi* Swirski & Ragusa 1976, *T. (Anthoseius) rarus* Wainstein, 1961, *T. (Anthoseius), T. (Typhlodromus) athiasae* (Phytoseiidae), *Raphignathus gracilis* (Rack, 1962) (Raphignathidae) and *Agistemus duzgunesae* Koc, Cobanoglu & Madanlar, 2005 (Stigmaeidae); one neutral mite, *Tydeus californicus* (Banks, 1904) (Tydeidae) were detected in the olive orchards sampled (Table 2).

Eriophyid mites, *A. oleae* and *T. hassani* were found in all locations of Balikesir, Manisa and Izmir Provinces in spring (April and May) and autumn (September and October) in 2015, 2016 and 2017 (Table 1). Both mite species were found in mixed populations and they occurred greenish-yellow spots and malformation of leaves, rust spots on buds and malformation of fruit (Figure 2). Tenuipalpid mites, *C. lineola* and *Brevipalpus* sp. were detected from one location in Izmir and nine locations from Balikesir, Manisa and Izmir Provinces, respectively. No damage to olive trees caused by tenuipalpid mites was observed. Phytoseiidae species were the most common predatory mites. *Typhlodromus athenas* was commonly observed throughout the year in the majority of the olive orchards, and this species is a first record for the Turkish fauna. *Agistemus duzgunesae* was recorded from 11 locations in Balikesir, Manisa and Izmir Provinces, and *A. duzgunesae* was observed to feed on eriophyid mites.

Table 2. Mite species obtained from olive orchards in Balikesir, Izmir and Manisa

Family	Species	Locality* (as referred in Table 1)
Eriopyhidae	*Aceria oleae*	I (a, b, c, d, e), II (a, b, c, d, e, f, g, h, i, j, k, l, m, n), III (a, b, c, d, e, f, g, h)
	Tegolophus hassani	I (a, b, c, d, e), II (a, b, c, d, e, f, g, h, i, j, k, l, m, n), III (a, b, c, d, e, f, g, h)
Tenuipalpidae	*Cenopalpus lineola*	II (b)
	Brevipalpus sp.	I (a, c, d, e), II (b, c, l), III (a, f)
Phytoseiidae	*Amblyseius andersoni*	II (f)
	Euseius stipulatus	I (c)
	Neoseiulus barkeri	I (a), II (c)
	Paraseiulus talbii	II (d)
	*Typhlodromus (Anthoseius) athenas***	I (a, b, c, d, e), II (a, b, d, g, k, j, m, o), III (a, c, d, e, f, g, h)
	Typhlodromus(Anthoseius) psyllakisi	I (c), II (b, d, g)
	Typhlodromus (Anthoseius) rarus	I (b), II (f), III (e, f, h)
	Typhlodromus (Anthoseius) recki	I (a), II (c, l)
	Typhlodromus(Typhlodromus) athiasae	I (c), II (b, c, g, l, n), III (a, b, c, e)
Raphignathidae	*Raphignathus gracilis*	II (b, d, g), III (a)
Stigmaeidae	*Agistemus duzgunesae*	I (b, c, e), II (b, d, g, j, l), III (a, f, h)
Tydeidae	*Tydeus californicus*	I (a, b, c, d), II (b, d, e, g, l), III (a, e, f)

* (I) Balikesir, (a) Ayvalik, (b) Burhaniye, (c) Edremit, (d) Gomec, (e) Havran; (II) Izmir, (a) Aliaga, (b) Bergama, (c) Bayindir, (d) Bornova, (e) Dikili, (f) Foca, (g) Kemalpasa, (h) Menderes, (i) Odemis, (j) Selcuk, (k) Seferihisar, (l) Tire (m) Torbali, (n) Urla; (III) Manisa, (a) Akhisar, (b) Ahmetli, (c) Belen, (d) Kirkagac, (e) Merkez, (f) Saruhanli, (g) Soma, (h) Salihli;

