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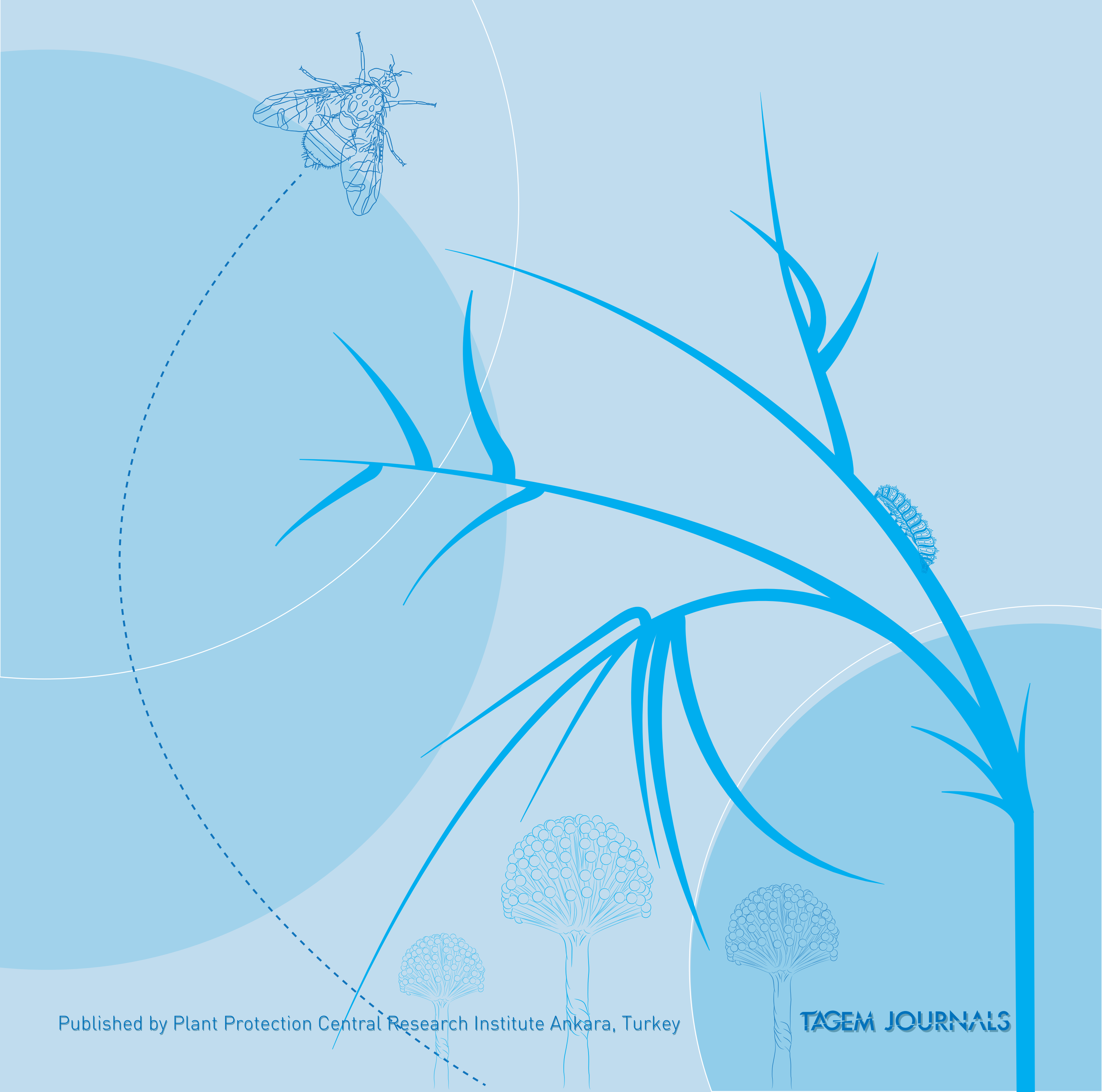

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Plant Protection Bulletin has been published by Plant Protection Central Research Institute since 1952. The journal is published four times a year with original research articles in English or Turkish languages on plant protection and health. It includes research on biological, ecological, physiological, epidemiological, taxonomic studies and control methods of disease, pest, weed, and natural enemies that cause damage in plants and plant products. In addition, studies on residue, toxicology and formulations of plant protection products and plant protection machinery are also included. Article evaluation process is based on double blind referee system and published as open access. Annual biological studies, short communication, first report and compilations do not publish in the journal.

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Volume 62	No 2	April-June 2022

Contents / İçindekiler

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Original article

Investigating the efficacies of the entomopathogenic nematodes on the last instar larvae of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) under laboratory conditions

Entomopatojen nematodların (Rhabditida: Heterorhabditidae ve Steinernematidae) Elma içkurdu *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)'nin son dönem larvaları üzerindeki etkinliklerinin laboratuvar koşullarında araştırılması

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A B S T R A C T

Codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is the main pest of apple all over the world. Besides the usage of the broad spectrum insecticides being the main control for the *C. pomonella*, also there is a potential of the usage of the entomapathogen nematodes(EPN) for the same control. In this study, the efficacies of four entomopathogenic nematode species [*Steinernema carpocapsae* (Tokat Bakisli05), S. *feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20), and *H. bacteriophora* (11KG)] have been investigated against the last instar of *C. pomonella* under laboratory conditions (*in vitro*). Three different EPNs concentrations [250, 500 and 1000 infective juvenile (IJ)/ml^{-1}] of the nematodes and distilled water as a control were used. Dead larvae were counted 48, 72 and 120 h after treatment, and mortality rates were calculated. *H. bacteriophora* (11KG) isolate was the most effective isolate causing 77.8% larval mortality at the highest concentration (1000 IJs/ml^{-1}) after 120 h. This was followed by *S. feltiae* (76.4%), *S. carpocapsae* (69.5%) and *H. bacteriophora* (TOK-20) (65.3%). The lowest mortality rates (respectively %5.3, 13.5, 2.7 and 10.8) were seen in all nematode species at a concentration of 250 IJs/ml^{-1}. The isolates are found effective on the last instar larvae of *C. pomonella* but should be supported with detailed field studies.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is a highly nutritious fruit from the Rosaceae family that is grown in a wide territory all over the world. It is accustomed to a broad of ecologies (Öztemiz et al. 2017). Turkey is among the most important apple producing countries in the world (FAOSTAT 2018). Produced 3.878,550 tons of apples in 2020 (TUIK 2020).

One of the most important pests of apple, pear and walnut is the Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Barnes 1991, Howell et al. 1992, Kuyulu and Genç 2019). It's larvae feed directly on fruit, causing significant quality and quantity losses. Broad spectrum insecticides are used to control codling moth. Due to the

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Codling moth larvae are known to be sensitive to EPNs (Nachtigall and Dickler 1992, Sledzevskaya 1987, Unruh and Lacey 2001, Weiser 1955). There is not a single report studied that shows resistance to the entomopathogenic nematodes used to control codling moth. Entomopathogenic nematodes could be used as a supplemental agent for reducing overwintering populations (Kaya et al. 1984, Unruh and Lacey 2001). Larvae that did not die from pesticide application during the previous season could die from the application of EPNs. For this reason, involving the EPNs in the integrated pest management programs could cause reduction in the population of *C. pomonella* (Odendaal et al. 2018). The objective of this study was to determine the efficacy of 4 EPN isolates on the last instar larvae of codling moth.

MATERIALS AND METHODS

Entomopathogenic nematodes cultures

EPN species [(*Steinernema feltiae* (Tokat-Emir), *S. carpocapsae* (Tokat-Bakışlı05), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11- KG)] were obtained from the Plant Protection Department of Tokat Gaziosmanpaşa University, Turkey. The infective juveniles were reared on *Galleria mellonella* (L.) last instar larvae according to Kaya and Stock (1997). Whatman paper was placed in small petri dishes 6 cm in diameter, soaked with distilled water, and ten larvae were lined up on the paper. The infective juveniles were removed from the water with a pipette and applied to the *G. mellonella* larvae. Then they were placed in the incubator at 28±2 °C and checked regularly. The infective juveniles were obtained by the "White trap" method (White 1927). The recovered larvae were kept in an incubator at +10 °C.

Galleria mellonella culture

A special diet was prepared according to the relevant literature (Haydak 1936, Mohammed and Coppel 1983). First, the prepared diet was transferred to glass jars, and then *G. mellonella* larvae were placed on it. Then the jars with the eggs were placed in the incubator (28±2 °C, 16/8 photoperiod).

Codling moth culture

Codling moth last instar larvae using in experiment were reared in climate cabinet (25±1 °C, 65% RH with a 16L:8D photoperiod). The larvae were fed on artificial diet until the last instar larvae were obtained. Artificial diet was bought from Southland Products Incorporated, USA. 50 adults were released in 2 l plastic container containing adult diets. Eggs were laid on polyethylene sheets by adults. Hatched larvae on polyethylene sheets were placed by a brush in artificial larval diets in petri dishes.

Laboratory bioassays of EPNs

The experiments were performed using plastic containers. The last instar larvae of codling moth were transferred to each container one by one with soft forceps. A piece of corrugated cardboard (1×3 cm) was also placed in plastic containers. Then, the EPN isolates prepared in distilled water [250, 500 and 1000 infective juvenile (IJ)/ ml-1] and these isolates were applied into the plastic container with a pipette. After the application, the mouth of the container was closed with tulle and rubber. Only distilled water was used in the control. Deltamethrin 2.5 EC (at a concentration of 25 g/l) was used as a positive control. The containers were incubated under the same conditions (24±5 °C and 65%±5% RH under a 16h light/8h dark cycle). The dead larvae in plastic containers were counted at the end of 48, 72 and 120 h. The studies were performed with 3 repetitions and 10 replications. Dead larvae were taken into the "White Trap" (White 1927). After one week, EPNs were obtained from infected *C. pomonella* larvae.

Table 1. Mortality (%) of Codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) last instar larvae caused by entomopathogenic nematode (EPN) isolates [*Steinernema carpocapsae* (Tokat-Bakışlı05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK-20), *H. bacteriophora* (11KG)] at the end of 48, 72 and 120 h

EPN Species	EPN Concentration	48 Hours	72 Hours	120 Hours
S. carpocapsae (Tokat Bakışlı05)	250	5.3±3.5d1B[2]	6.7±3.5efB	34.7±5.0ghA
	500	9.5±3.4cdB	22.0±5.6c-eB	52.7±5.5 d-hA
	1000	16.3±2.1cdB	37.0±6.5bcB	69.4±5.0b-dA
S. feltiae (Tokat Emir)	250	13.5±5.2cdB	14.9±4.6d-fB	51.3±5.0 d-hA
	500	23.2±2.3bcB	30.1±0.9b-dB	61.1±7.7b-fA
	1000	34.2±2.5bB	45.2±2.4bB	76.3±5.0bcA
H.bacteriophora (Tok20)	250	2.7±1.3dB	8.1±2.2 efB	33.3±2.4hA
	500	5.4±2.7dB	13.7±1.4d-fB	40.2±2.7f-hA
	1000	12.3±4.2cdB	24.6±2.0c-eB	65.2±2.7b-eA
H.bacteriophora (KG11)	250	10.8±3.5cdB	15.0±1,2d-fB	47.2±2.7 e-hA
	500	23.2±3.3bcB	27.2±4,5b-dB	55.5±5.0c-gA
	1000	34.1±4.6bB	38.3±4.8bcB	77.7±1.3bA
	Control (Water) (-)	0.0±0.0dA	0.0±0. 0fA	0.00±0.0iA
	Control (Insecticide) (+)	97.2±1.3aA	100.0±0.0aA	100.0±0.0aA
		F=58.8; df=13,2; P<0.05	F=48.8; df=13,2; P<0.05	F=32.9; df=13,2; P<0.05

[1]Different lowercase letters following means in the same column indicate statistical significance from each other (Anova P<0.05, Tukey test)

[2]Different uppercase letters in the same line indicate statistically different from each other (Anova P<0.05, Tukey test

Statistical analysis:

The mortality data recorded in assays were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 18) computer program.

RESULTS AND DISCUSSION

According to statistical analyzes mortality rates increased in all entomopathogenic nematode species as the concentration increased (Table 1).

Deltamethrin which was used as a positive control, was in group A. In the assessments after 48 hours, 250 IJs/ml^{-1} doses of *S. carpocapsae* and *H. bacteriophora* (TOK-20) were included in the same group as the negative control. In all experiments, the highest mortality rate was observed at a concentration of 1000 IJs/ml^{-1}, while the lowest mortality rate was obtained at the lowest concentrations of 250 IJs/ml^{-1} . According to the results, the isolate *H. bacteriophora* (KG11) (77.8%) was the most effective isolate at the highest concentration compared to the other EPN isolates. After 120 hours, this isolate was included in group B. In addition, the mortality rates were found for other isolates such as (76.3%) for *S. feltiae* (Tokat-Emir), (69.5%) for *S. carpocapsae* (Tokat Bakışlı05) and (65.3%) for *H. bacteriophora* (TOK-20). The mortality rates at 250 IJs /ml^{-1} concentration were in *S. feltiae* (Tokat-Emir) isolate with (51.3%), followed by H. bacteriophora (11KG) with (47.2%), *S. feltiae* (Tokat Bakışlı05) with (34.7%) and *H. bacteriophora* (TOK-20) with (33.3%). No mortality were observed in controls treated with distilled water alone (Table 1).

There are numerous studies demonstrating the success of EPNs as a biological control agent. Orchard pests are also considered in these studies (Gaugler 2002, Kaya et al. 1984, Shapiro-Ilan et al. 2005, Unruh and Lacey 2001). *S. feltiae* has been shown to be effective against codling moth larvae in March or mid-October (Lacey et al. 2006a, 2006b, Reggiani et al. 2008). *S. carpocapsae* (Nemasys® C) provides a high level of efficacy in controlling *C. pomonella* overwintering in apple orchards (Curto et. al. 2008, Unruh and Lacey 2001). Lacey and Unruh (1998) conducted a study with *S. carpocapsae*, *S. riobrave*, and *H. bacteriophora* and reported that the most effective species was *S. carpocapsae* with 99%, followed by *S. riobrave* (83%) and *H. bacteriophora* (80%).

In another study on *C. pomonella* conducted by Odendaal et al. (2016), *S. feltiae* and *H. bacteriophora* (Hb1 and Hb2) (commercial preparations) and two indigenous species (*S. jeffreyense* and *S. yirgalemense*) were used. As a result of the study, it was reported that *S. jeffreyense* was 67% effective, followed by *H. bacteriophora* (Hb1) with 42% and *S. yirgalemense* with 41%. In laboratory bioassays involving spray application under simulated field conditions, it was found that the most effective EPN was *S. feltiae* (67%) followed by *S. yirgalemense* (58%) (Odendaal et al. 2016).

Heterorhabditis pakistanensis NBAIR H-05 strain was found to be effective against diapause larvae of codling moth (Ahmad et al. 2020). In another study investigating the efficacy of EPNs on *C. pomonella*, it was reported that pupal susceptibility was low and cocoon larvae were more susceptible than non-cocoon larvae (Navaneethan et al. 2010). *S. feltiae* was applied to *C. pomonella* larvae in four different apple fields. At the end of the study, damage was 33% less when a lower dose was applied and, correspondingly, the higher the dose, the less damage (Peters et al. 2008). It is thought that entomopathogenic nematodes can be used in biological control programs against *C. pomonella*, but more detailed field studies are needed.

The mortality data recorded in assays were converted to percent mortality and then transformed by arcsine transformation. One-way analysis of variance was used to test the significance, and treatment means were separated by Tukey's multiple comparison test. The statistical analyses were carried out on MINITAB (Release 18) computer program.

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ÖZET

Elma içkurdu *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) dünya genelinde elmanın başlıca zararlısıdır. Mücadelesinde ağırlıklı olarak geniş spektrumlu insektisitler kullanılmakla birlikte, zararlıya karşı uygulanan biyolojik mücadele etmenleri arasında entomopatojen nematodların (EPN) kullanılabilme potansiyelleri bulunmaktadır. Bu çalışmada, dört adet EPN izolatı [*Steinernema carpocapsae* (Tokat Bakisli05), *S. feltiae* (Tokat-Emir), *Heterorhabditis bacteriophora* (TOK20) ve *H. bacteriophora* (11KG)]'nın *C. pomonella*'nın son dönem larvaları üzerindeki etkinliği laboratuvar koşullarında (*in vitro*) araştırılmıştır. Denemelerde nematodlar üç farklı konsantrasyonda [250, 500 ve 1000 enfektif larva (EL)/ml^{-1}] hazırlanmış ve uygulanmıştır. Kontrol olarak saf su kullanılmıştır. Nematod uygulamasından 48, 72 ve 120 saat sonra ölü larvalar sayılmış ve ölüm oranları hesaplanmıştır. *H. bacteriophora* (11KG) izolatı 120 saat sonra en yüksek konsantrasyonda (1000 EL/ml^{-1}) %77.8 ölüme neden olan en etkili izolat olmuştur. Bu izolatı sırası ile *S. feltiae* (Tokat-Emir) (%76.4), *S. carpocapsae* (Tokat Bakisli05) (%69.5) ve *H. bacteriophora* (TOK20) (%65.3) izlemiştir. En düşük ölüm oranları (sırasıyla %5.3, 13.5, 2.7 ve 10.8) tüm nematod türlerinde 250 EL/ml^{-1} konsantrasyonunda görülmüştür. İzolatlar *C. pomonella*'nın son dönem larvaları üzerinde etkili bulunmuş ancak, detaylı arazi çalışmaları ile bu sonuçların desteklenmesi gerekmektedir.