** New record for Turkish fauna.

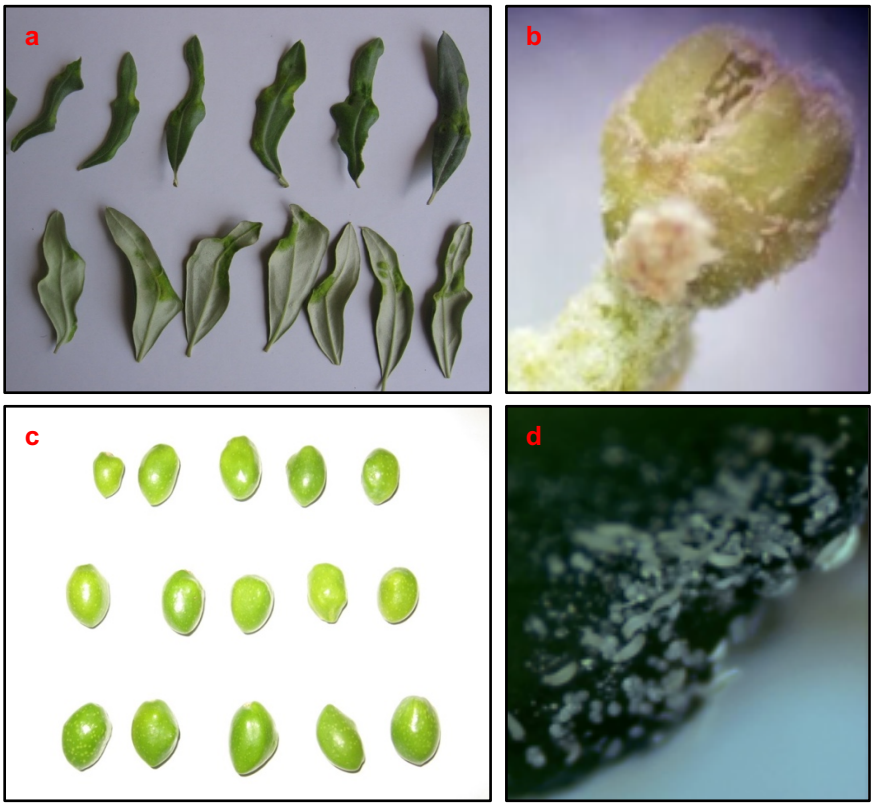

Figure 2. Damage caused by eriophyid mites to leaves, buds and fruit: a) greenish-yellow spots and malformation of leaves, b) rust spots on a bud, c) malformation of fruit, and d) eriophyid mites on a fruit.

Population density of phytophagous mites

The population density of eriophyid mites, *A. oleae* and *T. hassani* in four olive orchards in Izmir (Bornova and Kemalpasa) and Manisa (Akhisar and Saruhanli) in 2016 and 2017 are given in Figures 3 and 4. In Bornova, eriophyid mites first appeared on 23 March 2016 on buds of olive trees with a density of 1.5 mites/bud (Figure 3a). The population density of eriophyids was the highest in late April 2016 (33.3 mites/bud). Subsequently, eriophyids appeared to spread to colonize leaves and fruit in early May 2016. The population of eriophyid mites peaked four times on leaves in mid-May (13.5 mites/leaf), early June (21.9 mites/leaf), late July (15.5 mites/leaf) and late September (3.9 mites/leaf) and peaked three times on fruit in late May (70 mites/fruit), early June (37.5 mites/fruit) and late September (10.4 mites/fruit). In 2017, the first occurrence of the eriophyids on bud was found in early April and the highest density was in mid-April (33.3 mites/bud). The population of eriophyid mites peaked three times on leaves in early May (4.6 mites/leaf), early June (5 mites/leaf) and late July (10 mites/leaf). The highest density on fruit was in early June (60 mites/fruit). Their population on fruit was higher than buds and leaves in both 2016 and 2017 (Figure 3a).

In Kemalpasa, eriophyid mites appeared between late March and early October in 2016 and between early May and early September in 2017 (Figure 3b). Eriophyid mites appeared the first time on buds in late March 2016 and the highest population was in late April (10 mites/bud). The maximum density on leaves and fruit was in mid-June with a density of 23.9 mites/leaf and 78.9 mites/fruit, respectively. In 2017, eriophyids were first observed on buds in late April and their density on buds and leaves peaked in late May (12 mites/bud and 89.5 mites/leaf). Eriophyid mites moved from leaves to fruit at the beginning of June. Their population peaked once in mid-June with a density of 115.5 mites/fruit (Figure 3b).

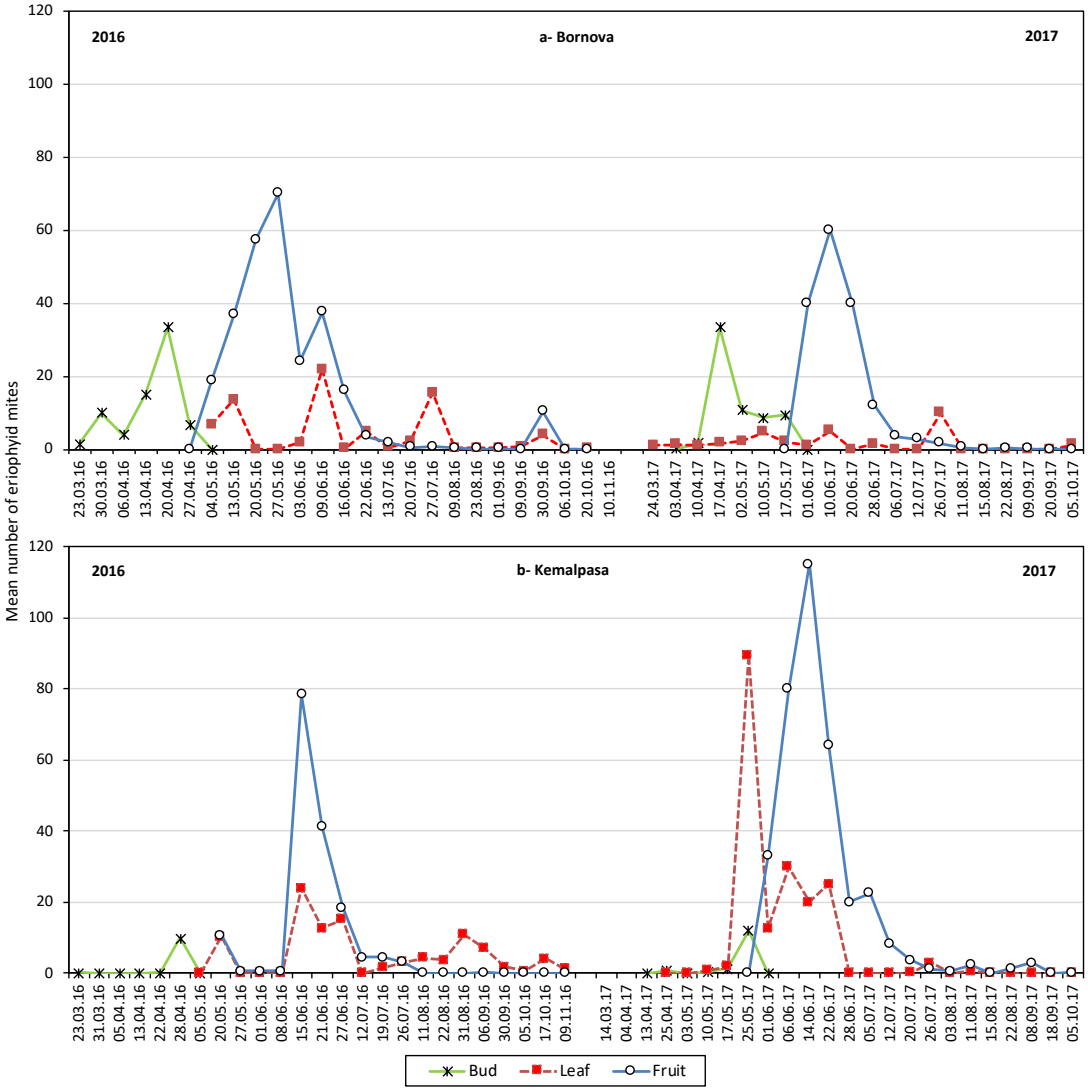

Figure 3. Population densities of eriophyid mites on olive orchards in Bornova (a) and Kemalpasa (b) districts of Izmir, Turkey during 2016-2017.

In Akhisar, eriophyid mites appeared on buds in early April 2016 and their population peaked in early June (44.4 mites/ bud) (Figure 4a). They migrate from buds to leaves in early May. The population of eriophyid mites peaked four times on leaves in early May (3 mites/leaf), mid-June (7 mites/leaf), late July (17.2 mites/leaf) and late August (23.9 mites/leaf) and peaked four times on fruit in mid-June (55.9 mites/fruit), late June (31.7 mites/fruit), late September (4.4 mites/fruit) and early October (5 mites/fruit). In 2017, eriophyid mites observed first on bud and the highest population on buds and leaves was in late May with a density of 2.8 mites/bud and 20 mites/leaf. At the beginning of June, they completely disappeared from the leaves. Then, they were observed on fruit and they peaked in late June (23.2 mites/fruit). The population of eriophyids on both leaves and fruit in 2016 was higher than in 2017 (Figure 4a).