Anahtar kelimeler: biyolojik mücadele, *Cydia pomonella*, entomopatojen nematodlar, *Heterorhabditis*, *Steinernema*

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Original article

New data on the tribe Osmiini (Hymenoptera: Megachilidae) fauna of Türkiye

Türkiye Osmiini (Hymenoptera: Megachilidae) faunası hakkında yeni bilgiler

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A B S T R A C T

Ten species belonging to the tribe Osmiini were collected and identified from the east of Türkiye during 2017-2021. The specimens were identifed as *Chelostoma emarginatum* (Nylander, 1856), *Heriades rubicola* Pérez, 1890, *Hoplitis leucomelana* (Kirby, 1802), *Hoplitis tridentata* (Dufour and Perris, 1840, *Hoplitis* (Anthocopa) *serainae* Müller, 2012, *Hoplitis (Hoplitis*) sp., *Osmia aurulenta* (Panzer, 1799), *Osmia gallarum* Spinola, 1808, *Osmia* (*Helicosmia*) *signata* Erichson, 1835 and *Osmia viridana* Morawitz, 1874. Distributions, illustrations and brief descriptions of all species are added to the study. No new records were obtained but new localities for some species have been reported.

INTRODUCTION

Megachilidae comprises approximately 4000 species classified into seven tribes and more than 70 genera (Ascher and Pickering 2020, Michener 2007). This group is a large family with specialized characteristics but they possess morphologically uniformity, as well. They can be found in a wide diversity of habitats on all continents except Antarctica, ranging from lowland tropical rain forests to deserts to alpine environments (Litman et al. 2011). There has been an unique pollen-collecting adaptation in which the scopa (the pollen collecting hairs) of a female is located on the ventral side of the metasoma in the family Megachilidae (Banaszak and Romasenko 1998). It has been reported that some species belonging to the Megachilidae are effective pollinators in some plants (Bosch and Blas 1994, Vicens and Bosch 2000). The osmiine bees constitute a tribe Osmiini within the family Megachilidae (Hymenoptera), which is one of the nine currently recognized families of bees (Engel 2005, Michener 2007). The tribe Osmiini Newman, 1834 which comprise 15 genera and roughly 1200 species worldwide, occur in North America, Africa and Eurasia (Müller 2019). They are especially diverse in Mediterranean and xeric climates of southern Africa, southwestern North America and the Palaearctic region. The Palaearctic Osmiine bee fauna is quite diverse with 10 genera and about 700 species (Proshchalykin and Maharramov 2020). In Türkiye, the tribe Osmiini comprises approximately 247 taxa from eight genera (Özbek 2013, Proshchalykin and Maharramov 2020). The osmiine bees have special importance for their often spectacular and very diverse nest-building behaviors as well as for their close relationships with flowering plants (Cane et al. 2007, Müller et al. 1997, Sedivy et al. 2008). Some Osmia species such as O. cornuta in Europe, O. cornifrons in Asia and Osmia lignaria in North America are commercially used to pollinate the flowers of fruit trees (Bosch and Kemp 2002).

In this study, we tried to assess the specimens belonging to this tribe from eastern Türkiye.

MATERIALS AND METHODS

The study was conducted in Diyarbakır and Bingöl provinces of eastern Türkiye (Figure 1). Osmiine bees were collected using insect net in the different flowering seasons during 2017-2021. Determinations of all species were made by Andreas Müller. Photographs of morphological characters and male genitalia of adults were taken by using a digital camera attached to a stereomicroscope. All collected specimens are deposited in the individual collection of Department of Plant Protection, Faculty of Agriculture, Bingöl University (Bingöl, Türkiye).

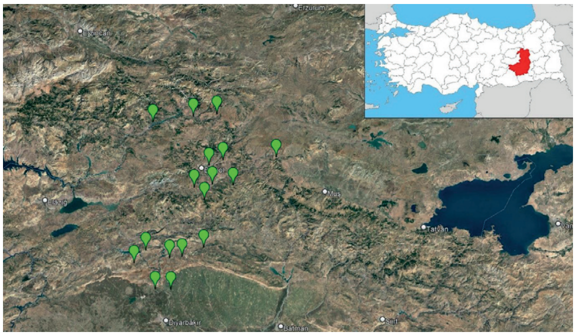

Figure 1. Map of the investigated area in Türkiye

RESULTS

Totally ten species from four genera (*Chelostoma Latreille*, 1809, *Heriades Spinola*, 1808, *Hoplitis* Klug, 1807, *Osmia* Panzer, 1806) of tribe Osmiini were listed. The list of species, distributional data and brief description are given below alphabetically.

Family: Megachilidae

Subfamily: Megachilinae

Tribe: Osmiini

Genus: *Chelostoma* Latreille, 1809

Chelostoma (*Chelostoma*) *emarginatum* (Nylander, 1856)

Material examined: Bingöl: Çayağzı, N 38° 47' 57.65", E 40° 33' 40.63", 999 m, 19.V.2021, ♀; Ekinyolu, N 38o 54' 00.00", E 40° 34' 17.58", 1036 m, 22.V.2021, ♀; Adaklı, Kamışgülü, N 39° 13' 04.16", E 40° 25' 56.89", 1215 m, 29.V.2021, ♀; Diyarbakır: Eğil, Yatır, N 38° 48' 21.94", E 40° 33' 16.56", 721 m, 24.IV.2021, ♀.

Description. Female: *Length*: 8–9 mm. *Colour*: Black. *Head*: Mandible long with two apical teeth and inner margin brownish hairy; labrum short; face slight white hairy (Figure 2b, d). *Thorax*: Pronotal lobes dense hairy; scutum and scutellum moderately punctuated; propodeum completely dull and punctuated (Figure 2a). *Metasoma*: Apical margin of terga 1–5 with white hair bands; pygidial plate V-shaped; sterna with dense grayish hairy (Figure 2a, c, e).

Previous records: Adıyaman, Afyonkarahisar, Amasya, Ankara, Antalya, Aydın, Bingöl, Bursa, Bilecik, Çanakkale, Çorum, İstanbul, Konya, Mersin, Muğla, Şanlıurfa, Şırnak, Yozgat (Özbek 2011, Güler et al. 2014).

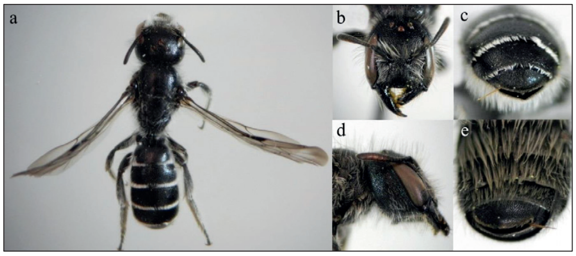

Figure 2. *Chelostoma emarginatum*, ♀; a) Dorsal view, b) Face, c) Pydigial plate, d) Lateral view of head, e) Sterna

Genus: *Heriades* Spinola, 1808

Heriades (*Heriades*) *rubicola* Pérez, 1890

Material examined: Bingöl: Büyükterkören, N 38o 49' 54.31", E 40o 34' 21.97", 1008 m, 05.VI.2021, ♂.

Description. Male: *Length*: 6 mm. *Colour*: Black. *Head*: Mandible long and slender; labrum short; face dense white hairy (Figure 3b). *Thorax*: Pronotal lobes dense hairy; scutum and scutellum punctuated; propodeum completely dull (Figure 3a). *Metasoma*: Apical margin of terga 1–2 with white hair bands laterally; terga dense deep punctuated; pygidial plate V-shaped; sterna with slight whitish hairy in places (Figure 3a, c, d). *Genitalia*: Length 0.9 mm, brownish, paramere narrow and broad on the upper and inferior sides respectively; aedeagus slender, penis valve dilated and with a widely central entrance (Figure 3e).

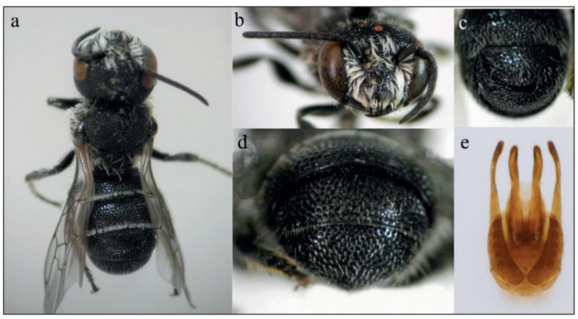

Figure 3. *Heriades* (*Heriades*) *rubicola*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

Previous records: Antalya, İçel (Özbek and Zanden 1992b, Özbek 2013).

Figure 3. *Heriades* (*Heriades*) *rubicola*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

Genus: *Hoplitis* Klug, 1807

Hoplitis (*Alcidamea*) *leucomelana* (Kirby, 1802)

Material examined: Bingöl: Ekinyolu, N 38o 54' 00.00", E 40o 34' 17.58", 1036 m, 01.VI.2021, ♂; Diyarbakır: Dicle, Meydan, N 38o 19' 14.62", E 40o 13' 54.99", 730 m, 24.IV.2021, ♂; Eğil, Yatır, N 38o 48' 21.94", E 40o 33' 16.56", 721 m, 24.IV.2021, ♂.

Description. Male: *Length*: 6–8 mm. *Colour*: Black. Head: Mandible and labrum short; face dense yellowish hairy (Figure 4b). *Thorax*: Pronotal lobes yellowish hairy; scutum and scutellum punctuated; propodeum completely dull (Figure 4a). *Metasoma*: Apical margin of terga 1–5 with white hair bands laterally; terga slight shallow punctuated; pygidial plate V-shaped; Apical margin of sterna 2–4 with brownish hairy (Figure 4a, c, d). *Genitalia*: Length 1.2 mm, yellowish, paramere narrow on the upper and inferior sides; aedeagus slender, penis valve dilated and with a slight central entrance (Figure 4e).

Previous records: Artvin, Bilecik, Erzincan, Erzurum, Hakkâri, Iğdır, Kars, Konya, Nevşehir (Özbek 1979a, Özbek and Zanden 1992a, Özbek 2013).

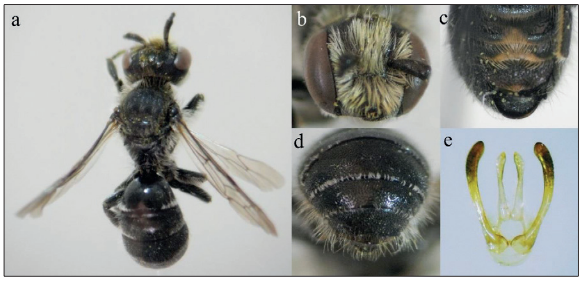

Figure 4. *Hoplitis* (*Alcidamea*) *leucomelana*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

Hoplitis (*Alcidamea*) *tridentata* (Dufour and Perris, 1840)

Material examined: Bingöl: Genç, Dilektaşı, N 38o 46' 06.45", E 40o 46' 27.64", 1653 m, 09.V.2021, ♂.

Description. Male: *Length*: 11 mm. *Colour*: Black. Head: Mandible and labrum short; face dense long brownish hairy (Figure 5b). *Thorax*: Pronotal lobes and pleuron long brownish hairy; scutum shallow punctuated; scutellum, metanotum and propodeum long brownish hairy (Figure 5a). *Metasoma*: terga and sterna with long brownish hairy in places; basal margin of terga dense shallow punctuated; pygidial plate groove-shaped (Figure 5c).

Previous records: Amasya, Artvin, Bilecik, Bitlis, Erzurum, Kars, Konya, Nevşehir, Tunceli (Özbek 1979a, Zanden 1980, Özbek and Zanden 1992a, Özbek 2013).

Figure 5. *Hoplitis* (*Alcidamea*) *tridentata*, ♂; a) Dorsal view, b) Face, c) Pydigial plate Hoplitis (Anthocopa) serainae Müller, 2012

Material examined: Diyarbakır: Dicle, Sergenli, N 38o 19' 30.64", E 40o 13' 38.46", 750 m, 24.IV.2021, ♂.

Description. Male: *Length*: 7 mm. *Colour*: Black. Head: Mandible and labrum short; face dense yellowish hairy (Figure 6b). *Thorax*: Pronotal lobes yellowish hairy; scutum and scutellum shallow punctuated; propodeum completely dull and long whitish hairy (Figure 6a). *Metasoma*: Apical margin of terga with white short hair bands; terga dense shallow punctuated; pygidial plate with two teeth; sterna with whitish hairy in places (Figure 6a, c, d). Genitalia: Length 1.1 mm; brownish, paramere narrow and broad on the upper and inferior sides respectively, apically pointed; aedeagus slender, penis valve dilated and with a slight central entrance (Figure 6e).

Previous records: Antalya, Kütahya, Nevşehir, Siirt, Hakkâri (Müller 2012)

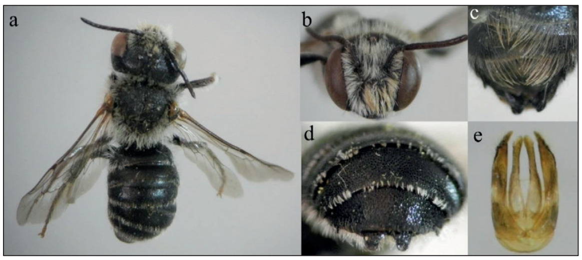

Figure 6. *Hoplitis* (*Anthocopa*) *serainae*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

Hoplitis (*Hoplitis*) species

Material examined: Diyarbakır: Hani, Çardaklı, N 38o 18' 56.31", E 40o 24' 06.29", 1057 m, 12.V.2017, ♂.

Description. Male: *Length*: 8 mm. *Colour*: Brownish black. *Head*: Mandible short and labrum long; face dense whitish hairy (Figure 7b). *Thorax*: Pronotal lobes whitish hairy; scutum and scutellum shallow punctuated and dull; propodeum completely dull and long whitish hairy (Figure 7a). *Metasoma*: Apical margin of terga with white hair bands; terga dense shallow punctuated; pygidial plate

U-shaped; apical margin of sterna brownish (Figure 7a, c, d). *Genitalia*: Length 1.3 mm; brownish, paramere very slender on upper sides and broad on the inferior; aedeagus robust and wide, penis valve dilated and with a slight central entrance (Figure 7e).

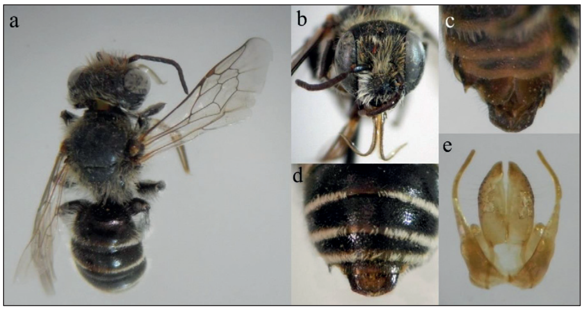

Figure 7. *Hoplitis* (*Hoplitis*) species, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

Genus: *Osmia* Panzer, 1806

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

Material examined: Bingöl: Adaklı, Kamışgülü, N 39o 13' 04.16", E 40o 25' 56.89", 1215 m, 29.V.2021, ♂; Yayladere, Yaylabağ, N 39o 10' 42.04", E 40o 05' 45.14", 1170 m, 30.V.2021, ♂.

Description. Male: *Length*: 11-12 mm. *Colour*: Black. Head: Mandible and labrum short; face dense yellowish hairy (Figure 8b). *Thorax*: Pronotal lobes yellowish hairy; scutum, scutellum and propodeum brownish hairy and completely dull (Figure 8a). *Metasoma*: Apical margin of terga and sterna 2–5 with white brownish hairy; pygidial plate flat and wide (Figure 8a, c, d). *Genitalia*: Length 1.6 mm; brownish black paramere narrow and broad on the upper and inferior sides respectively; aedeagus slender, penis valve dilated and with a widely central entrance (Figure 8e).

Previous records: Ağrı, Ankara, Artvin, Bayburt, Bilecik, Bitlis, Burdur, Erzincan, Erzurum, Eskişehir, Hakkâri, Hatay, Karaman, Kayseri, Konya, Mersin, Nevşehir, Siirt, Tokat, Tunceli (Friese 1921, Özbek 1979b, Özbek and Zanden 1992a, Güler and Çağatay 2006, Özbek 2014).

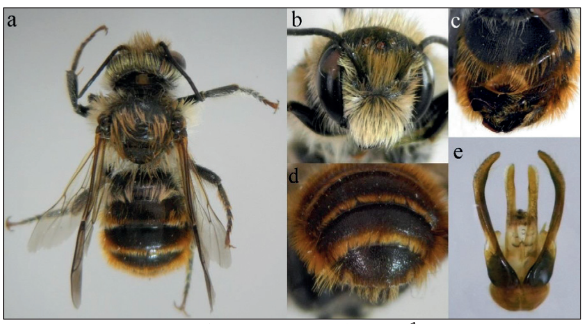

Figure 8. Osmia (*Helicosmia*) *aurulenta*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

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

Material examined: Bingöl: Çayağzı, N 38o 47' 57.65", E 40o 33' 40.63", 999 m, 05.VI.2021, ♀.

Description. Female: *Length*: 6 mm. *Colour*: Black. *Head*: Mandible and labrum short; face slight whitish hairy (Figure 9b, d). *Thorax*: Pronotal lobes whitish hairy; scutum and scutellum shallow punctuated; propodeum shiny and slightly hairy (Figure 9a). *Metasoma*: First abdominal tergite with white hair in laterally; terga dense shallow punctuated; pygidial plate V-shaped and hairy; sterna with whitish long hairy in places (Figure 9a, c, e).