In Saruhanli, eriophyids were first detected in mid-April 2016 on buds and their population peaked two times in late April (4.7 mites/bud) and late May (4 mites/bud). Their population on the leaves was the highest in late May (50 mites/leaf). At the beginning of June, the population was the highest on fruit with a density of 25.7 mites/fruit. In 2017, eriophyid mites appeared first on buds in mid-April and their population peaked in mid-May (2.4 mites/bud). The population of eriophyid mites peaked three times on leaves in late May (25 mites/leaf), mid-June (5 mites/leaf) and late June (8.2 mites/leaf) and peaked once on fruit in late June (51.5 mites/fruit) (Figure 4b).

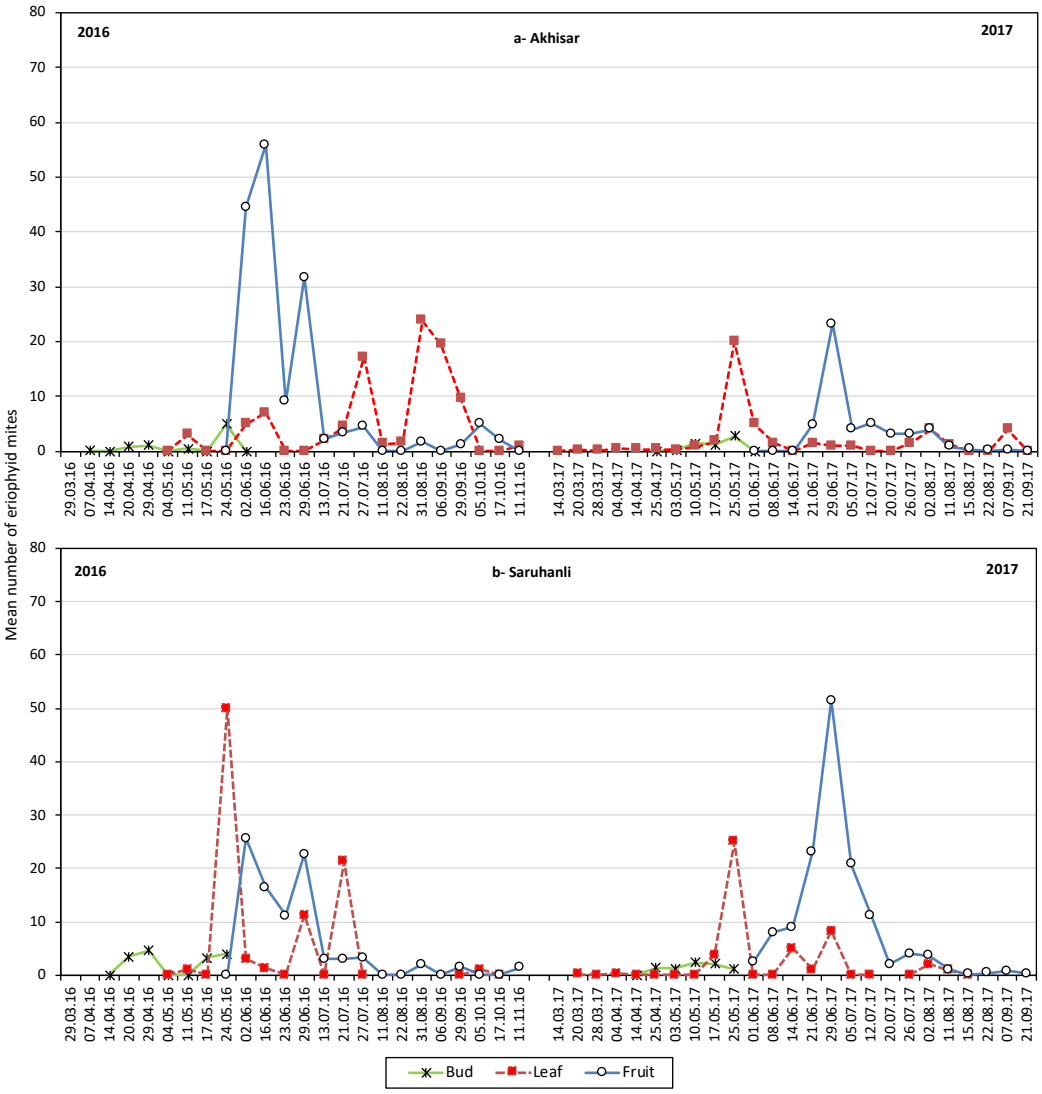

Figure 4. Population densities of eriophyid mites on olive orchards in Akhisar (a) and Saruhanli (b) districts of Manisa, Turkey during 2016-2017.