Previous records: Ankara, Antalya, Artvin, Aydın, Bilecik, Erzincan, Erzurum, Eskişehir, Gümüşhane, Hakkâri, Isparta, İstanbul, İzmir, Kars, Konya, Mersin, Muğla, Muş, Nevşehir, Niğde, Siirt, Şanlıurfa (Özbek 1979a, Warncke 1992, Güler et al. 2014, Özbek 2014).

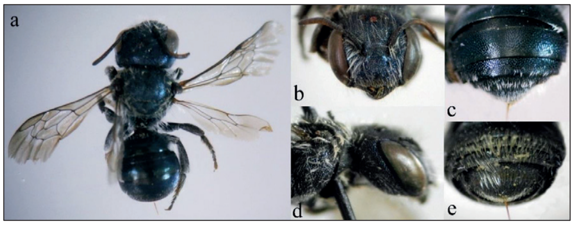

Figure 9. *Osmia (Pyrosmia) gallarum*, ♀; a) Dorsal view, b) Face, c) Pydigial plate, d) Lateral view of head, e) Sterna

Osmia (*Helicosmia*) *signata* Erichson, 1835

Material examined: Diyarbakır: Dicle, Meydan, N 38o 19' 14.62", E 40o 13' 54.99", 730 m, 24.IV.2021, ♀.

Description. Male: *Length*: 11 mm. *Colour*: Black. *Head*: Mandible long with three apical teeth and labrum short; apical margin of clypeus dense brownish hairy; face dense whitish hairy (Figure 10b, c). *Thorax*: Pronotal lobes yellowish hairy; scutum and scutellum punctuated with dense brownish hairy; propodeum completely dull (Figure 10a). *Metasoma*: Apical margin of terga with white hair bands; terga dense deep punctuated; pygidial plate flat; sterna with dense blackish hairy (Figure 10a, c, e)

Previous records: Adana, Afyonkarahisar, Aksaray, Ankara, Antalya, Aydın, Bayburt, Bingöl, Birecik, Bitlis, Bursa, Çanakkale, Diyarbakır, Erzincan, Erzurum, Eskişehir, Hakkâri, Hatay, İstanbul, Iğdır, Kahramanmaraş, Karaman, Kars, Kayseri, Kilis, Konya, Mardin, Mersin, Muş, Nevşehir, Şanlıurfa, Şırnak, Uşak, Van, Yalova (Friese 1921, Alfken 1935, Zanden 1980, Warncke 1988, Özbek and Zanden 1992a, Özbek 2014).

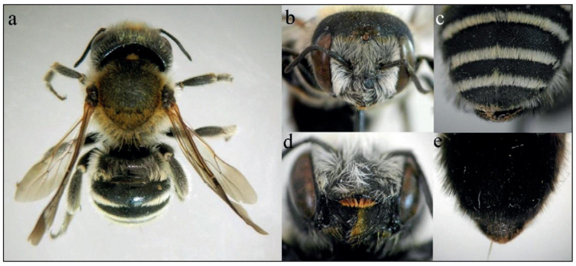

Figure 10. *Osmia* (*Helicosmia*) *signata*, ♀; a) Dorsal view, b) Face, c) Pydigial plate, d) Mouth parts, e) Sterna

Osmia (*Pyrosmia*) *viridana* Morawitz, 1874

Material examined: Bingöl: Solhan, Arakonak, N 38o 56' 49.39'', E 41o 07' 40.76'', 1639 m, 20.VII.2017, ♂; Diyarbakır: Lice, Angül, N 38o 24' 23.81'', E 40o 33' 50.23'', 866 m, 25.IV.2021, ♂.

Description. Male: *Length*: 6–7 mm. *Colour*: Metallic greenish. Head: Mandible with two apical teeth and labrum short; face long yellowish hairy (Figure 11b). *Thorax*: Pronotal lobes yellowish hairy; scutum and scutellum deep punctuated; propodeum shiny (Figure 11a). *Metasoma*: First abdominal tergite with white hair in laterally; pygidial plate with two teeth; sterna with slight whitish hairy in places (Figure 11a, c, d). *Genitalia*: Length 1.1 mm; brownish; paramere narrow and broad on the upper and inferior sides respectively; upper part of paramere notched; aedeagus slender, penis valve dilated and with a widely central entrance (Figure 11e).

Previous records: Adana, Adıyaman, Ankara, Antalya, Aydın, Diyarbakır, Erzurum, Hakkâri, Kahramanmaraş, Mardin, Mersin, Muğla, Nevşehir, Siirt, Şanlıurfa (Zanden 1984, Özbek and Zanden 1992a, Warncke 1992, Güler et al. 2014, Özbek 2014).

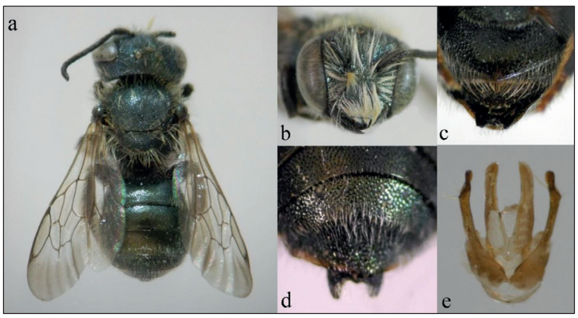

Figure 11. Osmia (*Pyrosmia*) *viridana*, ♂; a) Dorsal view, b) Face, c) Sterna, d) Pydigial plate, e) Male genitalia

DISCUSSION

In the present publication, Osmiine bees collected from eastern Türkiye are morphologically diagnosed, and the male genitalia was explained. Among the identified species, six species are the first record for the study area: *Heriades rubicola* Pérez, 1890, *Hoplitis leucomelana* (Kirby, 1802), *H. tridentata* (Dufour and Perris, 1840), H. *serainae* Müller, 2012, *Osmia aurulenta* (Panzer, 1799), *O. gallarum* Spinola, 1808. Other species (*Chelostoma emarginatum* (Nylander, 1856), *O. signata Erichson*, 1835 *O. viridana Morawitz*, 1874) are an additional record for the study area. The results of this research (with ten species) together with other works on tribe Osmiine of Türkiye (e.g. Özbek 2013, Proshchalykin and Maharramov 2020) indicate that eastern Türkiye is quite rich in terms of insect diversity. Although the fauna of Osmiine of Türkiye was studied rather well (see references), the fauna of provinces Bingöl and Diyarbakır eastern of Türkiye was poorly studied. With the diverse flora in eastern Türkiye, we expect much more species of Osmiine in this area. Osmiine bees are both ecologically and economically important; they include many pollinators of natural, urban and agricultural vegetation (Gonzalez et al. 2012). It has also been reported in previous studies that these species can be used commercially as first pollinators in some cases. (Güler and Çağatay 2006, Richards 1997,). However, many osmiin species are still unidentified, as the identification and classification of many important pollinator groups is inadequate. Consequently, identifying these bees which are quite considerable in plant pollination from the study area will guide future studies.

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I would like to thank Dr. Andreas Müller (Zürich, Switzerland) for the identification of all species.

ÖZET

Türkiye'nin doğusundan 2017-2021 yılları arasında Osmiini tribüsüne ait on tür toplanmış ve tanımlanmıştır. Örnekler *Chelostoma emarginatum* (Nylander, 1856), *Heriades rubicola* Pérez, 1890, *Hoplitis leucomelana* (Kirby, 1802), *Hoplitis tridentata* (Dufour and Perris, 1840, *Hoplitis* (*Anthocopa*) *serainae* Müller, 2012, *Hoplitis* (*Hoplitis*) sp., *Osmia aurulenta* (Panzer, 1799), *Osmia gallarum* Spinola, 1808, *Osmia* (*Helicosmia*) *signata* Erichson, 1835 ve *Osmia viridana* Morawitz, 1874 olarak tanımlandı. Çalışmaya tüm türlerin dağılımları, illüstrasyonları ve kısa tanımları eklenmiştir. Yeni kayıt elde edilmemiş ancak bazı türler için yeni lokaliteler bildirilmiştir.

Anahtar kelimeler: Hymenoptera, Megachilidae, Osmiini, dağılım, fauna, sistematik, Türkiye

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Original article

The effectiveness of mass trapping of *Ceratitis capitata* (Wiedemann,1824) (Diptera: Tephritidae) in peach orchards in İzmir, Aydın and Mersin

Ceratitis capitata (Wiedemann,1824) (Diptera: Tephritidae)'nin İzmir, Aydın ve Mersin şeftali bahçelerinde kitle yakalama tekniğinin etkinliğinin belirlenmesi

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A B S T R A C T

Ceratitis capitata (Wiedemann,1824) (Diptera: Tephritidae) is a species which is known as one of the most important quarantine pests with a zero-tolerance. Due to its high capacity of reproduction and direct damages on fruits, it is not possible to reach higher yield rates without management of this pest. Mass trapping, which is a biotechnical control method, is an alternative control method that is considered to be successful in low populations of this pest. This study carried out in peach orchards simultaneously and the effectiveness of traps were determined according to population differences between Aegean and Mediterranean regions. Experiments were conducted in 2018 in Kuşadası (Aydın), Selçuk (İzmir) and Erdemli (Mersin) in peach orchards. The population dynamics and the effectiveness of mass-trapping were detected during this study. Pheromone traps, Decis Trap (Bayer), were used to monitor the population change of the pest. The daily number of adult individuals was 4.8, 149.1 and 166.9 in Kuşadası, Selçuk and Erdemli, respectively, in July. Accordingly, effectiveness of traps was 94.19% in Kuşadası, 95.6% in Selçuk and 56.35% in Erdemli. Due to the higher population in the Mediterranean region than the Aegean region and longer duration of the peach vegetation in the Mediterranean Region, required control level of success was not provided.

INTRODUCTION

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann,1824) (Diptera: Tephritidae), has been causing economic losses by spreading to all tropical and subtropical regions since 1829 it was first noticed as a pest (Headrick 1996) until nowadays. Several researchers have reported that the Mediterranean fruit fly is a polyphagous species and causes economically important damages on hundreds of agricultural products (Elekçioğlu 2009, 2013, Orono 2006, Satar and Tiring 2016, Satar et al. 2016). Zümreoğlu (1986) reported that this species was found in 21 host plant species and varieties in Turkey, and it causes significant damages in 17 products. Among these 17 plant species and varieties, it is the main pest of citrus fruits. Peaches, apples, quinces, apricots, persimmons, plums, pomegranates and avocados are among its hosts (Demirdere 1961, Demirel 2016, İleri 1961, Karsavuran et al. 1988, Kaya and İpekdal 2018, Tiring and Satar 2017, Tunçyürek 1972, Zümreoğlu 1986, 1990), while it leads to significant economic losses in these products. Today, this pest may cause widespread epidemics especially in Mediterranean and Aegean regions in Turkey, and it may cause significant economic losses in almost unexpected locations (Satar et al. 2016). According to İleri (1961), C. capitata has entered Turkey in the 1890s, according to CABI (2019) citing Fimiani (1989) and 1915 according to Demirdere (1961) citing Bodenheimer (1951). Ceratitis capitata is a quarantine pest, and its tolerance is assumed to be zero. Due to the suspicions that there was the Mediterranean fruit fly on mandarin fruits exported to the Russian Federation, the products were sent back to Turkey (Özbay 2011). It is not possible to achieve production without the control of this pest.

There are four different approaches to control the Mediterranean fruit fly. These are sterile insect technique, mass trapping, protein bait spraying and foliar pesticide applications (Yayla and Satar 2017). The control methods that are prevalently used in Turkey are chemical pesticide application and mass trapping. In chemical control, in the case that insecticides are not applied at the suitable dose and on time, issues of residues in fruits are encountered. In residue screenings of insecticides that were used to control Mediterranean fruit fly in Satsuma mandarin and pomegranate, the residue value of Malathion was found to be higher than the MRL levels of the European Union (Dinçay et al. 2017). High MRL values of insecticides firstly pose a risk for human health, and they lead to problems in international trade. In addition to these control methods, for the first time in Turkey, the infestation of the pest could be prevented by perimetric trapping around the transportation and attachment source of the pest outside agricultural areas in the district of Çivril in Denizli (Tolga et al. 2018).

Instead of traditional chemical control, biotechnical methods integrated with alternative control programs have been studied and utilized all over the world for years. The biotechnical methods that are the most frequently used against this pest and provide successful results from the mass trapping, and attract and kill methods. The objective of mass trapping and ‘Attract and Kill’ is to eliminate the usage of insecticides or minimizing the number of insecticide applications by combining the method with other control methods within the framework of an integrated control program (Layık and Kısmalı 1994).

To increase the usage of alternative control methods, starting with 2010, the Turkish Ministry of Agriculture and Forestry has been providing producers with incentives in different products under the declaration of "Payment for Supporting Biological and/or Biotechnical Control in Plant Production" (Declaration no.: 2018/22)". However, utilization of the assistance by producers and usage of traps are not on the desired level. There are several studies conducted in Turkey on trapping against Mediterranean fruit fly (Akman and Zümreoğlu 1973, Akyol 2014, Başpınar et al. 2009, Delrio and Zümreoglu 1983, Elekçioğlu et al. 2011, Kahyaoğlu and Gürkan 2010, Satar and Tiring 2016, Sierras et al. 2012, Yayla and Satar 2017). However, these studies were carried out at different times, in different regions and separately. There are no data on which results were shared in the same year that were studied simultaneously in two different regions. This study aimed to determine the usability of traps employed in the same numbers per hectare at peach orchards in İzmir, Aydın and Mersin in Turkey. Additionally, trials of biological effectiveness were conducted to contribute the increase in usage of traps and minimization of costs.

MATERIALS AND METHODS

Determination of the population of the Mediterranean fruit fly

The trials were carried out on the peach variety of extreme Great in 1 ha of area in Kuşadası (Aydın) and 2 ha of area in Selçuk (İzmir) and on the Hale variety of peach in 1 ha of area in Erdemli (Mersin) in 2018. The trials were conducted according to ‘Large Parcel’ experimental design. The characters of experiments were Mass Trapping and Control parcels. Experiments were 10 da and the numbers of traps were determined as 5 traps per da. Control parcel was determined as 1 da and there was at least 100 m distance between control and trial parcels (Anonymous 2020).

The land was divided into 10 plots of 0.1 ha each, and trial traps were placed in Kuşadası-Aydın. A total of 50 Decis traps were installed and there would be 50 traps per ha. The counting was done on a total of 25 trial traps, including at least 2 in each plot. A control plot of 0.1 ha was left at a 100 m of distance from the Decis trap plots, and one delta-type pheromone (Trimedlure) trap was hanged for observation purposes. All traps were hanged on 11 June 2018 when the fruits were in their green period, and with the harvest on 19 July 2018, the trial was ended.

The land was divided into 20 plots of 0.1 ha each, and trial traps were placed in Selçuk-İzmir. A total of 100 Decis traps were hanged therefore there would be 50 traps per ha. Counting was made on a total of 40 trial traps. A control plot of 0.1 ha was left at a 100 m of distance from the Decis trap plots, and one delta-type pheromone (Trimedlure) trap was hanged for observation purposes. All traps were hanged on 13 June 2018 when the fruits were in their green period, and with the harvest on 25 July 2018, the trial was ended.

The land was divided into 10 plots of 0.1 ha each, and trial traps were placed in Erdemli-Mersin. A total of 50 Decis traps were installed so that there would be 50 traps per ha. Counts were made on a total of 10 trial traps, including 1 in each plot. A control plot of 0.1 ha was left at a 100 m of distance from the Decis trap plots, and one delta-type pheromone (Trimedlure) trap was hanged for observation purposes. All traps were hanged on 6 June 2018 when the fruits were in their green period, and with the harvest on 13 August 2018, the trial was ended.

For biological activity, counting were made and recorded weekly in the trial traps, and the individuals of C. capitata in the trap were removed after counting. In the pheromone traps, the capsules were replaced once every 4-5 weeks, and the trays were replaced every two weeks. All traps were installed at a height of 1.5-1.8 meters from the ground and on the southern side of the trees. Counting was done at the pheromone traps weekly, and it was aimed to determine the population change of the pest. The results of the pheromone traps and Decis traps were presented in figures.

Determination of the effectiveness of the mass trapping product

One or two trees in the middle of each plot were marked, and dents were checked on an average of 50 fruits on the tree and all fruits that fell off the tree in trial areas (Kuşadası/Aydın, Selçuk/İzmir, Erdemli/Mersin). The counts were carried out by checking the fruits of 12 trees in Kuşadası, 40 trees in Selçuk and 10 trees in Erdemli. In the control plots, the fruits on and those that fell off one or two trees were checked each week, and the dented and intact ones were recorded. The effectiveness in percent was determined with Abbott's formula [(Percentage effect = (% intact in control - % intact in trap plot)/(% intact in control) x 100)] (Abbott 1925, Karman 1971), while the statistical difference was determined with the Chi-Squared analysis method. The SPSS 23.0 package software was utilized for the statistical analyses.

Comparison of populations among the districts

The daily numbers of flies (DNF) per pheromone and Decis trap in the trial orchards of each district were calculated with the formula given below. The calculations that were made to determine the population differences among the districts were statistically analysed. The results that were obtained based on the DNF values were subjected to ANOVA in the SPSS 20.0 package software. The statistical differences were determined by using Tukey's HSD test (P=0.05) (Radonjic et al. 2013).

DNF=TNF/NTxNDT

DNF: the daily number of flies caught per each trap

TNF: total number of flies caught in all traps

NT: total number of traps

NDT: number of days traps stay in the orchard

RESULTS AND DISCUSSION

Population change of the Mediterranean fruit fly

The population changes were shown based on the mean numbers of adult individuals per trap in the Decis traps in all studied districts and the Mediterranean fruit fly numbers in all pheromone traps hanged at the control plots. The population changes in Kuşadası, Selçuk, and Erdemli were given in Figure 1, 2, and 3, respectively.