Discussion

This study showed that eriophyid mites, *A. oleae* and *T. hassani* can be found at all sampled locations in Balikesir, Manisa and Izmir Provinces. Both mite species were found in mixed populations and caused deformation of leaves, buds and fruit. Similarly, in the studies conducted by other researchers, *A. oleae* was reported to be an important pest that feeds on the vegetative parts and fruit of olive trees (Cetin & Alaoglu, 2006; Kacar et al., 2010; Chatti et al., 2017). Kacar et al. (2010) observed that there were dense spots on the upper surface of the leaf which had a greenish-yellow color and slightly collapsed inwards when *A. oleae* fed on the leaves of olive. Also, it has been observed that buds and flowers developed blackening, deformation and drying of the buds (Lindquist et al., 1996; Elhadi & Birger, 1999; Cetin & Alaoglu, 2006; Chatti et al., 2017). During the fruit development period, fruit deformation, rust-like appearance and fruit drop occur in the early period as a result of feeding on fruit stem pits and fruit (Kacar et al., 2010). Similarly, it was found that *T. hassani* caused deformation of olive leaves and fruit and caused color change in rust appearance especially in fruit (Zaher & Hanna, 1965; Jeppson et al., 1975; Abou-Awad et al., 2005; Shahini et al., 2009; Kacar et al., 2010). In the present study, the tenuipalpid mites, *C. lineola*

and *Brevipalpus* sp., were detected from one location in Izmir and nine locations from Balikesir, Manisa and Izmir Provinces, respectively. No damage to olive trees caused by these tenuipalpid mites was observed. These species are generally pests in fruit trees and ornamental plants and are reported to be important plant virus vectors (Miranda et al., 2007).

In this study, Phytoseiidae species were the most common predatory mites. *Typhlodromus athenas* was commonly observed throughout the year in the majority of the olive orchards, and this species is a first record for the Turkish fauna. The primary food sources of *Neoseiulus* and *Typhlodromus* species obtained in this study are tetranychid mites, and they can also feed on phytophagous mites such as eriophyid and tarsonemid mites (Lindquist, 1983; McMurtry et al., 1984). As no tetranychid mites were found in the olive orchards, the phytoseiids most likely feed on eriophyid mites. To confirm this, more detailed studies are needed. Stigmaeidae is the second-largest family of predatory mites after Phytoseiidae (Santos & Laing, 1985; Thistlewood et al., 1996). These mites feed on scale insects, whiteflies and some phytophagous mites of the families Tetranychidae, Tenuipalpidae and Eriophyidae (Santos & Laing, 1985).

In this study it was observed that *Agistemus duzgunesae* feed on eriophyid mites. Similarly, many researchers showed that *Agistemus* species fed on different eriophyid species and completed their development. For example, Momen (2012) found that *Agistemus olivi* Romeih successfully developed and reproduced on all eriophyid mites tested; *Aceria mangiferae* Sayed, 1946, *Aculops lycopersici* (Massee, 1937) and *Aculus fockeui* (Nalepa et Trouessart, 1891). Leiva et al. (2013) reported that *Agistemus aimogastaensis* Leiva, Fernandez, Theron & Rollard, 2013 is an important predator of two eriophyid mites, *A. oleae* and *Oxycenus maxwelli* (Keifer, 1939), in olive orchards in Argentina. That study showed that *A. oleae* and *T. hassani* were found throughout the growing period from April to November in all orchards and their highest population densities were found on buds in April and leaves and fruit in May and June. Similarly, Cetin & Alaoglu (2006), and Leiva et al. (2013) reported that eriophyid mites actively damaged olives from early April to November. In the present study, there were differences in the population densities of eriophyid mites in some olive orchards in 2016 and 2017 (Figures 3 and 4). For instance, the population density of eriophyid mites on fruit was similar in Bornova in both 2016 and 2017, it was higher in Kemalpasa and Saruhanli in 2017 than in 2016, and their density in Akhisar was higher in 2016 than in 2017. Climatic factors, especially temperature and humidity, affect the population density of eriophyid mites (Lindquist et al., 1996). However, there are no huge differences in the average temperature and relative humidity between 2016 and 2017 (Figure 1). Eriophyid mites are locally more abundant on some leaves, buds or fruit. This may be the reason for their high number in some years.

The results of the study showed that the population of eriophyid mites is mostly found on fruit in May and June. For this reason, the control of eriophyid mites for March and April would be recommended as this is when they move from the buds to the leaves. In this way, the populations of eriophyid mites will be reduced before they migrate to the fruit. The prey range of predatory mites, *T. athenas* and *A. duzgunesae,* and their potential as biological control agents of eriophyid mites deserves further studied.

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