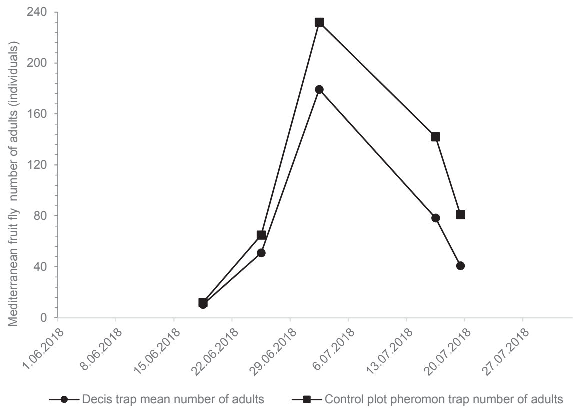

Figure 1. Population changes based on the mean number of adults in Decis traps and the number of adults in pheromone traps in the control plot in the district of Kuşadası

The mean of adults was 10.4 in Decis traps and 12.0 adults in the pheromone trap in the control plot were counted on 18 June 2018 in Kuşadası. The population raised to the highest level on 2 July 2018 in Decis traps and control plot pheromone trap respectively as 179.2 individuals/trap and 232.0 individuals/trap during this study (Figure 1).

The mean number of adults was 0.7 individuals/trap counted in Decis traps on 20 June 2018 in Selçuk, while there was no adult in the pheromone trap on the same date. The population raised to the highest level on 25 July 2018 in Decis traps (the number of the adult was 78.2 per trap). The control plot reached the highest level with 49 individuals/trap on 1 August 2018 in the pheromone trap (Figure 2).

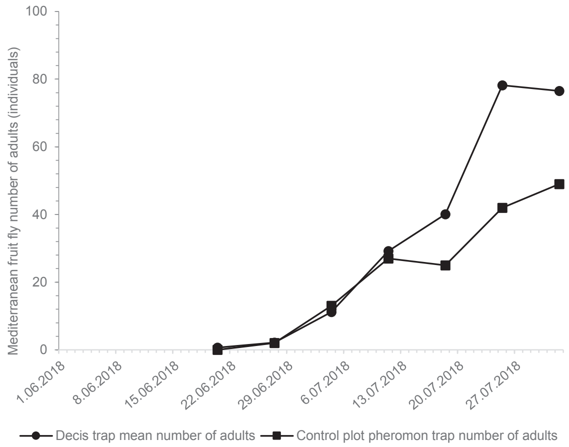

Figure 2. Population change based on the mean number of adults in Decis traps and the number of traps in pheromone traps in the control plot in the district of Selçuk

The mean of adults was 57.5 individuals/trap on 11 June 2018 in the Decis traps and 250 individuals/trap in the pheromone trap in the control plot in Erdemli. The highest population was observed in the pheromone trap on 09 July 2018 by 1025 individuals/trap. The highest level of population in Decis traps was found as 132.5 individuals/ trap on 30 July 2018 (Figure 3).

To reveal the differences among the populations, the daily numbers of flies caught in the Decis and pheromone traps were calculated. The calculations were made based on the counts made during the trial months of June and July in Kuşadası and Selçuk and June, July and August in Erdemli.

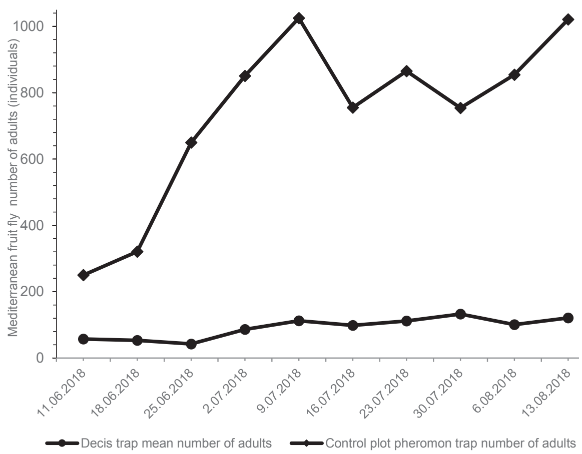

Figure 3. Population change based on the mean number of adults in Decis traps and the number of traps in pheromone traps in the control plot in the district of Erdemli

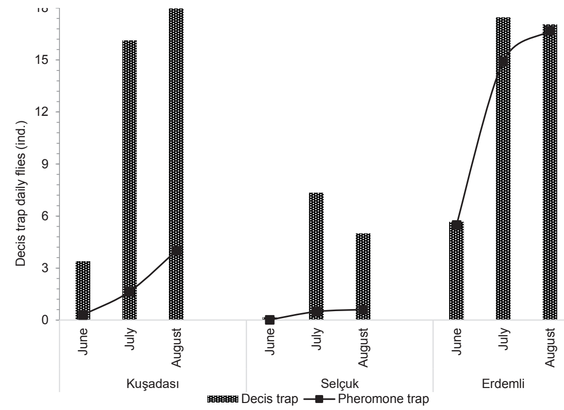

Figure 4. Daily numbers of flies caught in the Decis and pheromone traps

The daily numbers of flies in the pheromone traps for Kuşadası and Selçuk were 2.9 and 0.1 in June and 16.4 and 4.8 in July, respectively. These numbers for June, July and August were 54.8, 149.1 and 166.9, respectively, in Erdemli. In July, where the population increased, the number of flies caught per day in Selçuk was lower than that in Kuşadası. The numbers of flies in the traps in Erdemli were much higher than those in the other districts, and 9-18 times more flies were caught per day within the season (Figure 4).

Fruit infestation rates and effects of traps

The results of fruit counting in Kuşadası, Selçuk and Erdemli were given respectively in Tables 1, 2 and 3.

As a result of the counting in Kuşadası, the infestation rate in the Decis trap plots was (N:3328) 0.9%, while that in the control plot was (N:756) 15.5% (Table 1). Accordingly, the

Table 1. Crosstabs on fruit counts, infestation rates in treatments in Kuşadası (fruit*treatment Crosstabulation)

			treatment		Total
			trap	control	
fruit	non-damaged	Count	3298	639	3937
		% within fruit	83.8%	16.2%	100.0%
		%within treatment	99.1%	84.5%	96.4%
		% of Total	80.8%	15.6%	96.4%
	damaged	Count	30	117	147
		% within fruit	20.4%	79.6%	100.0%
		%within treatment	00.9%	15.5%	3.6%
		% of Total	0.7%	2.9%	3.6%
Total		Count	3328	756	4084
		% within fruit	81.5%	18.5%	100.0%
		%within treatment	00%	100.0%	100.0%
		% of Total	81.5%	18.5%	100.0%

effectiveness of the trap was calculated as 94.19%. It was determined that there was a significant difference between the treatment plots and the control plot in the trial area (x2: 377.14; P < 0.05; df:1).

As a result of the counting in Selçuk, the infestation rate in the Decis trap plots was (N:6740) 0.9%, while that in the control plot was (N:622) 18.6% (Table 2). Accordingly, the effectiveness of the trap was calculated as 95.16%. It was determined that there was a significant difference between the treatment plots and the control plot in the trial area (x2: 747.95; P < 0.05; df:1).

Table 2. Crosstabs on fruit counts, infestation rates in treatments in Selçuk (fruit*treatment Crosstabulation)

			treatment		Total
			trap	control	
fruit	non-damaged	Count	6676	639	7182
		% within fruit	93.0%	7.0%	100.0%
		%within treatment	99.1%	81.4%	97.6%
		% of Total	90.7%	6.9%	97.6%
	damaged	Count	64	116	180
		% within fruit	35.6%	64.4%	100.0%
		%within treatment	00.9%	1.6%	2.4%
		% of Total	0.7%	2.9%	3.6%
Total		Count	6740	622	7362
		% within fruit	91.6%	8.4%	100.0%
		%within treatment	100.0%	100.0%	100.0%
		% of Total	91.6%	8.4%	100.0%

As a result of the counting in Erdemli, the infestation rate in the Decis trap plots was (N:6392) 15.89%, while that in the control plot was (N:4195) 36.2% (Table 3). Accordingly, the effectiveness of the trap was calculated as 56.35%. The effectiveness of the control trap in the counts made in Erdemli was lower than those of the other districts. The main reason for this situation was the population densities and numbers of offspring were different among the regions. The population density in Erdemli was higher than Kuşadası and Selçuk. It was determined that there was a significant difference between the treatment plots and the control plot in the trial area (x2: 582.47; P < 0.05; df:1).

The mean numbers of flies caught in Erdemli in terms of both the Decis traps and the pheromone trap were higher than Kuşadası and Selçuk's results. As it can be seen in the data that were obtained here, it was also reported by El-Gendy (2014) that population could increase in peach orchards between regions and years based on the presence of hosts and prevalence of offspring. The population of the pest in Erdemli showed an increase in July and August in this study. Different researchers have reported in Adana, which has similar climate characteristics to those in the studied regions, that populations of the pest increased in peach orchards between the last week of May and the first week of July, in grapefruit orchards between May and September, in persimmon orchards in July, September and November and in pomegranates in September, October and November (Kasap and Aslan 2016, Satar et al. 2016, Tiring and Satar 2017). In a different study that we carried out in Karaburun and Menderes districts of İzmir, it was observed that the pest was seen between April and November, and its population increased especially in August and September (Tolga et al. 2019).

Table 3. Crosstabs on fruit counts, infestation rates in treatments in Erdemli (fruit*treatment Crosstabulation)

			treatment		Total
			trap	control	
fruit	non-damaged	Count	5383	2675	8058
		% within fruit	66.8%	33.2%	100.0%
		%within treatment	84.2%	63.8%	76.1%
		% of Total	50.8%	25.3%	76.1%
	damaged	Count	1009	1520	2529
		% within fruit	39.9%	60.1%	100.0%
		%within treatment	15.8%	36.2%	23.9%
		% of Total	9.5%	14.4%	23.9%
Total		Count	6392	4195	10587
		% within fruit	60.4%	39.6%	100.0%
		%within treatment	100.0%	100.0%	100.0%
		% of Total	60.4%	39.6%	100.0%

The control process with traps provided success rates of 94.19% in Kuşadası and 95.15% in Selçuk, while it provided a success rate of only 56.35% in Erdemli. As a result of examining these data, it was determined that the success of control decreases in areas where populations are high levels. Likewise, Hafsi et al. (2016) examined the activities of two different bait stations in early and mid-late peach varieties and reported that the number of adult flies on the late varieties was two times higher than those caught in the early varieties, and therefore, the effectiveness dropped in late varieties. Additionally, they recommended increasing the number of traps per hectare for the control process to be successful due to the high populations observed in the late varieties. Tiring and Satar (2017) determined that the population of the pest was not dense in the varieties that were harvested in June-July, and there was no problem, but populations increased in the varieties that were harvested later, and this constituted a thread for peaches. Penarrubia (2010) reported that control processes carried out in peach orchards in Spain by mass trapping were successfully effective on low populations, but these should be supported with chemical control in the case of high populations. They also recommended increasing the number of traps to be used in the control process so that there would be no damage in years where the population increases by two times. Elekçioğlu et al. (2011) found that the population of the pest was high in Adana in August and September, and in the study, they carried out with traps containing Trimedlure and DDVP capsules, they managed to decrease the number of control processes with insecticides from nine to five. Papadopoulos et al. (2001) investigated the effects of low-density (1.5 traps/ha) and high-density (15 traps/ha) by using traps containing attractants with the same properties as Decis traps. They reported that traps hanged in peach orchards with high intensity attracted more individuals, traps that were hanged with high density at fruit areas caught Mediterranean fruit fly earlier, and the type of trap and the host were key factors in early monitoring of the pest. In parallel to the results and recommendations proposed by different researchers, in areas like Erdemli where the population is constantly high or in years where the population is determined to increase, the number of traps per hectare should be increased to prevent damage in the fruits. In cases where it is not possible to increase the number of traps, the control process should be supported by at least one application of insecticides. Yayla and Satar (2017) emphasized that there is a need to apply integrated fruit fly control techniques in cases of high population levels. The traps were kept in the land for 5 weeks in Kuşadası, 6 weeks in Selçuk and 10 weeks in Erdemli, and during these times, these traps were observed to catch the adults of C. capitata. As in the case that was observed during our study, dry traps such as Decis trap and similar ones that are used against the Mediterranean fruit fly are effective for 6-10 weeks (Jang et al. 2007).

Biotechnical methods are some of the most significant practices that are among the integrated control methods. However, it was presented with this study that biotechnical control alone would not be sufficient in cases where pest populations are higher levels. However, the number of traps may be increased to reduce damage levels of this pest. Similarly, in this study, it was determined that the density of the pest was high during this study especially in the Erdemli district of the province of Mersin in Turkey, and trap control was not sufficient by itself. It is considered that usage of different numbers of traps (traps/ha) in areas where the population of the pest is high and those where it is low may be effective in suppressing this population. In cases where it is not possible to increase the numbers of traps, it is recommended to carry out the control process by combining biotechnical control with methods such as chemical control, early harvest, and especially cultural control. The rates of damages, especially on the economic concerns of the producers, may increase in products that are harvested late. For this reason, the harvesting process should be completed without delay, and the pest should be controlled with an integrated approach.

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ÖZET

Ceratitis capitata karantina zararlısı ve toleransı sıfır olarak kabul edilen bir türdür. Üreme kapasitesinin yüksek olması ve doğrudan meyvede zarar yapması nedeniyle zararlı ile mücadele yürütülmeden üretim yapmak mümkün olamamaktadır. Kimyasal mücadeleye alternatif olarak biyoteknik mücadele yöntemi olan kitle halinde tuzakla yakalama, zararlının çok yüksek olmayan popülasyonlarında başarılı sayılan bir mücadele şeklidir. Tuzaklar ile mücadelede farklı besin cezbedici materyaller kullanılarak bireylerin tuzağa çekilmesi sonucu öldürülmesi sağlanmaktadır. Bu çalışma, Ege ve Akdeniz Bölgelerinde şeftali bahçelerinde eş zamanlı yürütülmüş ve bölgeler arasındaki popülasyon farklılığına göre tuzakların etkinliğinin belirlenmesi amaçlanmıştır. Denemeler 2018 yılında Kuşadası (Aydın), Selçuk (İzmir) ve Erdemli (Mersin) ilçelerinde yürütülmüştür. Zararlının feromon tuzaklar ile popülasyon değişimi ve kitle halinde yakalama tuzaklarının etkinliği saptanmıştır. Mücadele tuzağı olarak Decis Trap (Bayer) isimli ürün kullanılarak tuzağın etkinlikleri saptanmıştır. Temmuz ayında Kuşadası, Selçuk ve Erdemli ilçelerinde sırasıyla 4.8, 149.1 ve 166.9 adet/tuzak ergin birey yakalamıştır. Buna göre Kuşadası ilçesinde %94.19, Selçuk ilçesinde %95.6 oranlarında etkili bir başarı sağlarken Erdemli ilçesinde %56.34 oranında etkili olmuştur. Akdeniz Bölgesi popülasyonunun Ege Bölgesine göre daha yüksek seyretmesi ve şeftali üretim sezonun daha uzun sürmesinden dolayı istenilen düzeyde başarı sağlanamadığı belirlenmiştir.

Anahtar kelimeler: biyoteknik mücadele, *Ceratitis capitata*, İzmir, Mersin, şeftali

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Original article

Determination of pesticide residues and risk assessment in some vegetables grown in Tokat province

Tokat ilinde üretilen bazı sebzelerde pestisit kalıntılarının tespiti ve risk değerlendirmesi

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ABSTRACT

This study was carried out to determine the pesticide residue levels and health risk assessments in tomato, pepper, and cucumber grown in Tokat province of Turkey. A residue analytical method was verified to determine 260 pesticides by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS). Pesticide solutions at 10 and 50 µg kg^{-1} doses were fortified with the pesticide-free cucumber matrix for verification of the method. The linearities (R^2), the limit of detection (LODs), the limit of quantification (LOQs), and mean recovery values of the pesticides ranged between 0.990-0.999, 0.71-2.96 µg kg^{-1}, 2.36-9.86 µg kg^{-1} and 77.25-117.61%, respectively. Pesticide residue levels of 28 vegetable samples collected from Tokat province were determined using the verified method. Seventeen different pesticides were detected in 16 samples, and pesticide concentrations in 3 samples were higher than the allowable limits of the European Union Maximum Residue Limits (EU-MRL). The pyridaben level in 1 pepper sample, metrafenone in 2 cucumber samples were higher than the threshold values stated by the EU-MRL. The results of health risk assessments indicated that famoxadone and pyridaben have chronic toxicity potential for consumers.

INTRODUCTION

Vegetables are an important part of the human diet as they provide essential specific nutrients for the human body (Keikotlhaile and Spanoghe 2011). Their consumption is recommended not only to prevent avitaminosis, but also to reduce the incidence of important diseases such as cancer, cardiovascular diseases, and obesity. Rapid urbanization and increase in human population caused changes in lifestyle and eating habits, and the food demands of the consumers have diversified. These approaches have strongly impressed food production and consumption; therefore, the producers have more carefully adopted plant protection measures to control pests. Pesticides are commonly used in pest management due to their rapid action, easy use, and affordability. However, misuse or unconscious use of pesticides may damage plants, the environment, wildlife, and public health. Because pesticides used in agricultural production often lead to residues. Contamination of crops, especially fruits and vegetables, is one of the current important issues. The majority of consumers are not familiar with pesticide residue in the food; therefore, monitoring the residue levels of pesticides in food is vital for human health.

Detailed studies on pesticide residue levels in vegetables have been reported in Turkey. Bakırcı et al. (2014), Balkan and Kara (2019), Çatak and Tiryaki (2020), Çiftçi (2019), Durmuşoğlu (2002), Ersoy et al. (2011), Golge and Kabak (2018) reported residue concentrations over MRL threshold in cucumber, tomato, and pepper samples. In contrast, the residue concentration in vegetables reported by Hepsağ (2019), Kaya and Tuna (2019), Polat and Tiryaki (2018), Zengin and Karaca (2017) were lower than the MRL threshold values.

Exposure to pesticides can cause health problems, such as nausea and headaches in a shorth term. In addition, neurotoxicity, cytogenetic damage, infertility, and endocrine system problems may occur chronically (Baldi et al. 2001), and leukemia, non-Hodgkin lymphoma, brain, bone, breast, ovarian, prostate, testicular, and liver cancers in the long term (Cantor et al. 1992). Therefore, health risk assessment is very important for pesticide residues. Although studies on detection of pesticide residues for important vegetables in Turkey have increased, the studies on health risk assessment are not at the desired level. Gölge and Kabak (2018) determined no health risk in tomato, Soydan et al. (2021) stated no health risk in tomato and pepper. Çatak and Tiryaki (2020) noted no risk of chronic exposure in cucumber.

The increase in living standards raised the awareness on pesticide residues in agricultural products. In addition, ensuring safe pesticide residue level is important in promoting agricultural product export of Turkey. Increasing residue studies is essential for both local agricultural development and raising awareness for consumers. This study aimed to determine the pesticide residue levels and the health risk assessments in tomato, pepper, and cucumber grown in Tokat province, which has a high agricultural potential.

MATERIALS AND METHODS

Sample collection and storage

Samples were collected in accordance with the Commission Directive 2002/63/EC on sampling for the official control of pesticide residues in and on products of plant and animal origin (EC 2002). Ten cucumbers, 8 tomatoes, and 10 peppers were collected randomly in Tokat province, Turkey. Collected samples were transported immediately to the laboratory and stored at -18 °C.

Reagents and chemicals

Pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile (MeCN > 99% purity), methanol (MeOH > 99% purity), anhydrous magnesium sulfate ($MgSO_4$ ≥ 99% purity), ammonium formate (CH_5NO_2 ≥ 99% purity), sodium acetate (NaOAc ≥ 99% purity), and acetic acid (AcOH > 99% purity) were supplied by Merck. Primary-secondary amine (PSA) was obtained from Supelco analytical.

Chromatographic analysis

This study was carried out using an LC-MS 8050 model (Shimadzu®) equipped with a UPLC: LC-30AD pump x 2, SIL-20A autosampler, a DGU-20A3R degasser, a CTO-20ACV column oven, and a triple quadrupole MS/MS detector. The LC column was inertsil (ODS IV) C18 column (2.1 mm x 150 mm, 3 µm particle size) of GL Sciences Inc (Tokyo, JAPAN). Chromatographic separation was performed using a gradient elution program with eluent A consisting of distilled H_2O + 5 mM ammonium formate and eluent B consisting of methanol + 5 mM ammonium formate. The analysis started with 5% eluent B, which was increased linearly to 60% in 3 min, 70% in 4 min, 80% in 6 min, and 95% in 7 min. The gradient elution was started with 5% of B (held 1 min), then increased linearly to reach 95% of B in 4 min (held 2 min), and decreased to the initial stage (5% of B) at 6 min, and kept until 9 min. The flow rate was 0.40 mL min^{-1}, and the injection volume was set to 10 µL. The column and autosampler temperatures were set to 35 °C and 4 °C, respectively. For MS/MS detection, the electrospray ionization (ESI) interface was used for positive polarity with the following: 3 kV of capillary voltage, 3V of extractor voltage, 350 °C of heat block temperature, 250 °C of desolvation line (DL) temperature, nitrogen (N_2) as nebulizer gas of 2.9 L min^{-1} and drying gas of 10 L min^{-1}. Nitrogen gas of 99% purity produced by a Peak Scientific nitrogen generator (Billerica, MA, USA) was used in the ESI source and the collision cell. Collision-induced dissociation (CID) gas was argon (Ar, 99.999%) of 230 kpa with a flow rate of 0.15 mL min^{-1}. All parameters of the instrument were controlled using LabSolution® software (version 4.91) (Balkan and Yılmaz 2022).

Sample extraction and clean up

The official QuEChERS AOAC Method 2007.01 was used for the extraction and clean-up procedures (Lehotay 2007). The steps in Figure 1 were followed for the QuEChERS process. Each sample was analyzed in triplicate with LC-MS/MS.

Calculation of risk assessment

In assessing the acute and chronic risk of pesticide residues; estimated dietary exposure was compared to toxicological values known as acute reference dose (ARfD, mg kg^{-1} bw day^{-1}) and acceptable daily intake (ADI, mg kg^{-1} bw day^{-1}). The acute/short-term consumer health risk (aHI) was calculated based on the estimated short-term intake (ESTI, mg kg^{-1} day^{-1})

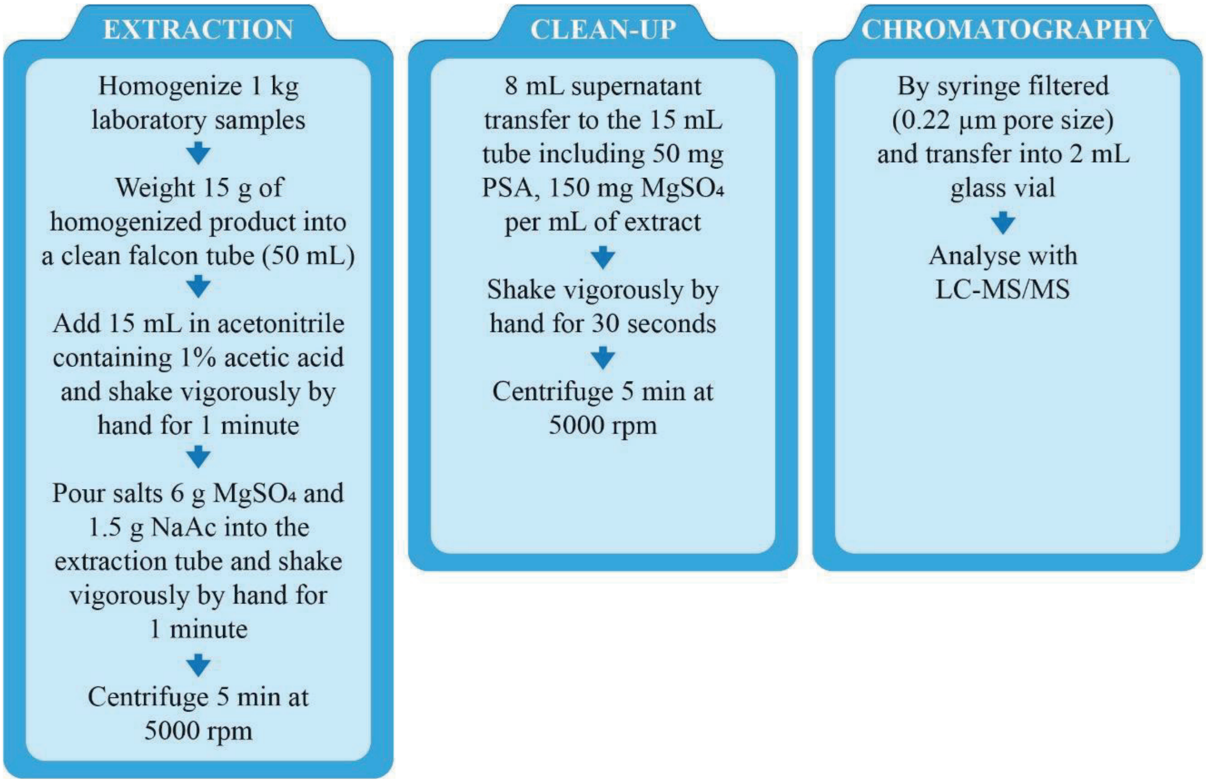

Figure 1. Analytical steps of the QuEChERS-AOAC Official Method 2007.01

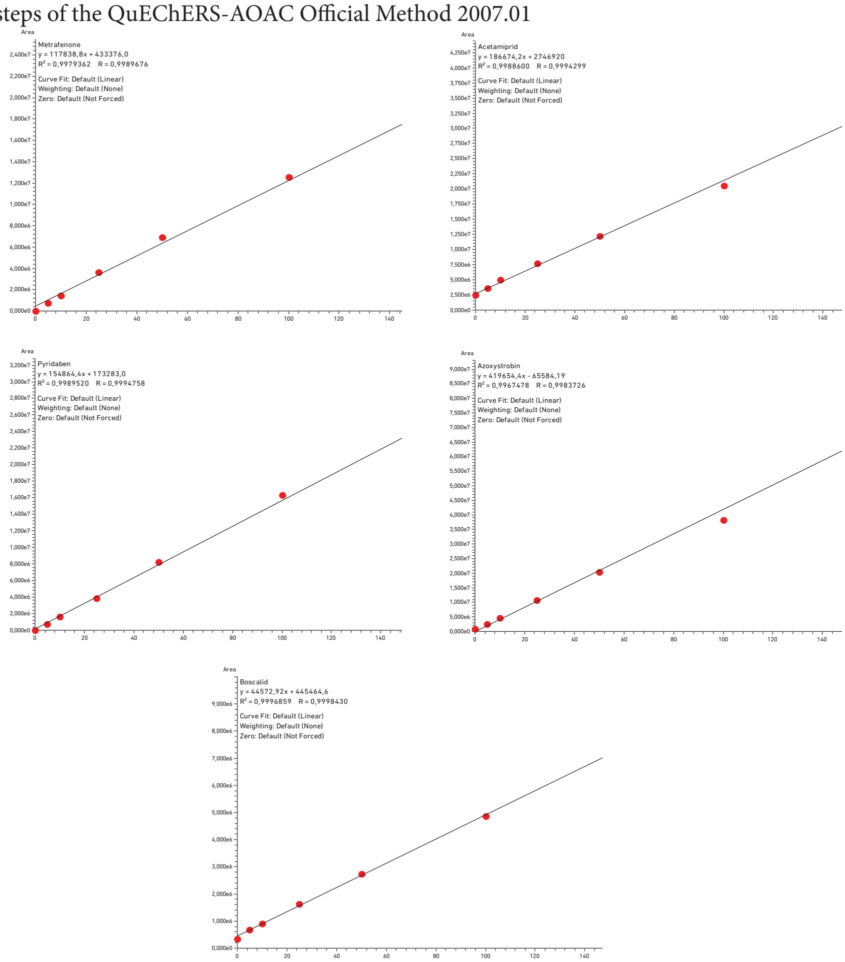

Figure 2. Calibration curves for five compounds in matrix-matched calibration

and the acute reference dose (ARfD). The chronic/long-term consumer health risk (chronic hazard index, cHI) was calculated based on the estimated daily intake (EDI, mg kg^{-1} day^{-1}) and the acceptable daily intake (ADI). The relevant formulas were as follows (Liu et al. 2016);

ESTI=(high residue level x food consumption)/body weight (1)

aHI=ESTI/ARfD x 100 (2)

EDI=(mean residue level x food consumption)/body weight (3)

cHI=EDI/ADI x 100 (4)

The food is considered a risk to the consumers when the health risk index >1. The food is considered acceptable when the index is <1 (Darko and Akoto 2008, Soydan et al. 2021). The average body weight of an adult was considered 73.5 kg. (TÜİK, 2019). Daily consumption of cucumber, tomato, and pepper for the general population in Turkey were used as 0.05, 0.31, and 0.07 kg^{-1}day^{-1}, respectively (TÜİK 2021).

RESULTS AND DISCUSSION

Method verification

The matrix calibration curves and calibration equations of 260 pesticides in the LC-MS/MS system were linear ($R^2 \geq 0.990$) in the calibration range of 5–200 µg mL^{-1}. Correlation coefficient (R^2) over 0.990 is an important criteria of linearity (Tiryaki et al. 2008). Calibration curves of metrafenone, pyridaben of which the concentration was over MRL, and acetamiprid, azoxystrobin, boscalid of which the residue concentration was lower than the MRL, were shown Figure 2.

Detection and quantification limit

The LOD and LOQ values in all target analytes of the representative cucumber matrix were extremely low (EC 2019). The LOD values ranged from 0.71 to 2.96 µg kg^{-1}, while LOQs were between 2.36 and 9.86 µg kg^{-1}. The lowest LOD and LOQ values were recorded in the methomyl, while the highest value was determined in the fenthion active substance. The LOD and LOQ values of acetamiprid, azoxystrobin, boscalid, metrafenone, and pyridaben were 0.99-3.32, 2.35-7.84, 1.98-6.61, 1.57-5.22, and 1.74-5.79 µg kg^{-1}, respectively (Figure 2). The values for all pesticides were lower than the MRL values determined by the European Union for pepper, tomato, and cucumber.

Precision and accuracy

Method of precision and accuracy is evaluated by repeatability (%RSD) and recovery (%Q) (EC 2019, Magnusson and Örnemark 2014, TURKAK 2019). The recovery tests were carried out using five replicates at two fortification levels of 10 and 50 µg kg^{-1}, respectively. The recovery rates of acetamiprid, azoxystrobin, boscalid, metrafenone, and pyridaben are given in Table 1. Figures obtained for the other 255 pesticides were within the values stated by SANTE recovery limits (70% ≤ Q ≤ 120%) and repeatability (≤ 20%) (EC 2019).

Current findings on recovery rates are consistent with method validation parameters for pesticide residue analysis (EC 2019, Magnusson and Örnemark 2014). The accuracy values, which are expressed as the closeness of the measured values to the actual values (Tiryaki 2016), are given in Table 1. The results showed that QuEChERS provides efficient recovery rates for 260 pesticides. Therefore, the current analytical method could offer a fast and accurate method for residue analysis in the studied matrices.

Residues of samples

Two hundred sixty pesticides belonging to different groups used in Turkey were discussed in the study. Analysis of 260 pesticides was carried out using an LC-MS/MS device. The LOD, LOQ, and EU-MRL values of 17 pesticides detected are given in Table 2.

Table 1. QuEChERS method verification data

Analyte	Concentration (µg kg^{-1})		Recovery % (As a tool for trueness)	RSD % (As a tool for precision)
	Spiked	Measured[a]		
Acetamiprid	10	11.18	111.83	7.41
	50	56.39	112.78	4.05
Azoxystrobin	10	10.48	104.78	10.48
	50	51.92	103.83	7.20
Boscalid	10	10.57	105.67	6.50
	50	54.08	108.15	2.69
Metrafenone	10	7.94	79.40	5.15
	50	53.25	106.49	3.45
Pyridaben	10	10.56	105.60	6.90
	50	49.05	98.09	5.03

[a] Mean of three analytical portions

A total of 28 samples were analyzed. The residue levels were between 7.93 and 1501.30 µg kg^{-1}. The results and frequency of pesticides are presented in Table 3.

Acetamiprid, azoxystrobin, and boscalid were the active substances detected in all three vegetables (Table 3). The concentration of acetamiprid, azoxystrobin, and boscalid were between 9.90- 51.53, 11.46- 56.69, and 16.39-664.88 µg kg^{1}, respectively.

Bifenazate and kresoxim-methyl were detected in pepper and cucumber, pyridaben in pepper and tomato, penconazole in tomato and cucumber, pyrimethanil in pepper, famoxadone in tomato and clofentezine, fluopyram, hexythiazox, metrafenone, pirimicarb+pirimicarb-desmethy, tebuconazole and thiacloprid in cucumber. The

Table 2. LOQ and EU-MRL values

Analyte	LOD μg kg^{-1}	LOQ μg kg^{-1}	EU-MRL μg kg^{-1}			ADI*(mg kg^{-1} bw day^{-1})	ARfD* (mg kg^{-1} bw day^{-1})
			pepper	tomato	cucumber		
Acetamiprid	0.99	3.32	0.3	0.5	0.3	0.025	0.025
Azoxystrobin	2.35	7.84	3	3	1	0.2	/
Boscalid	1.98	6.61	3	3	4	0.04	/
Bifenazate	1.61	5.36	3		0.5	0.01	/
Clofentezine	2.24	7.45			0.2	0.02	/
Famoxadone	2.02	6.73		2		0.012	0.2
Fluopyram	1.92	6.39			0.5	0.012	0.5
Hexythiazox	2.30	7.66			0.5	0.03	/
Kresoxim-methyl	2.26	7.52	0.8		0.5	0.4	/
Metrafenone	1.57	5.22			0.5	0.25	/
Penconazole	2.31	7.72		0.1	0.06	0.03	0.5
Pirimicarb+ Pirimicarb-desmethy	1.41 1.08	4.70 3.59			1	0.035	0.1
Pyridaben	1.74	5.79	0.3	0.15		0.01	0.05
Pyrimethanil	2.26	7.52	2			0.17	/
Tebuconazole	2.21	7.38			0.6	0.03	0.03
Tetraconazole	1.84	6.13			0.2	0.004	0.05
Thiacloprid	1.60	5.32			0.5	0.01	0.03

*ARfD and ADI were adopted from IUPAC pesticides properties database (IUPAC 2022)
The symbol of "/" represented that there was no authorized value for ARfD

results were evaluated according to the threshold values stated by the EU-MRL. The residual values from 1 (10%) of pepper and 2 (20%) of cucumber samples were over MRL. Metrafenone and pyridaben concentrations were higher than the MRL values.

Metrafenone is used against powdery mildew, and pyridaben is used against plant-feeding mites and whitefly. Eleven samples contained more than one pesticide, and 8 of them contained more than three pesticide active ingredients. These samples (10.7%) had pesticide residues above EU-MRL values. The residue level in tomato samples was not higher than the EU-MRL values. Similarly, Szpyrka et al. (2015) reported that the residual values detected in 40 cucumbers and 42 tomato samples collected in southeast Poland were below the MRL values. Adeniyi et al. (2016) reported that the residual values detected in 6 tomato samples in Louisiana, USA was below the MRL values. Zengin and Karaca (2017), Polat and Tiryaki (2018), and Hepsağ (2019) indicated that the residue levels in tomato samples were collected from open and greenhouse tomato growing areas in Uşak, Çanakkale, and the Mediterranean region, respectively were below the MRL. The residue values in tomato and cucumber samples from İzmir province were below the MRL, while no pesticides were detected in pepper samples (Kaya and Tuna 2019). Algharibeh and Al Fararjeh (2019) reported that the residual values detected in 40 tomato samples were below the MRL, while pesticide content in 17 of 32 pepper samples in Jordan was above the MRL. Velioglu et al. (2019) stated that the residual values in tomato samples taken from Tekirdag province, Turkey were close to the EU-MRL values. Gölge and Kabak (2018) determined that the pesticide content of 5 tomato samples from Mersin and Antalya provinces was higher than the MRL value. Loughlin et al. (2018) reported that the residue level in 2 of 10 tomato samples and 7 of 23 pepper samples in Argentina were above the MRL. Elmastaş (2018) determined that the residual values of 2 tomato samples from Diyarbakır province, Turkey were above the MRL, and the residue level in cucumber samples was lower than the MRL values. Salamzadeh et al. (2018) reported that the residue levels in 4 of 150 tomato samples in Iran were above the MRL. Çiftçi (2019) detected residues in 10 tomato samples and 14 pepper samples from Çanakkale province, Turkey higher than the maximum residue limits (MRL) specified in the Turkish Food Codex (TGK 2021). Hu et al. (2020) reported that the residual values detected in 22 dried cucumbers and 40 pepper samples in Jilin, China were below the MRL, while the residue level in 3 of 31 tomato samples were above the MRL. Yi et al. (2020) reported that the residue values in 638 cucumber samples in Korea were below the MRL, and 1 of 149 tomato and 638 pepper samples had residue above the MRL. Osaili et al. (2020) reported that the residue levels in 87 of 233 cucumber samples, 41 of 205 tomato samples, and 130 of 316 pepper samples in the United Arab Emirates were above the MRL value. Ramadan et al. (2020) indicated that detectable pesticide residues in 44 samples (20.9%) were above MRLs, and residue level in 145 samples (68.7%)

Table 3. Pesticide residue levels and frequencies

Matrix	No. of samples detectable residues (%)	No. of samples >MRL (%)	Pesticides	Frequency of detection	Pesticide residue (µg kg^{-1})	No. of samples >MRL
Pepper	6 (60)	1 (10)	Acetamiprid	3	14.58- 27.33- 28.83	
			Azoxystrobin	1	11.46	
			Bifenazate	1	21.23	
			Boscalid	2	16.39- 664.88	
			Kresoxim methyl	1	53.35	
			Pyridaben	4	11.06- 37.61- 44.18- 684.36	1
			Pyrimethanil	1	60.07	
Tomato	3 (37.5)	-	Acetamiprid	1	21.74	
			Azoxystrobin	1	34.17	
			Boscalid	1	37.21	
			Famoxadone	1	58.99	
			Penconazole	1	10.87	
			Pyridaben	2	13.73- 40.43	
Cucumber	7 (70)	2 (20)	Acetamiprid	2	9.90- 51.53	
			Azoxystrobin	3	17.36- 35.45- 56.69	
			Bifenazate	2	18.71- 28.21	
			Boscalid	1	522.95	
			Clofentezine	2	25.95- 67.54	
			Fluopyram	3	18.20- 37.02- 49.35	
			Hexythiazox	1	8.47	
			Kresoxim-methyl	2	107.30- 419.48	
			Metrafenone	2	1412.11- 1501.30	2
			Penconazole	1	12.76	
			Pirimicarb+ Pirimicarb- desmethy	1	65.73	
			Tebuconazole	2	7.93- 22.70	
			Tetraconazole	2	45.37-60.19	
			Thiacloprid	1	7.68	

was lower than MRLs. The MRL values in chili pepper (14 samples) and cucumber (10 samples) were high. Soydan et al. (2021) found the residual values determined in peppers grown in the Aegean region lower than the MRL. The residue values in 2 cucumber samples were above the MRL. Çatak and Tiryaki (2020) determined the MRL value of 1 cucumber sample collected from Çanakkale open markets was above the MRL.

Residues of samples

Risk analysis was carried out for 27 pesticides and the results are given in Table 4.

The short-term risk assessment revealed that pyridaben level possesses high risk with a value of 1.3139, while all other aHI values were less than 1 which indicates a negligible acute risk. In the long-term risk assessment, the chronic risk index (cHI) values were considerably higher than the aHI values. The results indicate that the chronic risk from pesticide exposure through the consumption of peppers, tomatoes, and cucumbers should be considered. The risk assessment considering the pesticide exposures in tomato and cucumber vegetables showed that the pesticide residues would not pose a risk for the Kazakh people (Lozowicka et al. 2015). However, the estimated dietary pesticide exposures considered only tomato and cucumber exposures. Yi et al. (2020) stated that pesticide residues may not be considered a serious public health problem in Korea. Theoretical maximum daily intake evaluation of Çatak and Tiryaki (2020) showed that the pesticides in cucumber did not pose a risk of chronic exposure. Soydan et al. (2021) reported that pesticide residue levels detected cannot be considered a serious public health problem. Darko and Akoto (2008)

Table 4. The results of long-term and short-term risk assessments

Matrix	Pesticide	Long-term risk EDI (mg kg^{-1}day^{-1})	cHI	Short-term risk ESTI (mg kg^{-1}day^{-1})	aHI
Pepper	Acetamiprid	2.26211E-05	0.0905	2.76751E-05	0,1107
	Azoxystrobin	1.0994E-05	0.0055	1.10009E-05	/
	Bifenazate	2.03667E-05	0.2037	2.03795E-05	/
	Boscalid	0.000326783	0.8170	0.000638245	/
	Kresoxim-methyl	5.11805E-05	0.0128	5.12128E-05	/
	Pyridaben	0.000186399	1.8640	0.000656944	1.3139
	Pyrimethanil	5.76272E-05	0.4802	5.76636E-05	/
Tomato	Acetamiprid	9.25963E-05	0.3704	2.08691E-05	0.0835
	Azoxystrobin	0.000145539	0.0728	3.28011E-05	/
	Boscalid	0.000158487	0.3962	3.57194E-05	/
	Famoxadone	0.000251254	2.0938	5.66268E-05	0.0283
	Penconazole	4.62981E-05	0.1543	1.04345E-05	0.0021
	Pyridaben	0.000115341	1.1534	3.88104E-05	0.0776
Cucumber	Acetamiprid	2.13811E-05	0.0855	4.94657E-05	0.1979
	Azoxystrobin	2.54122E-05	0.0127	5.4419E-05	0.1814
	Bifenazate	1.63334E-05	0.1633	2.70799E-05	/
	Boscalid	0.000364091	0.9102	0.000502	/
	Clofentezine	3.25415E-05	0.1627	6.48343E-05	/
	Fluopyram	2.42634E-05	0.2022	4.7373E-05	0.0095
	Hexythiazox	6.085E-06	0.0203	8.38987E-06	/
	Kresoxim-methyl	0.000183379	0.0458	0.000402675	/
	Metrafenone	0.001014191	0.4057	0.001441157	/
	Penconazole	8.88383E-06	0.0296	1.22488E-05	0.0024
	Pirimicarb+ Pirimicarb-desmethy	4.57629E-05	0.1308	6.30968E-05	0.0631
	Tebuconazole	1.06592E-05	0.0355	2.17906E-05	0.0726
	Tetraconazole	3.67467E-05	0.9187	5.77788E-05	0.1156
	Thiacloprid	5.34701E-06	0.0535	7.37234E-06	0.0246

The symbol of "/" represented that there was no authorized value for ARfD, and the corresponding risk index could not be computed.

reported that some health risks may occur due to the OP residues detected in tomatoes and eggplants collected from a market in Ghana Kumasi. Bolor et al. (2018) stated that the residues on vegetables consumed by children may pose both carcinogenic and non-carcinogenic health risks, while the residue level detected may not pose health risks for adults. Kumari and John (2019) reported that methyl parathion and triazophos residues detected in fruit and vegetable samples collected from the West Indian Himalayan region pose a potential threat to human health, especially in children. Odewale et al. (2021) reported that α-HCH and γ-HCH in tomato and watermelon samples may pose carcinogenic health risks in child consumers, and α-HCH in adult consumers. Odewale et al. (2021) reported that the consumption of tomatoes and watermelon containing α-HCH and γ-HCH may pose carcinogenic health risks in child consumers, and α-HCH in adult consumers.

This study was conducted to reveal the pesticide residues in tomatoes, cucumbers, and peppers produced in Tokat province, and the health risk assessments related to pesticide residues. Seventeen different pesticides were detected in 16 of 28 samples. The residue content of 2 pesticides exceeded the MRL levels. Risk analysis was conducted for 27 pesticides in the study. The short-term risk assessment revealed that the highest risk was in pyridaben with a 1.3139 aHI value, and aHI values for other active substances were at a negligible level. The long-term risk assessment showed that cHI values were much higher than the aHI values. The results indicated that the chronic risk arising from pesticide exposure in tomatoes and cucumbers should be taken into account. Potential risks are possible due to prolonged dietary exposure; therefore, reside level should constantly be monitored. Some precautions should be taken into account to minimize such risks. The precautions suggested can be listed as follows. Adopting IPM and GAP approaches to producers is important to reduce both pesticide residue and health risks. Pest control should be carried out regularly by experts, and appropriate doses and application time between the last spraying and harvest should be determined. Farmers should be trained and precautions should be taken regarding the sale of pesticides. Manufacturers should be informed of the consequences of incorrect pesticide use. The use of banned preparations should be strictly avoided. The use of highly toxic pesticides should be limited. Agricultural products should be marketed after the residue analysis. Products with residue problems must be destroyed, and dealers who advise these manufacturers should be punished. To monitor appropriate measures and thus minimize the associated health risks, a national monitoring program and database should be established for locally consumed agricultural products and processed food. Agricultural production cannot be carried out without using pesticides. However, pesticide residue levels should be kept at values below the MRL. Compounds used in agricultural activities have a high tendency to accumulate in human tissues as biomass. Since vegetables are widely consumed, pesticide residues in vegetables may cause serious health problems. Overall, the findings of the study are important as a reference point for future research.

ÖZET

Bu çalışma, Tokat ve çevresinde üretilen domates, biber ve hıyarda pestisit kalıntı düzeylerini tespit etmek ve bu ürünler için sağlık risk değerlendirmelerini belirlemek amacıyla yapılmıştır. Sıvı kromatografi-tandem kütle spektrometresi (LC–MS/MS) ile bu sebzelerdeki 260 pestisit kalıntısının belirlenmesi için analitik metot doğrulaması yapılmıştır. Bunun için, pestisit içermeyen salatalık matrisine 10 ve 50 µg kg^{-1} seviyelerinde pestisit çözeltisi eklenmiştir. Pestisitlerin doğrusallıkları (R^2), tayin limitleri (LOD), tespit limitleri (LOQ) ve ortalama geri kazanımları sırasıyla 0.990-0.999, 0.71-2.96 µg kg^{-1}, 2.36-9.86 µg kg^{-1} ve %77.25-117.61 arasında bulunmuştur. Bu yöntemle, Tokat (Türkiye) ilinden toplanan 28 sebze örneği analiz edilmiştir. 16 örnekte 17 farklı pestisit tespit edilmiş ve bunların 3'ünde pestisit konsantrasyonları Avrupa Birliği Maksimum Kalıntı Limitlerinden (AB-MRL) yüksek bulunmuştur. 1 biber örneğinde pyridaben ve 2 hıyar örneğinde metrafenone için AB-MRL değerleri aşılmıştır. Örneklerde tespit edilen pestisitlere yönelik risk değerlendirmesinde famoxadone ve pyridaben'in tüketiciler için kronik toksisite potansiyeline sahip olduğu kanaatine varılmıştır.

Anahtar kelimeler: akut risk, kronik risk, LC-MS/MS, metot doğrulama, QuEChERS

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Original article

Using yellow sticky traps in control to *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) on pear trees

Armut ağaçlarında *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) mücadelesinde sarı yapışkan tuzakların kullanımı

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A B S T R A C T

This study investigates the applicability of a mass trapping method using yellow sticky traps for controlling of the pear psyllid *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae), which is harmful to pear trees. Studies involving Ankara pear-variety saplings were carried out in the Ankara province in three different pear orchards in two different locations in 2012. In the first stage of the study, experiments were conducted using visual yellow sticky traps to determine trap efficiency in the pear orchard. Afterwards, mass trapping studies were carried out with the number of traps determined to be most effective in two different orchards. At the end of the study, it was observed that the yellow sticky traps had a very high ability to attract *C. pyri* adults, but were insufficient to suppress the pest as time progressed. As a result, it was concluded that the use of yellow sticky traps alone in the control of *C. pyri* would not be sufficient. However, it has been concluded that the traps can be used as a monitor in the early spring period when overwintered *C. pyri* adults are present and the beneficial population is inactive.

INTRODUCTION

Pear (*Pyrus communis* L.) ranks second after apple in terms of amount of production among pome fruits grown in Turkiye. Pear is one of the leading export products of Turkiye. There are many pests that threaten pear production. One of them, *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae), is an important pest in pear growing regions. Mixed populations of two *Cacopsylla* species have caused significant economic damage in pear orchards. *C. pyri* is the main pest in Europe (Civolani and Pasqualini 2003, Erler et al. 2007, Jenser et al. 2010) and *Cacopsylla pyricola* (Foerster, 1848) is in North America (Alston and Murray 2007, Horton 1994). Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and pear psylla *Cacopsylla pyricola* (Foerster) (Hemiptera: Psyllidae) are the major insect pests attacking commercial pears in North America. It is estimated that 50 to 80% of the costs is associated with controlling arthropod pests in pear orchards (Horton 2004). In Türkiye, Er (2008) has identified the species most harmful to the Ankara variety of pear grown in the Ankara province as *C. pyri*. Kovancı et al. (2000) investigated the population fluctuation of species *C. pyri* and *C. pyricola* that infested pears in the Bursa province and found that these species overwintered as adults, became active in mid-March, and gave 3-4 generations per year, depending on meteorological conditions. Adult and nymph *C. pyri* are particularly dense in certain regions and cause damage to pear orchards. The nymphs of this pest cause damage mainly by feeding on leaves and shoots, with heavy infestations resulting in inhibited tree growth, leaf and fruit fall, as well as fruit deformation. A sooty mold (fumagine) develops on secreted honeydew that inhibits respiration and photosynthesis, causes overall weakness in the tree, and lowers the market value of the fruit, with the market value of blackened fruit decreasing considerably. Other than this direct damage, it is known to have also indirect effects by acting as a vector of certain plant diseases, such as fire blight and viral disease (Erler 2004). *C. pyri* is defined by Brunner (1982) as an extremely difficult pest to control, and effective management requires an investment in efficient and timely sampling for adults and immature. Summer management of pear psylla is very difficult if the overwintered population is not controlled. Pear psylla is now resistant to many insecticides (Croft et al. 1989, Pree et al. 1990). Cultural measures and biological controls are recommended for the first-line management of this pest, which has the potential to cause significant damage in the presence of an increased population. In recent years increasing problems with pear psylla management in pear orchards treated with broad-spectrum insecticides, have necessitated chemical control which can be compatible with the preservation of natural enemies (Erler 2004). Insecticides have been reported to decrease the activities of the parasites and predators of this pest (Solomon et al. 1989). It has been reported that natural predators are not sufficient to suppress the pest in the early period, and so alternative control strategies are needed for the control of pear psyllid (Erler 2004). Yellow sticky traps are used to determine the accurate time of pest control and to serve as an early warning by predetermining the spreading time of the bug populations (Horton 1999, Horton and Lewis 1997, Krysan and Horton 1991). The only study conducted in Turkiye to date is the study bu Kosovaeri et al. (2014) in which they investigated the use of pheromone yellow sticky traps for the control of *C. pyri*. In this study, which was carried out in Ankara in 2012, the applicability of the mass trapping method using yellow sticky traps was investigated in order to create an alternative control method for the chemical control of *C. pyri* adults.

MATERIALS AND METHODS

The study materials included *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) adults, Ankara pear-variety saplings, and yellow sticky traps (20×25 cm). The 2012 study was carried out in three different orchards containing pear saplings. Two orchards were located in the Atatürk Forest Farm (AFF) and third one was located in the other experimental area in Ankara. The study was planned in two stages. In the first stage of the study, trap activity was determined, while the second stage involved a mass trapping study to determine the number of traps that could be considered effective.

Determination of trap efficiency

Yellow sticky traps (20 × 25 cm) were used to determine trap efficiency for use in *C. pyri* (L.) control, with studies initiated in 2012 in an orchard of Ankara pear-variety saplings aged 3–4 years in the experimental area. Accordingly, two yellow sticky traps were hung to monitor the emergence of the first adult, and after the first adult was captured on the trap, the experiment to monitor yellow sticky trap efficiency began. The experiment was initiated on 22 May 2012 when the first adult was identified in the monitor traps in the orchard selected for the experiment. The experimental setup was established according to the paired design and involved 10 replications with 1 trap/tree and 2 traps/tree. The traps were hung on the trees at a height of 1–1.5 m above the ground. The experiment considered two opposing trees as one replication. The traps were hung 1 m above the ground on two parallel lines. Then crossed over to form a transverse shape (in order of A1B1, B2A2, A3B3, B4A4, etc. and A and B represent respectively: 1 trap/tree and 2 traps/tree). A 15 m safety distance was left between the set-up tested traps (Anonymous 2010). The adults caught in the traps were counted weekly, and their numbers were recorded. Counting continued for nine weeks until 17.07.2012. Dirty traps were replaced with new traps.

Mass trapping studies

The study was conducted to investigate the applicability of the mass trapping method using yellow sticky traps for the control of *C. pyri* and was carried out in 2012 in the pear sapling orchard of the Atatürk Forest Farm (AFF). A 2-trap per tree application was found to be effective in the trap efficiency experiment, and was tested by comparing with control parcel to conduct mass trapping studies. After the first adult was identified in the yellow monitoring trap, the mass trapping studies began. Studies were carried out on two different parcels containing 450 saplings each within the AFF. One of the parcels was used for the mass trapping experiment, while the other was 500 m away, and was kept as a control. In the experimental parcel, two traps were hung on each sapling, 100 cm above the ground. Mass trapping trial was set up according to the large plot trial design. The experimental design consisted of 10 replications. In the control parcel, two yellow sticky traps were hung on two saplings to monitor presence the pest population. The traps were checked weekly, the number of trapped adults were recorded and any dirty traps were replaced with fresh traps. Data on temperature, relative humidity, and precipitation for 2012 in the Ankara-Center, where the studies were conducted, were obtained from the General Directorate of Meteorology. 10 saplings were considered as 10 replications and on each sapling 10 shoots (2 sprouts in each of 4 different directions and in the middle) were counted weekly. By this way 100 sprouts were counted totally on each sapling to determine the effect of mass trapping in decreasing the level of *C. pyri* infestation on the pear saplings. Sprouts with honeydew dripping were considered to be infested. The number of damaged sprouts was determined for the calculation of the infestation rate. The collected data was assessed using an appropriate statistical analysis to evaluate the success of the application.

Statistical assessment

In the trap efficiency experiments, the numbers of adults caught in the test traps were subjected to a t test to identify any difference in activity between the two trap set-ups (one trap/tree and two traps/tree). Following an analysis of variance, Duncan's test was used to determine the level of significance of the differences between set-ups. A count was made of the damaged sprouts in the mass trapping experimental parcel and in the control parcel, and the results were analyzed with a Chi-square test to assess whether the difference between set-ups was significant. The statistical assessment of the collected data was made using SPSS software.

RESULTS AND DISCUSSION

Trap efficiency

In the first stage of the trapping study, conducted in 2012 to identify an alternative approach to the control of *Cacopsylla pyri* (L.), trap efficiency was established. For this purpose, an experimental setting was created with a paired design on 22 May in the experimental area. The mean numbers of *C. pyri* adults caught in the yellow sticky traps in the trap activity (2 traps x 1 trap) experiment are presented in Table 1 and Figure 1. As seen in Table 1 and Figure 1, the adult population of *C. pyri* peaked on 26 June and 3 July among the counting dates. Collected data revealed no statistically significant difference between the traps in terms of the total number of *C. pyri* adults caught in the parcels throughout the season (t= -0.862; p>0.05).

No difference was identified in the number of adults caught in the traps between the one trap/tree and two traps/tree set-ups, based on weekly counts. Although no statistically significant difference was found between the two set-ups, the two traps/tree approach was preferred for the mass trapping studies, as a greater number of adults in totally were caught in the two traps/tree set-up. Since the traps will be used when deciding to control of *C. pyri* in mature trees in the pear orchards in early period, two traps per tree character were preferred during the mass trapping studies. Yellow sticky traps might be used to determine the population status prior to making a decision on the control of *C. pyri*.

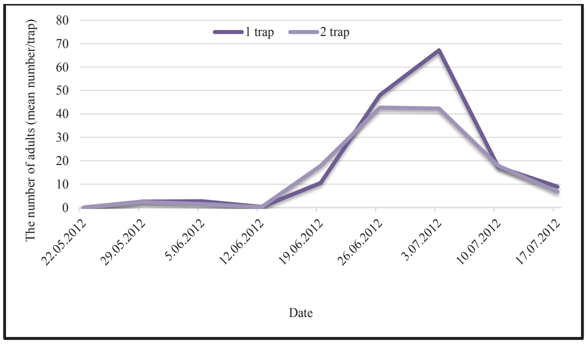

Figure 1. The number of *Cacopsylla pyri* (L.) adults caught in the yellow sticky traps in the trap experiment conducted in the Ankara province in 2012

Table 1. Average number of *Cacopsylla pyri* (L.) adults caught on traps in trap efficiency experiment in 2012

Counting Dates	Average number of adults caught in the trap (number/1 trap)	Number of adults caught in the trap (number/2 traps)
	Mean±St. Error (Min-Max)	Mean±St. Error (Min-Max)
29.05.2012	2.60±0.89 (0-10)	2.45±0.42 (0-10)
05.06.2012	2.70±0.78 (0-8)	1.5±0.38 (0-11)
12.06.2012	0.30±0.30 (0-3)	0.15±0.10 (0-3)
19.06.2012	10.50±3.22 (0-27)	17.80±4.27 (2-126)
26.06.2012	48.10±10.60 (7-100)	42.70±7.36 (18-197)
03.07.2012	67.20±14.30 (0-141)	42.35±11.66 (17-328)
10.07.2012	17.30±5.77 (0-63)	17.85±4.87 (2-142)
17.07.2012	8.90±1.19 (4-16)	6.75±1.04 (2-27)

Table 2. The number of *Cacopsylla pyri* (L.) adults caught in yellow sticky traps in the mass trapping parcel in 2012

Counting Dates	Number of adults caught in the yellow sticky traps in the mass trapping parcel (total number/2 traps)										
	Trap number										
	1	2	3	4	5	6	7	8	9	10	Total
12.06.2012	Traps hung										
18.06.2012	116	100	66	54	17	52	49	64	39	31	588
26.06.2012	47	185	23	44	21	217	274	76	87	37	1011
02.07.2012	454	367	292	148	233	324	350	255	216	195	2834
09.07.2012	752	753	553	680	226	880	567	556	341	575	5813
16.07.2012	445	452	397	416	479	493	541	487	464	475	4649
23.07.2012	367	359	245	473	367	350	562	449	311	423	3906
30.07.2012	351	268	270	195	200	292	327	286	232	133	2554
Total	2532	2484	1846	2010	1543	2608	2670	2173	1690	1869	21425

It is reported that sticky traps can be useful when deciding upon the means of control of *C. pyricola*, although there are other factors affecting the *C. pyricola* density in the trap counts. The number of adults caught in traps hung on large mature trees has been found to be higher than those hung on young trees, which, it has been reported, may be attributed to the different light intensities to which small and large trees are exposed (Horton and Lewis 1997). A study using yellow sticky traps was conducted to establish the optimum spraying time against Diaphorina citri Kuwayama (Hemiptera: Psyllidae), which is harmful to citrus fruit and to estimate population density. Accordingly, 10 trees were selected to apply one trap/one tree and three traps/one tree set-ups, and weekly counts were performed. The population level was reported to be low in the three traps/one tree experiment, and changes in temperature and solar radiation besides sunlight were found to be effective in decreasing the number of adults caught in the traps (Hall 2009). In another study conducted with traps, it was reported that the number of winter form *C. pyri* adults caught in traps increased between morning and noon, were highest at midday, and decreased in the afternoon. Accordingly, *C. pyri* was reported to engage in greater flight activity in warm and sunny conditions than in cool and cloudy conditions (Brown et al. 2009, Horton 1994). In another study about use of sticky traps was referred that care must be taken in interpreting the results, used sticky traps as monitoring tools could underestimate the actual insect population in the field. Sticky traps are cumulative, but catching efficacy is affected by the position in the orchard and thus hamper the acquisition of correct results (Adams et al. 1983, Adams and Los 1989).

Mass trapping

The efficiency of two traps/tree set-up that was envisaged to be effective in mature pear orchard in the trap activity experiment was tested for management of *C. pyri* by mass trapping. The mass trapping experiment was carried out in two orchards in the Atatürk Forest Farm. A mass trapping experimental set-up was established in one of the orchards, while the other one was kept as a control. Yellow sticky traps were used for the mass trapping studies for the alternative control of *C. pyri*, with 20 traps hung on 10 trees, with 2 traps/tree, in the experimental orchard located in the AFF on 12 June 2012, when the pear trees were leafy. The numbers of adults caught in the traps during the mass trapping activity are provided in Table 2, in which it can be seen that the highest number of adults was caught and the population of summer generations peaked on 9 July in all replications. The data collected during the mass trapping study were assessed and the time x trap interaction could not be established ($p>0.05$).

For mass trapping studies only 2 yellow sticky traps were hung on the control character to monitor adults. The numbers of adults caught in the monitor trap in the control parcel are provided in Table 3.

Table 3. The numbers of *Cacopsylla pyri* (L.) adults caught in the yellow sticky traps in the control parcel in the mass trapping activity in 2012

Counting Dates	The number of adults caught in sticky traps in the control parcel (number/trap)
12.06.2012	Trap hung
18.06.2012	55
26.06.2012	73
02.07.2012	97
09.07.2012	36
16.07.2012	83
23.07.2012	56
30.07.2012	48
Total	448

The infestation rate counting was done during harvest. The infestation rate was determined as 87.67%±3.97 and 35.00%±4.98 in the mass trapping parcel and control parcel, respectively. The infestation rates in the mass trapping parcel and the control parcel are presented in Figure 2. The infestation rate in the control parcel was found to ve lower than in the mass trapping parcel, which we believe may be because the traps attracted *C. pyri* adults, while the increased adult density in the area led to an increase in the infestation rate.

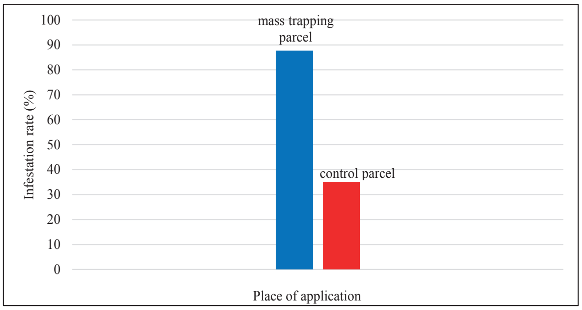

Figure 2. The infestation rate of *Cacopsylla pyri* in the mass trapping parcel and control parcel

Figure 3 presents the temperature, relative humidity and precipitation data for March-August 2012 in the Ankara province, where the study was conducted. The climate conditions were not observed to have a negative effect on the population development of the pest during our studies.

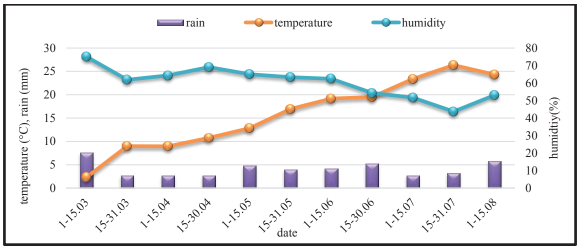

Figure 3. Temperature, relative humidity and precipitation data for March–August 2012 in the Ankara province

At the end of the study an analysis was done to make comparison. It was determined that the number of adults caught in the traps and the infestation rate revealed a negative correlation between the two ($r= -0.415$; $p<0.05$). In line with the findings of the present study, Cooper et al. (2010) found yellow sticky traps to have the potential to aid in the estimation of population size and in the determination of approaches to the control of *C. pyricola* before bud opening in the spring. Among the factors affecting sticky trap counts are such factors as the sex and reproductive status of the insect, the trap color and absence of foliage all of which are reported to affect population density (Horton 1999). Yellow sticky traps have been found to be helpful in estimating the population size of *C. pyricola* and in steering decisions on its control before bud opening in the spring (Cooper et al. 2010). There have also been studies conducted using yellow sticky traps to estimate population size (Hall 2009). A previous study utilized yellow sticky traps, transparent traps, and the beating method to sample the pear psyllid *C. pyricola*, and it was found that more adults were caught in the transparent trap on pear trees before bud opening in the early spring, while more adults were caught in the yellow trap after the green parts became evident. The captured adults were found to be mostly male (Krysan and Horton 1991). In the study by Kosovaeri et al. (2014) conducted in Turkiye regarding the use of yellow sticky traps alone for the control of *C. pyri*, it was demonstrated that yellow sticky traps were highly effective in catching *C. pyri* (L.) and Agonoscena pistaciae Burckhardt&Lauterer (Hemiptera: Psyllidae) adults. The authors, however, reported being unable to achieve a promising outcome in the control of *C. pyri* and *A. pistaciae* when yellow sticky traps and the pheromone formulation were tested together.

The present study, which was conducted to establish an alternative control method, found yellow sticky traps were considerably successful in attracting *C. pyri* adults, although they were also found to attract natural predator species from the Coccinellidae, Chrysopidae, Syrphidae and Vespidae families, as well as pollinator bees. Intended to enlighten an alternative approach to the control of *C. pyri*, the present study found yellow sticky traps lack the ability to sufficiently control the pest after population grew up, and did not lead to a decrease in the infestation rate in the bunches of flowers. It was observed during the study that the traps also attracted beneficial species, such as Coccinellidae. It was concluded that it would be appropriate to use yellow sticky traps only to monitor the first emergence of the adult and the population size in the early period. Use of traps would be appropriate when beneficial species are inactive and have a low population size, and when the *C. pyri* overwintering adults start to become active, and to use them before bud opening in the spring. As a conclusion, yellow sticky traps might be used to establish the population density prior to making a decision on the control of *C. pyri*. We believe that the findings of the present study will contribute to the implementation of integrated pest management programs in pear orchards to decrease the number of sprayings for the successful control of *C. pyri*.

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ÖZET

Bu çalışma ile armut ağaçlarında zararlı armut psillidi *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae) mücadelesinde sarı yapışkan tuzakların kullanımı ve kitlesel yakalama yönteminin uygulanabilirliği araştırılmıştır. Çalışmalar 2012 yılında Ankara ilinde Ankara armudu cinsi fidanların dikili olduğu iki farklı alanda bulanan üç ayrı bahçede yürütülmüştür. Çalışmanın ilk aşamasında tuzak etkinliğini belirlemek için görsel sarı yapışkan tuzaklarla deneme yapılmıştır. Etkili olduğu belirlenen tuzak sayısı ile kitle halinde tuzakla yakalama çalışmalarına geçilerek iki farklı bahçede denemeler yürütülmüştür. Çalışmanın sonucunda sarı yapışkan tuzakların *C. pyri* erginlerini çekme kapasitesinin oldukça yüksek olduğu, ancak dönem ilerledikçe zararlıyı baskı altına almada yetersiz kaldığı görülmüştür. Sonuç olarak, sarı yapışkan tuzakların tek başına *C. pyri*´nin mücadelesinde kullanılmasının yeterli olamayacağı, ancak faydalı populasyonunun aktif olmadığı ve kışlayan psillid erginlerinin bulunduğu erken ilkbahar döneminde ergin popülasyon yoğunluğunu takip etmek amacıyla monitör olarak kullanılabileceği kanısına varılmıştır.

Anahtar kelimeler: *Cacopsylla pyri*, armut, kitle yakalama, sarı yapışkan tuzak

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Original article

Seedling resistance of some bread wheat genotypes to *Fusarium pseudograminearum*

Bazı ekmeklik buğday genotiplerinin *Fusarium pseudograminearum*'a fide dönemi dayanıklılıkları

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A B S T R A C T

Fusarium pseudograminearum is one of the most damaging *Fusarium* species that causes root, crown, and foot rots in wheat. Identification of resistant germplasm is one of the most efficient and environmentally sound control methods. However, up to date, limited wheat genotypes with partial resistance are available. Therefore, in this study, the seedling resistance reaction of 200 bread wheat genotypes plus 6 control genotypes obtained from CIMMYT to *Fusarium pseudograminearum* was determined under growth room conditions. Out of the 200 tested genotypes; 1 (0.5%), 35 (17.5%), 112 (56%), 45 (22.5%), and 7 (3.5%) were resistant, moderately resistant, moderately susceptible, susceptible and very susceptible to *Fusarium pseudograminearum*, respectively. Resistant and moderately resistant genotypes could be used in breeding studies for developing crown rot-resistant cultivars.

INTRODUCTION

Wheat (*Triticum* spp.) is the main source of human nutrition and is grown in large areas in the world. Wheat grain is one of the most important carbohydrate sources used in human nutrition. Today, the main food source of nearly half of humanity is wheat. Flour, pasta, bulgur, and starch obtained from wheat are used in human nutrition. Wheat stems are used in the paper-cardboard industry, and as bran and straw in animal nutrition. Underground parts of wheat and stubble residues left in the field are an important source of organic matter. Wheat, the most planted plant type in the world, is the most planted and the most grain-produced cereal type in Türkiye (Geçit 2016, Süzer 2008).

India, China, Russia, the USA, Canada, Australia, Türkiye, Kazakhstan, and Ukraine are the world's largest wheat-producing countries (Anonymous 2019). In 2020, wheat is planted in 219 006 893 hectares of land with a total yield of 760 925 831 tonnes worldwide (Anonymous 2022a). In the same year in Türkiye wheat is planted in 6 922 236 hectares of land with a total yield of 20 500 000 tonnes (Anonymous 2022b).

There are many biotic and abiotic diseases factors affecting the wheat plant. Many fungi, bacteria, viruses, and nematodes can cause diseases in wheat plants (Bockus et al. 2010). *Fusarium*, causes diseases root, crown, and foot rot in cool climate cereals, has a large number of species and infects wide host ranges. Economically, they are very important and can be found in most parts of the world (Bockus et al. 2010, Booth 1971). It has been determined that *Fusarium* species cause root and crown rot disease as well as head blight (Bockus et al. 2010). Species belonging to *Fusarium* can be transported by soil and seeds. Factors such as climate, soil conditions, and ecological characteristics of the production area are important factors affecting the severity of the disease and yield. In addition, factors such as crop pattern in the production area, tolerance of the cultivars to the disease, tillage, fertilization, and fungicide use also affect the damage potential of the disease. The plant is most likely to become infected in an area contaminated with the pathogen. The severity of the disease increases in cases where the air temperature is high, the water content in the soil is low and the plant is under water stress (Ahmadi et al. 2022, Dababat et al. 2018, Smiley and Patterson 1996).

A number of *Fusarium species* are associated with the root, crown, and foot rots of wheat plants. *Fusarium pseudograminearum*, *F. culmorum*, and *F. graminearum* infect the stem base of wheat causing dry rot of roots, basal stem, and crown tissues. Necrosis is also observed (Bockus et al. 2010). The root, crown, and foot rot agents increase their effect with stress factors. Drought-stressed plants during anthesis are the most affected (Liddell et al. 1986). When suitable conditions occur root rot, crown rot, foot rot, and head blight cause significant yield reductions (Smiley and Patterson 1996). The most important sign of the disease is the browning of the roots, crowns, and stems of the infected plants. Honey brown necrosis can be observed on the leaf sheaths, crowns, and sub-crown internode regions of the plants. Pink-colored hyphal growth can also be seen in plant parts under humid conditions. The disease can also be distinguished in adult plants by the presence of whiteheads (Burgess et al. 2001).

Although *F. pseudograminearum* and *F. graminearum* are fungi that cause root rot in wheat, *F. graminearum* is mostly the causative agent of ear blight in wheat, while *F. pseudograminearum* is more dominant as a root rot agent (Chakraborty et al. 2006). *Fusarium* root, crown, and foot rot, caused by *F. pseudograminearum* (formerly *F. graminearum* group 1) (Aoki and O'Donnell 1999), is a cereal disease that occurs in many arid and semi-arid cropping regions of the world. Yield losses due to this disease have been recorded up to 35% in the Pacific Northwest (PNW) region of the USA (Smiley et al. 2005) and 25-58% in Australia (Chakraborty et al. 2010). Seedling blight can also occur (Bockus et al. 2010, Kazan and Gardiner 2018). This disease is also present in Türkiye (Gebremariam et al. 2018, Hekimhan and Boyraz 2011, Tunali et al. 2008, Yıldırım et al. 2016). Ölmez and Tunalı (2019) reported that *F. pseudograminearum* and *F. culmorum* were the most important crown rot pathogens in the Southeastern Anatolia region of Türkiye. These isolates constituted 13% of the isolated *Fusarium species.* Hekimhan and Boyraz (2011) and Gebremariam et al. (2018) also reported *F. pseudograminearum* causing root rot from the Thrace and Central Anatolia wheat fields in Türkiye. Management of *Fusarium* root, crown, and foot rots is difficult. Genetic resistance is the most promising and efficient way to control the diseases caused by soil-borne pathogens (Erginbas-Orakci et al. 2013, Gebremariam et al. 2020, Wallwork et al. 2004).

In this study, two hundred bread wheat (*Triticum aestivum* L.) genotypes obtained from CIMMYT, Mexico were screened under growth room conditions and their seedling resistance status was determined. In addition, 6 control genotypes (2-49, Altay 2000, Seri 82, Sunco, Süzen 97, Carisma) were also used in this study. We aimed to find new sources for resistance for *F. pseudograminearum* in bread wheat genotypes and to contribute to the usage of cultivars, especially in breeding programs.

MATERIALS AND METHODS

This study was carried out under controlled environment conditions at the Transitional Zone Agricultural Research Institute located in Eskişehir, Türkiye. *Fusarium pseudograminearum* isolate was obtained from International Maize and Wheat Improvement Center (CIMMYT)-Türkiye. Two hundred bread wheat genotypes were obtained from CIMMYT, Mexico. In addition, 6 control genotypes (2-49, Altay 2000, Seri 82, Sunco, Süzen 97, Carisma) were also used.

For inoculum production, oven bags (25 cm x 38 cm) were filled with 200 g wheat bran and humidified with 30 ml water, and autoclaved at 121 °C for 20 min for 3 consecutive days. Sterilized wheat bran was inoculated with *F. pseudograminearum* propagules and incubated for 4 weeks at 23 °C. Seeds were washed under running tap water and were placed into 1% NaOCl solution for 3 min and rinsed three times with sterile distilled water. Surface sterilized 8 wheat seeds were placed on the moistened blotting paper in sterilized Petri dishes and left for 3 days at 19 °C for germination. Germinated seeds were planted into the plastic tubes (16 cm height x 2.5 cm diam.) (Stuewe and Sons, Corvallis, OR, USA) containing sand: soil: animal manure (50:40:10 v/v/v). During the seeding, seeds were inoculated with wheat bran colonized by *F. pseudograminearum*. Each tube received 1 g of wheat bran containing $1x10^6$ *F. pseudograminearum* spores. Then these tubes were transferred to a controlled growth room. Each treatment was replicated 6 times and arranged in a randomized complete block design. The trial was repeated once for data validation.

Experiments were terminated 4 weeks after fungal inoculation. Roots were washed and evaluated for the resistance status using the Wildermuth and Mc Namara (1994) scale modified by Erginbas Orakci et al. (2018) based on the percentage of the browning of the crown region. In this scale, browning and rotting percentages were classified as followed: 1-9%= 1 (resistant), 10-29%= 2 (moderately resistant), 30-69%= 3 (moderately susceptible), 70-89%= 4 (susceptible) and 90-99%= 5 (very susceptible). Results were subjected to statistical analysis. Scale values were square-root transformed, and an analysis of variance was performed (JMP software (v 11), SAS Institute). For separation of means, LSD test was used.

RESULTS AND DISCUSSION

Wheat genotypes tested showed different reactions to the *Fusarium pseudograminearum* (Figure 1). Out of the 200 wheat genotypes tested; 1 (0.5%), 35 (17.5%), 112 (56%), 45 (22.5%) and 7 (3.5%) were resistant, moderately resistant, moderately susceptible, susceptible and very susceptible to *F. pseudograminearum*, respectively. The majority of the

Figure 1. Resistant (left) and susceptible bread wheat genotypes (right) at seedling stage under growth room conditions

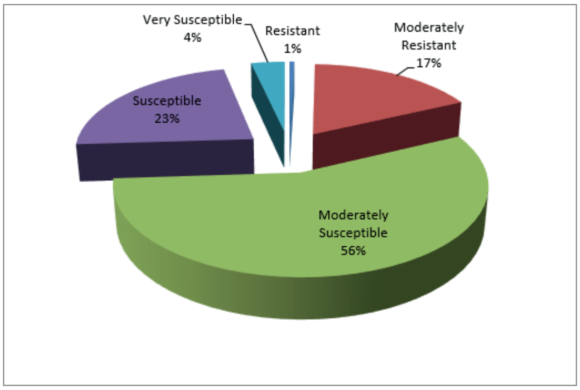

Figure 2. Seedling reaction percentages of 200 bread wheat genotypes to *Fusarium pseudograminearum*

genotypes showed moderately susceptible reactions to *F. pseudograminearum* (Table 1 and Figure 2).

Only one genotype (genotype no: 68) showed resistant reaction to *F. pseudograminearum* (scale value= 1.3). Genotypes 4, 117, 128, 183 (scale values of 2,3), 1, 2, 9, 30, 60, 86, 98, 102, 124, 141, 153, 162, 166, 177 (scale values 2.2), 123 (scale value 2) 29, 115, 157, 175, 187 scale values 1.8), 42, 63, 82, 89, 90, 150 (scale values 1.7), 8, 104, 154, 179, 180 (scale values 1.5) were placed in moderately resistant group. Control genotypes 2-49 (genotype no: 201), Altay 2000 (genotype no: 202) and Sunco (genotype no: 204) received scale values 2.2. Control genotype Carisma (genotype no: 206) received scale value of 1.8. These control genotypes were also placed in the moderately resistant group (Table 1).

Genotypes 3, 5, 7, 10, 11, 12, 15, 21, 24, 26, 27, 28, 32, 33, 34, 35, 38, 40, 41, 45, 46, 48, 49, 50, 51, 52, 53, 54, 56, 57, 58, 61, 62, 64, 65, 66, 67, 69, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 83, 84, 87, 91, 92, 93, 95, 96, 97, 99, 100, 101, 103, 106, 109, 110, 111, 112, 113, 114, 116, 118, 119, 125, 126, 127, 129, 130, 132, 133, 137, 138, 139, 140, 144, 146, 147, 149, 151, 156, 158, 160, 161, 163, 164, 165, 167, 168, 169, 170, 172, 178, 181, 184, 189, 190, 191, 193, 195, 196, 197, 198 and 200 exhibited moderately susceptible reactions to *F. pseudograminearum*. Majority of the genotypes were placed in this group (Table 1). Bread wheat genotypes 4, 6, 13, 16, 17, 18, 19, 23, 25, 31, 36, 39, 43, 44, 47, 55, 59, 71, 85, 88, 94, 105, 107, 108, 120, 121, 122, 134, 135, 136, 142, 143, 155, 159, 171, 173, 174, 176, 182, 185, 186, 188, 192, 194, and 199 and control wheat genotypes Seri 82 (genotype no: 203) and Süzen 97 (genotype no: 205) showed susceptible reactions to *F. pseudograminearum* and genotypes 20, 22, 37, 131, 145, 148, and 152 exhibited very susceptible reactions to *F. pseudograminearum*.

Finding new sources of resistance to the root rot pathogens

Table 1. Seedling resistance of 200 bread wheat genotypes and 6 control genotypes to *Fusarium pseudograminearum* under growth room conditions. Wildermuth and Mc Namara (1994) scale modified by Erginbas-Orakci et al. (2018) (1-5 scale) was used for disease assessment

Genotype+	Scale value[1]	Resistance[2,3]
22	5[a]	VS
37, 145, 148	4.8[ab]	VS
20, 131, 152	4.5[abc]	VS
142, 143	4.3[bcd]	S
36, 107, 122, 176	4.2[cde]	S
71, 188	4[cdef]	S
19, 23, 43, 44, 94, 135, 159, 171, 192	3.8[defg]	S
203 (Seri 82)	3.8[defg]	S
6, 16, 31, 39, 55, 59, 88, 105, 121, 136, 155, 185,	3.7[efgh]	S
4, 13, 17, 18, 25, 47, 85, 108, 120, 134, 173, 174, 182, 186, 194, 199	3.5[fghi]	S
205 (Süzen 97)	3.5[fghi]	S
3, 28, 35, 38, 40, 66, 67, 75, 76, 80, 81, 91, 97, 137, 163, 165, 167, 170, 190, 193	3.3[ghij]	MS
11, 21, 56, 57, 61, 64, 69, 77, 84, 93, 95, 100, 103, 106, 113, 114, 118, 127, 130, 133, 138, 144, 147, 158, 160, 172,195	3.2[hijk]	MS
10, 12, 27, 32, 41, 50, 51, 52, 58, 74, 78, 79, 87, 92, 99, 110, 112, 126, 129, 139, 146, 149, 168, 189, 191, 200	3[ijkl]	MS
5, 15, 24, 34, 46, 48, 49, 53, 54, 62, 72, 73, 96, 116, 119, 151, 161, 164, 169, 181, 196, 197, 198	2.8[jklm]	MS
7, 70, 83, 109, 111, 125, 140, 184	2.7[klmn]	MS
26, 33, 45, 65, 101, 132, 156, 178	2.5[lmno]	MS
14, 117, 128, 183	2.3[mno]	MR
1, 2, 9, 30, 60, 86, 98, 102, 124, 141, 153, 162, 166, 177	2.2[nop]	MR
201 (2-49)	2.2[op]	MR
202 (Altay 2000)	2.2[op]	MR
204 (Sunco)	2.2[opq]	MR
123	2[opqr]	MR
29, 115, 157, 175, 187	1.8[pqrs]	MR
206 (Carisma)	1.8[pqrs]	MR
42, 82, 90	1.7[qrst]	MR
63, 89, 150	1.7[rst]	MR
8, 104, 154, 179, 180	1.5[st]	MR
68	1.3[t]	R

* Means followed by the different letters are statistically significant (P= 0.05)
1 Numbers are mean of 6 replications
2 R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, H= Susceptible, VS= Very susceptible
3 Resistant= 1-1.4, Moderately resistant = 1.5-2.4, Moderately susceptible = 2.5-3.4, Susceptible = 3.5-4.4,
Very susceptible = 4.5-5
+= 201= 2-49 control genotype, 202= Altay 2000 control genotype, 203= Seri 82 control genotype, 204= Sunco control genotype, 205= Süzen 97 control genotype, 206= Carisma control genotype

has been limited. In our current study, 1 and 35 genotypes exhibited resistant and moderately resistant reactions, respectively. Similar results were obtained with the seedling test done with another important wheat root and crown rot pathogen *Fusarium culmorum* (Gebremariam et al. 2020). They tested the seedling reactions of 165 spring wheat lines obtained from CIMMYT, Mexico under growth room conditions using an aggressive isolate of *F. culmorum*. In their study, 2 and 20 lines exhibited resistant and moderately resistant reactions, respectively. Similar to our current results, the majority of the lines showed moderately susceptible and susceptible reactions to *F. culmorum*.

Farmers will benefit from growing resistant and tolerant cultivars and genotypes. Resistance to this disease is limited and some genotypes show tolerant reactions (Kazan and Gardiner 2018). Resistance breeding should focus on obtaining resistant cultivars preferably containing resistance to a few root rot pathogens at the same time. In this study, we identified some bread wheat genotypes showing resistant or moderately resistant responses to *F. pseudograminearum*.

Different researchers investigated the resistance status of wheat plants against root and crown rot disease caused by *F. pseudograminearum* in their studies using different wheat genotypes. Wildermuth and Mc Namara (1994) determined the resistance of 28 different wheat genotypes against *F. pseudograminearum*. They used a scale of 0-4 in their study to determine resistance and the line 2-49 received a scale value of 1.7 and was determined as resistant. In our current study, 2-49 bread wheat line, which was also used as a control genotype, received a 2.2 scale value and was placed into a moderately resistant group to *F. pseudograminearum* under controlled conditions. Wallwork et al. (2004) observed the resistance status of bread and durum wheat genotypes against *F. pseudograminearum* and *F. culmorum*. In their study, the bread wheat line 2-49 showed good resistance against *F. pseudograminearum*. It was also determined that the bread wheat cultivar Sunco was sufficiently resistant to *F. pseudgoraminearum*. In the present study, Sunco and 2-49 bread wheat genotypes were determined as moderately resistant to *F. pseudograminearum*.

In another study carried out by Mitter et al. (2006), the resistance status of 19 different wheat genotypes to *F. pseudograminearum* was determined. Sunco and Lang cultivars were determined as the most resistant cultivars against the disease.

Li et al. (2008) evaluated different wheat genotypes using different inoculation methods of *F. pseudograminearum*. They found that the two bread wheat genotypes, Sunco and 2-49, were resistant with scale values of 2.16 and 2.05, respectively. This was in agreement with our current study for both genotypes.

Erginbas Orakci et al. (2016) reported in their study that Sunco, Altay 2000, and 2-49 genotypes were moderately resistant; Seri 82 genotype was susceptible to *F. pseudograminearum*. These responses agreed with the results obtained from our current study where Seri 82 control genotype had a scale value of 3.8 and was found as susceptible. In our current study, control genotypes 2-49, Altay 2000, and Sunco received scale values of 2.2 and showed a moderately resistant reaction against *F. pseudograminearum*.

In another study carried out by Demirci (2003), it was determined that *F. graminearum* caused high disease severity in 10 wheat cultivars, and only the Mızrak cultivar was moderately susceptible with a slight difference.

In conclusion, wheat genotypes resistant and moderately resistant to *F. pseudograminearum* were determined in our current study. One and 35 bread wheat genotypes were found resistant and moderately resistant to *F. pseudograminearum*, respectively. These genotypes are recommended for crosses in breeding programs.

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ÖZET

Fusarium pseudograminearum buğdayda kök ve kök boğazı çürüklüğüne sebep olan en tahripkar *Fusarium* türlerinden birisidir. Dayanıklı genotiplerin seçilmesi en etkili ve çevre ile dost bir mücadele yöntemlerinden birisidir. Günümüzde kısmi dayanıklılık gösteren sınırlı sayıda genotip bulunmaktadır. Bu çalışmada CIMMYT'den temin edilen 200 adet ekmeklik buğday genotipinin ve 6 adet kontrol genotipinin *Fusarium pseudograminearum*'a karşı dayanıklılık durumları iklim odası şartlarında tespit edilmiştir. 200 adet ekmeklik buğday hattının *Fusarium pseudograminearum*'a karşı 1 adedinin (%0.5) dayanıklı, 35 adedinin (%17.5) orta derecede dayanıklı, 112 adedinin (%56) orta derecede hassas, 45 adedinin (%22.5) hassas ve 7 adedinin (%3.5) ise çok hassas olduğu bulunmuştur. Dayanıklı ve orta derecede dayanıklı olarak bulunan genotipler ıslah çalışmalarında kök ve kök boğazı çürüklüğü hastalığına karşı dayanıklı çeşitler geliştirmede kullanılabilinir.

Anahtar kelimeler: ekmeklik buğday, *Triticum aestivum*, *Fusarium pseudograminearum*, hastalığa dayanıklılık, kök ve kök boğazı çürüklüğü

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